SDU
UNIVERSITY OF SOUTHERN DENMARK

HONEY

Analysis of phenolic acids and flavonoids in honey by HPLC

K-PTE-4 Semester project

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Submission date – 18-05-2017

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Abstract

The basis of this report is the analysis of different types of honey with HPLC in order to figure out the concentration of flavonoids and phenolic acids. The theory section includes concepts about the flavonoids and phenolic acids, theory about the HPLC machine and background theory for the statistical analysis. The experiment was made on four different honeys and the optimized method was performed only on one honey. Of the four different honeys, three different extractions were made, and of each extraction two test-samples were taken. The data was collected from HPLC analysis. The results were attained with statistical calculations to compare the concentration of phenolic acids and flavonoids in the honeys. These results have proven the coherence between the theory and the practical result.

Resumé

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1 Abbreviations/Symbols

In the following table is all the symbols and abbreviations, which are used in the report.

Symbol	Meaning
n	Numbers
μ_i	Mean
σ	Standard deviation
CV	Coefficient of Variation
α	Significance level
H_0	Null hypothesis
H_i	Alternative hypothesis
M_i	Mean
MS_w	Mean squared within
HSD	Honest significance difference
UV	Ultraviolet
HPLC	High-performance liquid chromatography
A	Absorbance
ε	Absorption coefficient
b	Light pathway
c	Concentration of compound
h	Peak height
w_b	Peak width
t_R	Retention time
t_M	Void time
t'_R	Adjusted retention time
R_c	Resolution
k	Retention factor
A_C	Asymmetry factor
A and B	Width from middle of peak to the edge of the peak
T_f	Tailing factor
TFA	Triflour acetic acid
ACN	Acetonitrile
S	Standard deviation
S^2	Standard deviation squared
R^2	Correlation coefficient
LOD	Lowest limit of detection

2 Introductory part

2.1 Introduction

This report is about phenolic acids and flavonoids in honey. The honey is analysed using HPLC-analysis and the aim is to optimize the HPLC-method. The optimization parameters are temperature, polarity of the gradient and the flow rate. The starting method was developed in cooperation with Xavier Fretté, and this method is optimized regarding the parameters stated above.
The optimized method and the results from the HPLC-analysis will be compared with the literature concerning the subject. The experimental progress is to optimize the method on only one honey, and then the optimized method will be used on four different kinds of honey. Two honeys from different origin, one from the city (C) and one from the country (D) and two honeys from the same origin but from different production year, one from 2010 (G) and one from 2015 (H). More details for the chosen honeys can be seen in appendix A.

With these results, there will be a discussion about the content of phenolic acids and flavonoids in the honeys, to see if there is any coherence or difference of those in the honeys.
The aim with the optimized method is to prove the presence of the phenolic acids and flavonoids in the four different honeys, and furthermore to quantify the phenolic acids and flavonoids. Determine accurately which phenolic acids and flavonoids the honeys contain with the six phenolic acid standards and two flavonoid standards. The phenolic acids used are 4-hydroxybenzoic acid, chlorogenic acid, syringic acid, caffeic acid, gallic acid and p-coumaric acid. The flavonoids are quercetin and rutin. These standards are analysed with the same optimized method as the honeys are run with.
The results will be analysed statistically by calculating the statistical error in the results with the null hypothesis, which states that the measurements from the HPLC analysis are equal to the mean of each group of honey. The alternative hypothesis is that the measurements are different from the mean.
The first HPLC analysis was run on May 2nd 2017 because of a broken UV lamp in the HPLC machines, it was not a possibility to run it any sooner.

In the end of the project, it will be considered how further work can be conducted in relation to the analysis, separation of the peaks in the chromatograms, and if it is possible to extract the phenolic acids and flavonoids with another method other than just dissolving it in water and acetonitrile.

2.2 Theoretical background

2.2.1 The chemical composition of honey

Honey derives from nectar. Nectar is a liquid and consists of sugar, acids, proteins, lipids, etc. As the composition of those compounds varies a lot it is difficult to measure the exact composition of honey. The type of sugars in honey varies a lot. In some honeys the main type of sugar is sucrose, in others glucose, and in others fructose. This depends on which flower the nectar originates from. The flavonoid content of honey also depends on what nectar it is made from. This means that honey from a specific origin has characteristic flavonoids from for instance a tree in that area. The amino acids and phenolic acids in the honey make the honey acidic. The pH value of honey is roughly 4. (Ball, 2007) (Tomás-Barberán, Ferreres, García-Viguera, & Tomás-Lorente, 1992)

2.2.2 Phenolic acids and flavonoids in general

Flavonoids:

Flavonoids are phenolic compounds. Flavonoids are major colouring component in flowering plants. The general structure for all flavonoids is showed in figure 1.

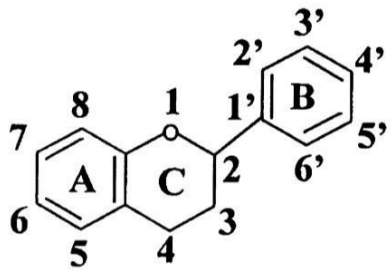
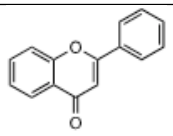
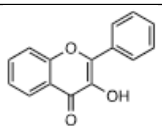
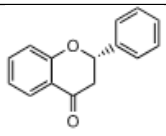
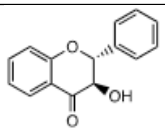
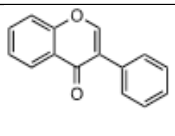
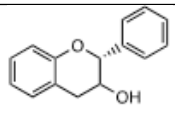


Figure 1 Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships (Heim, Tagliaferro, & Bobilya, 2002)*

Flavonoids are separated into different classes according to the arrangement of hydroxy- and methoxy groups. Table 1 below shows the different types and the backbone structure of the different flavonoids. In food flavonoids are often responsible for the colour, taste, prevention of fat, oxidation and protection of vitamins and enzymes. (Heim, Tagliaferro*, & Bobilya, 2002)

Table 1 Chemistry and biological activities of flavonoids: an overview

Group of flavonoid	Flavones	Flavonols	Flavonones	Flavononol	Isoflavones	Flavan-3-ols
Backbone structure						

Phenolic acid:

Phenolic acid is a subclass to a larger group called phenolics. Phenolics are aromatic rings with at least one hydroxy group, while a phenolic acid also contains an acid group that can differ as showed in figure 2. Phenolic acids are aromatic secondary plant metabolites. (Robbins, 2009)

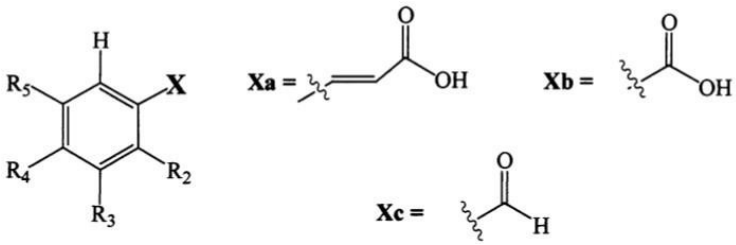


Figure 2 General structure of phenolic acids (Robbins, 2009)

2.2.3 Content of Phenolic acids and flavonoids

As described in the section about flavonoids in general, it is stated that flavonoids are the compounds that give yellow plants their colour, and this is the case with honey as well, this has a rich yellow colour, and the darker the honey is the higher the concentration of phenolic components (Pyrzynska & Biesaga, 2009). Furthermore, honeys content of phenolic acids and flavonoids, which are commonly defined as polyphenols, also serves as antioxidants in honey and it is also used to determine the geographical origin of the honey, because different flowers contain different concentrations of polyphenols (Pyrzynska & Biesaga, 2009).

Honey contains lots of sugars and these sugars surround the polyphenols, especially the flavonoids, which makes it difficult to isolate the polyphenols, because removing the sugars will remove a big part of the phenolic acids and flavonoids. (Campone, et al., 2014) The honey will therefore, only be diluted for the HPLC machine to run. The method of the HPLC analysis is seen in the section "Starting method", but the main aspect is that the mobile phase must be polar because phenolic acids and flavonoids are polar. Table 2, shows some of the polyphenols that honey can contain.

Phenolic acids	Flavonoids
Cis, trans-abscisic acid	Apigenin
Caffeic acid	Chrysin
Ferulic acid	Hesperetin
p-coumaric acid	Pinobanksin
Syringic acid	Quercetin
Vanillic acid	3-methylquercetin
p-hydroxybenzoic acid	Galangin
Gallic acid	Kaempferol
Vanillic acid	8-methoxykaempferol
Chlorogenic acid	Luteolin
Cinnamic acid	Myricetin
Ellagic acid	Pinocembrin
o-coumaric acid	Rutin
m-coumaric acid	Isohamnetin
p-coumaric acid	Tricetin
	Genkwanin

Table 2 Table of phenolic acids and flavonoids in honey. It might not be a complete table, but this table contains the most documented compounds in honey. (Campone, et al., 2014), (Pyrzynska & Biesaga, 2009), (Andrade, Ferreres, & Amaral, 2006), (Michalkiewicz, Biesaga, & Pyrzynska, 2008)

In the literature, science reports and journals, there were used different columns throughout their experiments, in this project though, the only used column is an C18-column. This may cause that some of the compounds listed in table 1 are not visible. Honeys from different geographical origins also do not contain all the same polyphenols, because this is, as mentioned in "The chemical composition of honey", defined from what plants grow in that particular area.

2.2.4 UV profile of phenolic acids and flavonoids

Ultraviolet (UV) spectroscopy uses electron transitions to determine bonding patterns.
The sample is irradiated with the broad spectrum of the UV radiation (185 to 400 nm.). If a particular electronic transition matches the energy of certain band of UV, it will be absorbed, hence there will be a "gap" (absorption spectrum). (Rouessac & Rouessac, 2007)
Figure 3 shows how the electronic transition absorbed the energy.

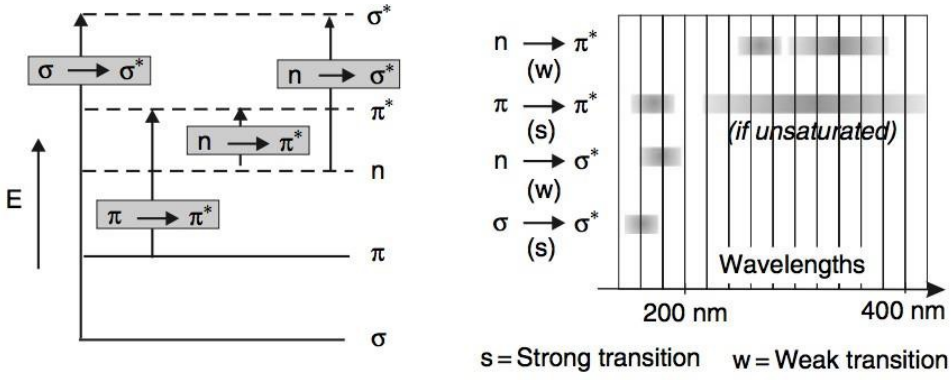


Figure 3 Four types of transition with the different spectra ranges. (Rouessac & Rouessac, 2007)

The instrument consists of three parts: a light source, a dispersive system and the detector. The light source is used for both the UV and the visible range of light. The dispersive system permits the extraction of a narrow interval of the emission spectrum, which makes the method more accurate. Finally, the detector converts the intensity of the light reaching it to an electrical signal. (Rouessac & Rouessac, 2007)

Using an UV-spectrum method, functional groups can be defined from the compound, which are analysed, by comparing the results obtained with the plots of the standards. Shown in figure 4 and 5.

Therefore, tables with the absorption values of different functional groups have been measured previously.

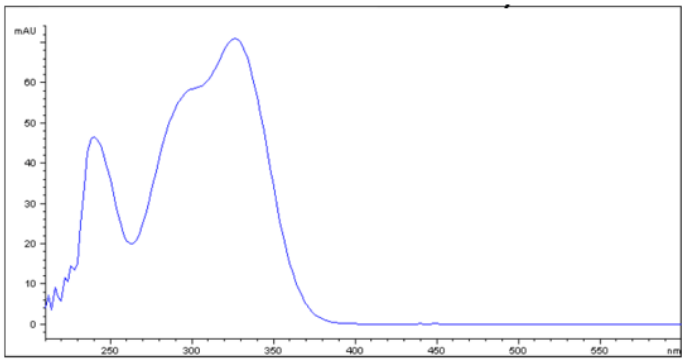


Figure 4., UV profile of the phenolic acid (Fretté, 2016)

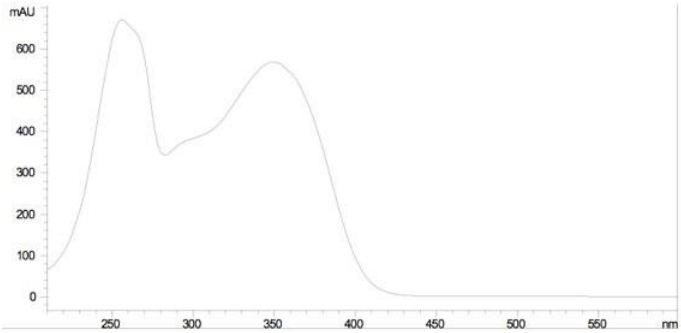


Figure 5., UV profile of flavonoid (Fretté, 2016)

2.3 Theoretical background for HPLC

2.3.1 Introduction

HPLC is an acronym for high pressure liquid chromatography, which is a method for analysing the composition of a mixture or identifying different compounds. HPLC is widely used in many big industries such as the pharmaceutical, food, chemical and environmental industry. Here it is used to account for purity, to identify or to separate compounds.

2.3.2 Description of HPLC equipment

A HPLC machine consists of three central parts - a mobile phase, a stationary phase and a detector.

Mobile phase

The mobile phase is the liquid, which carries the sample through the stationary phase and into the detector. The mobile phase can be made from different liquids with different polarities, and the composition often depends on the method used in the analysis. For this project, a combination of water and acetonitrile is used as mobile phase. A more specific description can be read in the section “Optimization of the method”. (Dong M. W., 2006)

Stationary phase

The stationary phase in HPLC is the column in which the sample compounds are separated. The column is a tube with a porous layer of sorbent on the inside, and the interaction between the sample compounds and the material of the column determines the retention time, and thereby the compound can be compared to a standard.

If the HPLC method used is supposed to be optimized, different parameters could be changed such as the flow rate, the pH of the mobile phase, the composition the mobile phase, the temperature of the column and the polarity of the column.

In the method, there are four kinds of modes that can be used regarding stationary and mobile phase. The modes are normal phase, reversed phase, ion exchange or size exchange. In the experiments carried out in this rapport, reverse phase is used. Reverse phase is set up with a nonpolar column and a polar mobile phase. Reverse phase chromatography is the most common mode for HPLC and is used in 70% of all analysis. The column used for the experiments is explained in the section "Material and methods". (Dong M. W., 2006)

Detector

When a compound exits the column, it enters the detector which detects the compound, measures the retention time and, depending on the detector type, other parameters.

The detector could for instance be an UV/VIS, that uses UV or visible light, which is the most common detector, because many compounds can absorb light. The detection with an UV/VIS detector takes basis in Lambert-Beer's law, which is shown below. A is the absorbance of the relevant compound, ε is the absorption coefficient, b is the light pathway and c is the concentration of the compound.

$$A = \varepsilon \cdot b \cdot c$$

The UV/VIS detector is very sensitive and can detect substances in the range of [ng] and [pg].

The UV detector creates a chromatogram containing UV profiles of the different compounds that are in the sample analysis, and these UV profiles can then be used to identify what kind of compound that is present. Theory on the UV profiles can be read in section "UV profiles of phenolic acids and flavonoids"

A detector extensively used in the detection of ions, acids and surface active compounds, is the conductivity detector, which measures the ability to lead an electric current of the sample compound. This detector is not suitable for this project. (Dong M. W., 2006)

Table 3 displays the coherence between different types of detectors and the substances they can detect.

Table 4.2. Common HPLC Detectors and Attributes

Detector	Analyte/attributes	Sensitivity
UV/Vis absorbance (UV/Vis)	Specific: Compounds with UV chromophores	ng–pg
Photo diode array (PDA)	Specific: Same as UV/Vis detectors, also provides UV spectra	ng–pg
Fluorescence (Fl)	Very specific: Compounds with native fluorescence or with fluorescent tag	fg–pg
Refractive index (RI)	Universal: polymers, sugars, triglycerides, organic acids, excipients; not compatible with gradient analysis	0.1–10 µg
Evaporative light scattering (ELSD)	Universal: nonvolatile or semivolatile compounds, compatible with gradient analysis	10 ng
Corona-charged aerosol (CAD)	Universal: use nebulizer technology like ELSD and detection of charges induced by a high-voltage corona wire	Low ng
Chemiluminescence nitrogen (CLND)	Specific to N-containing compounds based on pyro-chemiluminescence	<0.1 ng of nitrogen
Electrochemical	Very specific: Electro-active compounds (Redox)	pg
Conductivity	Specific to anions and cations, organic acids, surfactants	ng or ppm–ppb
Radioactivity	Specific, radioactive-labeled compounds	Low levels
Mass spectrometry (MS)	Both universal and specific, structural identification; very sensitive and specific	ng–pg
MS/MS		pg–fg

Table 3 The different kind of detectors and their ability to analyze different kinds of compounds. (Dong M. W., 2006, s. 88).

2.3.3 Parameters

The HPLC machine generates chromatograms based on the sample. In figure 6 a chromatogram with some of the important parameters used to optimize and then characterize the sample are shown.

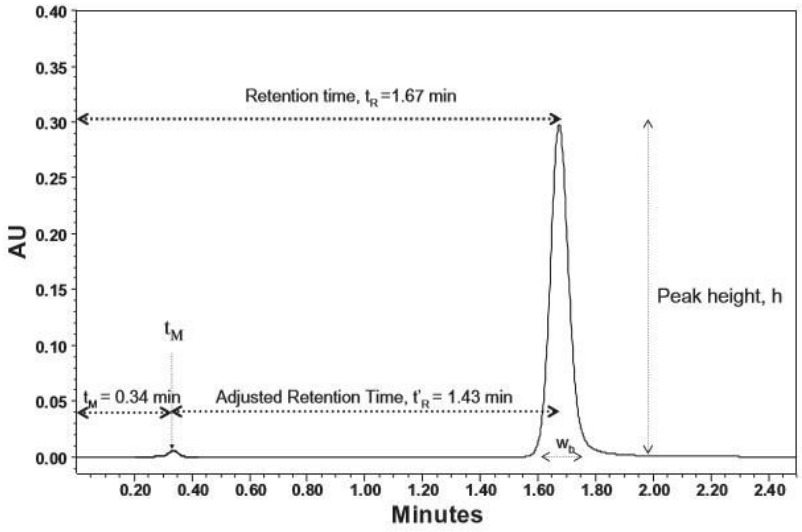


Figure 6 Chromatogram showing some of the basic parameters in HPLC (Dong M. W., 2006, s. 17)

The chromatogram is absorbance as a function of time. In the chromatogram, there is a line at absorbance equal to 0 and this line is called the baseline. When measuring from the baseline to the top of the peak the peak height (h) is found. The peak width (w_b) is found by measuring from the

start of the peak to the end of the peak on the baseline. Instead of using the peak width, the half peak width ($w_{½}$) is often used, because it is easier to measure, and this is the width at half the total height of the peak.

In the chromatogram, the retention time (t_R) is given as the total time for one compound from when the sample is injected to when it is carried out from the column (stationary phase) onto the detector by the mobile phase. The retention time can be calculated as followed.

$$t_R = t_M + t_R'$$

t_M is the void time, also known as the hold up time, and t'_R is the adjusted retention time. The void time is the time before the first baseline disturbance is observed. The void time is also the time the first compound takes (the mobile phase) to reach the detector and therefore also a factor for the time the compound takes to run through the stationary phase. The adjusted retention time is the time from the void time to the first peak. Each compound has different retention times because they interact differently with the stationary phase in the column.

Running a sample with different compounds through a HPLC machine, will typically give rise to several peaks depending on the separation and the retention time. Therefore, there are some parameters, which can be changed to create an optimal chromatogram. Typically, the chromatogram is good when the peaks look like a Gaussian peak. This means that they follow the Gaussian equation (Barnes, Jacques, & Vickers, 2006). The peaks also need to be well separated in order to see all the compounds. The run-time also needs to be as short as possible but still keeping in mind that the peaks need to be separated.

The separation of the peaks is, as explained, an important factor in a chromatogram to measure, because if the peaks are not well separated the compounds can not be quantified and it can be difficult to determine if there is more than one compound in the peak. This separation between the peaks is called the resolution (R_S) and is calculated with following equation.

$$R_S = \frac{2 \cdot (t_{R2} - t_{R1})}{W_{b2} + W_{b1}}$$

The resolution refers to the baseline separation, which means that if the resolution is good, the peak with retention time t_{R1} is "done" before the peak with retention time t_{R2} starts. The ideal value for the resolution is between 1.5 – 2.0. If the two peaks are not separated, the resolution will be equal to 0. If the resolution is equal to 0.6 the separation between the peaks is small. The minimum separation required for quantification is 1, which indicates a partial separation of the peaks. When the

resolution is 1.5 there is baseline separation. If the values are higher than 2 it will be required to optimize the method because the method is probably then unnecessary long.

Another way to look at the separation of the peaks is by looking at the selectivity (α), but to do this the retention factor (k) of the peaks needs to be calculated.

$$k = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M}$$

The retention factor is a value that describes the interaction between the compounds in the stationary phase compared to the mobile phases void time. Theoretically, the value of k should be between 1 and 20, and a value of over 20 would indicate that the compound is highly retained. With the retention factor the selectivity can be calculated.

$$\alpha = \frac{k_2}{k_1}$$

The value of α needs to be higher than 1. The selectivity factor is not the best method to find the separation on the baseline, because it only depends on the time takes the compounds to run through the column and not the peak width. By changing different parameters, like the mobile phase, the stationary phase or the temperature, the resolution and the selectivity can be manipulated.

A different factor to remember while looking at HPLC chromatograms is the shape of the peaks. If the peaks are not symmetrical, it is uncertain if there are more peaks hiding in the same peak. It is important that the peak follows the Gaussian peaks symmetry as much as possible. In figure 7 a variety of different peak shapes are shown.

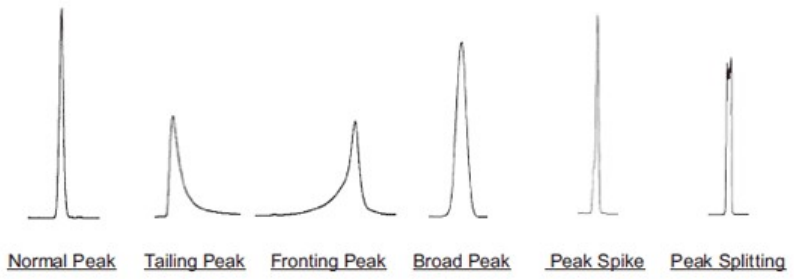
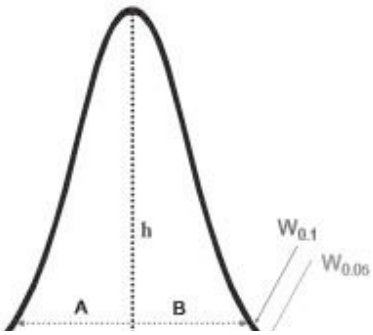


Figure 7 Different kinds of peak illustrations. The normal peak is the Gaussian peak. (T1. Poor peak shape, u.d.)

Normally the shapes of the peaks are not perfect. To measure the degree of symmetry in the peak the asymmetry factor (A_S) is calculated as followed:

$$A_S = \frac{B}{A}$$

How to measure the A and B value is shown in figure 8, and it is the width from the middle of the peak to the edge of the



Figur 8 Peak with the different parameters used to calculating the peak asymmetry and tailing factor (Dong M. W., 2006, s. 25)

peak to each side. A and B are measured at 10% ($W_{0.1}$) of the total peak height.

It can be seen in figure 7 that peaks also can be tailing or fronting. When looking at the degree of that the tailing factor (T_f) is used and can be calculated as followed:

$$T_f = \frac{W_{0.05}}{2 \cdot f}$$

Where the peak width at 5% ($W_{0.05}$) of the total peak height is used, and f is the total peak width at the baseline. If the tailing factor are equal to 1 the peak follows the Gaussian shaped peak. If the value is lower than 1 the peak is tailing, whilst if it is higher than 1 the peak is fronting. (Dong M. W., 2006)

Area of peaks in chromatogram

When a chromatogram is created by the HPLC machine each compound creates a specific peak depending on the compound's retention time. The area under the peak of the compound is therefore also directly coherent with the concentration of the compound in the sample analysed.

2.3.4 Parameters for optimization

Regarding the method used in the HPLC analysis, there are a lot of different parameters to vary. This could for instance be the mobile phase, the column temperature, the flow rate etc. Optimizing on the different parameters can be a good way of reaching method goals, which could be shorter run time or minimal sample work-up. The overall optimization goal should always be to get an adequate result for the lowest price.

Mobile phase

The mobile phase can be changed, to either running the column under isocratic or gradient mode. Isocratic is running one eluent during the whole analysis, where gradient is an ongoing changing in concentrations during the analysis. Isocratic works well if the analysis sample is simple in relation to constituents. If the sample is more complex, the gradient analysis is commonly used, because the polarity of the different compounds in the given sample might differ. The different concentrations over time can vary a lot when gradient analysis is used and it is often a method which takes longer time. The advantage of the gradient mode is that it enables the separation of every peak. This makes them easier to analyse. In the same time this method will make the resolution for early and late eluting peaks clearer. With the gradient analysis, it is also possible to have more peaks in the same chromatogram. (Dong M. W., 2006)

Temperature

Regarding the column temperature, enhancement of the temperature will reduce the retention of the peaks and will likely have a negative effect on selectivity. This negative effect on the chromatogram is ascribed to the lowering of viscosity caused by the temperature. (Dong M. W., 2006)

Flow rate

The flow rate affects the selectivity and retention in gradient, since more polar compounds will travel faster through the column if the mobile phase is polar too. If the analysis is carried out in the isocratic mode, the flow rate will not have any effect. (Dong M. W., 2006)

Injection volume

The injection volume can be changed if the concentration of the compound that one wants to find is not high enough. The higher injection volume, the higher amount of compound, and therefore a larger injection volume can lead to a better visualization of the peaks. (Dong M. W., 2006)

2.3.5 Starting method

In order to find out which temperature was the best to analyze honey at, a method was developed and then run at three different temperatures: 40 °C, 45 °C and 50 °C. Honey D was selected to test and optimize the method, simply because honey D were the most in quantity.
The method was run with the settings and parameters described below.

The honey was diluted 1:1 wt.% with a mixture of water and acetonitrile (ACN) 70:30, and heated for easier dilution. Gradient method was used with ACN, and water containing 0.05 wt.% TFA. Mobile phase gradient method ran at 3 min at 1% ACN which then continuously increases from 1% ACN to 99% ACN in 30 min thereafter it ran 5 min at 99% ACN, afterwards 1 min decreasing from 99% ACN to 1% ACN and finally 3 min at 1% ACN. Injection volume was performed with 10.000 [µL] and the flow rate of the mobile phase was 0.6 [mL/min]. The method was developed and discussed in cooperation with Xavier Fretté. Wavelength and bandwidth for the starting method is seen in table 4.

Signal	Wave length	Band width
A	250	4
B	254	4
C	300	4
D	320	4
E	360	4

Table 4 Table with the different wavelength run on the HPLC machine.

The honey solution was heated for better fluidization and filtered through a syringe filter 0.2 [µm] to remove any solid particles. The high content of sugar makes it difficult to analyse the flavonoids in honey by HPLC analysis. (Martos, Ferreres, & Toma´s-Barberan, 2000) Other studies in honey phenolic acids and flavonoids use extraction before analysing their samples. In this project it was decided in cooperation with Rime Bahij El-Houri not to use any extraction method and only dilute the samples with water and ACN because extraction might remove some of the flavonoids. (Andrade, Ferreres, & Amaral, 1997) (Martos, Ferreres, & Toma´s-Barberan, 2000) (Ferreres, et al., 1994) All other studies on the same subject used a C18-column so it was decided to use a C18-column. (Ferreres, et al., 1994) For more specific information about the equipment used look at chapter "Materials and method".

The main argument why the analysis was not run with an extraction of the phenolic acids and flavonoids was that if the sugars and other polar components were removed from the honey the some of the flavonoids might be removed as well, because sugar molecules surround the flavonoids. (Andrade, Ferreres, & Amaral, 1997)

2.3.6 Standards used for the HPLC analysis

For determination of some phenolic acids and some flavonoids, six phenolic acids and two flavonoids are used. These standards were used to determine a small amount of the phenolic acids and flavonoids by retention time, the others are only determined if they are a phenolic acid or a flavonoid using the UV profile.

The concentrations of the standards were calculated based on the weighed amount of the standards, which are then dissolved in 1000 g ultrapure water. Only quercetin was dissolved in ethanol. The amount of standard dissolved are decided on the basis of the solubility of each of the standards. Table 5 and 6 below shows the phenolic acids and the flavonoids used in the analysis:

Table 5. Phenolic acids used in the analysis as standards.

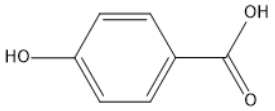
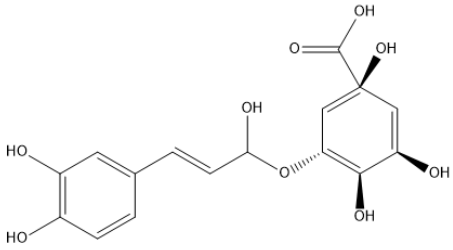
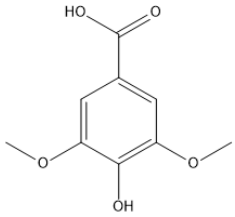
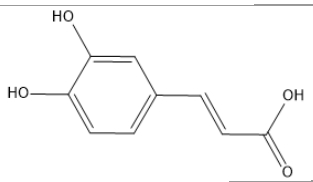
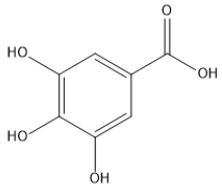
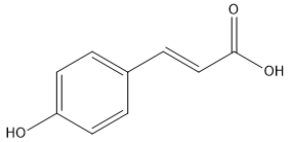
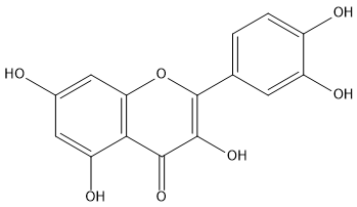
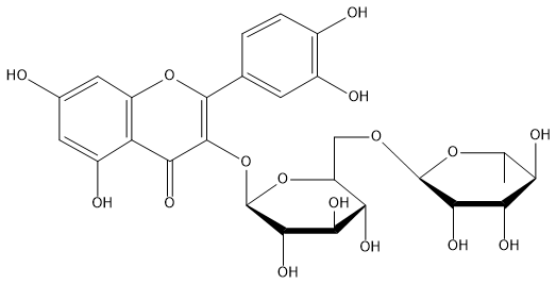
Phenolic acids	Chemical structure	Concentration of the standard
4-Hydroxybenzoic acid		4.5716 $\frac{mg}{g\ water}$
Chlorogenic acid		0.0393 $\frac{mg}{g\ water}$
Syringic acid		1.0727 $\frac{mg}{g\ water}$
Caffeic acid		0.5028 $\frac{mg}{g\ water}$
Gallic acid		1.0217 $\frac{mg}{g\ water}$
p-Coumaric acid		0.1044 $\frac{mg}{g\ water}$

Table 6. Flavonoids used in the analysis as standards.

Flavonoid	Chemical structure	Concentration of the standard
Quercetin		$0.0740 \frac{mg}{g\ ethanol}$
Rutin		$0.1229 \frac{mg}{g\ water}$

2.4 Theoretical background for statistics

First the basics of statistical analysis, descriptive measures, is described with the purpose of explaining the statistical analysis used for this project.

2.4.1 Descriptive measures

In this project, the data will come out as descriptive measures, which is data that describe a specific measurement. All data points from the data sets are random in the way that none of the parameters, set for the method used, are influencing the data to come out with a specific number and the experiment do not search for any specific numbers.

When getting a dataset of some sort, the mean distribution is a good way to start analysing the data.

$$\mu = \frac{\sum_{i=1}^{n} x_i}{n}$$

This is the sum of the data divided by the number of observations, n. If n is the total number of observations in the population, the mean of the population is denoted μ. If the mean is calculated for several observations, that is not the entire mean, the mean is denoted x. (Ellison, Barwick, & Duguid Farrant, 2009)

The data can deviate from the mean and this is called variance, a specific sample variance is the deviation of the data point from the mean, this indicates if the sample variance is big, the overall spread of the data is.

$$S^2 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$$

This also means that if the data spread is diverging from the mean it is not a good representation of the data used. (Ellison, Barwick, & Duguid Farrant, 2009)

The standard deviation is calculated by the square root of the sample deviation, as the following equation shows.

$$S = \sqrt{\frac{\sum^{n}_{i=1} (x_i - \bar{x})^2}{n-1}}$$

The practical value of the standard deviation compared to the sample variance is that the standard deviation has the same unit as the data used for the analysis. Sample variances unit is the squared unit of the data, which makes it harder to understand in complex data. (Ellison, Barwick, & Duguid Farrant, 2009)

The standard deviation is denoted as σ when it is for the whole population of the dataset. Another useful analysis is the standard deviation of the mean (Standard error) which calculates the variation of the mean of a standard variation.

$$s(\mu) = \frac{s}{\sqrt{n}}$$

This value is the variation of the mean from the data used for the standard deviation, and can also be explained as the average variation for the whole dataset instead of each data point. (Ellison, Barwick, & Duguid Farrant, 2009)

With this standard error the coefficient of variation, CV, can be calculated to measure the spread of the data.

$$CV = \frac{s}{\mu}$$

CV is a fraction or a percentage and shows how widespread the data used for calculating the mean is. (Ellison, Barwick, & Duguid Farrant, 2009)

2.4.2 Hypothesis testing

Hypothesis testing is a common way to check if obtained data has the same distribution or if the data represents the same population. Hypothesis testing can be used to determine whether there is a difference in the means or the variance, either among one or more groups. The result can be used to conclude if the difference in the variables are due to a systematic variation e.g. variation in temperature or if the variation is random. Hypothesis testing depend on how many samples there are. If there is one sample the hypothesis testing is based on the test of one mean or variance against a fixed value. If there are 2 samples the test is based on two means or variance. If there are more than

2 samples an ANOVA test is applied. In the start of the testing it is necessary to set a null hypothesis (H_0) which is a statement for the test data that typically says the data sheets are the same. After stating the null hypothesis an alternative hypothesis (H_1) is established which is applied if the null hypothesis is rejected. To determine which of the hypotheses to reject the significance level (α) is used. The α value is typically 0.05 and is also the highest value of uncertainty the data can have before the null hypothesis can be rejected. When the H_0 is rejected the probability of a certain outcome from the data is lower than the α value. Then the null hypothesis is not true and can be rejected. Rejecting the null hypothesis means that the alternative hypothesis will be accepted which means that statistical the decision is a better fit compared to the decision if the H_0 was accepted. (Pedersen, KPTE-4 ASED 8. lecture, 14.03.2017)

2.4.3 ANOVA – Analysis of Variance

ANOVA is a statistical method to determine the variance between groups of data. The basis of ANOVA is that the data set has similar factors that define the experiment data. The term factor describes the groups of the data. For instance, the factor in a data set could be a defined temperature or other parameter that is fixed for the experiment. Thereby an ANOVA analysis can account for the variance between an experiment carried out at 40 °C and one at 50 °C. The method used in ANOVA is comparison of the means in the groups. The zero hypothesis is therefore $\mu_1 = \mu_2 = \mu_i \ldots = \mu_k$ and the alternative hypothesis is $\mu_1 \neq \mu_k$, which means that the means differ from each other.

Degrees of freedom in ANOVA are, for the total number of samples, N, given as N-1. The number of degrees of freedom for the number of groups, p, are p-1.

In ANOVA, the square means of the groups are compared using an F-test, the principle of an F-test is comparison of variances in groups. In an F-test the typical zero-hypothesis is that the variances are equal, whereas the alternative hypothesis is that the variances are different. The F-ratio from the F-test are compared with a critical F-value, which is found in a table, for instance in Edison - Practical Statistics for the Analytical Scientist. (Ellison, Barwick, & Duguid Farrant, 2009, s. 212)

If the calculated F-ratio is smaller than the critical F-value, which is looked up in an appendix, the zero-hypothesis is accepted, and for the analysis of variance, this means that the means are equal.

The coherence between F-ratio versus critical F-value is given below in figure 9. Here the F-value is displayed at the x-axis, and it is showed that when the experimental F-value surpasses the critical f-value, the zero-hypothesis is rejected.

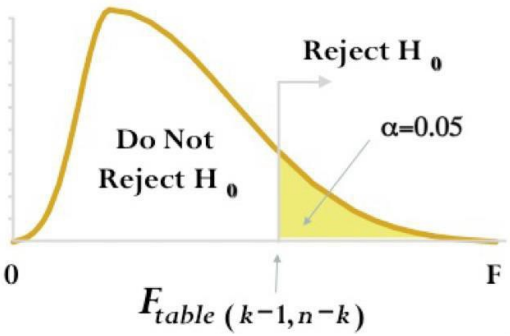


Figure 9 figure of f-value (Pedersen, KPTE-4 ASED 10. lecture, 31.03.2017)

The example with the two temperatures above represents a single factor ANOVA, here one parameter is fixed, in this case the temperature. If the experiment was carried out with variation on two parameters, for example temperature and pH, one could analyse this case with a two factor ANOVA. Here the output tells if the two parameters influences the samples, but also if the parameters affect each other. (Ellison, Barwick, & Duguid Farrant, 2009) (Pedersen, KPTE-4 ASED 8. lecture, 14.03.2017) (Pedersen, KPTE-4 ASED 10. lecture, 31.03.2017)

The parameters mentioned above can be summed up in an analysis of variance table as shown in table 7.

Table 7 ANOVA table (Ellison, Barwick, & Duguid Farrant, 2009)

Source of variation	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F**
Between-group	S_b	$p-1$	$M_b = S_b/(p-1)$	M_b/M_w
Within-group	S_w	$N-p$	$M_w = S_w/(N-p)$	
Total	$S_{tot} = S_b + S_w$	$N-1$		

2.4.4 Tukey test

The Tukey test is a post-test common used after applying ANOVA test. Since ANOVA test shows how significant the results are overall, but not clearly where these different means are. Thereafter, Tukey test is performed in order to compare specific means, to determine which specific groups of means are different, so it is possible to compare all means against each other.

Tukey test can be performed easily with R program, the mechanics behind following this equation:

$$HSD = \frac{\bar{X}_B - \bar{X}_A}{\sqrt{\frac{s^2}{n}}}$$

Where $\bar{X}_B - \bar{X}_A$ is the difference between of the pair of means. S^2 is the error mean square

- ANOVA in R program and n is number of groups. (Zar, 2014)

2.4.5 Linear regression

Linear regression is a method which establishes a relationship between two variables. In analytical chemistry, carrying this method out is very common to create the standard curves. (Ellison, Barwick, & Duguid Farrant, 2009) Therefore, a relationship is set between area of the peak of the standards and the concentration of them following this equation:

$$y = a + bx$$

The correlation coefficient (R^2), which measures the degree of linear association between the area and the concentration in the standards, should be pointed out. It is calculated by the chosen software e.g. Excel or R-studio. The closer R^2 value is to 1, the greater the relationship between the variables is. If 10% is the range of relative uncertainties then R^2 value must be above 0.99, in the same way If 1% is used R^2 value will be above 0.9999. (Ellison, Barwick, & Duguid Farrant, 2009)

2.4.6 Error in sampling

All measurement methods are subject to minor errors that affect the data produced. The errors arise from a number of different sources and cannot often easily be removed.
Stored materials have a risk of physical segregation, which means that the material is capable of packing in layers (stratification). When a sample is taken for analysis, it is important to ensure the material is as homogeneous as possible for the most valid data. Other effects may also lead to variation throughout the samples; storage, preparation, volatility, viscosity of the different components and so on are all parameters that may influence sampling. (Haines, 2002, pp. 10-13 and 21-26).

The number of measurements taken from the same sample may also vary and this variation is the instrumental error. The variations between different done samples by the same analyst give the error from the analyst, which is why for this project only one analyst made all the samples, so the error should be consistent. In addition, if there has been an error in producing a sample and subsamples from that sample is made, the error is carried through all the samples, so to ensure this did not happen a new sample was made for each HPLC test that was run.

2.4.7 Method of validation

Precision

Precision is defined as the similarity of the replication method and the original method and is often showed as standard deviation. The precision is determined by the conditions the replications are made in. There are different types of precision such as repeatability, intermediate precision and reproducibility. In this section only repeatability and intermediate precision will be described, because these are the precision used in this report.

The conditions under repeatability are that it should be the same analyst, using the same machine and all the results have to be collected over a short period, approximately only a day. This method is often used to find the variation in a single batch.

In intermediate precision, the results collected in the same laboratory but under more variable conditions than the repeatability precision. In intermediate precision the results can be collected over several days but it has to be in the same laboratory. It is not necessary that the same analyst makes all the samples.

Bias

Precision only describes the similarity of the replications but it does not tell how far it is from the true concentration. To tell how far the results are from the true value bias is used. Bias is evaluated from comparing the mean value from the experimental data to a reference value.

Accuracy

Accuracy is a combination of bias and precision. Accuracy describes how well grouped the test results are and how close they are to the true value.

Limit of detection

Limit of detection is the lowest reliable concentration of analyte that can be detected on the HPLC machine. The method to determine the limit of detection on the HPLC machine is preparing standards at different concentrations. The chromatogram shows if there is a detection of the wished analyte, but if there are no peaks from the analyte, the limit of detection is reached.

Limit of quantification

The limit of quantification is the lowest level of analyte in a sample that still can be quantified.

Limit of quantification is not always the same as the limit of detection. There could be a peak in the chromatogram but it can be quantified.

3 Main part

3.1 Materials and preparation of samples

Chemicals

Acetonitrile is used as solvent and purchased at Sigma-Aldrich Denmark and the standards of flavonoids and phenolic acids 4-Hydroxybenzoic acid, Chlorogenic acid, Syringic acid, Caffeic acid, Gallic acid, p-Coumaric acid, Quercetin and Rutin are also purchased at Sigma-Aldrich Denmark. The ultrapure water is obtained from Veolia water solutions & technologies the model is PURELAB chorus. The safety sheets for the used chemicals can be seen in appendix B.

Figure 10. Picture of the HPLC machine used for the project. HPLC 4..

Instrumentation

The HPLC machine used to analyse is an Agilent 1200 series, which can be seen at figure 10. The auto sampler is an Agilent 1200 series. The used column is a LiChrospher 5 µ RP-18 100A, 250·4.60 mm five micron and the column thermostat is an Agilent 1200 series G1316A. The thermostat is an ALS Thermostat G1330B. The binary pump is from Agilent 1200 series G1312A. The detector is a Diode Array and Multiple Wavelength Detector G1315D. The degasser is an Agilent 1200 series G1379B.

Honey preparation

For this project, each honey is dissolved in 70 w% ultrapure water and 30 w% acetonitrile (ACN) in the ratio 1:1, which means 10 g honey dissolves in 10 g of solvent. The solution is heated up in a warm water bath to get a better solubility of the different honeys and at the same time they were under magnetic stirring. The honey solutions are filtered through a 0.2 [µm] into vials for the HPLC machine.

3.2 Results

3.2.1 Optimization of the method

Method 1 - 3 is the first optimization run. This is an optimization of the temperature of the column. A sample was run at 40.0 °C, 45.0 °C and 50.0 °C and the optimal temperature was found to be 50.0 °C. In figure 11 the chromatogram at 50.0 °C is shown. The peaks are not fully separated and the method is therefore optimized.

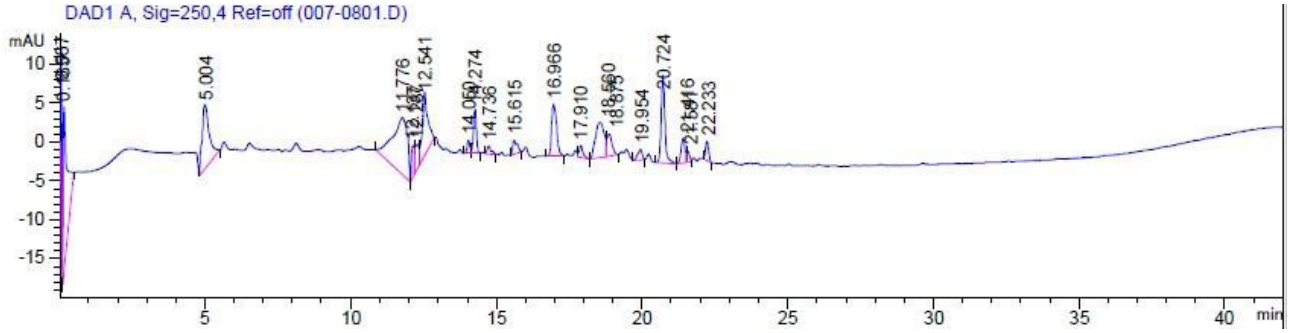


Figure 11 Chromatogram for the starting method at 50 °C

Method 4 - 13 was the optimization on the gradient. The start gradient was changed to get a better separation of the peaks. Method 7 had the best separation. However, the run time was too long compared to the time that it took all the compounds to travel through the column.

Method 14 and 15 optimized on the run time by changing the gradient to a shorter time. Before, the runtime at 1 % ACN was 6 minutes and it was changed to 2 minutes, and the runtime when raising the ACN conc. from 50% ACN to 99% ACN was 15 minutes. This was changed to 3 minutes. However the runtime at 1% ACN in the end was raised to 5 minutes instead of 3 minutes. The influence of the flow rate was tested with one analysis at 0.6 [mL/min] and 1 [mL/min]. The optimal flow rate was 0.6 [mL/min]. In these two methods one of the wavelength is also changed from 254 [nm] to 260 [nm] for better detection.

The optimized method was method 14 and is described below. The rest of the methods can be find in appendix C.

Method 14

50.0 °C – H_2O + Acetonitrile (ACN) – 1% - 99% gradient.

2 min 1 % ACN, 40 min 1% - 50% ACN, 3 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 5 min 1% ACN

Flow rate: 0.6 [mL/min]

Wavelengths: 250, 260, 300, 320 and 360 [nm].

3.2.2 The peak separation and symmetry

To check the seperation of the peaks of the chromatograms, some of the peak parameters are calculated in the next section. The peaks used for this are from the first chromatogram in D14T50, and it is seen in figure 12. The peaks indicated by the red box, are the peaks used.

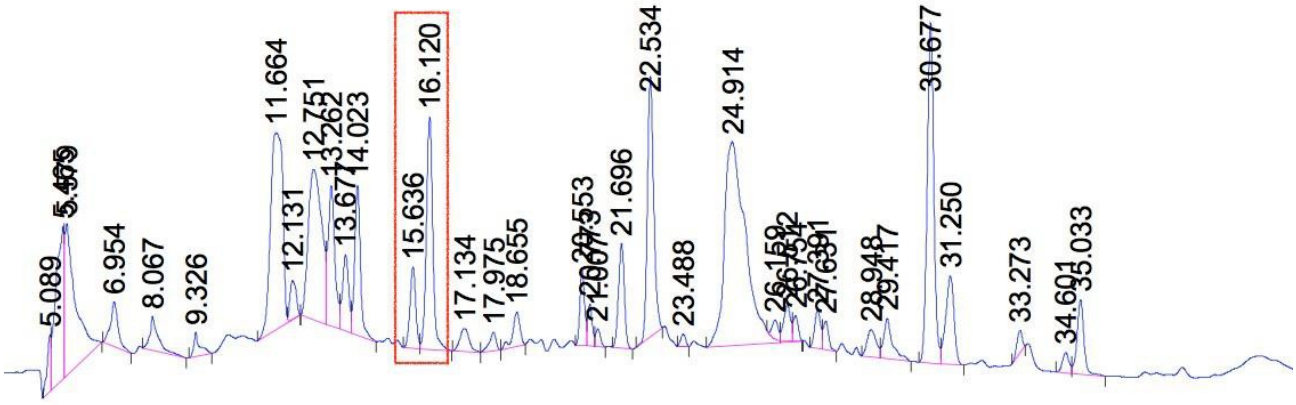


Figure 12 Chromatogram for method 14

Below the resolution of the two peaks is calculated. The resolution is an expression for the seperation of the peaks at the baseline.

$$R_S = \frac{2 \cdot (t_{R2} - t_{R1})}{W_{b2} + W_{b1}}$$

$$R_S = \frac{2 \cdot (16.120 - 15.636)}{0.57 + 0.44} = 0.96$$

The calculated resolution is a little below one, and as stated in the theoretical HPLC section, the resolution should be one, to be sufficient for quantification. These two peaks do not meet that requirement, but the value is close, so it is used.

Next the retention factors for the two peaks are calculated. The retention factor describes the relationship between the retention time and the void time. The void time read off the chromatogram as 5.089.

$$k_1 = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M}$$

$$k_1 = \frac{15.636 - 5.089}{5.089} = 2.07$$

$$k_2 = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M}$$

$$k_2 = \frac{16.120 - 5.089}{5.089} = 2.17$$

The value means that the compounds are not very retained, compared to peaks later in the chromatogram.

The separation of two peaks could also be looked at with the selectivity (α) and as stated in the theory section, the selectivity must be over one for separation of peaks.

$$\alpha = \frac{k_2}{k_1}$$

$$\alpha = \frac{2.17}{2.07} = 1.05$$

This value indicates that the peaks are separated.

To account for the symmetry of the peaks, the asymmetry factor, A_S, is calculated for both peaks.

$$A_S = \frac{B}{A}$$

$$A_{S-15.636} = \frac{0.81}{0.8} = 1.01$$

$$A_{S-16.12} = \frac{0.87}{0.8} = 1.09$$

This indicates that the peak with the retention time of 15.636 has a quite good symmetry, while the peak of 16.12 has a drag, which means that the peak is tailing.

In continuation of the asymmetry of the peak at 16.12, the tailing factor for this peak is calculated below. The properties of the peak are height = 0.8 and width = 0.1

$$T_f = \frac{W_{0.05}}{2 \cdot f}$$

$$T_f = \frac{0.095}{2 \cdot 0.1} = 0.475$$

This indicated, at seen in the asymmetry calculations that the peak is tailing.

3.2.3 Retention times for the standards and determination of these in the honeys

The standards were run through the HPLC machine with the optimized method and the retention times retained from these are seen in table 8. All standards expect S5 were dissolved in ultrapure water. S5 was dissolved in ethanol because it is not dissolvable in water.

Table 8. Retention times for the standards.

Standard	Standard number	Retention time [min] – at 300 [nm]
4-Hydroxybenzoic acid	S1	21.555
Chlorogenic acid	S2	22.037
Syringic acid	S3	24.259
Caffeic acid	S4	23.707
Gallic acid	S6	14.271
p-Coumaric acid	S7	27.597
Quercetin	S5	36.976
Rutin	S8	28.081

The chromatograms for the standards can be seen in appendix H.

All the upcoming pictures of chromatograms can be seen fully in appendix G. Each compound concentration was calculated from the standard curves, and these can be seen in section "Standard curves". The total amount of the phenolic acids and the flavonoids in the honeys is described in the section "Quantifications of phenolic acids and flavonoids the honeys", here there is only a description about which of the available standards there were in the analysed honeys. Only the chromatograms at 250 [nm] will be shown, the rest can be seen in the appendix, and the analysis on which standards that were in the honeys was done on sample 2 on each honey, so the results can differ a little bit from sample to sample within the same honey.

As it will be seen below, the standards (that means at the same retention time) found in the honey are not taken as a phenolic acid or a flavonoid because the UV profile did not match the UV profiles of either a phenolic acid or a flavonoid. The peaks that were determined to be a phenolic acid or a flavonoid can be seen in appendix E with the area and the concentration.

For **honey C** it will now be determined what kind of compounds there are in the honey. The chromatogram of honey C can be seen in figure 13

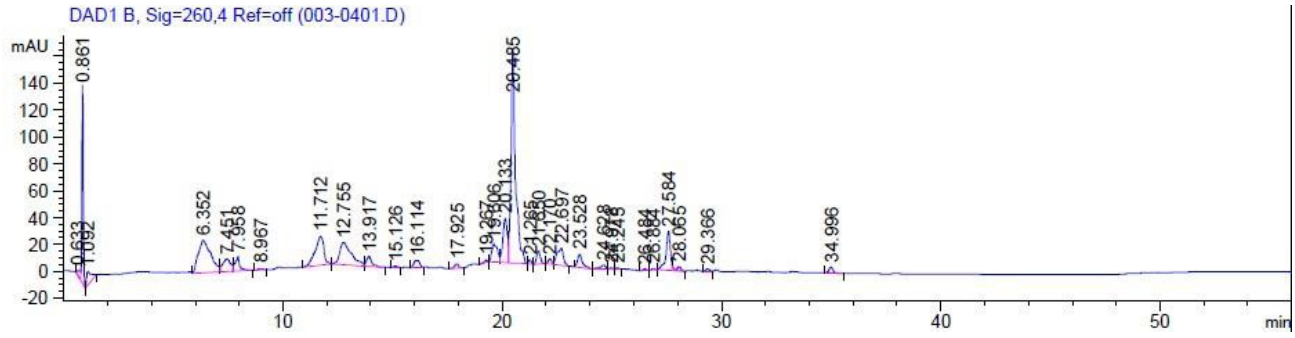


Figure 13. Chromatogram for honey C.

In honey C these standards were found as seen in table 9:

Table 9. Content of the standards in honey C.

Compound found	Retention time in chromatogram [min]	Area [mAU]	Concentration [mg/g water]
p-Coumaric acid	27.584	179.837	0.006
Rutin	28.070	138.377	0.004

Only p-Coumaric acid and rutin were found in the honey.

On the chromatogram, each peak was analysed to see if the peak was a phenolic acid, a flavonoid or a third compound. They were determined by their UV profiles. Some of these UV profiles for some of the peaks in honey C are shown in figures 14-17 here:

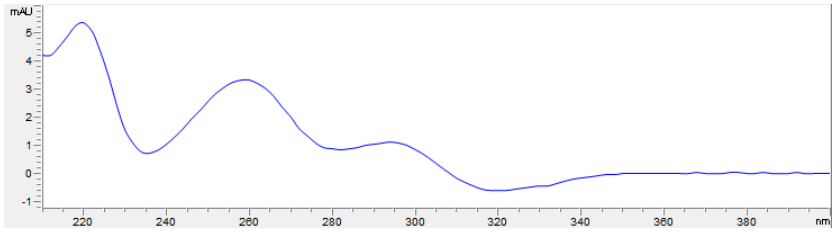


Figure 14. Peak with retention time 17.924 - Flavonoid.

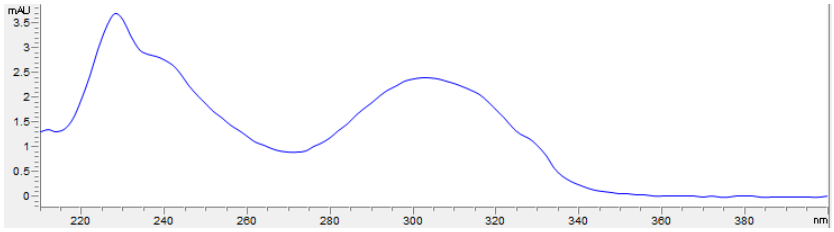


Figure 15. Peak with retention time 25.241 - Flavonoid.

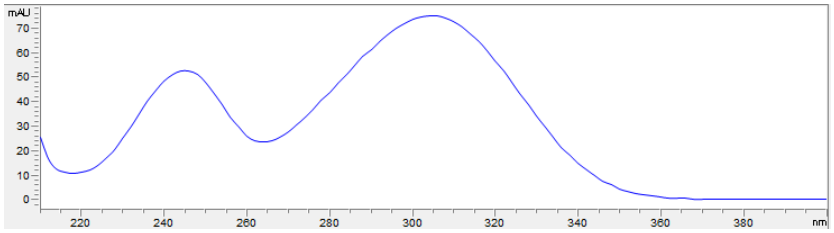


Figure 16. Peak with retention time 20.703 - Phenolic acid.

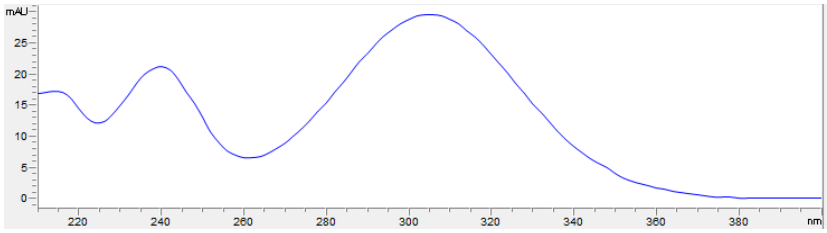


Figure 17. Figure 4. Peak with retention time 22.475 - Phenolic acid.

None of these peaks were determined as one of the standards.

In table 10 the rest of the standards in the different types of honey are shown. The rest of the chromatograms are shown in appendix D.

Table 10. Content of the standards in honey D, G and H

Honey	Compound found	Retention time in chromatogram [min]	Area [mAU]	Concentration [mg/g water]
D	**p-Coumaric acid**	27.598	21.447	0.002
G	**p-Coumaric acid**	27.605	14.205	0.002
H	**p-Coumaric acid**	27.609	247.842	0.007
	Rutin	28.095	77.123	0.003

Even though only p-Coumaric acid and rutin were found in the honeys, this does not mean there are not any other phenolic acids or flavonoids in the honeys. These were the only standards available for the project. The total amount of the phenolic acids and flavonoids are measured in section "Quantifications of phenolic acids and flavonoids the honeys" as mentioned before.

3.2.4 Standard curves

To quantify the total amount of phenolic acids and flavonoids in the honeys two standard curves were developed, one for the phenolic acids made with caffeic acid and one for the flavonoids with quercetin. Caffeic acid was dissolved in ultrapure water and quercetin was dissolved in ethanol due to better solubility. The standard curve for caffeic acid was used to calculate the total amount phenolic acids and the standard curve for quercetin was used to calculate the total amount of flavonoids.

The samples used to make the standard curves were made with different concentration spanned as wide as possible, from the highest to the lowest concentration the HPLC machine can detect. The standard curve samples were made quite a few times to detect the lowest limit on the HPLC machine.

The data from the HPLC machine for each of the analysis made for the standard curves can be seen in appendix I and J

The standard curve for caffeic is created from the data in table 11.

Table 11. Data for the standard curve on caffeic acid.

Concentration $[\frac{mg}{g\ water}]$	**Area of HPLC peak** $[mAU \cdot s]$
0.5028	$2.13 \cdot 10^4$
0.2514	$1.01 \cdot 10^4$
0.0503	2142.20
0.0050	207.19
0.0005	21.34

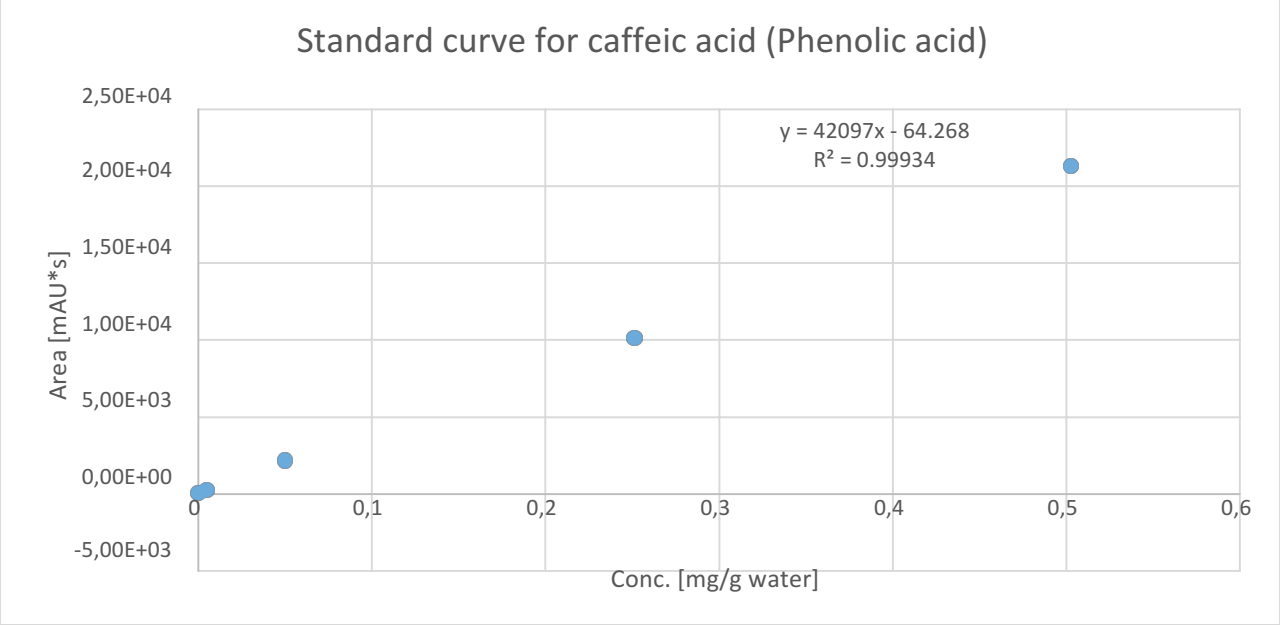


Figure 18. Standard curve for caffeic acid (Phenolic acids).

This standard curve looks proper as it has an R^2 value close to 1. The correlation has the function:

$$f(x) = 42097 \cdot x - 64.268$$

The standard curve for quercetin is created on the data in table 12 and these data create the standard curve in graph:

Table 12. Data for the standard curve on quercetin.

Concentration $[\frac{mg}{g\ ethanol}]$	**Area of HPLC peak** $[mAU \cdot s]$
0.0739	2960.7918
0.0369	1450.4911
0.0185	687.7634
0.0093	351.1922
0.0074	268.4907

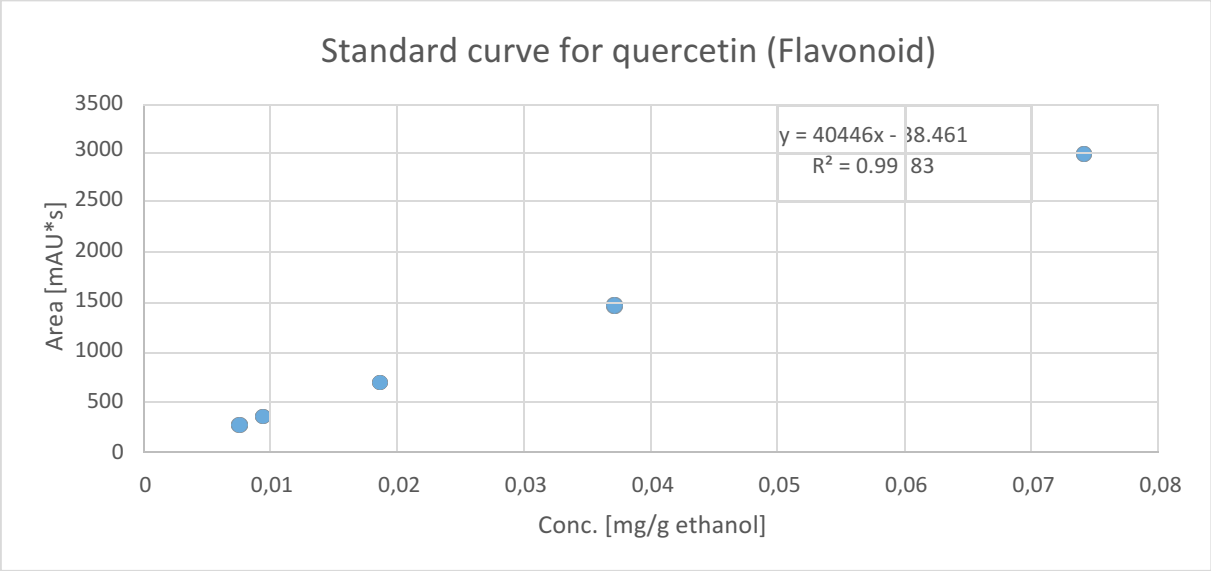


Figure 19. Standard curve for quercetin (Flavonoids).

This standard curve looks proper as it has an R^2 value close to 1. The correlation has the function:

$$f(x) = 40446 \cdot x - 38.461$$

3.2.4.1 LOD – lowest limit of detection

Synchronously with the making of the standard curve the limit of detection of the used HPLC machine were determined. The lowest concentration detected on the chromatogram was for quercetin, and the LOD can be seen in table 13.

Table 13. Lowest LOD on the HPLC machine by analysing samples above and under the lowest LOD.

Concentration $\left[\frac{mg\ quercetin}{g\ ethanol}\right]$	**Area**	**Detected? Yes/No**
0.0093	351.192	Yes
0.0074	268.491	Yes
0.00074	N/A	No
0.000074	N/A	No

The chromatogram for the concentration 0,00074 $\left[\frac{mg\ quercetin}{g\ ethanol}\right]$ can be seen in figure 20.

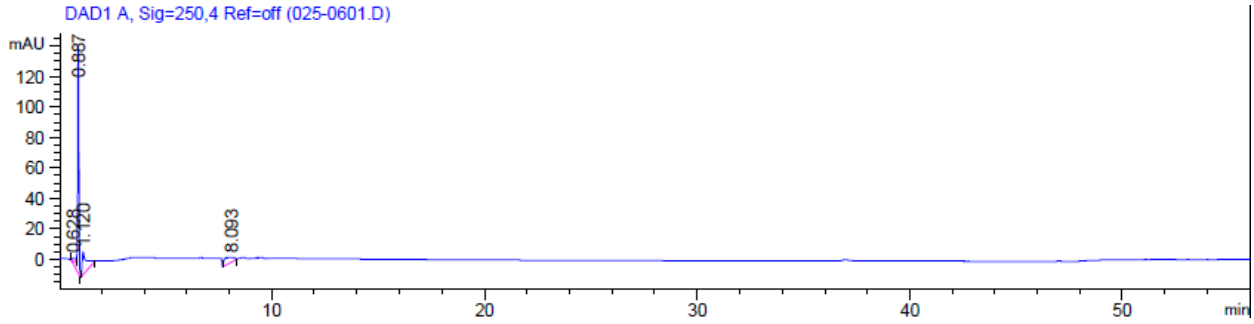


Figure 20. Chromatogram for quercetin at a concentration of 0.00074 mg quercetin/g ethanol.

3.2.4.2 Quantifications of phenolic acids and flavonoids in the honeys

For creating enough data to calculate the statistical difference in the honeys there were made three samples (Three vials) of each honey diluted as explained in section "Materials and method". From each of these vials there were made two tests, which means that each honey was analysed with six HPLC tests with the optimized method. The total amount of phenolic acids was calculated on the basis of the standard curve for phenolic acids created with caffeic acid. The total amount of flavonoids was calculated on the basis of the standard curve for flavonoids created with quercetin.
The datasheet with all the peaks that were distinguished as either a phenolic acid or a flavonoid can be seen in appendix E.

Honey C

In table 14 the amount of phenolic acids and flavonoids in each of the taken tests can be seen:

Table 14. Total content of phenolic acids and flavonoids in honey C.

Honey sample	Total area [mAU]	Total concentration [mg/g honey]
C1-1	Phenolic acids: 1058.7 Flavonoids: 202.3	Phenolic acids: 0.0267 Flavonoids: 0.0059
C1-2	Phenolic acids: 723.6 Flavonoids: 142.3	Phenolic acids: 0.0187 Flavonoids: 0.0045
C2-1	Phenolic acids: 902.6 Flavonoids: 115.9	Phenolic acids: 0.0229 Flavonoids: 0.0038
C2-2	Phenolic acids: 684.9 Flavonoids: 161	Phenolic acids: 0.0178 Flavonoids: 0.0049
C3-1	Phenolic acids: 671.5 Flavonoids: 149.7	Phenolic acids: 0.0175 Flavonoids: 0.0047
C3-2	Phenolic acids: 673.7 Flavonoids: 149.7	Phenolic acids: 0.0175 Flavonoids: 0.0047
Average conc. phenolic acids [mg/g honey]	0.0202	
Average conc. flavonoids [mg/g honey]	0.0047	

As it can be seen there is a big difference in the concentrations from sample to sample, and this is discussed and explained in both the discussion and in the statistical analysis. There are a lot more phenolic acids in honey C than flavonoids.

Honey D

Below in table 15 the amount of phenolic acids and flavonoids in each of the taken tests can be seen:

Table 15. Total content of phenolic acids and flavonoids in honey D.

Honey sample	Total area [mAU]	Total concentration [mg/g honey]
D1-1	Phenolic acids: 393.6 Flavonoids: 949.8	Phenolic acids: 0.0109 Flavonoids: 0.0244
D1-2	Phenolic acids: 394.0 Flavonoids: 943.7	Phenolic acids: 0.0109 Flavonoids: 0.0243
D2-1	Phenolic acids: 403.4 Flavonoids: 927.3	Phenolic acids: 0.0111 Flavonoids: 0.0239
D2-2	Phenolic acids: 402.0 Flavonoids: 925.0	Phenolic acids: 0.0111 Flavonoids: 0.0238
D3-1	Phenolic acids: 412.6 Flavonoids: 943.4	Phenolic acids: 0.0113 Flavonoids: 0.0243
D3-2	Phenolic acids: 411.8 Flavonoids: 951.4	Phenolic acids: 0.0113 Flavonoids: 0.0245
Average conc. phenolic acids [mg/g honey]	0.0111	
Average conc. flavonoids [mg/g honey]	0.0242	

As it can be seen above there are about twice as much flavonoids as phenolic acids in honey D.

Honey G

Below in table 16 the amount of phenolic acids and flavonoids in each of the taken tests can be seen:

Table 16. Total content of phenolic acids and flavonoids in honey G.

Honey sample	Total area [mAU]	Total concentration [mg/g honey]
G1-1	Phenolic acids: 641.1 Flavonoids: 51.4	Phenolic acids: 0.0168 Flavonoids: 0.0022
G1-2	Phenolic acids: 644.4 Flavonoids: 54.8	Phenolic acids: 0.0168 Flavonoids: 0.0023
G2-1	Phenolic acids: 640.4 Flavonoids: 49.7	Phenolic acids: 0.0167 Flavonoids: 0.0022
G2-2	Phenolic acids: 641.3 Flavonoids: 49.8	Phenolic acids: 0.0168 Flavonoids: 0.0022
G3-1	Phenolic acids: 651.2 Flavonoids: 53.6	Phenolic acids: 0.0169 Flavonoids: 0.0023
G3-2	Phenolic acids: 647.0 Flavonoids: 55.2	Phenolic acids: 0.0169 Flavonoids: 0.0023
Average conc. phenolic acids [mg/g honey]	0.0168	
Average conc. flavonoids [mg/g honey]	0.0022	

As it can be seen above there are about twice as much phenolic acids as flavonoids in honey G.

Honey H

Below in table 17 the amount of phenolic acids and flavonoids in each of the taken tests can be seen:

Table 17. Total content of phenolic acids and flavonoids in honey H.

Honey sample	Total area [mAU]	Total concentration [mg/g honey]
H1-1	Phenolic acids: 191.5 Flavonoids: 922.1	Phenolic acids: 0.0061 Flavonoids: 0.0237
H1-2	Phenolic acids: 193.3 Flavonoids: 918.2	Phenolic acids: 0.0061 Flavonoids: 0.0237
H2-1	Phenolic acids: 306.9 Flavonoids: 819.7	Phenolic acids: 0.0088 Flavonoids: 0.0212
H2-2	Phenolic acids: 189.3 Flavonoids: 942.3	Phenolic acids: 0.0060 Flavonoids: 0.0242
H3-1	Phenolic acids: 194.2 Flavonoids: 945.9	Phenolic acids: 0.0061 Flavonoids: 0.0243
H3-2	Phenolic acids: 195.1 Flavonoids: 951.1	Phenolic acids: 0.0062 Flavonoids: 0.0245
Average conc. phenolic acids [mg/g honey]	0.0066	
Average conc. flavonoids [mg/g honey]	0.0236	

As it can be seen above there are about four times as much flavonoids as phenolic acids in honey G.

The only outlier is honey sample H2-1, were there are more phenolic acids compared to the other samples, but that is because one peak (Retention time 21.69 min) is a phenolic acid in sample H2-1 but are a flavonoid in the other honey samples when looking at the UV profile.

3.2.5 Method of statistics analysis

The aim of the statistical analysis was to compare the data from the different honeys and to determine how accurate the data were compared to each other and if it was necessary to remove those data-points that differed a lot to figure out if the error could be minimized.

To calculate the error within the extractions and between the extraction single factor ANOVA test with two levels of replication was used. Here it was seen that extractions C1, C2 and H2 had a very big error regarding phenolic acids concentrations, which means that the instrumental error was very big. It is assumed that the error within the extractions (Instrumental error) is smaller than the error between the extractions (Analyst error), because the instrument is very precise, and with the data from extraction C1, C2 and H2 this was not the case. Therefore, to make this error as small as possible the data for these three data points were removed.

Once the data was removed, a single factor ANOVA test was used again, and this case the error within the extractions was smaller than the error between the extractions.
To compare the honeys mean values of phenolic acids and flavonoids to each other Tukey test was used. The means of the extractions were compared so that honey C and honey D were compared and honey G and honey H were compared, to see if there was any difference in the mean values. If the p-value of the tukey test is lower than the α-value, then the null hypothesis can be rejected and that means there is a difference in the mean values.

3.2.6 Statistical analysis of data

The statistical analysis which is seen below, is conducted in R. The output from the script is seen in appendix F and the most important values are given in tables below.

Phenolic acids

An ANOVA test with two levels of replication is carried out in order to figure out the analyst error and the error among the instrument regarding the concentration of phenolic acids. As it can be seen in the results of the ANOVA in the R program. The mean Square of the residuals, which is the same as the variance, is $3.69 \cdot 10^{-6}$ for the error among the analysts and $4.08 \cdot 10^{-6}$ for the instrumental error, which can be seen in table 18. These values are quite low, and this leads up to a p-value of $8.21 \cdot 10^{-6}$ and therefore the null hypothesis can be rejected. The error can be considered small enough, which tells that the results are acceptable.

Table 18. Results from ANOVA test in R phenolic acids concentrations.

Error: extract	**Mean Sq. (Variance)**	**Pr (<F)**
Source	$2.196 \cdot 10^{-4}$	$8.21 \cdot 10^{-6}$
Residuals	$3.690 \cdot 10^{-6}$	
Error: Within		
Residuals	$4.081 \cdot 10^{-6}$	

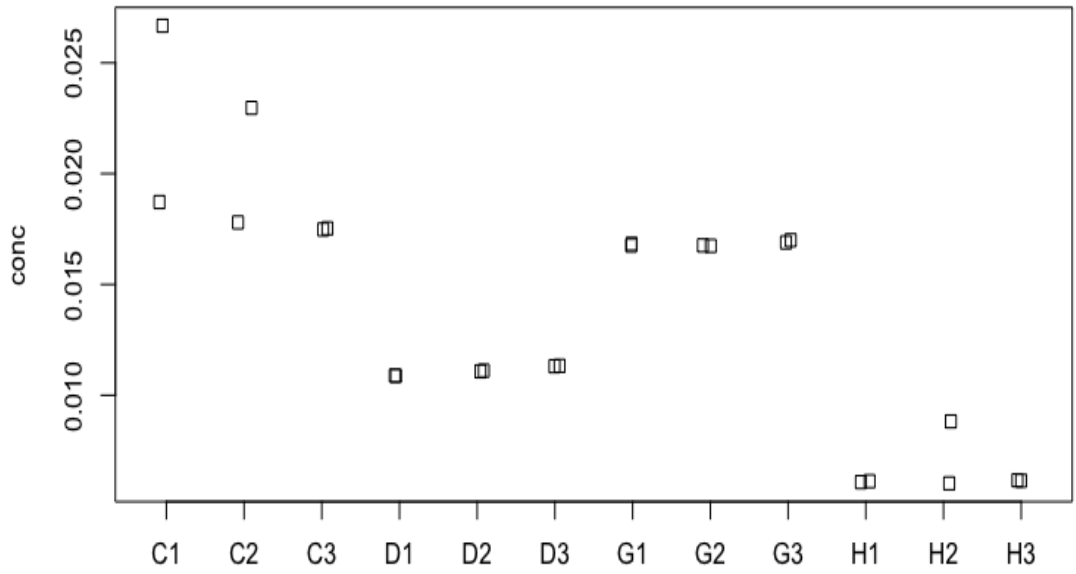


Figure 21. Stripchart of the extractions with their concentrations – phenolic acids.

Comparison of the two mean squares for the residuals and the error within, shows that they are very close. The instrumental error ought to be smaller than the analyst, because the machine should be more accurate than the person. As seen in figure 21 above, C1, C2 and H2 are outliers and below there are conducted another statistical analysis without these points.

The ANOVA test is carried out once more, but this time without these extractions, and the results can be seen in table 19 below. In this case, the results show the mean square of the error within, error regarding the injections, is lower than the error among the extractions. That makes good sense, as it is assumed that the HPLC machine is more precise than the analyst is. Here the p-value is also below 0.05 which means the null hypothesis also is rejected with extractions C1, C2 and H2 removed.

Table 19. Results from ANOVA test in R for phenolic acids concentrations without C1, C2 and H2.

Error: extract	**Mean Sq. (Variance)**	**Pr (<F)**
Source	$1.123 \cdot 10^{-4}$	$2.66 \cdot 10^{-8}$
Residuals	$5.000 \cdot 10^{-8}$	
Error: Within		
Residuals	$1.279 \cdot 10^{-9}$	

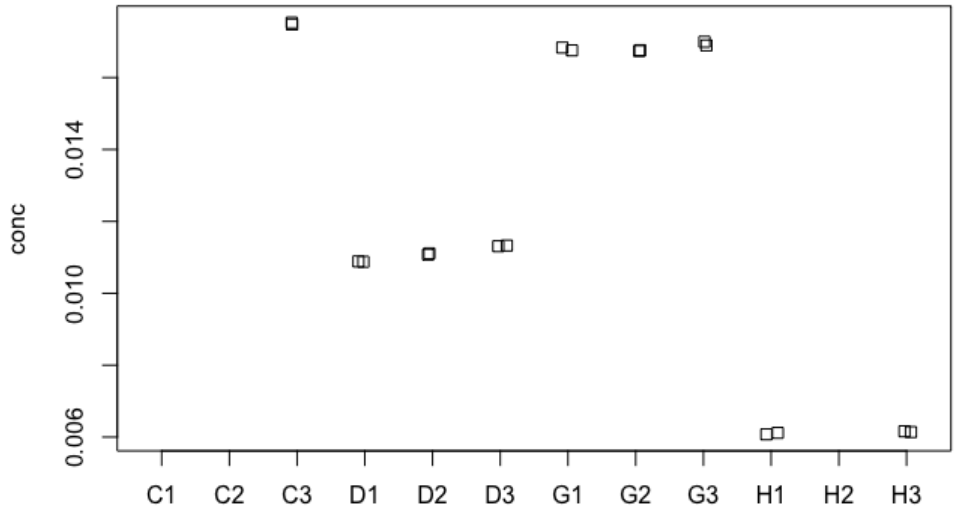


Figure 22. Stripchart of extractions with their concentration and without C1, C2 and H2 – phenolic acids.

Thereafter, a Tukey test is performed by R program in order to compare the honeys with each other. In this project, only the comparison between C and D, and the comparison between G and H is taken into consideration.

Regarding the honeys C and D, the obtained p-values are $1\text{-}7 \cdot 10^{-4}$ and $2.2 \cdot 10^{-6}$, respectively, if C1 and C2 are removed, both values are small enough to reject the null hypothesis. Therefore, the conclusion is that the means are different.

On another hand, the honeys H and G have a p-value of respectively $6.9 \cdot 10^{-5}$ and $5 \cdot 10^{-7}$ without H2 sample, hence null hypothesis can also be rejected.

Moreover, the obtained difference is negative in all cases, that means the concentration of phenolic acids in honey D is smaller than the concentration in honey C, in the same way the concentration of phenolic acids in honey H is smaller than concentration in honey G. This can be seen in table 20, which is the results from the tukey test before removing C1, C2 and H2, and in table 21, which is the results from the tukey test after removing C1, C2 and H2. Table 22 show the mean values of the phenolic acids in the extraction, which the tukey test is based on, can be seen.

Table 20. Results of the tukey test for the whole dataset – phenolic acids.

Honeys	**diff**	**P adj**
D-C	-0.009096667	$1.69 \cdot 10^{-4}$
H-G	-0.010274500	$6.97 \cdot 10^{-5}$

Table 21. Results of the tukey test without C1, C2 and H2 values – phenolic acids.

Honeys	diff	P adj
D-C	-0.0064066667	$2.2 \cdot 10^{-6}$
H-G	-0.0107065833	$5 \cdot 10^{-7}$

Table 22. Table of means for Tukey test, without C1, C2 and H2 taken to account – phenolic acids.

Source (Honey)	Mean with C1, C2 and H2	Mean without C1, C2 and H2
C	0.020194000	0.01750400
D	0.011097333	0.01109733
G	0.016830333	0.01683033
H	0.006555833	0.00612375

Flavonoids

In the case of the concentration of flavonoids in the honeys, ANOVA test with two levels of replications is carried out again, as before, and the results show that the variance within the extractions is $5.3 \cdot 10^{-7}$, which is quite good, while the variance between the extractions is $5 \cdot 10^{-7}$. This can be seen in table 23. The two mean squares are very close each other, which indicates that there is no big difference in the analyst and instrumental error. This is also seen in figure 23, where the only outlier point is H2. Removing H2 is done in order to check if it has a significant effect on the variance. This leads up to a very low p-value, $1.36 \cdot 10^{-11}$, which means the null hypothesis can be rejected.

Table 23. Results from ANOVA test in R flavonoids concentrations.

Error: extract	Mean Sq. (Variance)	Pr (<F)
Source	0.0008398	$1.36 \cdot 10^{-11}$
Residuals	0.0000005	
Error: Within		
Residuals	$5.308 \cdot 10^{-7}$	

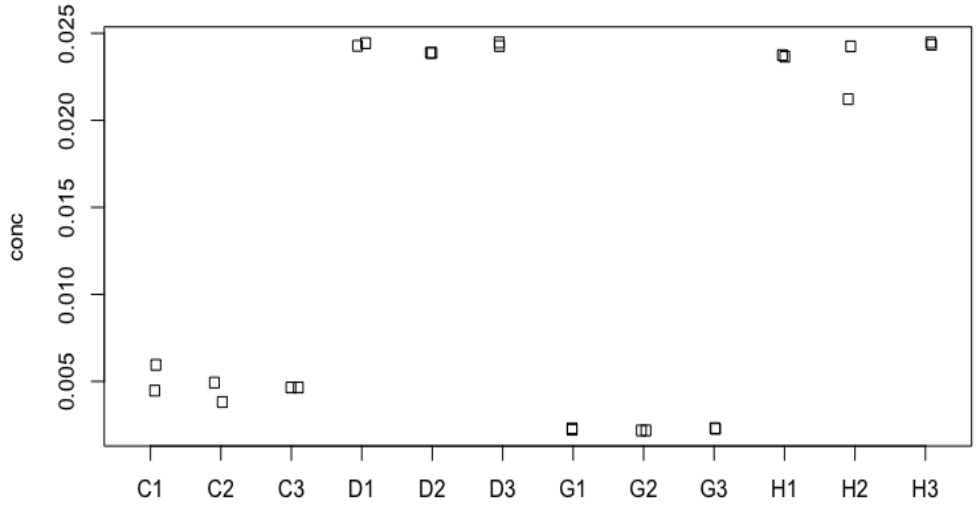


Figure 23. Stripchart of the extractions with their concentrations - flavonoids.

When deleting extraction H2 the comparison is $1.61 \cdot 10^{-7}$ for the variance within the extractions and $2 \cdot 10^{-7}$ for the variance between the extractions. The removal of H2 had no significant effect on the mean squares, and therefore, H2 has no major influence on the overall p-value. Because of the low p-value the null hypothesis is still rejected. The variances can be seen in table 24 and figure 24 shows the stripchart of the concentrations of flavonoids without H2.

Table 24. Results from ANOVA test in R for flavonoids concentrations without H2.

Error: extract	**Mean Sq. (Variance)**	**Pr (<F)**
Source	0.0007813	$1.73 \cdot 10^{-11}$
Residuals	0.0000002	
Error: Within		
Residuals	$1.611 \cdot 10^{-7}$	

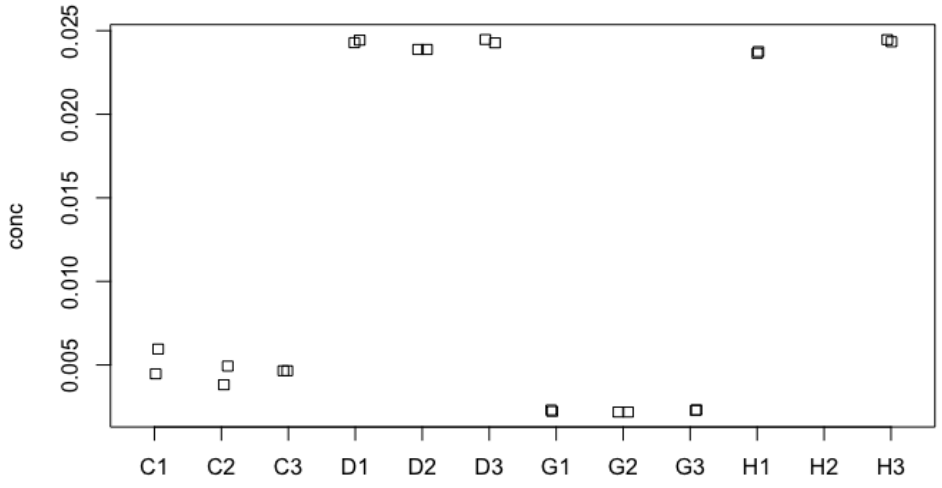


Figure 24. Stripchart of extractions with their concentration and without H2 – flavonoids.

Again, a Tukey test is used to compare honey C and D with each other and to compare honey G and H.

Regarding the honeys C and D, the obtained p-value is respectively 0.0 and 0.0 without H2 extraction, both values are small enough to reject the null hypothesis. Therefore, the conclusion is that the means are different.

On another hand, the honeys H and G have p-values of respectively 0.0 and 0.0 without H2 extraction, hence null hypothesis can also be rejected.

Moreover, the obtained difference is positive in all the cases, which means that the concentration of flavonoids in honey D is higher than the concentration in honey C, in the same way the concentration of flavonoids in honey H is higher than concentration in honey G. This can be seen in table 25, which is the results from the Tukey test before removing H2, and in table 26, which shows the results from the Tukey test after removing H2. Table 27 shows the mean values of the flavonoids in the extraction, which the Tukey test is based on.

Table 25. Results of the tukey test for the whole dataset – flavonoids.

Honeys	diff	P adj
D-C	0.0194576667	0.0000000
H-G	0.0213650000	0.0000000

Table 26. Results of the tukey test without H2 – flavonoids.

Honeys	diff	P adj
D-C	0.0194576667	0.0000000
H-G	0.0218045000	0.0000000

Table 27. Table of means for Tukey test for the whole dataset– flavonoids.

Source (Honeys)	Mean with H2	Mean without H2
C	0.004745667	0.004745667
D	0.024203333	0.024203333
G	0.002247000	0.002247000
H	0.023612000	0.024051500

3.3 Discussion

The purpose of this project was to compare the amount of flavonoids and phenolic acids in different types of honey, and to optimize the method to analyse these with the HPLC machine. Optimizing the method for the HPLC was done by looking at the separation of the peaks, and it was found that 50.0 ºC was the best temperature from the three temperatures tested. The gradient needed to be 2 min 1% ACN, 40 min 1% ACN to 50% ACN, 3 min 50% to 99% ACN, 5 min 99% ACN, 1 min 99% to 1% ACN and last 5 min 1% ACN. For better separation of the most polar compounds, which runs through first, a flow rate of both 0.6 [mL/min] and 1.0 [mL/min] were used. It was found that 0.6 [mL/min] was best.
The peak symmetry and resolution of the peaks were calculated on two peaks from the optimized method, and the actual value is close to the optimal supposed value, which indicated that the optimization has worked, because a too big resolution value would waste time.

To quantify the phenolic acids and flavonoids in the honeys, standard curves for both caffeic acid and quercetin were made. The HPLC machine makes a chromatogram with the UV profile of the analysed compounds. UV profile was used to decide which compounds were considered as phenolic acids or flavonoids and by using the linear regression from the standard curves it was possible to calculate the concentration of phenolic acids and flavonoids in the honeys. This is not a totally valid method as the standard curves actually cannot be used for all the different types of flavonoids and phenolic acids. To make the method totally acceptable a standard curve should be made for each of the flavonoids and phenolic acids types, but this would have taken too much time and moreover all the flavonoids and phenolic acids were not available. Therefore, the standard curves for respectively the caffeic acid and the quercetin were used for all of the found phenolic acids and flavonoids.

The total amount of phenolic acids and flavonoids in the honeys were calculated, and it can be seen that honey C (City honey) nearly has double as much phenolic acids in it than honey D (Countryside honey). It is the same case regarding the flavonoids. Honey C has about five times as much flavonoids as honey D.
Honey G and H come from the same producer but from different years and the difference between their content of phenolic acids and flavonoids are quite significant. The content of flavonoids are 10 times as high in honey H as in honey G. The content of phenolic acids is two and half times as high

in honey G than in honey H. This would show as a visual appearance as honey H is more red/orange than honey G. These numbers can be seen in appendix E.
In conclusion, it can be seen that among all the honeys, honey D has the highest content of flavonoids and honey C, even though the data differs much from each other, has the highest content of phenolic acids.

Eight standards were available, six phenolic acids and two flavonoids, and these were all run in the HPLC machine with the optimized method in order to find the retention times for these standards. These standards retention times were compared with the chromatograms of each of the honey to determine if there were any of the standards in the honeys. Both honey C and honey H had p-Coumaric acid and rutin in them and honey D and honey G had only p-Coumaric acid in them, regarding the standards available. This means, within all the phenolic acids and flavonoids, only two were determined with the standards and the others cannot be determined, as their standards are not available.

To make statistical analysis on the data three extractions of each honey were made and from each extraction two test-samples were taken, thereafter with ANOVA testing the error within each extraction was found and the error between the extractions. It was found that extraction C1, C2 and H2 each had a big error with the extraction of phenolic acids and by removing these from the dataset, the error within the extraction got smaller than the error between the extractions. The error within the extraction is the error on the HPLC machine and the error between the extraction are the errors from the analysts, and hence this, the error within the extraction theoretically should be the smallest. In flavonoids analysis testing only extraction H2 showed to have a larger error within the extraction and this was removed, but it did not have a big impact on the p-values or on the errors within or between the extractions.

From the stripchart and the means, it can also be seen that honey C has the highest concentration of phenolic acids, next honey G, next D and at last honey H, which has the smallest concentration of phenolic acids. Of flavonoids honey D has a slightly higher concentration than honey H, next honey C and at last honey G, which has the smallest concentration of flavonoids.
With ANOVA testing and Tukey testing it was found that none of the honeys means of phenolic acids or of flavonoids are equal to each other, therefore the null hypothesis must be rejected.

4 Conclusion

A HPLC method with a gradient of ultrapure water and acetonitrile was developed and optimized for the analysis of phenolic acids and flavonoids in four honeys. The optimized method was performed at 50.0 °C, on a C_{18}-column and with a gradient at 2 min 1% ACN, 40 min 1% - 50% ACN, 3 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN and last 5 min 1% ACN.

With this HPLC method four different honeys were analysed, and from this analysis it can be concluded that by just comparing the content of phenolic acids and flavonoids in the honeys there is a big difference in the content of the two compounds. Honey C consists of 0.0202 $[\frac{mg}{g\ honey}]$ phenolic acids and 0.0048 $[\frac{mg}{g\ honey}]$ flavonoids. Honey D consists of 0.0111 $[\frac{mg}{g\ honey}]$ phenolic acids and 0.0024 $[\frac{mg}{g\ honey}]$ flavonoids. Honey G consists of 0.0168 $[\frac{mg}{g\ honey}]$ phenolic acids and 0.0023 $[\frac{mg}{g\ honey}]$ flavonoids. Honey H consists of 0.0066 $[\frac{mg}{g\ honey}]$ phenolic acids and 0.0236 $[\frac{mg}{g\ honey}]$ flavonoids. Honey H has the highest concentration of flavonoids and honey C has the highest concentration phenolic acids. All the phenolic acid concentrations were determined with a standard curve made on p-Coumaric acid and all flavonoid concentrations were determined with a standard curve made on quercetin. The only determined phenolic acid was p-Coumaric acid and this was found to be in all of the honeys. The only determined flavonoid was rutin and this was found in honey C and honey H. These two compounds were only in trace concentrations.

Statistically the data from each honey was tested with both ANOVA test and Tukey test. Of each honey three extractions were made, and from each extraction two samples for the HPLC machine were taken, so for each honey six analysis were made. For the phenolic acids extractions C1, C2 and H2 were removed because they differed a lot from the mean of the extraction, and with ANOVA testing the p-value $2.66 \cdot 10^{-8}$ was found, which means that none of the honeys mean values of the phenolic acid concentrations were equal, hence the null hypothesis must be rejected. The same relating to the flav0noids. Only extraction H2 differed a lot from the mean and with ANOVA testing the p-value $1.73 \cdot 10^{-11}$ was found and the null hypothesis must therefore berejected. Comparing honey C with D and honey G with H using Tukey test, this also showed that none of them are equal, and that honey D has the highest concentration of flavonoids and honey G has the lowest concentration of flavonoids. Honey C has the highest concentration of phenolic acids and honey H has the lowest concentration of phenolic acids.

5 Perspectives

As it is mentioned earlier, the content of phenolic acids and flavonoids in honeys varies quite a lot. This variation depends on the flowers, which the honey is made from. According to the obtained results, the origin of the honeys affects the composition of honey C and D. On the other hand, the production year of the honey also has a significant effect as seen on the results from honey H and G.

Therefore, for future studies on honey, it is important to keep in mind that the amounts of phenolic acids and flavonoids can change considerably.

In comparison to previous studies, the majority of them mention the idea of how much composition of honey can vary depending on the origin of the flowers. (Kaskoniene & Venskutonis, 2010)

As a future study, flavonoids in the honey could be specified and compared with the flavour of the honey. The standard curves could be made from the exact flavonoid or phenolic acid, so that the results would be closer to the true value. At the same time, it would be possible to compare the amount of phenolic acids to the pH in the honey. A lower pH value induces a less beneficial environment for microorganisms and hereby prolong shelf life for the honey. This could minimize food waste and thereby reduce the production of plastic and glass for honey containers.

Another future study could be to change the column. For better statistical results, it would be suitable to have different containers of each honey, from the same producer and same year. That way the honeys, that theoretically are the same, could be compared.

6 Reference

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Appendix A

Honey chosen

For this project four different honeys from around Denmark were chosen. These four honeys come from both the city, the countryside and from different periods of time (2010 and 2015). In table 28 of the four different honeys, and honey D is the honey that the HPLC method is optimized on.

Table 28. Table with information of the four different honeys chosen.

Name of honey	**Flower**	**Origin and year**	**Manufacturer**	**Picture of honey**	**Colour**
(C) Eventyrhonning – City honey	Mixed	Odense C	-		Orange
(D) Late summer honey – Countryside honey	Mixed	Holmstrup	Solens hjerte		Orange
(G) Højbo honey	White clover	Gedser Odde 2010	-		Clear orange
(H) White clover honey	White clover	Gedser Odde 2015	-		Cloudy yellow

Each honey should hopefully contain different kinds of phenolic acids and flavonoids but because the Højbo honey and the white clover honey comes from the same floral origin and the same producer there should be some similarities. Eventyrhonning and the late summer honey should also contain different honeys.

Appendix B

Safety sheets for used chemicals in lab

Compound	MW $[\frac{g}{mol}]$	Density $[\frac{g}{cm^3}]$	M.p. / B.p. [°C]	Carcinogen. teratogen. mutagenic	Toxic	Harmful to health / Irritating	Explosive	Self-ignitable (pyrofort)	Very flammable / Flammable	Oxidizing	Corrosive	Tear gas (lakrymator)	Others
Acetonitrile	41.06	0.78	-45 / 81-82	**(X)**	**X**	**X**			**X**				
Rutin (Not investigated)	610.57	1.82	195 / 983±65		**X**								
Quercetin	302.25	0.24	314 decomp / >200 subl.	**(X)**	**X**	**X**							
p-Courmarin acid	164.17	1.33	214 / 346±17			**(X)**							
4-Hydroxybenzoic acid	138.13	1.46	213-214 / n/a			**(X)**							
Chlorogenic acid (Not investigated)	354.34	-	208 / n/a			**(X)**							
Caffeic acid	180.17	1,48	194-225 / 417±35			**(X)**							
Syringic acid	198.19	-	205 / n/a	**(X)**		**X**							
Gallic acid	170.13	1.694	251 / 501±50			**(X)**							
Trifluoroacetic acid	114.03	1.49	-15.4 / 72.4	**(X)**		**(X)**							
Ethanol	46.07	0.8	-114 / 78.0	**(X)**		**(X)**			**(X)**				

Reaction conditions (heat. cooling. pressure. vacuum. *etc.*): Heat for dissolving the standards.

Gas generation ? Precautions: None

Exo/endothermic? -

Working practices:	Safety glasses: X	Lab coat: X	Fume cupboard: X
(Cross)	Gloves (types): X	Dust mask:	Other:
	Wash bottle (content):	Face shield:	

Neutralization of excess chemicals:

Waste management:

B, H

(kemibrug, 2017)

Appendix C

Used methods

Here are all the used methods in the project showed.

Method 1, 40 °C

H_2O + Acetonitrile - 99% gradient.

3 min 1% ACN, 30 min 1% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN.

Flow rate: 0.6 [mL/min]

Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 2, 45 °C

H_2O + Acetonitrile - 99% gradient.

3 min 1% ACN, 30 min 1% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]

Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 3, 50 °C

H_2O + Acetonitrile - 99% gradient.

3 min 1% ACN, 30 min 1% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]

Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 4, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 40 min 1% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]

Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 5, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 25 min 1% - 50% ACN, 3 min 50%, 25 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 6, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

10 min 1% ACN, 50 min 1% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 7, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 40 min 1% - 50% ACN, 15 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 8, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 35 min 1% - 40% ACN, 20 min 40% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 9, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 30 min 1% - 30% ACN, 25 min 30% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 10, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 10 min 1% - 10% ACN, 3 min 10%, 40 min 10% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 11, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 15 min 1% - 20% ACN, 3 min 20%, 35 min 20% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 12, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 20 min 1% - 30% ACN, 3 min 30%, 30 min 30% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 13, gradient

50 °C - H_2O + Acetonitrile - 99% gradient.

6 min 1% ACN, 25 min 1% - 40% ACN, 3 min 10%, 25 min 40% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 3 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 254, 300, 320 and 360 [nm]

Method 14, gradient, flow rate and wavelength

50 °C - H_2O + Acetonitrile - 99% gradient.

2 min 1% ACN, 40 min 1% - 50% ACN, 3 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 5 min 1% ACN

Flow rate: 0.6 [mL/min]
Wavelength: 250, 260, 300, 320 and 360 [nm]

Method 15, gradient, flow rate and wavelength

50 °C - H_2O + Acetonitrile - 99% gradient.

2 min 1% ACN, 40 min 1% - 50% ACN, 3 min 50% - 99% ACN, 5 min 99% ACN, 1 min 99% - 1% ACN, 5 min 1%

Flow rate: 0.6 [mL/min]
Wavelength: 250, 260, 300, 320 and 360 [nm]

Appendix D

Determination of the standards in the honeys

For **honey D** it will now be determined what kind of compounds there is in the honey. The chromatogram of honey D can be seen in figure 25:

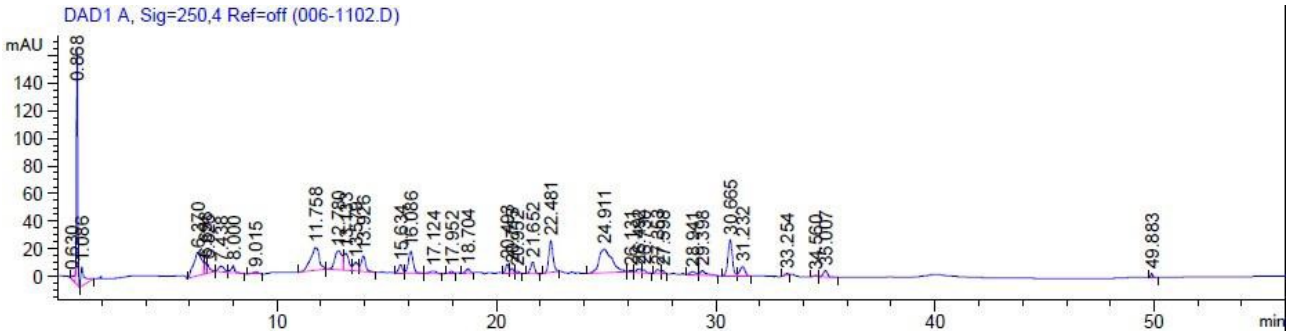


Figure 25. Chromatogram for honey D.

In honey D the found standards can be seen in table 29:

Table 29. Content of the standards in honey D.

Compound found	Retention time in chromatogram [min]	Area [mAU]	Concentration [mg/g water]
p-Coumaric acid	27.598	21.447	0.002

Only p-Coumaric acid were found in honey D of the available standards.

For **honey G** it will now be determined what kind of compounds there is in the honey. The chromatogram of honey G can be seen in figure 26:

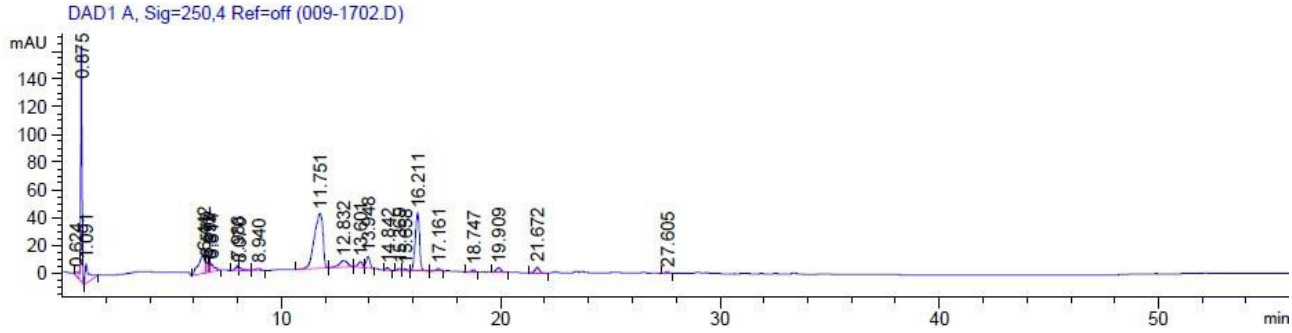


Figure 26. Chromatogram for honey G.

In honey G there were found these standards as seen in table 30:

Compound found	Retention time in chromatogram [min]	Area [mAU]	Concentration [mg/g water]
p-Coumaric acid	27.605	14.205	0.002

Table 30. Content of the standards in honey G.

Only p-Coumaric acid was found in honey G of the available standards.

For **honey H** it will now be determined what kind of compounds there is in the honey. The chromatogram of honey H can be seen in figure 27:

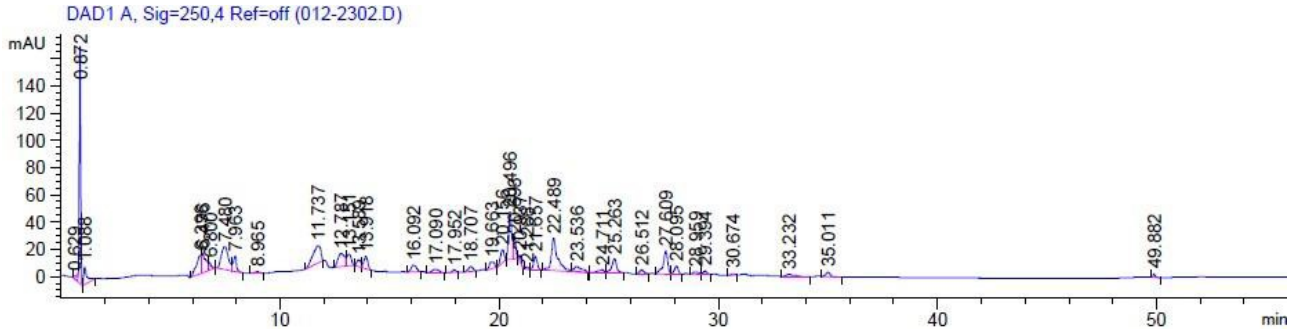


Figure 27. Chromatogram for honey H.

In honey H there were found these standards as seen in table 31:

Compound found	Retention time in chromatogram [min]	Area [mAU]	Concentration [mg/g water]
p-Coumaric acid	27.609	247.842	0.007
Rutin	28.095	77.123	0.003

Table 31. Content of the standards in honey H.

Only p-Coumaric acid and rutin were found in the honey.

Appendix E

Amount of phenolic acids and flavonoids in the honeys

In the tables below the retention time and area for each detected phenolic acid and flavonoid is shown. Also the total area and concentration of them are shown. For each type of honey 3 solutions are made and for each solution 2 injections are taken and therefore there are 2 chromatograms. The notation e.g. for type C solution 3 injection 2 is C3-2. The retention time is in minutes, areal is in [mAU] and the concentration is [mg/pr. g honey].

Honey C - C1-1		
Ret. Time	**Compound**	**Area**
16.204	Phenolic acid	103.5
18.029	Flavonoid	28
19.328	Phenolic acid	50.1
20.736	Phenolic acid	638.1
20.964	Flavonoid	149.5
21.289	Phenolic acid	69.5
22.536	Phenolic acid	197.5
25.301	Flavonoid	24.8
Total areal of flavonoid:		202.3
Total areal of phenolic acid:		1058.7
Total conc. of flavonoid:		0.006
Total conc. of phenolic acid:		0.0267

Honey C - C1-2		
Ret. Time	**Compound**	**Area**
16.545	Phenolic acid	10.9
17.928	Flavonoid	29.2
19.295	Flavonoid	36.2
20.709	Phenolic acid	450.7
20.931	Flavonoid	57.5
21.643	Phenolic acid	99.5
22.487	Phenolic acid	162.5
25.25	Flavonoid	19.4
Total areal of flavonoid:		142.3
Total areal of phenolic acid:		723.6
Total conc. of flavonoid:		0.0045
Total conc. of phenolic acid:		0.0187

Honey C - C2-1		
Ret. Time	**Compound**	**Area**
16.059	Phenolic acid	71.9
16.539	Phenolic acid	12.1
17.924	Flavonoid	30.5
20.167	Phenolic acid	195.9
20.703	Phenolic acid	445.7
20.926	Flavonoid	57.5
22.13	Phenolic acid	12.9
22.475	Phenolic acid	164.1
25.241	Flavonoid	27.9
Total areal of flavonoid:		115.9
Total areal of phenolic acid:		902.6
Total conc. of flavonoid:		0.0038
Total conc. of phenolic acid:		0.023

Honey C - C2-2		
Ret. Time	**Compound**	**Area**
16.071	Phenolic acid	73.4
16.543	Phenolic acid	12
17.936	Flavonoid	30
20.708	Phenolic acid	446.5
20.932	Flavonoid	57.7
22.494	Phenolic acid	165
22.887	Flavonoid	46.3
25.256	Flavonoid	27
Total areal of flavonoid:		161
Total areal of phenolic acid:		684.9
Total conc. of flavonoid:		0.0049
Total conc. of phenolic acid:		0.0178

Honey C - C3-1		
Ret. Time	**Compound**	**Area**
16.076	Phenolic acid	71.3
16.567	Phenolic acid	11.3
17.948	Flavonoid	29.6
19.304	Flavonoid	35.4
20.716	Phenolic acid	426.6
20.939	Flavonoid	57.2
22.497	Phenolic acid	162.3
25.256	Flavonoid	27.5
Total areal of flavonoid:		149.7
Total areal of phenolic acid:		671.5
Total conc. of flavonoid:		0.0047
Total conc. of phenolic acid:		0.0175

Honey C - C3-2		
Ret. Time	**Compound**	**Area**
16.078	Phenolic acid	72.4
16.551	Phenolic acid	11.2
17.952	Flavonoid	29.7
19.3	Flavonoid	35.9
20.711	Phenolic acid	427.2
20.934	Flavonoid	57
22.492	Phenolic acid	162.9
25.251	Flavonoid	27.1
Total areal of flavonoid:		149.7
Total areal of phenolic acid:		673.7
Total conc. of flavonoid:		0.0047
Total conc. of phenolic acid:		0.0175

Honey D - D1-1		
Ret. Time	**Compound**	**Area**
17.959	Flavonoid	19.5
20.712	Phenolic acid	25.9
22.475	Phenolic acid	367.7
23.436	Flavonoid	14.2
24.888	Flavonoid	809.8
26.124	Flavonoid	22.5
26.481	Flavonoid	51.1
26.716	Flavonoid	32.7
Total areal of flavonoid:		949.8
Total areal of phenolic acid:		393.6
Total conc. of flavonoid:		0.0244
Total conc. of phenolic acid:		0.0109

Honey D - D1-2		
Ret. Time	**Compound**	**Area**
17.95	Flavonoid	18.8
20.724	Phenolic acid	25.6
22.489	Phenolic acid	368.4
23.446	Flavonoid	14.3
24.901	Flavonoid	805.8
26.127	Flavonoid	22.1
26.481	Flavonoid	50
26.717	Flavonoid	32.7
Total areal of flavonoid:		943.7
Total areal of phenolic acid:		394
Total conc. of flavonoid:		0.0243
Total conc. of phenolic acid:		0.0109

Honey D - D2-1		
Ret. Time	**Compound**	**Area**
17.96	Flavonoid	19.1
20.727	Phenolic acid	24.8
22.486	Phenolic acid	378.6
23.445	Flavonoid	14.2
24.911	Flavonoid	790.8
26.131	Flavonoid	21.7
26.489	Flavonoid	49.1
26.726	Flavonoid	32.4
Total areal of flavonoid:		927.3
Total areal of phenolic acid:		403.4
Total conc. of flavonoid:		0.0239
Total conc. of phenolic acid:		0.0111

Honey D - D2-2		
Ret. Time	**Compound**	**Area**
17.952	Flavonoid	18.8
20.713	Phenolic acid	24.7
22.481	Phenolic acid	377.3
23.443	Flavonoid	14
24.991	Flavonoid	789.4
26.131	Flavonoid	21.6
26.492	Flavonoid	48.9
26.73	Flavonoid	32.3
Total areal of flavonoid:		925
Total areal of phenolic acid:		402
Total conc. of flavonoid:		0.0238
Total conc. of phenolic acid:		0.0111

Honey D - D3-1		
Ret. Time	**Compound**	**Area**
17.964	Flavonoid	19.4
20.733	Phenolic acid	25.1
22.496	Phenolic acid	387.5
23.451	Flavonoid	13.5
24.918	Flavonoid	807.3
26.131	Flavonoid	21.3
26.485	Flavonoid	49
26.72	Flavonoid	32.9
Total areal of flavonoid:		943.4
Total areal of phenolic acid:		412.6
Total conc. of flavonoid:		0.0243
Total conc. of phenolic acid:		0.0113

Honey D - D3-2		
Ret. Time	**Compound**	**Area**
17.954	Flavonoid	19.1
20.711	Phenolic acid	25.2
22.479	Phenolic acid	386.6
23.436	Flavonoid	13.6
24.907	Flavonoid	805.5
26.122	Flavonoid	21.2
26.483	Flavonoid	59.2
26.72	Flavonoid	32.8
Total areal of flavonoid:		951.4
Total areal of phenolic acid:		411.8
Total conc. of flavonoid:		0.0245
Total conc. of phenolic acid:		0.0113

Honey G - G1-1		
Ret. Time	**Compound**	**Area**
14.832	Phenolic acid	22.5
15.358	Phenolic acid	22.1
16.202	Phenolic acid	575.6
18.742	Flavonoid	20.4
23.458	Flavonoid	12.5
28.998	Flavonoid	18.5
33.384	Phenolic acid	20.9
Total areal of flavonoid:		51.4
Total areal of phenolic acid:		641.4
Total conc. of flavonoid:		0.0022
Total conc. of phenolic acid:		0.0168

Honey G - G1-2		
Ret. Time	**Compound**	**Area**
14.844	Phenolic acid	23.5
15.375	Phenolic acid	22.4
16.218	Phenolic acid	577.3
18.756	Flavonoid	20.3
23.463	Flavonoid	15.4
29.001	Flavonoid	19.1
33.368	Phenolic acid	21.2
Total areal of flavonoid:		54.8
Total areal of phenolic acid:		644.4
Total conc. of flavonoid:		0.0023
Total conc. of phenolic acid:		0.0168

Honey G - G2-1		
Ret. Time	**Compound**	**Area**
14.837	Phenolic acid	20.5
15.362	Phenolic acid	22.1
16.208	Phenolic acid	577.2
18.756	Flavonoid	19.6
23.457	Flavonoid	12.1
28.996	Flavonoid	18
33.368	Phenolic acid	20.6
Total areal of flavonoid:		49.7
Total areal of phenolic acid:		640.4
Total conc. of flavonoid:		0.0022
Total conc. of phenolic acid:		0.0167

Honey G - G2-2		
Ret. Time	**Compound**	**Area**
14.842	Phenolic acid	20.2
15.369	Phenolic acid	22.2
16.211	Phenolic acid	577.8
18.747	Flavonoid	19.7
23.455	Flavonoid	12.1
28.992	Flavonoid	18
33.374	Phenolic acid	21.1
Total areal of flavonoid:		49.8
Total areal of phenolic acid:		641.3
Total conc. of flavonoid:		0.0022
Total conc. of phenolic acid:		0.0168

Honey G - G3-1		
Ret. Time	**Compound**	**Area**
14.821	Phenolic acid	20.4
15.353	Phenolic acid	22.1
16.195	Phenolic acid	585.8
18.735	Flavonoid	20.3
23.447	Flavonoid	13.2
28.994	Flavonoid	20.1
33.368	Phenolic acid	22.9
Total areal of flavonoid:		53.6
Total areal of phenolic acid:		651.2
Total conc. of flavonoid:		0.0023
Total conc. of phenolic acid:		0.017

Honey G - G3-2		
Ret. Time	**Compound**	**Area**
14.834	Phenolic acid	20.5
15.361	Phenolic acid	22.1
16.206	Phenolic acid	582.4
18.743	Flavonoid	20.1
23.446	Flavonoid	15.8
28.983	Flavonoid	19.3
33.358	Phenolic acid	22
Total areal of flavonoid:		55.2
Total areal of phenolic acid:		647
Total conc. of flavonoid:		0.0023
Total conc. of phenolic acid:		0.0169

Honey H - H1-1		
Ret. Time	**Compound**	**Area**
17.948	Flavonoid	24.7
18.707	Phenolic acid	58.1
20.703	Phenolic acid	133.4
20.944	Flavonoid	34.9
21.654	Flavonoid	107.8
22.486	Flavonoid	473.5
24.708	Flavonoid	31.9
25.261	Flavonoid	192.6
28.956	Flavonoid	37.9
30.673	Flavonoid	18.8
Total areal of flavonoid:		922.1
Total areal of phenolic acid:		191.5
Total conc. of flavonoid:		0.0237
Total conc. of phenolic acid:		0.0061

Honey H - H1-2		
Ret. Time	**Compound**	**Area**
17.953	Flavonoid	24.3
18.707	Phenolic acid	59.2
20.69	Phenolic acid	134.1
20.932	Flavonoid	35
21.651	Flavonoid	110.4
22.48	Flavonoid	472.2
24.7	Flavonoid	32.3
25.252	Flavonoid	193.9
28.941	Flavonoid	31.2
30.662	Flavonoid	18.9
Total areal of flavonoid:		918.2
Total areal of phenolic acid:		193.3
Total conc. of flavonoid:		0.0237
Total conc. of phenolic acid:		0.0061

Honey H - H2-1		
Ret. Time	**Compound**	**Area**
17.993	Flavonoid	24.7
18.743	Phenolic acid	59.7
20.705	Phenolic acid	129.2
20.947	Flavonoid	35.1
21.689	Phenolic acid	118
22.51	Flavonoid	467.8
24.711	Flavonoid	32.9
25.265	Flavonoid	197.3
28.968	Flavonoid	35.5
30.684	Flavonoid	26.4
Total areal of flavonoid:		819.7
Total areal of phenolic acid:		306.9
Total conc. of flavonoid:		0.0212
Total conc. of phenolic acid:		0.0088

Honey H - H2-2		
Ret. Time	**Compound**	**Area**
17.952	Flavonoid	24.7
18.707	Phenolic acid	60.2
20.696	Phenolic acid	129.1
20.937	Flavonoid	34.9
21.657	Flavonoid	113.6
22.489	Flavonoid	478.1
24.711	Flavonoid	33.8
25.263	Flavonoid	199
28.959	Flavonoid	36.5
30.674	Flavonoid	21.7
Total areal of flavonoid:		942.3
Total areal of phenolic acid:		189.3
Total conc. of flavonoid:		0.0242
Total conc. of phenolic acid:		0.006

Honey H - H3-1		
Ret. Time	**Compound**	**Area**
17.946	Flavonoid	24.6
18.703	Phenolic acid	60.2
20.691	Phenolic acid	134
20.932	Flavonoid	35.2
21.641	Flavonoid	111.5
22.471	Flavonoid	484.1
24.691	Flavonoid	33.7
25.24	Flavonoid	200.9
28.935	Flavonoid	35.7
30.652	Flavonoid	20.2
Total areal of flavonoid:		945.9
Total areal of phenolic acid:		194.2
Total conc. of flavonoid:		0.0243
Total conc. of phenolic acid:		0.0061

Honey H - H3-2		
Ret. Time	**Compound**	**Area**
17.957	Flavonoid	25.2
18.714	Phenolic acid	60.6
20.697	Phenolic acid	134.5
20.937	Flavonoid	35.2
21.658	Flavonoid	114.3
22.486	Flavonoid	484.7
24.7	Flavonoid	336
25.251	Flavonoid	201.4
28.949	Flavonoid	36.1
30.685	Flavonoid	20.6
Total areal of flavonoid:		951.1
Total areal of phenolic acid:		195.1
Total conc. of flavonoid:		0.0245
Total conc. of phenolic acid:		0.0062

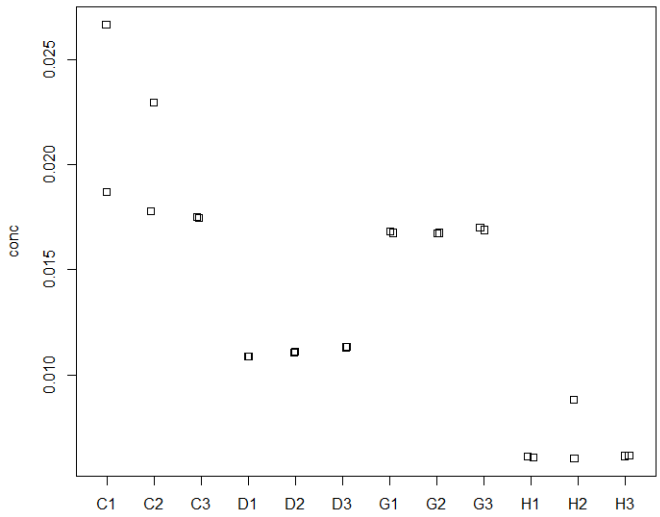
Appendix F

R coding for Results of statistical analysis

Below the R coding can be seen from the section “Results of statistical analysis”.

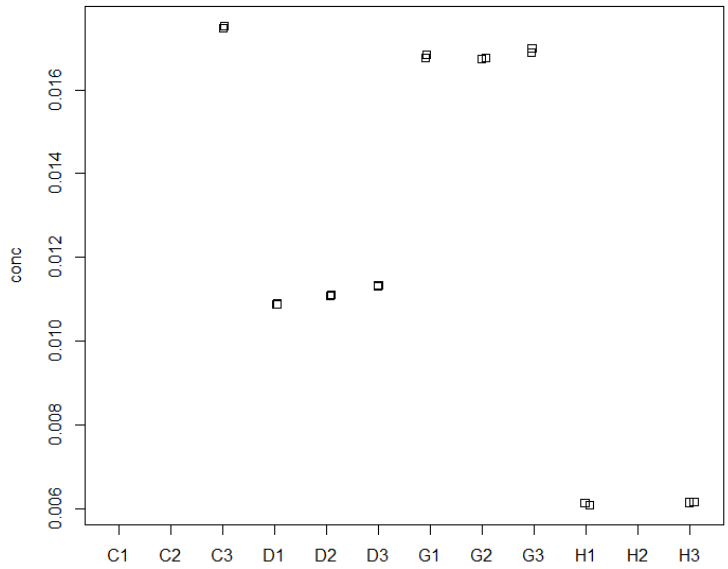
Phenolic acids

```
#Analysis of Phenolic acids
install.packages("dplyr")
library(dplyr)
pheno<-read.csv("../Statistics/phenolic.csv")
> head(pheno)
  source extract     conc
1      C      C1 0.026676
2      C      C1 0.018716
3      C      C2 0.022968
4      C      C2 0.017796
5      C      C3 0.017478
6      C      C3 0.017530


table(pheno$source, pheno$extract)

   C1 C2 C3 D1 D2 D3 G1 G2 G3 H1 H2 H3
C  2  2  2  0  0  0  0  0  0  0  0  0
D  0  0  0  2  2  2  0  0  0  0  0  0
G  0  0  0  0  0  0  2  2  2  0  0  0
H  0  0  0  0  0  0  0  0  0  2  2  2

> stripchart(conc~extract, data=pheno, vertical = TRUE, method = "jitter")
```



```
source("dfsumm.R")
> dfsumm(pheno)

 24 rows and 3 columns
 24 unique rows
                  source extract    conc
Class             factor  factor numeric
Minimum                C      C1 0.00602
Maximum                H      H3  0.0267
Mean                   D      D3  0.0137
Unique (excld. NA)     4      12      24
Missing values         0       0       0
Sorted              TRUE    TRUE   FALSE

mod1 <- aov(conc ~ source + Error(extract), data = pheno)
> summary(mod1)

Error: extract
          Df    Sum Sq   Mean Sq F value   Pr(>F)
source     3 0.0006587 2.196e-04   59.46 8.21e-06 ***
Residuals  8 0.0000295 3.690e-06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
          Df    Sum Sq   Mean Sq F value Pr(>F)
Residuals 12 4.897e-05 4.081e-06

pheno2 <- as.data.frame(summarise(group_by(pheno, source, extract), pheno.mean=
mean(conc), pheno.n=length(conc)))
> head(pheno2)
  source extract pheno.mean pheno.n
1      C      C1  0.0226960       2
2      C      C2  0.0203820       2
3      C      C3  0.0175040       2
4      D      D1  0.0108810       2
5      D      D2  0.0110925       2
6      D      D3  0.0113185       2

pheno5 <- as.data.frame(summarise(group_by(pheno2, source), pheno.mean=mean(phe
no.mean), pheno.v=var(pheno.mean)))
> head(pheno5)
  source  pheno.mean pheno.v
1      C 0.020194000      NA
2      D 0.011097333      NA
3      G 0.016830333      NA
4      H 0.006555833      NA

mod2 <- aov(pheno.mean ~ source, data = pheno2)
> summary(mod2)
            Df    Sum Sq   Mean Sq F value   Pr(>F)
source       3 0.0003293 1.098e-04   59.46 8.21e-06 ***
Residuals    8 0.0000148 1.850e-06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

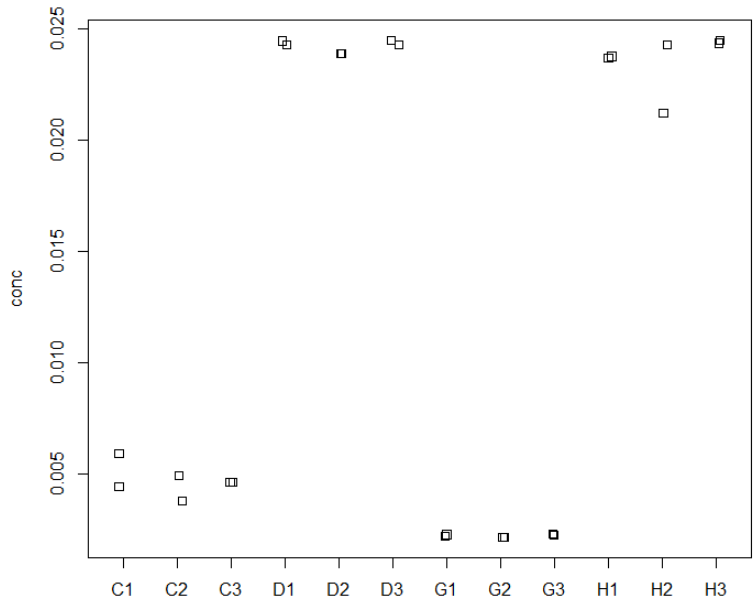
```
TukeyHSD(mod2)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pheno.mean ~ source, data = pheno2)

$source
             diff          lwr           upr     p adj
D-C -0.009096667 -0.012649435 -0.0055438987 0.0001687
G-C -0.003363667 -0.006916435  0.0001891013 0.0635834
H-C -0.013638167 -0.017190935 -0.0100853987 0.0000084
G-D  0.005733000  0.002180232  0.0092857679 0.0037769
H-D -0.004541500 -0.008094268 -0.0009887321 0.0146971
H-G -0.010274500 -0.013827268 -0.0067217321 0.0000697

model.tables(mod2, "means")
Tables of means
Grand mean

0.01366938

 source
source
       C        D        G        H
0.020194 0.011097 0.016830 0.006556

pheno3<-subset(pheno, !extract %in% c("C1","C2","H2"))
> head(pheno3)
   source extract     conc
5       C      C3 0.017478
6       C      C3 0.017530
7       D      D1 0.010876
8       D      D1 0.010886
9       D      D2 0.011109
10      D      D2 0.011076

> stripchart(conc~extract, data=pheno3, vertical = TRUE, method = "jitter")
```



```
mod3 <- aov(conc ~ source + Error(extract), data = pheno3)
> summary(mod3)

Error: extract
          Df    Sum Sq   Mean Sq F value   Pr(>F)
source     3 0.0003368 1.123e-04    2373 2.66e-08 ***
Residuals  5 0.0000002 5.000e-08
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
          Df    Sum Sq   Mean Sq F value Pr(>F)
Residuals  9 1.151e-08 1.279e-09

pheno4 <- as.data.frame(summarise(group_by(pheno3, source, extract), pheno.mean
=mean(conc), pheno.n=length(conc)))
> head(pheno4)
  source extract pheno.mean pheno.n
1      C      C3  0.0175040       2
2      D      D1  0.0108810       2
3      D      D2  0.0110925       2
4      D      D3  0.0113185       2
5      G      G1  0.0167950       2
6      G      G2  0.0167500       2

pheno6 <- as.data.frame(summarise(group_by(pheno4, source), pheno.mean=mean(phe
no.mean), pheno.v=var(pheno.mean)))
> head(pheno6)
  source pheno.mean pheno.v
1      C 0.01750400      NA
2      D 0.01109733      NA
3      G 0.01683033      NA
4      H 0.00612375      NA

mod4 <- aov(pheno.mean ~ source, data = pheno4)
> summary(mod4)
            Df    Sum Sq   Mean Sq F value   Pr(>F)
source       3 1.684e-04 5.613e-05    2373 2.66e-08 ***
Residuals    5 1.200e-07 2.000e-08
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD(mod4)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = pheno.mean ~ source, data = pheno4)

$source
              diff          lwr           upr     p adj
D-C -0.0064066667 -0.007061908 -5.751426e-03 0.0000022
G-C -0.0006736667 -0.001328908 -1.842579e-05 0.0451552
H-C -0.0113802500 -0.012075238 -1.068526e-02 0.0000005
G-D  0.0057330000  0.005269675  6.196325e-03 0.0000007
H-D -0.0049735833 -0.005491597 -4.455570e-03 0.0000024
H-G -0.0107065833 -0.011224597 -1.018857e-02 0.0000005
```

Flavonoids

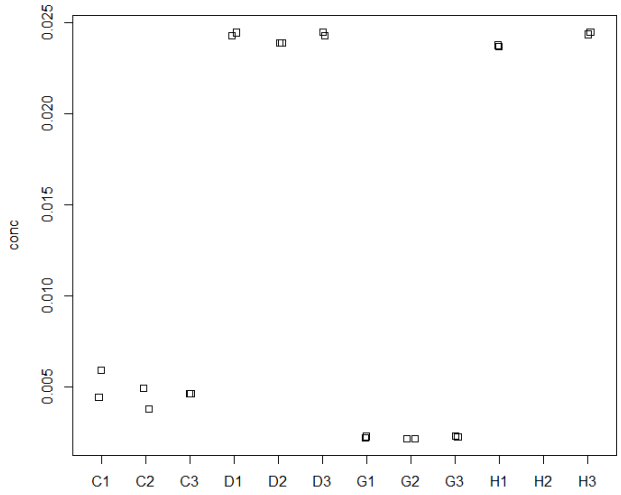
```
#Analysis of Phenolic acids
> install.packages("dplyr")
> flavo<-read.csv("../Statistics/Flavonoids.csv")
> head(flavo)
  source extract     conc
1      C      C1 0.005953
2      C      C1 0.004469
3      C      C2 0.003816
4      C      C2 0.004932
5      C      C3 0.004652
6      C      C3 0.004652
>
> table(flavo$source, flavo$extract)

   C1 C2 C3 D1 D2 D3 G1 G2 G3 H1 H2 H3
  C  2  2  2  0  0  0  0  0  0  0  0  0
  D  0  0  0  2  2  2  0  0  0  0  0  0
  G  0  0  0  0  0  0  2  2  2  0  0  0
  H  0  0  0  0  0  0  0  0  0  2  2  2
>
> stripchart(conc~extract, data=flavo, vertical = TRUE, method = "jitter")
```




```
> source("dfsumm.R")
> dfsumm(flavo)

 24 rows and 3 columns
 22 unique rows
                   source extract    conc
Class              factor  factor numeric
Minimum                 C      C1 0.00218
Maximum                 H      H3  0.0245
Mean                    D      D3  0.0137
Unique (excld. NA)      4      12      22
Missing values          0       0       0
Sorted               TRUE    TRUE   FALSE
```

```
>
> mod1 <- aov(conc ~ source + Error(extract), data = flavo)
> summary(mod1)

Error: extract
          Df   Sum Sq   Mean Sq F value   Pr(>F)
source     3 2.52e-03 0.0008398    1737 1.36e-11 ***
Residuals  8 3.90e-06 0.0000005
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
          Df    Sum Sq   Mean Sq F value Pr(>F)
Residuals 12 6.369e-06 5.308e-07
>
> library(dplyr)
>
> flavo2 <- as.data.frame(summarise(group_by(flavo, source, extract), flavo.mea
n=mean(conc), flavo.n=length(conc)))
> head(flavo2)
  source extract flavo.mean flavo.n
1      C      C1  0.0052110       2
2      C      C2  0.0043740       2
3      C      C3  0.0046520       2
4      D      D1  0.0243585       2
5      D      D2  0.0238780       2
6      D      D3  0.0243735       2
> flavo2
   source extract flavo.mea  flavo.n
1       C      C1  n                2
                   0.0052110
2       C      C2  0.0043740       2
3       C      C3  0.0046520       2
4       D      D1  0.0243585       2
5       D      D2  0.0238780       2
6       D      D3  0.0243735       2
7       G      G1  0.0022640       2
8       G      G2  0.0021810       2
9       G      G3  0.0022960       2
10      H      H1  0.0237010       2
11      H      H2  0.0227330       2
12      H      H3  0.0244020       2
>
> flavo5 <- as.data.frame(summarise(group_by(flavo2, source), flavo.mean=mean(f
lavo.mean), flavo.v=var(flavo.mean)))
> head(flavo5)
  source  flavo.mean flavo.v
1      C 0.004745667      NA
2      D 0.024203333      NA
3      G 0.002247000      NA
4      H 0.023612000      NA
>
> mod2 <- aov(flavo.mean ~ source, data = flavo2)
> summary(mod2)
            Df   Sum Sq   Mean Sq F value   Pr(>F)
source       3 1.26e-03 0.0004199    1737 1.36e-11 ***
Residuals    8 1.90e-06 0.0000002
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
>
> TukeyHSD(mod2)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = flavo.mean   source, data = flavo2)
                   ~

$source
             diff          lwr           upr     p adj
D-C  0.0194576667  0.018172055  0.0207432783 0.0000000
G-C -0.0024986667 -0.003784278 -0.0012130550 0.0011410
H-C  0.0188663333  0.017580722  0.0201519450 0.0000000
G-D -0.0219563333 -0.023241945 -0.0206707217 0.0000000
H-D -0.0005913333 -0.001876945  0.0006942783 0.4940966
H-G  0.0213650000  0.020079388  0.0226506117 0.0000000

>
> flavo3<-subset(flavo, !extract %in% c("H2"))
> head(flavo3)
  source extract     conc
1      C      C1 0.005953
2      C      C1 0.004469
3      C      C2 0.003816
4      C      C2 0.004932
5      C      C3 0.004652
6      C      C3 0.004652
> flavo3
   source extract     conc
1       C      C1 0.005953
2       C      C1 0.004469
3       C      C2 0.003816
4       C      C2 0.004932
5       C      C3 0.004652
6       C      C3 0.004652
7       D      D1 0.024434
8       D      D1 0.024283
9       D      D2 0.023878
10      D      D2 0.023878
11      D      D3 0.024273
12      D      D3 0.024474
13      G      G1 0.002222
14      G      G1 0.002306
15      G      G2 0.002180
16      G      G2 0.002182
17      G      G3 0.002276
18      G      G3 0.002316
19      H      H1 0.023749
20      H      H1 0.023653
23      H      H3 0.024338
24      H      H3 0.024466
>
> stripchart(conc~extract, data=flavo3, vertical = TRUE, method = "jitter")
```




```
> mod3 <- aov(conc ~ source + Error(extract), data = flavo3)
> summary(mod3)

Error: extract
          Df    Sum Sq   Mean Sq F value   Pr(>F)
source     3 0.0023439 0.0007813    3528 1.73e-11 ***
Residuals  7 0.0000016 0.0000002
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
          Df    Sum Sq   Mean Sq F value Pr(>F)
Residuals 11 1.773e-06 1.611e-07

> flavo4 <- as.data.frame(summarise(group_by(flavo3, source, extract), flavo.me
an=mean(conc), flavo.n=length(conc)))
> head(flavo4)
  source extract flavo.mean flavo.n
1      C      C1  0.0052110       2
2      C      C2  0.0043740       2
3      C      C3  0.0046520       2
4      D      D1  0.0243585       2
5      D      D2  0.0238780       2
6      D      D3  0.0243735       2
> flavo4
   source extract flavo.mean flavo.n
1       C      C1  0.0052110       2
2       C      C2  0.0043740       2
3       C      C3  0.0046520       2
4       D      D1  0.0243585       2
5       D      D2  0.0238780       2
6       D      D3  0.0243735       2
7       G      G1  0.0022640       2
8       G      G2  0.0021810       2
9       G      G3  0.0022960       2
10      H      H1  0.0237010       2
11      H      H3  0.0244020       2
```

```
>
> flavo6 <- as.data.frame(summarise(group_by(flavo4, source), flavo.mean=mean(f
lavo.mean), flavo.v=var(flavo.mean)))
> head(flavo6)
  source  flavo.mean flavo.v
1      C 0.004745667      NA
2      D 0.024203333      NA
3      G 0.002247000      NA
4      H 0.024051500      NA
>
> mod4 <- aov(flavo.mean ~ source, data = flavo4)
> summary(mod4)
            Df    Sum Sq   Mean Sq F value   Pr(>F)
source       3 0.0011719 0.0003906    3528 1.73e-11 ***
Residuals    7 0.0000008 0.0000001
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> TukeyHSD(mod4)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = flavo.mean   source, data = flavo4)
                   ~

$source
              diff          lwr           upr     p adj
D-C  0.0194576667  0.018558327  0.0203570068 0.0000000
G-C -0.0024986667 -0.003398007 -0.0015993265 0.0001640
H-C  0.0193058333  0.018300340  0.0203113262 0.0000000
G-D -0.0219563333 -0.022855673 -0.0210569932 0.0000000
H-D -0.0001518333 -0.001157326  0.0008536595 0.9565552
H-G  0.0218045000  0.020799007  0.0228099929 0.0000000
```

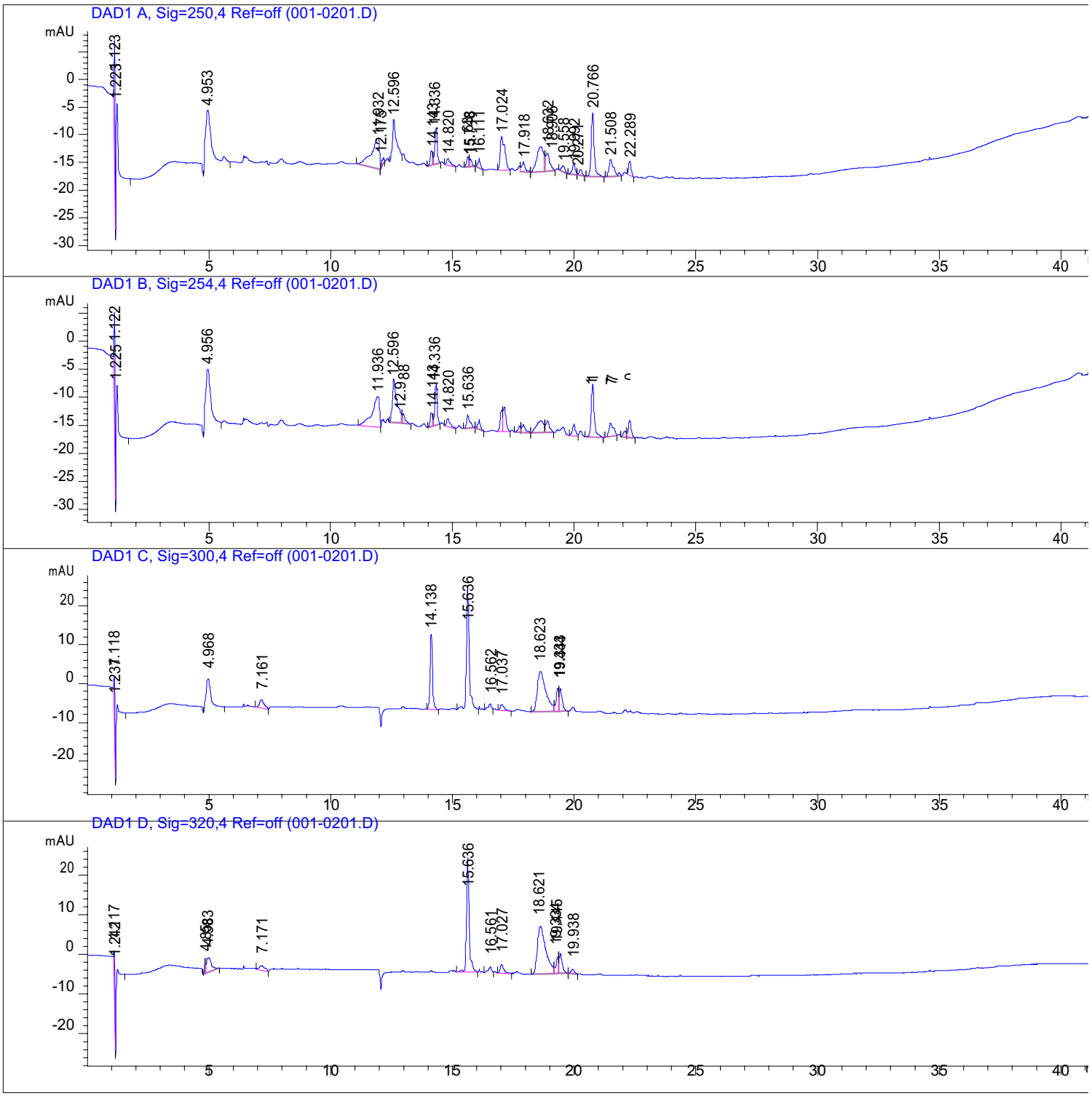
Appendix G

Method optimization HPLC chromatograms

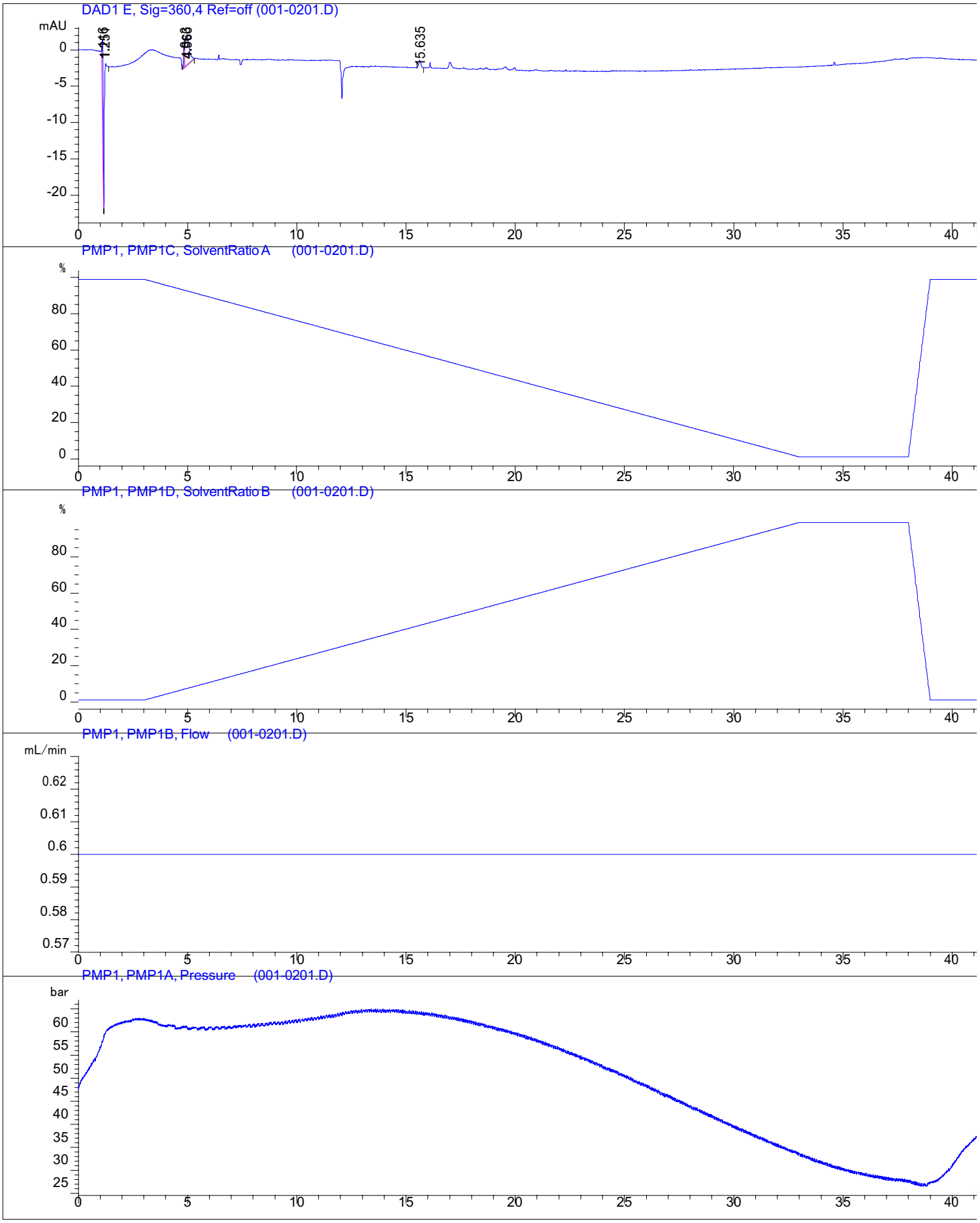
mple Name: A11T40

==========Method 1==========

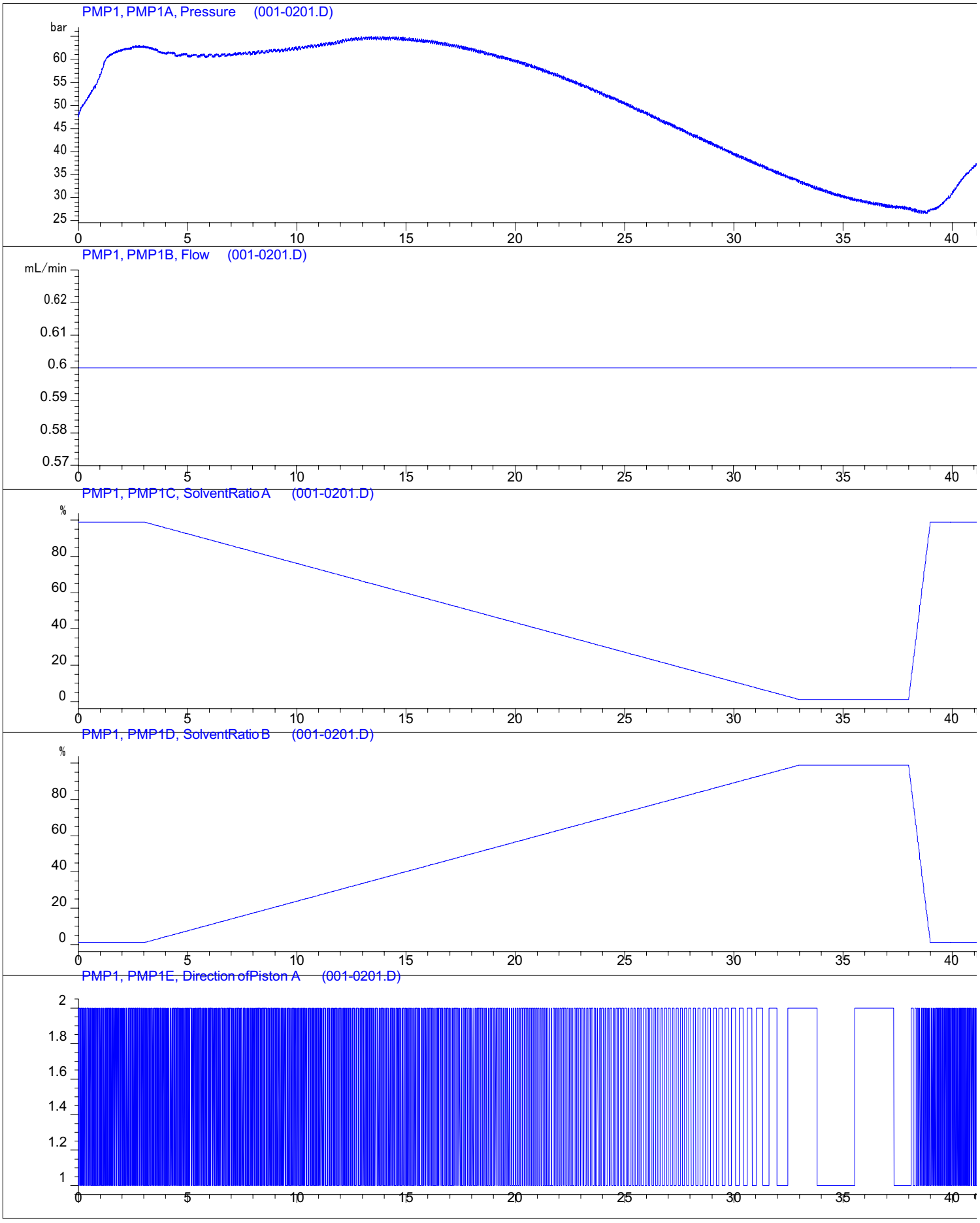
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 1
Injection Date : 5/1/2017 5:06:10 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start sequence.S
Method : C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start 40.M (Sequence Method)
Last changed : 5/1/2017 4:19:55 PM by SYSTEM
Method Info : Start 40



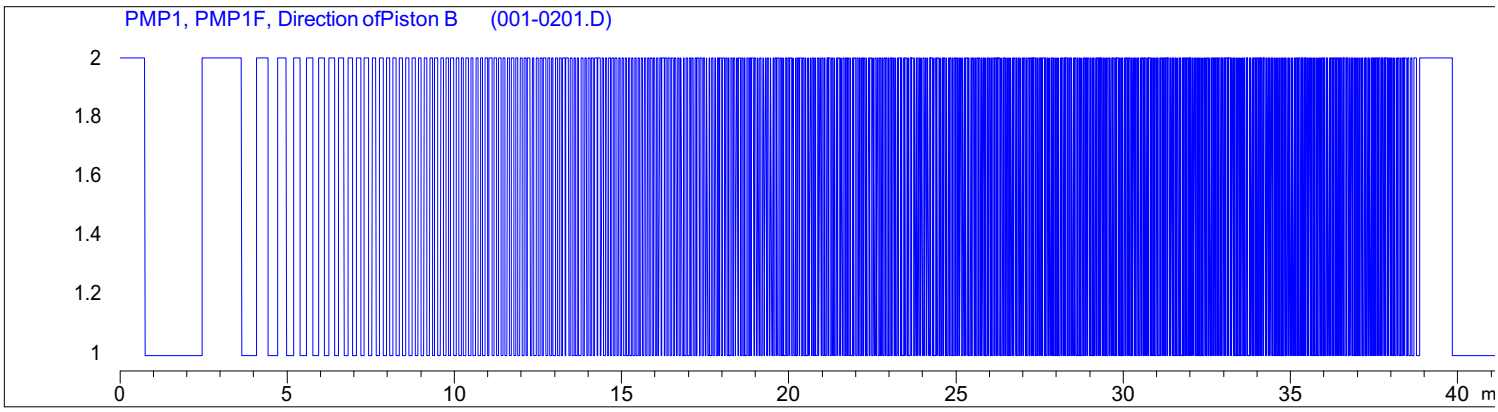
mple Name: A11T40



mple Name: A11T40



mple Name: A11T40



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.123	BB	0.0411	56.51979	22.66758	4.4511
2	1.223	BB	0.1460	255.05457	23.61543	20.0862
3	4.953	BV R	0.2741	226.06032	11.46088	17.8028
4	11.932	BB	0.3401	125.69981	5.39012	9.8992
5	12.175	BB	0.1060	8.93258	1.15738	0.7035
6	12.596	BB	0.1463	77.61326	7.16856	6.1122
7	14.143	BV	0.1037	17.38473	2.54636	1.3691
8	14.336	VB	0.1046	44.61226	6.46057	3.5133
9	14.820	BB	0.1401	11.18279	1.14311	0.8807
10	15.638	BV	0.0940	11.55301	1.82038	0.9098
11	15.748	VB	0.0954	8.53025	1.32054	0.6718
12	16.111	BB	0.0829	10.62393	1.74876	0.8367
13	17.024	BB	0.1775	78.16410	6.02065	6.1556
14	17.918	VB	0.1360	16.06586	1.73494	1.2652
15	18.632	BV	0.3076	89.62249	4.43748	7.0580
16	18.906	VB	0.1668	38.09201	3.24875	2.9998
17	19.558	BB	0.1377	10.04658	1.13041	0.7912
18	19.992	BV	0.1147	16.25101	1.88193	1.2798
19	20.271	VB	0.1261	8.64228	1.04954	0.6806
20	20.766	BB	0.1308	99.40329	11.50943	7.8282
21	21.508	BV R	0.1887	41.90406	3.08029	3.3000
22	22.289	BB	0.1136	17.84363	2.49003	1.4052
Totals :				1269.80260		123.08311

mple Name: A11T40

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.122	BB	0.0418	53.42464	20.90294	4.7428
2	1.225	BB	0.1492	233.81311	21.11693	20.7569
3	4.956	BB	0.2273	182.01598	11.45922	16.1586
4	11.936	BB	0.3043	113.03416	5.39793	10.0347
5	12.596	BV	0.1808	106.03946	7.78820	9.4137
6	12.988	VB	0.1174	13.54092	1.65517	1.2021
7	14.143	BV	0.1017	15.99543	2.40034	1.4200
8	14.336	VB	0.1033	49.07763	7.21755	4.3569
9	14.820	BB	0.1344	13.17616	1.41784	1.1697
10	15.636	BB	0.1265	21.14220	2.35979	1.8769
11	16.111	BB	0.0811	10.28929	1.73778	0.9134
12	17.029	BV	0.1031	25.68581	3.78918	2.2803
13	17.142	VB	0.1183	35.31810	4.37167	3.1354
14	17.762	BV	0.1164	8.80145	1.11166	0.7814
15	17.922	VB	0.1416	14.08883	1.42199	1.2507
16	18.646	BV	0.2961	44.60047	2.09728	3.9594
17	18.911	VB	0.1649	23.73049	2.08262	2.1067
18	19.994	BV	0.1131	17.00456	2.00229	1.5096
19	20.766	BB	0.1321	82.13100	9.38724	7.2912
20	21.501	BB	0.1834	30.27543	2.33279	2.6877
21	22.097	BV	0.1192	8.81889	1.01710	0.7829
22	22.289	VB	0.1258	24.43154	2.97552	2.1689
Totals :				1126.43554	116.04304	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.118	BB	0.0436	37.73127	13.94402	3.2414
2	1.237	BB	0.1646	213.38963	17.45681	18.3315
3	4.968	BB	0.2225	129.08315	8.43728	11.0891
4	7.161	BB	0.2004	28.66774	2.08490	2.4627
5	14.138	BB	0.1047	133.90227	19.36261	11.5030
6	15.636	VB R	0.1114	244.28653	31.89009	20.9857
7	16.562	BB	0.1219	11.96699	1.42628	1.0280
8	17.037	BB	0.1730	17.63325	1.57041	1.5148
9	18.623	BV	0.3617	252.70779	10.31820	21.7092
10	19.333	VV	0.1136	46.57986	5.93163	4.0015
11	19.444	VB	0.1186	48.11066	5.81299	4.1330
Totals :				1164.05913	118.23522	

mple Name: A11T40

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.117	BB	0.0470	33.81796	11.99879	3.5471
2	1.242	BB	0.1580	208.48032	17.89080	21.8673
3	4.858	BV	0.0576	10.66231	2.61490	1.1184
4	4.983	VB	0.2101	54.66419	3.88599	5.7337
5	7.171	BB	0.2198	18.02628	1.14411	1.8908
6	15.636	VV R	0.1115	217.09209	28.93227	22.7706
7	16.561	BB	0.1205	11.01181	1.33198	1.1550
8	17.027	BB	0.1563	22.32971	2.16635	2.3421
9	18.621	BV	0.3605	290.95764	11.93178	30.5183
10	19.334	VV	0.1108	31.78516	4.17771	3.3339
11	19.445	VB	0.1253	44.64698	5.04378	4.6830
12	19.938	BB	0.1244	9.91383	1.10814	1.0399
Totals :				953.38827	92.22661	

Signal 5: DAD1 E, Sig=360,4 Ref=off

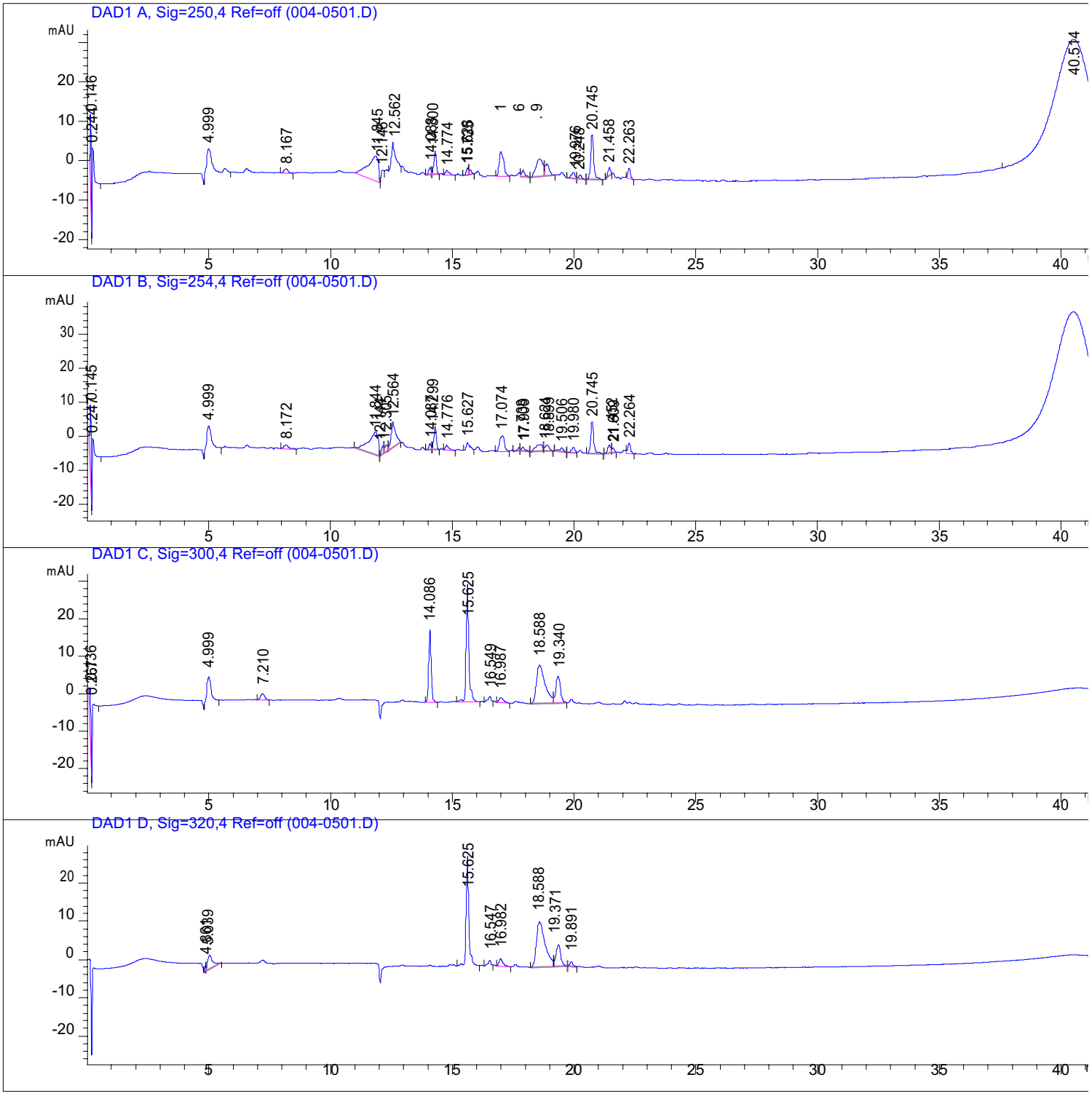
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.116	BB	0.0484	39.89515	12.78591	18.0463
2	1.251	BB	0.1199	109.89819	13.37062	49.7116
3	4.863	BV	0.0602	13.61952	3.29580	6.1607
4	4.966	VB	0.1890	50.64571	4.07633	22.9092
5	15.635	BB	0.0999	7.01311	1.07805	3.1723
Totals :				221.07168	34.60670	

*** End of Report ***

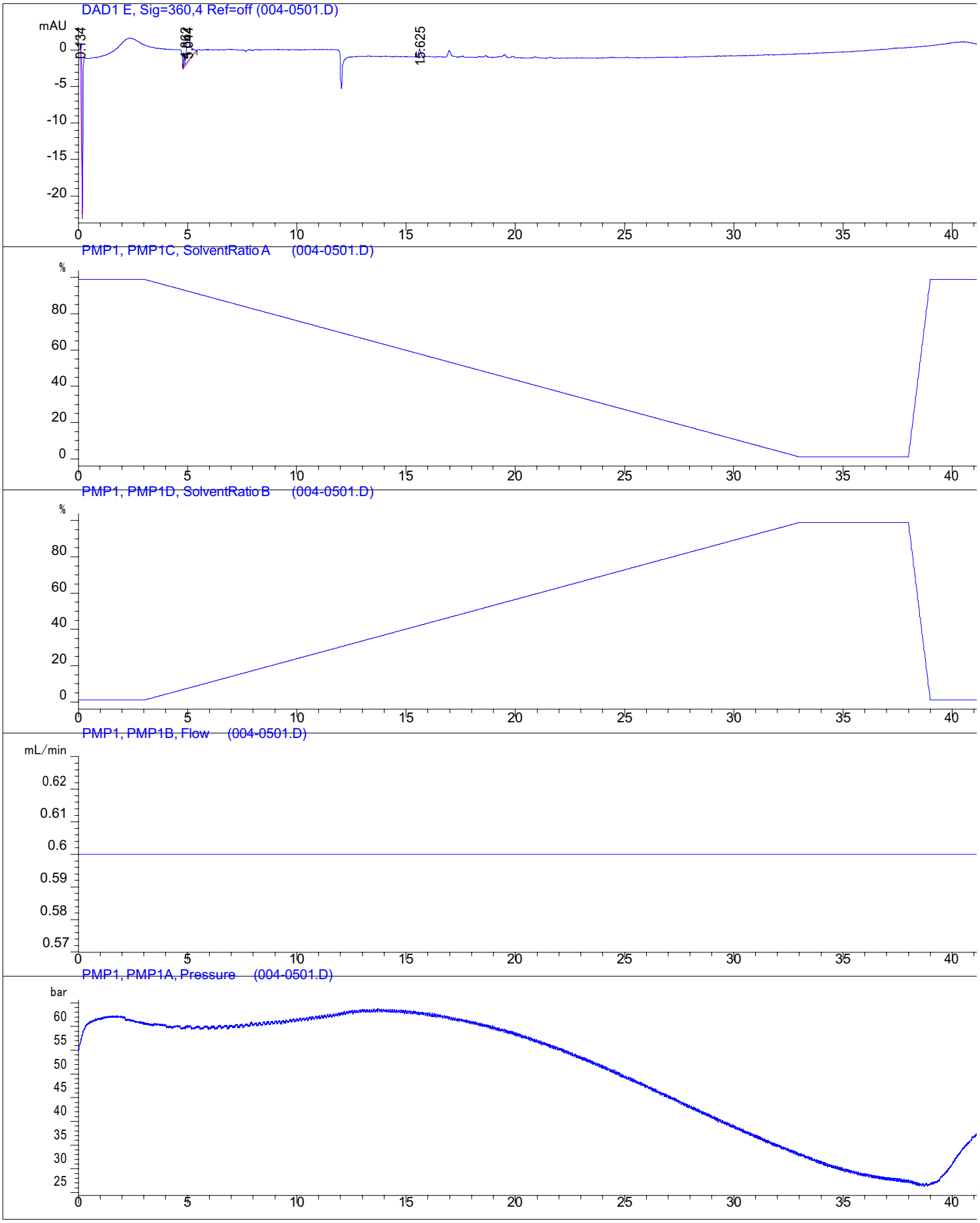
mple Name: A11T45

==========Method 2==========

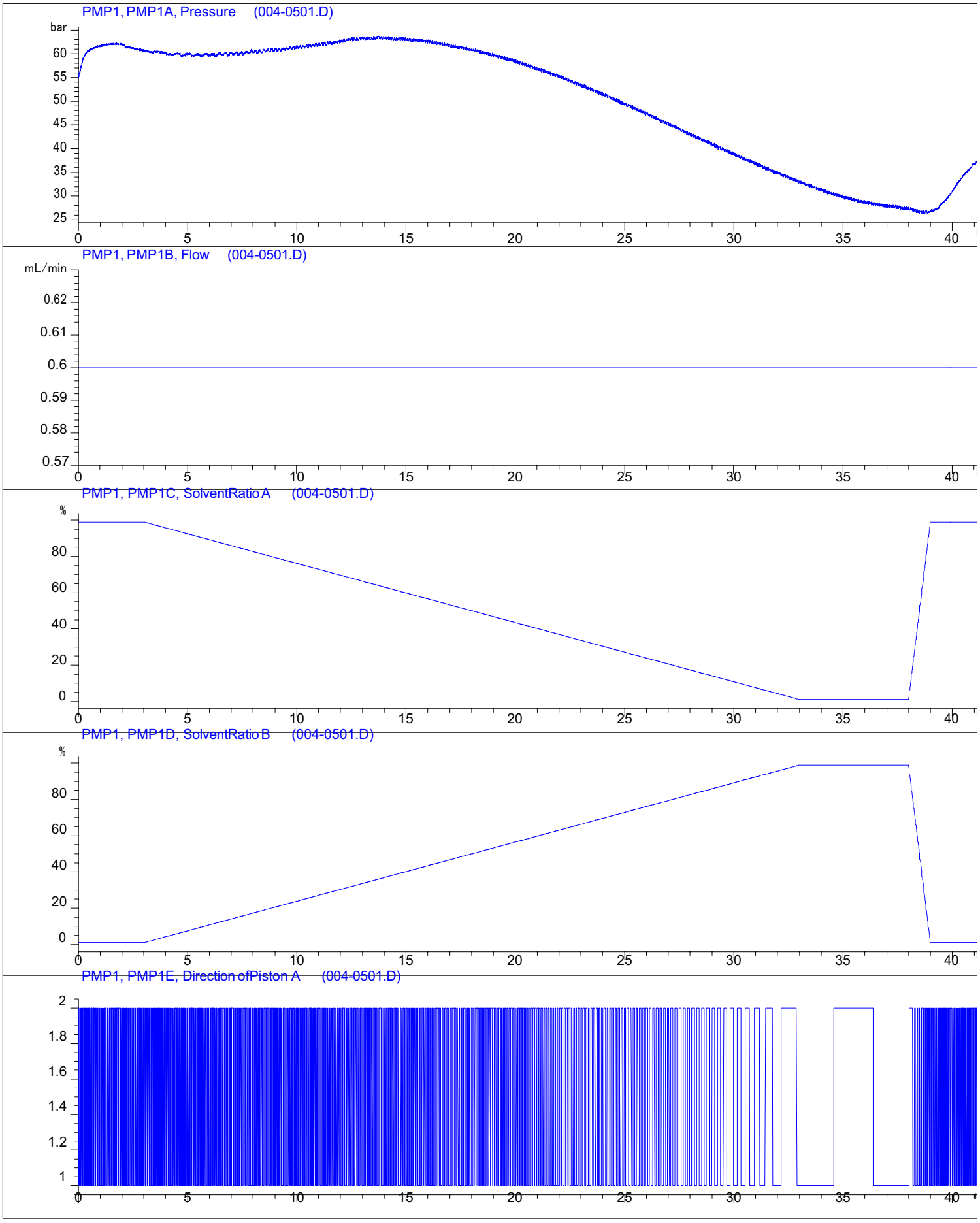
Acq. Operator	: SYSTEM	Seq. Line	: 5
Acq. Instrument	: HPLC 4	Location	: 4
Injection Date	: 5/1/2017 7:16:12 PM	Inj	: 1
		Inj Volume	: 10.000 µl
Sequence File	: C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start sequence.S		
Method	: C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start 45.M (Sequence Method)		
Last changed	: 5/1/2017 4:19:55 PM by SYSTEM		
Method Info	: Start 45		



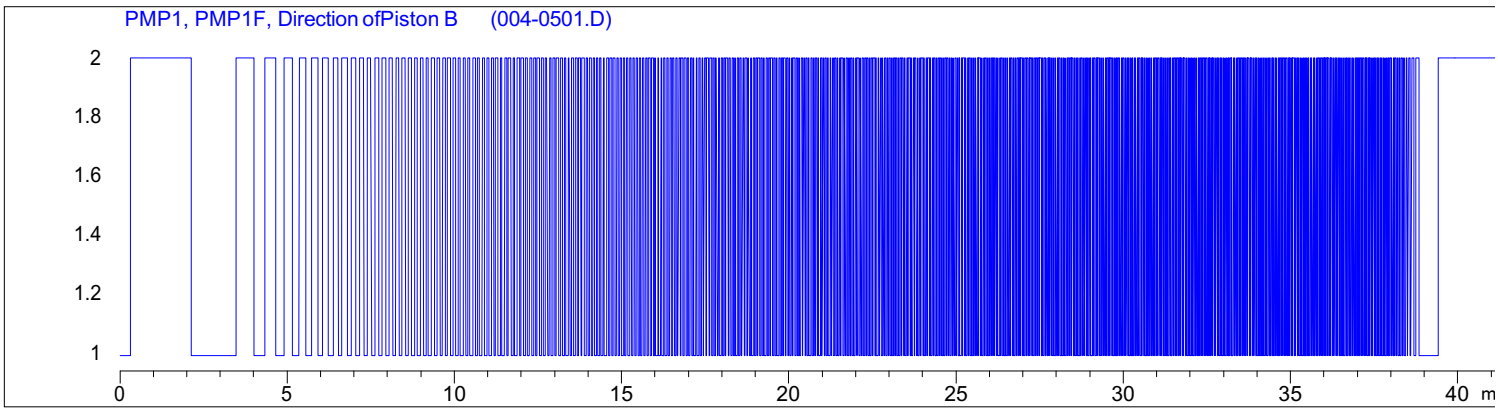
mple Name: A11T45



mple Name: A11T45



mple Name: A11T45



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.146	BB	0.0371	48.35300	20.81125	1.2162
2	0.244	BB	0.1184	175.34711	20.78193	4.4103
3	4.999	BV R	0.2980	186.10481	8.61429	4.6809
4	8.167	BB	0.1845	12.48026	1.02173	0.3139
5	11.845	BB	0.4133	184.73625	6.27847	4.6465
6	12.146	BB	0.1191	10.16041	1.39235	0.2556
7	12.562	BB	0.1495	75.10983	6.87437	1.8892
8	14.088	BV	0.1048	12.81333	1.89703	0.3223
9	14.300	VB	0.1035	40.40278	5.93007	1.0162
10	14.774	BB	0.1433	10.99825	1.09427	0.2766
11	15.628	BV	0.0956	11.79048	1.81948	0.2966
12	15.735	VB	0.0900	7.44929	1.24123	0.1874
13	16.985	BB	0.1719	78.57505	6.28526	1.9763
14	17.912	VB	0.1364	15.96073	1.71778	0.4014
15	18.590	BV	0.3143	91.90596	4.46169	2.3116
16	18.894	VB	0.1741	36.33220	3.02528	0.9138
17	19.976	BV	0.1471	16.31090	1.57136	0.4103
18	20.248	VB	0.1300	8.64122	1.00830	0.2173
19	20.745	BB	0.1312	98.68708	11.37858	2.4822
20	21.458	BB	0.1151	16.33038	2.23830	0.4107
21	22.263	BB	0.1122	17.84191	2.47348	0.4488
22	40.514	BBA	1.1811	2819.49390	28.57603	70.9159
Totals :				3975.82514	140.49254	

mple Name: A11T45

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.145	BB	0.0458	71.11301	23.20140	6.2451
2	0.247	BB	0.1352	185.74149	18.81045	16.3118
3	4.999	BB	0.2330	140.71153	8.77870	12.3573
4	8.172	BB	0.1820	13.49006	1.12388	1.1847
5	11.844	BB	0.3984	181.57539	6.57051	15.9459
6	12.144	BV	0.1206	17.61530	2.37333	1.5470
7	12.305	VV	0.1215	18.42462	2.03659	1.6180
8	12.564	VB	0.1711	98.32565	7.69466	8.6349
9	14.087	BV	0.1023	11.93484	1.77855	1.0481
10	14.299	VB	0.1034	45.54473	6.69360	3.9997
11	14.776	BB	0.1285	11.92493	1.35699	1.0472
12	15.627	BB	0.1238	20.10162	2.30308	1.7653
13	17.074	BB	0.1803	61.04152	4.55517	5.3607
14	17.738	BV	0.1166	8.91814	1.12417	0.7832
15	17.909	VB	0.1400	14.04162	1.41236	1.2331
16	18.624	BV	0.2733	45.00818	2.10647	3.9526
17	18.899	VB	0.1748	23.36302	1.96357	2.0517
18	19.506	BB	0.1974	16.89377	1.17740	1.4836
19	19.980	BV	0.1485	17.11596	1.68483	1.5031
20	20.745	BB	0.1321	81.29063	9.28951	7.1389
21	21.452	BV	0.1375	21.31890	2.35727	1.8722
22	21.609	VB	0.1080	9.23909	1.34654	0.8114
23	22.264	VB	0.1212	23.96203	2.93625	2.1043
Totals :				1138.69603	112.67528	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.136	BB	0.0516	48.58694	14.32097	4.4954
2	0.267	BB	0.1334	145.59825	15.25195	13.4712
3	4.999	BB	0.2070	109.59312	7.94049	10.1399
4	7.210	BB	0.1750	19.50579	1.66110	1.8047
5	14.086	BB	0.1098	138.93890	19.33192	12.8551
6	15.625	VB R	0.1139	242.85214	31.50487	22.4695
7	16.549	BB	0.1254	11.99023	1.40772	1.1094
8	16.987	BB	0.1705	16.83445	1.35943	1.5576
9	18.588	BV	0.3661	252.03989	10.20777	23.3195
10	19.340	VB	0.2057	94.87040	7.10666	8.7777
Totals :				1080.81011	110.09287	

mple Name: A11T45

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.861	BV	0.0579	6.30383	1.75414	0.9043
2	5.039	VB	0.2317	59.82178	3.84164	8.5814
3	15.625	VB R	0.1129	218.74077	28.69262	31.3781
4	16.547	BB	0.1227	11.15880	1.31933	1.6007
5	16.982	BB	0.1496	21.38071	1.98587	3.0670
6	18.588	BV	0.3646	292.73160	11.83573	41.9921
7	19.371	VB	0.2069	76.90188	5.71854	11.0315
8	19.891	BB	0.1292	10.07252	1.16107	1.4449
Totals :				697.11189	56.30894	

Signal 5: DAD1 E, Sig=360,4 Ref=off

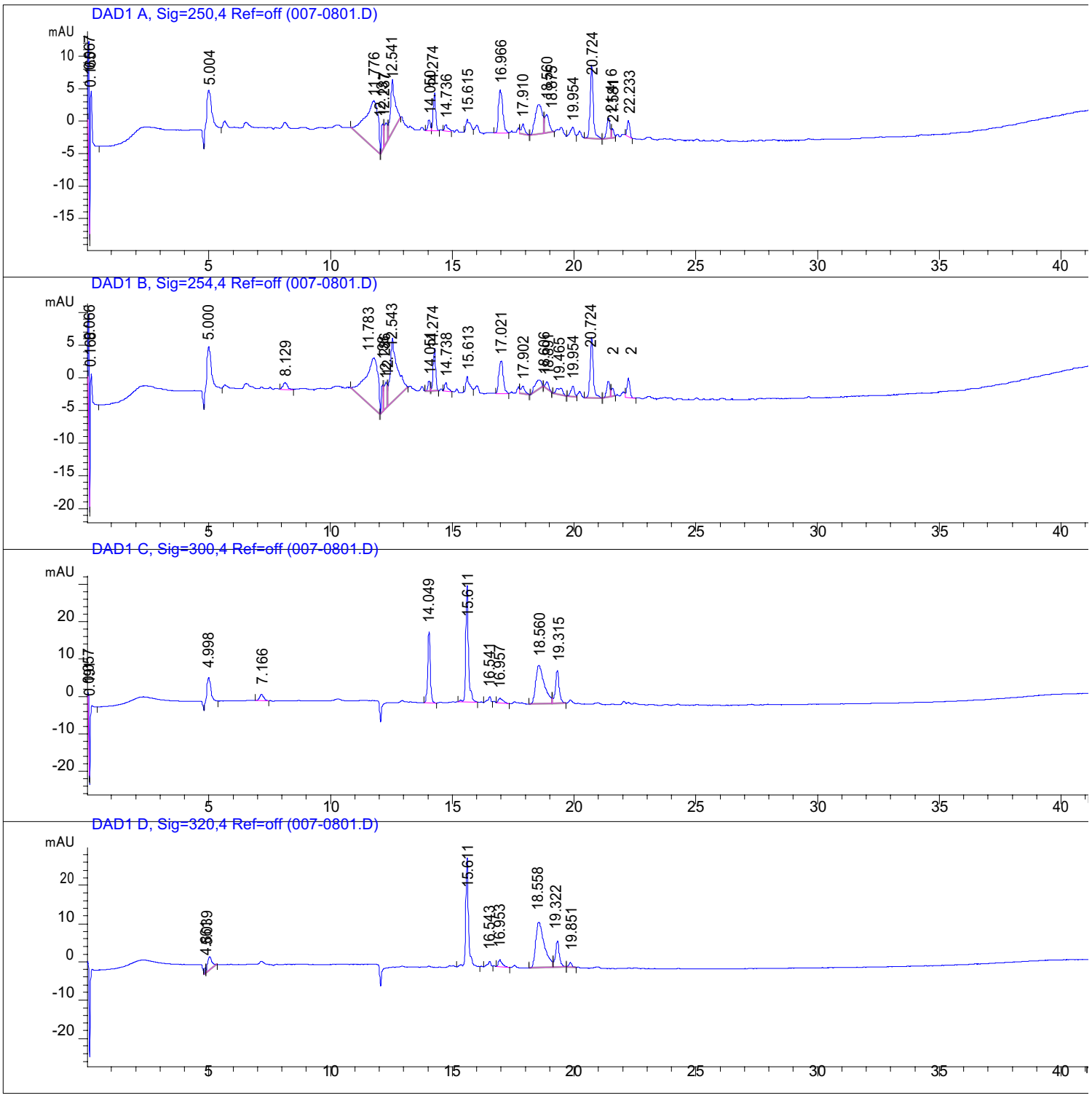
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.134	BB	0.0526	34.23290	10.90480	35.0580
2	4.862	BV	0.0554	6.38380	1.79767	6.5377
3	5.044	VB	0.2158	49.97212	3.47515	51.1766
4	15.625	BB	0.1010	7.05757	1.06867	7.2277
Totals :				97.64640	17.24628	

*** End of Report ***

mple Name: A11T50

==========**Method 3**==========

Acq. Operator	: SYSTEM	Seq. Line	: 8
Acq. Instrument	: HPLC 4	Location	: 7
Injection Date	: 5/1/2017 9:26:25 PM	Inj	: 1
		Inj Volume	: 10.000 µl
Sequence File	: C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start sequence.S		
Method	: C:\Chem32\1\Data\Start sequence 2017-05-01 16-19-55\Start 50.M (Sequence Method)		
Last changed	: 5/1/2017 4:19:55 PM by SYSTEM		
Method Info	: Start 50		



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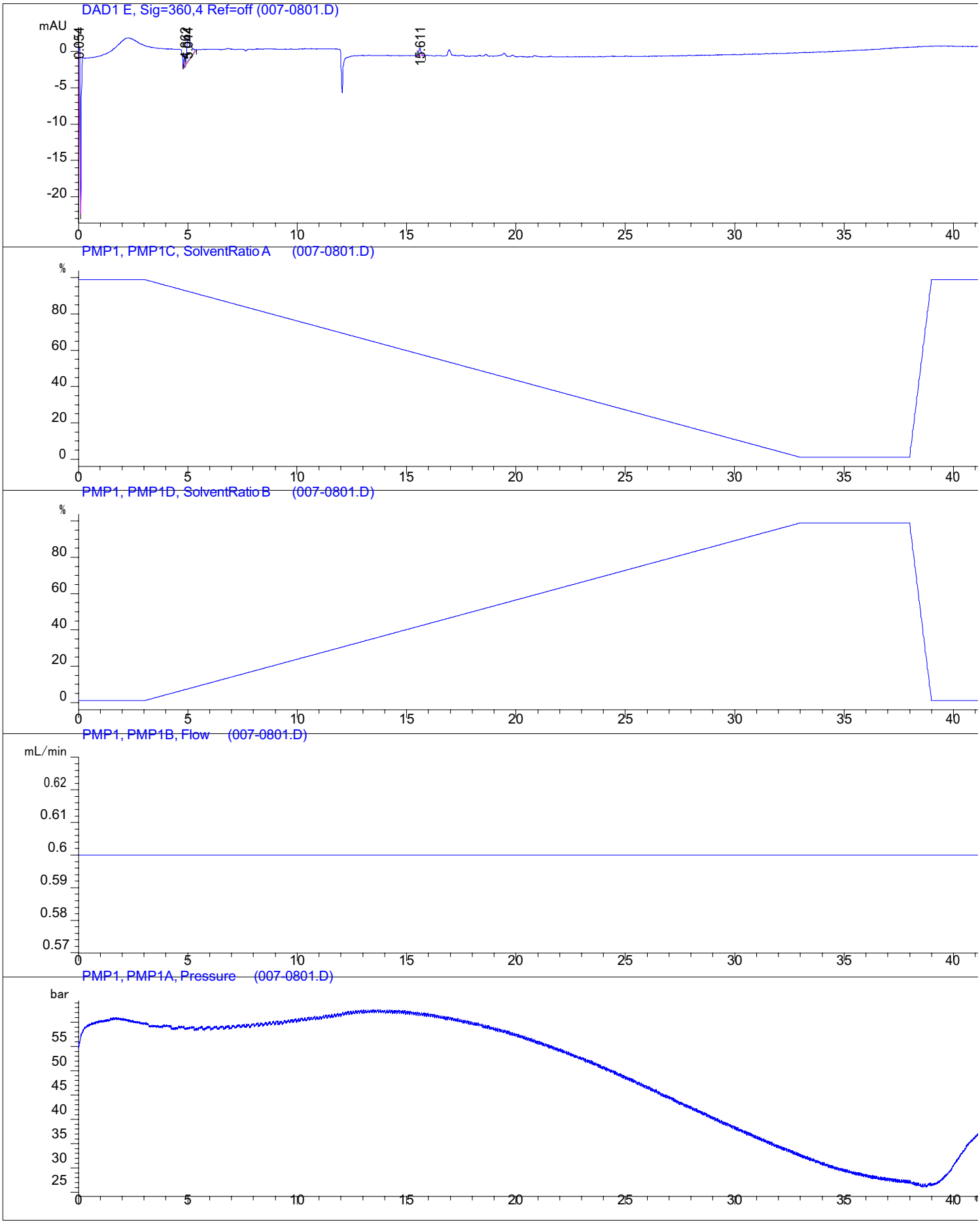

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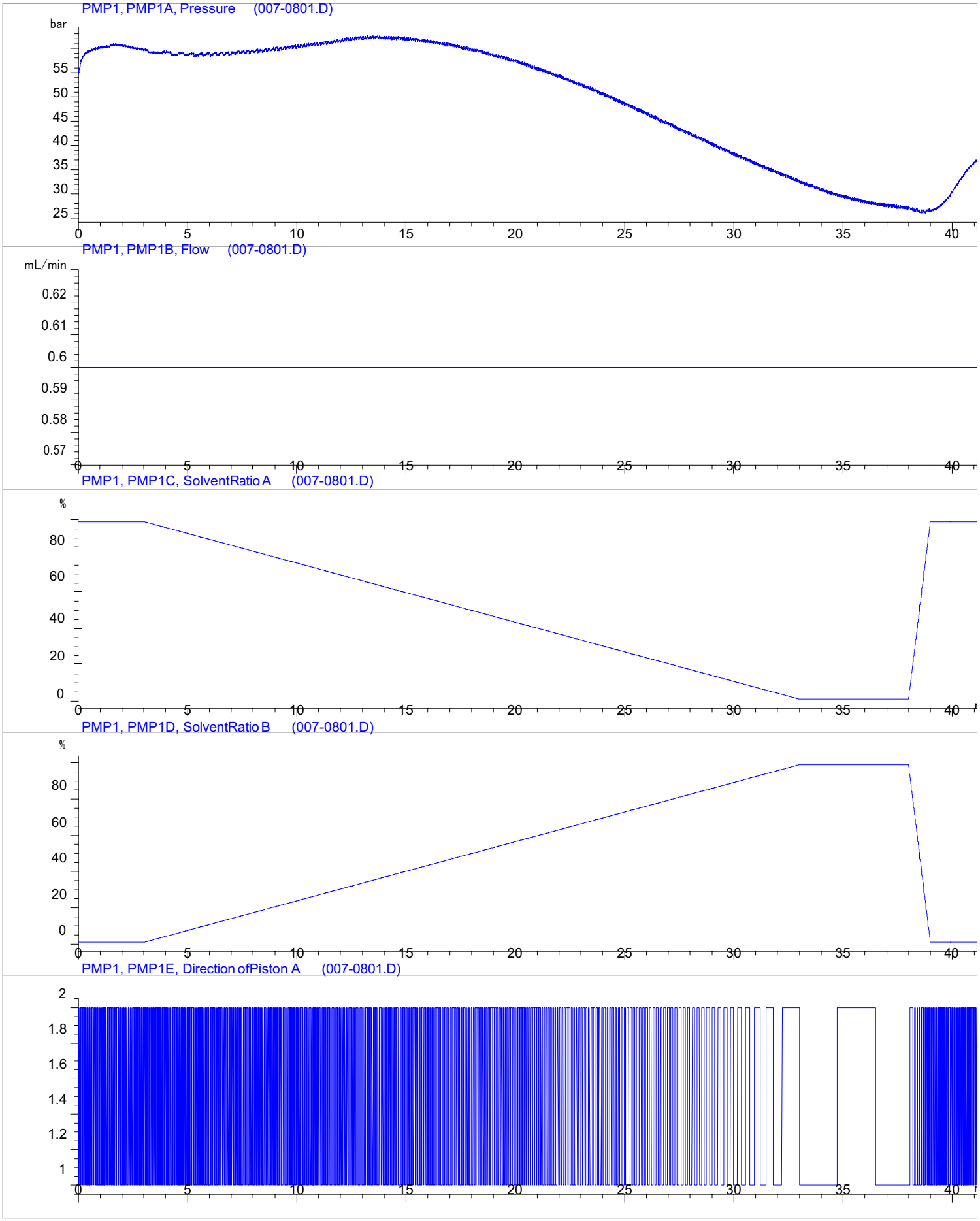

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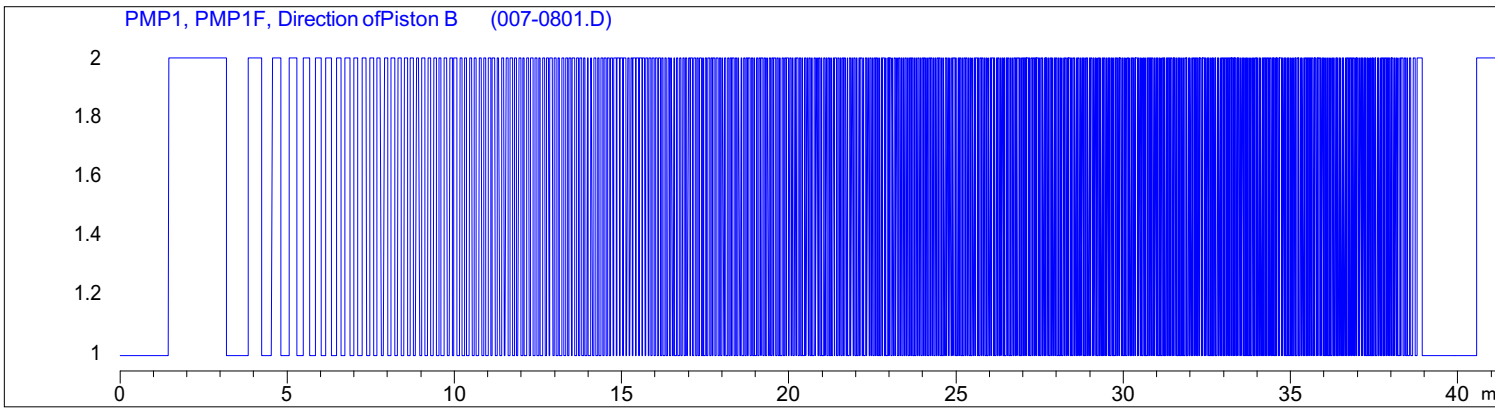

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.067	BB	0.0371	48.43140	20.88703	3.8323
2	0.165	BB	0.1223	182.59758	20.82521	14.4486
3	5.004	BB	0.2420	138.17354	8.14135	10.9334
4	11.776	BB	0.4748	257.34787	7.30856	20.3634
5	12.137	BV	0.1021	27.09149	4.04842	2.1437
6	12.287	VV	0.1255	29.64850	3.21894	2.3460
7	12.541	VB	0.1802	108.74583	8.12006	8.6048
8	14.050	BV	0.1039	11.54331	1.68453	0.9134
9	14.274	VB	0.1069	39.81015	5.74232	3.1501
10	14.736	BB	0.1254	8.75234	1.00664	0.6926
11	15.615	BB	0.1453	18.69190	1.76933	1.4791
12	16.966	BB	0.1938	79.91932	6.67110	6.3239
13	17.910	VB	0.1340	13.78977	1.54746	1.0912
14	18.560	BV	0.3135	94.61674	4.49669	7.4868
15	18.875	VB	0.1797	34.33590	2.78733	2.7169
16	19.954	BB	0.1488	13.68437	1.36677	1.0828
17	20.724	BB	0.1330	99.44572	11.26247	7.8689
18	21.416	BV	0.1416	29.31057	3.11874	2.3193
19	21.581	VV	0.1044	9.46374	1.37274	0.7488
20	22.233	BB	0.1129	18.37530	2.52487	1.4540
Totals :				1263.77533	117.90057	

mple Name: A11T50

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.066	BB	0.0366	40.81793	17.90983	3.1968
2	0.168	BB	0.1352	184.63779	18.70056	14.4605
3	5.000	BB	0.2311	139.39867	8.69048	10.9175
4	8.129	BB	0.1703	12.85439	1.03919	1.0067
5	11.783	BB	0.4449	263.26761	7.77778	20.6187
6	12.138	BV	0.0955	25.49395	4.15825	1.9966
7	12.286	VV	0.1344	39.95980	4.07451	3.1296
8	12.543	VB	0.2599	199.45309	9.86351	15.6208
9	14.051	BV	0.1022	10.50463	1.56675	0.8227
10	14.274	VB	0.1068	45.06445	6.50655	3.5294
11	14.738	BB	0.1192	10.48508	1.28539	0.8212
12	15.613	BB	0.1257	19.92858	2.28707	1.5608
13	17.021	BB	0.2007	61.79132	4.91487	4.8394
14	17.902	VB	0.1453	12.49683	1.24286	0.9787
15	18.606	BB	0.2764	28.81314	1.34223	2.2566
16	18.891	BB	0.1425	10.17208	1.11471	0.7967
17	19.465	BB	0.2145	17.61818	1.10230	1.3798
18	19.954	BV	0.1455	16.78195	1.66672	1.3143
19	20.724	BB	0.1357	81.95620	9.22179	6.4187
20	21.412	BV	0.1431	22.46507	2.40138	1.7594
21	21.584	VB	0.1029	8.66094	1.28103	0.6783
22	22.235	VB	0.1245	24.21971	2.92801	1.8968
Totals :				1276.84137	111.07574	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.057	BB	0.0421	19.09332	7.39913	1.8079
2	0.191	BB	0.1425	157.06863	15.46975	14.8725
3	4.998	BB	0.2018	106.31113	7.96078	10.0664
4	7.166	BB	0.1794	19.66209	1.66903	1.8618
5	14.049	BB	0.1119	140.06920	19.01460	13.2629
6	15.611	VB R	0.1128	236.83849	31.10651	22.4258
7	16.541	BB	0.1246	12.27957	1.42417	1.1627
8	16.957	BB	0.1732	16.21151	1.26790	1.5350
9	18.560	BV	0.3599	252.60361	10.23706	23.9186
10	19.315	VB	0.1640	95.96117	8.74570	9.0864
Totals :				1056.09873	104.29463	

mple Name: A11T50

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.861	BV	0.0567	5.63694	1.61773	0.8249
2	5.039	VB	0.1966	45.71080	3.54210	6.6893
3	15.611	VB R	0.1143	220.27283	28.45847	32.2347
4	16.543	BB	0.1254	11.59946	1.30855	1.6975
5	16.953	BB	0.1524	21.11492	1.91843	3.0900
6	18.558	BV	0.3585	293.83282	11.88373	42.9995
7	19.322	VB	0.1655	75.54481	6.80406	11.0552
8	19.851	BB	0.1274	9.62734	1.17686	1.4089
Totals :				683.33991	56.70993	

Signal 5: DAD1 E, Sig=360,4 Ref=off

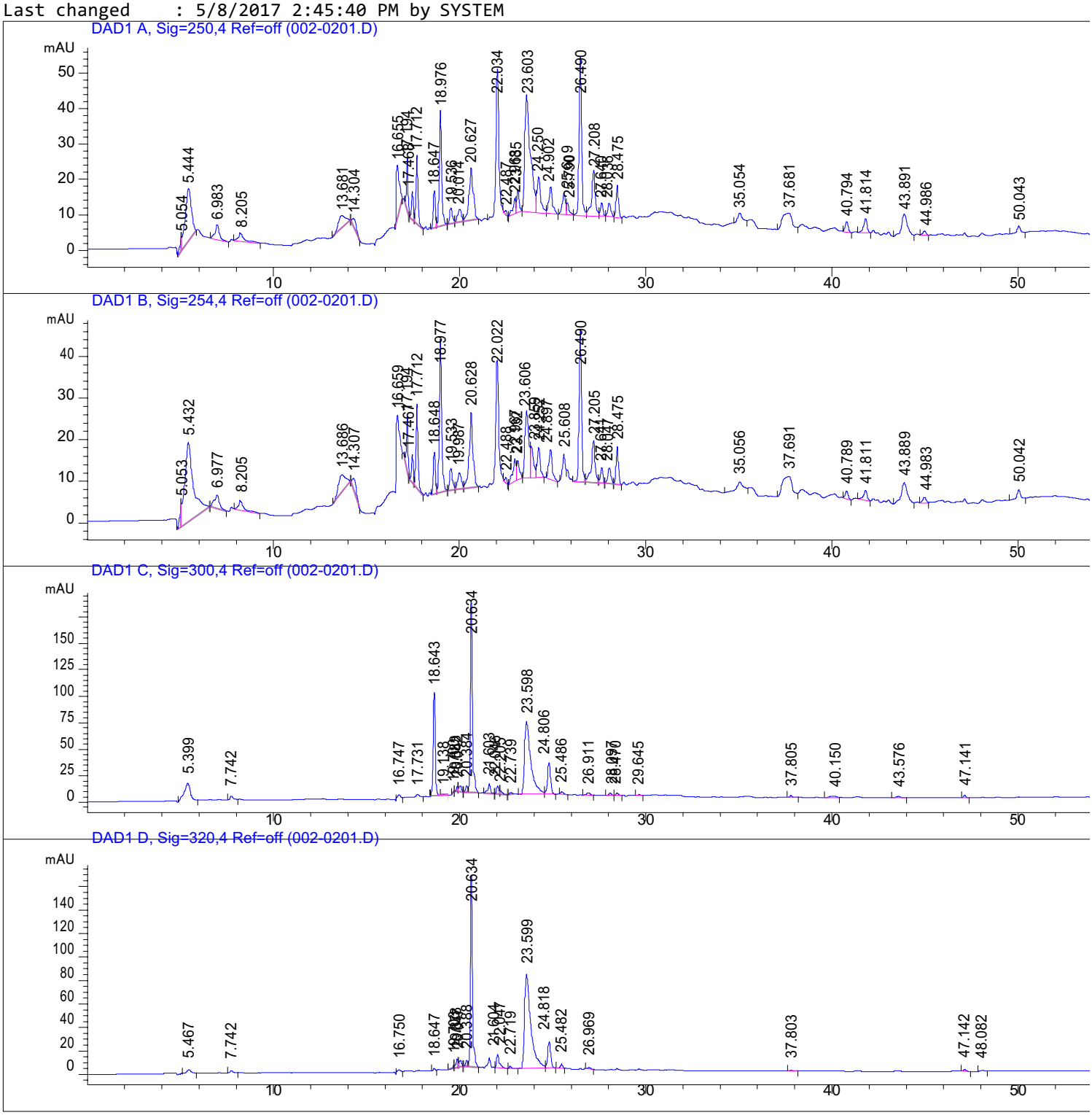
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.054	BB	0.0551	38.25588	11.40172	39.2859
2	4.862	BV	0.0536	5.40310	1.59042	5.5486
3	5.044	VB	0.1854	46.84994	3.42854	48.1114
4	15.611	BB	0.1039	6.86915	1.02856	7.0541
Totals :				97.37808	17.44924	

*** End of Report ***

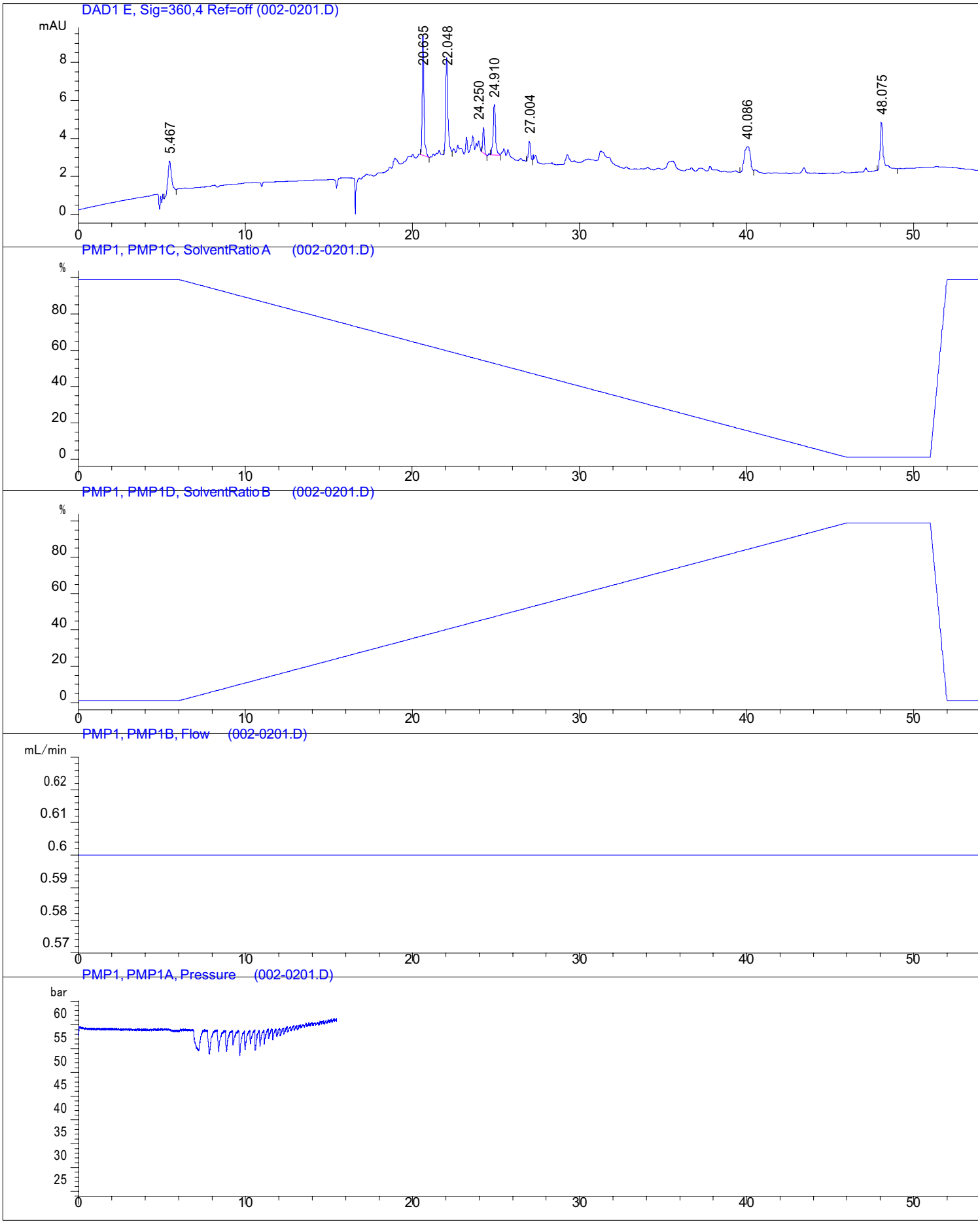
mple Name: D2T50

==========**Method 4**==========

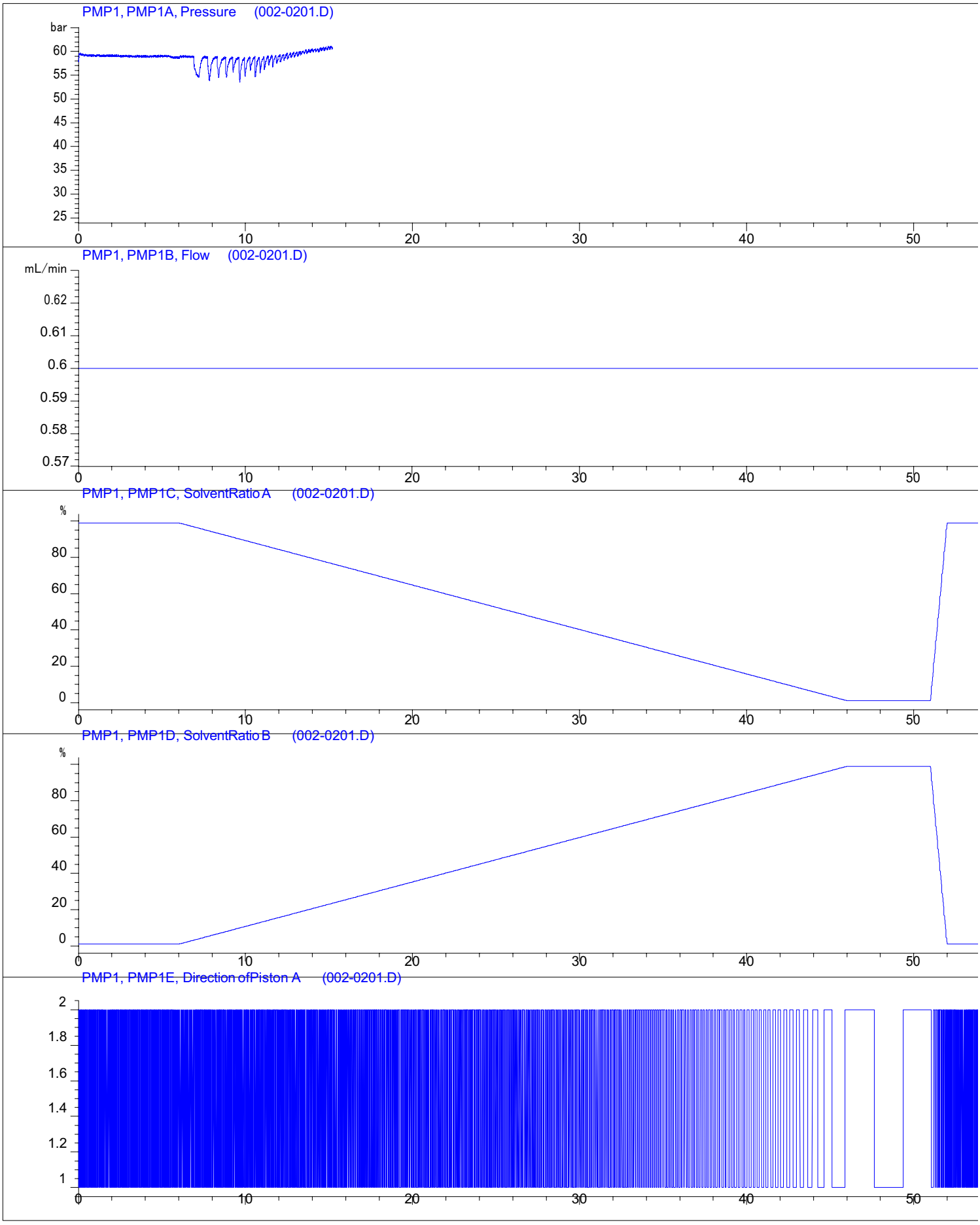
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/8/2017 2:57:39 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 2 2017-05-08 14-45-40\Sequence - method 2.S
Method : C:\Chem32\1\Data\Sequence - method 2 2017-05-08 14-45-40\Optimizing method 2.M (Sequence Method)
Last changed : 5/8/2017 2:45:40 PM by SYSTEM



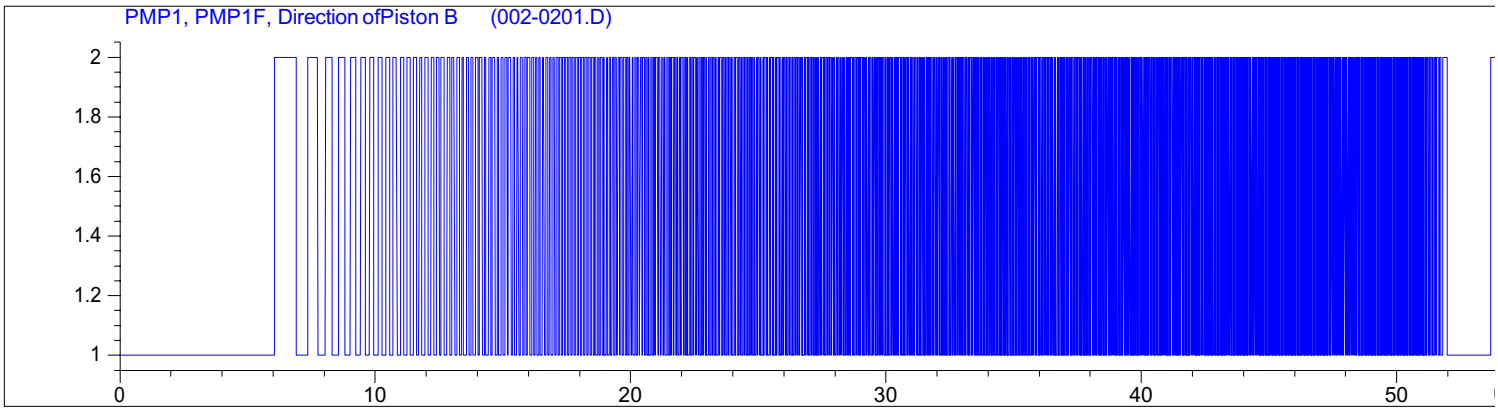
mple Name: D2T50



mple Name: D2T50



mple Name: D2T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.054	BV	0.1024	31.53468	4.25732	0.6823
2	5.444	VB	0.3158	345.16226	14.81789	7.4680
3	6.983	BB	0.2282	66.27102	4.15230	1.4338
4	8.205	BB	0.2431	45.12196	2.31757	0.9763
5	13.681	BB	0.4230	104.60294	3.70915	2.2632
6	14.304	BB	0.2682	39.82048	2.36784	0.8616
7	16.655	BB	0.1680	182.33633	15.87840	3.9450
8	17.194	BB	0.0862	79.44813	14.43128	1.7189
9	17.468	BV	0.1118	54.08212	7.71215	1.1701
10	17.712	VB	0.1143	142.02214	19.18978	3.0728
11	18.647	BV	0.1282	86.70847	10.51518	1.8760
12	18.976	VB	0.1314	284.21744	32.70850	6.1493
13	19.536	BV	0.1658	46.53690	4.38666	1.0069
14	20.014	VV	0.2138	54.18833	3.68012	1.1724
15	20.627	VB	0.2079	236.24045	14.82931	5.1113
16	22.034	VV R	0.1719	491.81723	42.22281	10.6410
17	22.487	VB E	0.1090	8.73158	1.25736	0.1889
18	22.968	BV	0.1521	47.47300	4.39366	1.0271
19	23.135	VB	0.1274	55.58347	6.39555	1.2026
20	23.603	BV	0.2989	692.13879	32.96773	14.9752
21	24.250	VV	0.2012	140.52550	10.04626	3.0404
22	24.902	VB	0.1916	101.81666	7.44342	2.2029
23	25.609	BV	0.1641	69.35890	6.03565	1.5007
24	25.790	VB	0.1485	33.50992	3.29900	0.7250
25	26.490	BV	0.1436	418.70886	44.58102	9.0592
26	27.208	VV	0.1861	169.00136	12.79885	3.6565
27	27.640	VB	0.1441	34.02411	3.60489	0.7361
28	28.036	BV	0.1754	43.46482	3.74558	0.9404
29	28.475	VB	0.1392	81.88798	9.08142	1.7717

mple Name: D2T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	35.054	BB	0.2290	39.03438	2.51592	0.8446
31	37.681	BB	0.4823	147.88704	4.26778	3.1997
32	40.794	BB	0.1709	32.67708	2.95773	0.7070
33	41.814	BB	0.1954	50.22097	3.92228	1.0866
34	43.891	BB	0.3129	129.75371	6.13164	2.8074
35	44.986	BB	0.1852	12.98644	1.10399	0.2810
36	50.043	BB	0.1716	23.01497	1.97977	0.4980
Totals :				4621.91042	365.70576	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.053	BV	0.1000	39.81898	5.66693	0.9539
2	5.432	VB	0.4166	608.98969	19.06926	14.5882
3	6.977	BB	0.2668	61.94897	3.21497	1.4840
4	8.205	BB	0.2245	41.48121	2.32064	0.9937
5	13.686	BB	0.4191	118.66515	4.20612	2.8426
6	14.307	BB	0.2662	46.80405	2.81215	1.1212
7	16.659	BB	0.1763	194.10126	16.13806	4.6496
8	17.194	BB	0.0848	65.27767	12.12466	1.5637
9	17.467	BV	0.1102	45.21198	6.57335	1.0830
10	17.712	VB	0.1149	149.52808	20.07888	3.5819
11	18.648	BV	0.1263	80.99946	10.02455	1.9403
12	18.977	VB	0.1317	320.90836	36.80545	7.6873
13	19.533	BV	0.1635	54.92796	5.19079	1.3158
14	19.987	VV	0.2085	54.92482	3.89606	1.3157
15	20.628	VB	0.1829	247.85365	17.96917	5.9373
16	22.022	BV R	0.1839	372.99118	30.23752	8.9349
17	22.488	VB E	0.1098	8.32310	1.21697	0.1994
18	22.967	BV	0.1647	63.09312	5.38289	1.5114
19	23.132	VB	0.1398	44.98690	4.53212	1.0776
20	23.606	BV	0.2131	227.42854	16.06944	5.4480
21	23.859	VV	0.1683	87.63287	7.50214	2.0992
22	24.252	VB	0.1702	81.42298	7.18684	1.9505
23	24.897	BB	0.1812	88.89618	7.04348	2.1295
24	25.608	BB	0.1278	40.68430	4.85152	0.9746
25	26.490	BV	0.1432	342.24216	36.55129	8.1983
26	27.205	VV	0.1978	141.20682	10.05632	3.3826
27	27.641	VB	0.1409	33.94671	3.70382	0.8132
28	28.047	BV	0.1901	44.29523	3.68966	1.0611
29	28.475	VB	0.1351	79.69296	9.01628	1.9090
30	35.056	BB	0.2652	33.81441	1.81625	0.8100
31	37.691	BB	0.4577	150.53671	4.37236	3.6061
32	40.789	BB	0.1675	19.36798	1.80027	0.4640
33	41.811	BB	0.2025	29.86851	2.25585	0.7155
34	43.889	BB	0.3259	110.20520	4.94631	2.6399
35	44.983	BB	0.1867	15.19928	1.26030	0.3641
36	50.042	BB	0.1795	27.27172	2.24814	0.6533

mple Name: D2T50

Totals : 4174.54817 331.83082

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.399	BB	0.3158	395.69128	16.98373	7.5821
2	7.742	BB	0.1499	35.36105	3.49765	0.6776
3	16.747	BB	0.2078	54.52039	4.42473	1.0447
4	17.731	BB	0.1551	26.34482	2.38120	0.5048
5	18.643	BV R	0.1242	790.71185	97.88929	15.1514
6	19.138	VB E	0.1490	13.95284	1.34553	0.2674
7	19.700	BB	0.0794	6.94551	1.41261	0.1331
8	19.883	BV	0.0946	30.81050	4.81620	0.5904
9	20.042	VB	0.1401	55.18476	5.95563	1.0574
10	20.384	BV E	0.1242	48.54435	6.14360	0.9302
11	20.634	VB R	0.1126	1364.47717	179.57730	26.1458
12	21.603	BV R	0.1438	96.07658	9.68357	1.8410
13	22.046	BV	0.1304	63.18553	7.34490	1.2107
14	22.205	VB	0.1325	28.60025	3.13160	0.5480
15	22.739	BB	0.1600	16.24702	1.72377	0.3113
16	23.598	VV R	0.3391	1624.07715	68.86467	31.1202
17	24.806	VB	0.1788	343.30661	29.70250	6.5784
18	25.486	BB	0.1416	23.73018	2.48122	0.4547
19	26.911	BV	0.2424	36.98212	2.36584	0.7086
20	28.097	BV	0.1563	28.97428	2.80958	0.5552
21	28.470	VB	0.1587	29.64833	2.81824	0.5681
22	29.645	BB	0.1351	10.71994	1.21341	0.2054
23	37.805	BB	0.1498	12.91628	1.32471	0.2475
24	40.150	BB	0.3055	31.82759	1.38829	0.6099
25	43.576	BB	0.2967	29.41937	1.51386	0.5637
26	47.141	BB	0.1372	20.46595	2.31438	0.3922

Totals : 5218.72171 463.10802

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.467	BB	0.2138	50.12050	3.08048	1.2549
2	7.742	BB	0.1483	17.07594	1.71322	0.4275
3	16.750	BB	0.2155	38.00287	2.92717	0.9515
4	18.647	BB	0.1140	9.31331	1.29359	0.2332
5	19.703	BV	0.0914	17.26966	2.82273	0.4324
6	19.878	VV	0.1198	58.34054	6.96303	1.4607
7	20.047	VB	0.1347	51.78986	5.76846	1.2967
8	20.388	BV E	0.1219	41.40507	5.25529	1.0367
9	20.634	VB R	0.1116	1224.21594	163.10593	30.6511
10	21.604	BV R	0.1409	83.39051	8.61877	2.0879
11	22.047	BB	0.1589	124.28722	11.24973	3.1118
12	22.719	BB	0.1362	18.61334	2.04416	0.4660

mple Name: D2T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
13	23.599	VV R	0.3418	1914.99133	80.40961	47.9463
14	24.818	VB	0.1792	261.89630	22.59395	6.5572
15	25.482	BB	0.1289	27.12488	3.20036	0.6791
16	26.969	BB	0.1901	17.89417	1.55708	0.4480
17	37.803	BB	0.1415	9.41992	1.02193	0.2358
18	47.142	BB	0.1415	17.32483	1.91564	0.4338
19	48.082	BB	0.1717	11.55634	1.02338	0.2893
Totals :				3994.03253	326.56450	

Signal 5: DAD1 E, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.467	BB	0.1995	26.34983	1.75118	11.6850
2	20.635	BB	0.1087	45.49894	6.26624	20.1769
3	22.048	BB	0.1391	46.29469	5.04144	20.5297
4	24.250	BB	0.1060	9.44462	1.37676	4.1883
5	24.910	BB	0.1507	26.43077	2.64139	11.7209
6	27.004	BB	0.1269	8.41818	1.01311	3.7331
7	40.086	BB	0.3302	30.92880	1.28780	13.7156
8	48.075	BB	0.1854	32.13482	2.50914	14.2504
Totals :				225.50065	21.88707	

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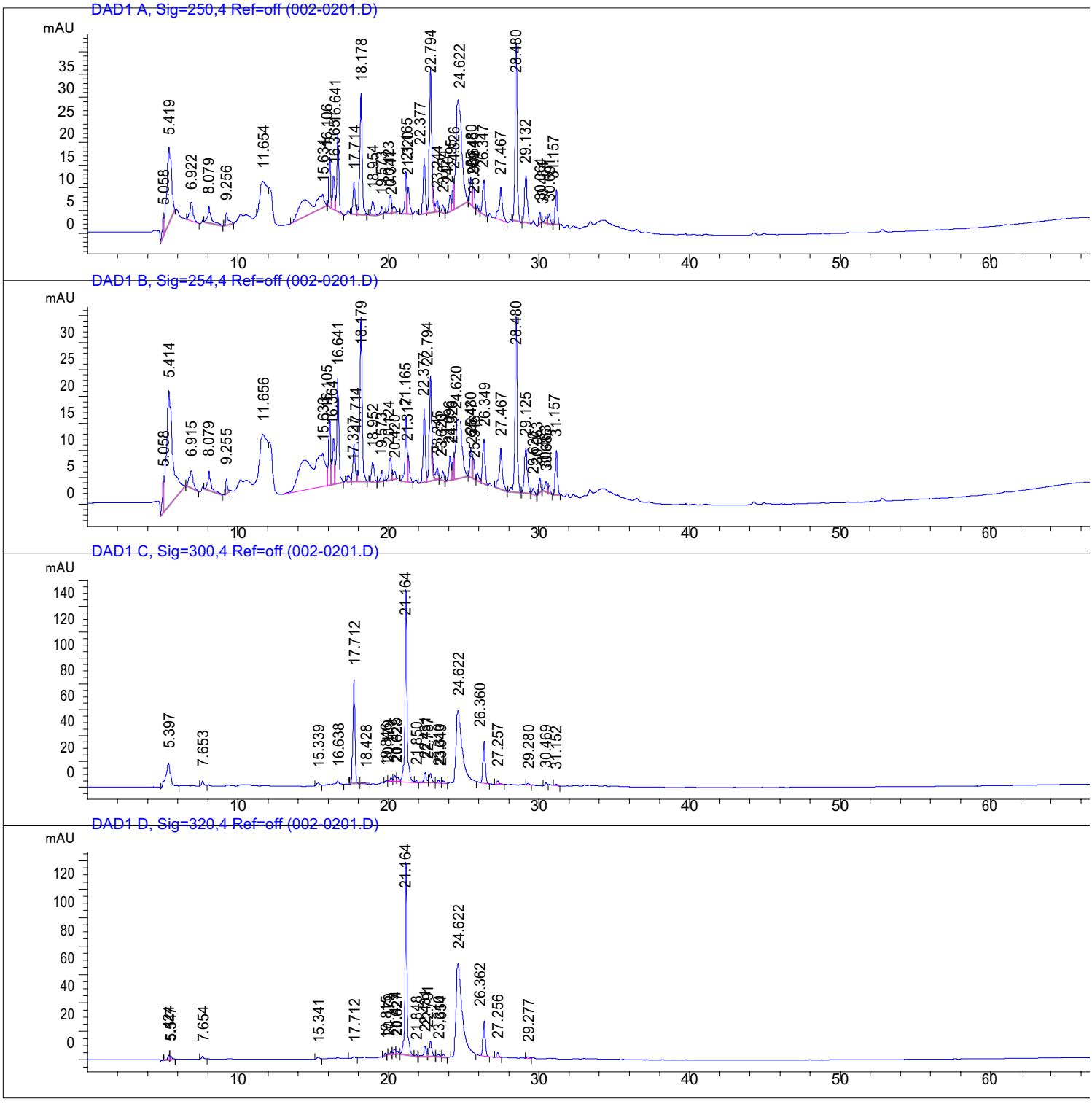
*** End of Report ***

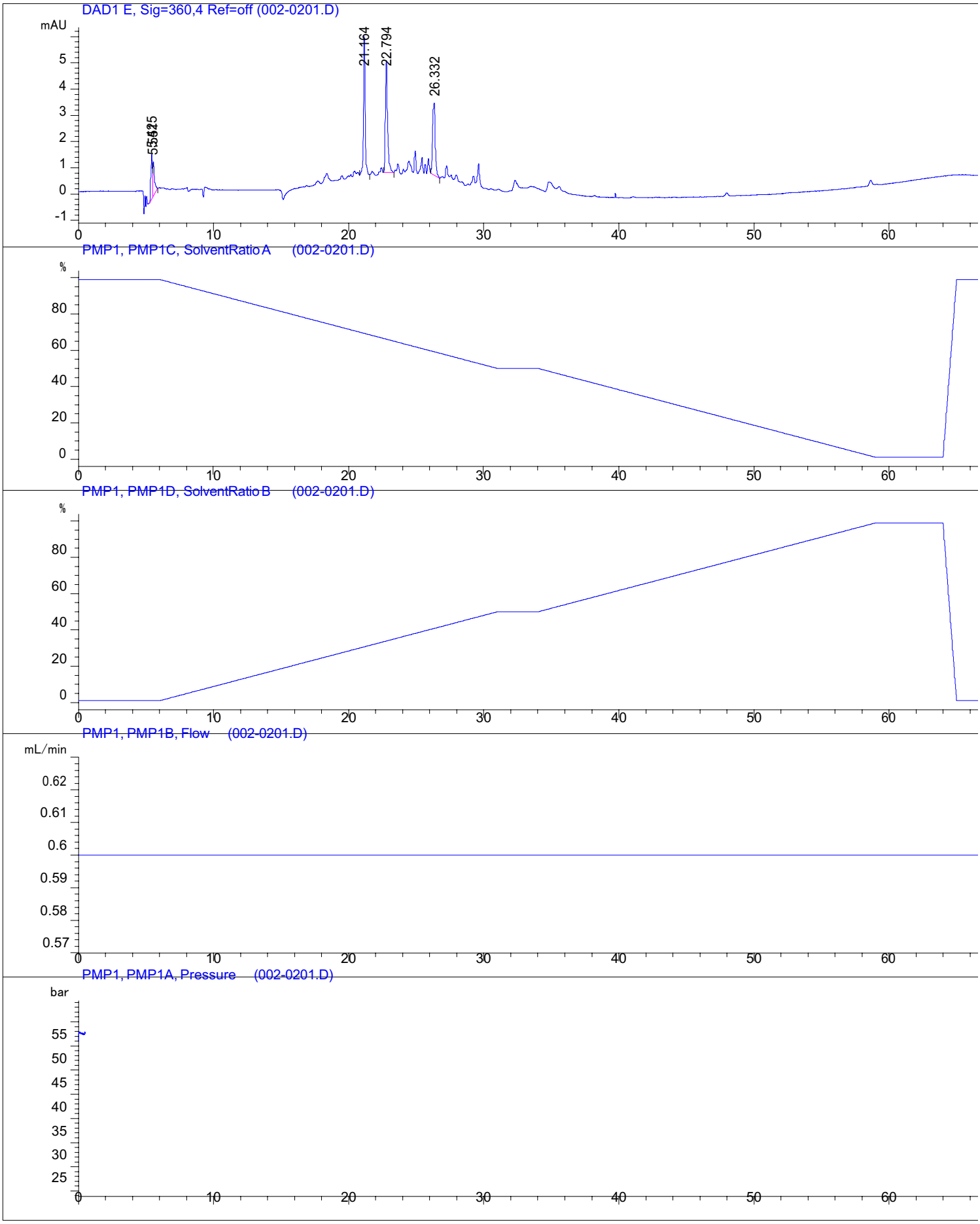
mple Name: D3T50

==========Method 5==========

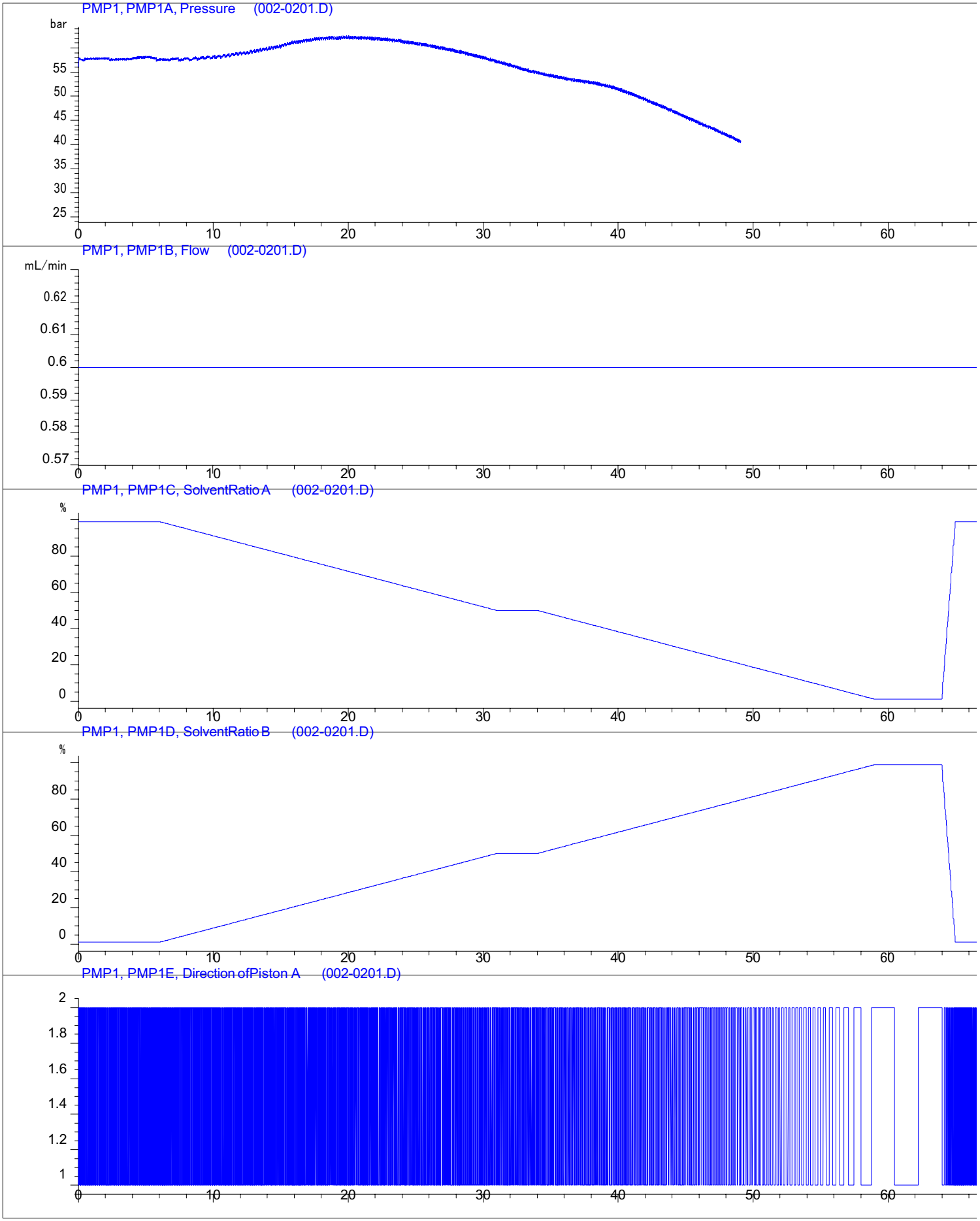
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/8/2017 4:40:54 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 3 2017-05-08 16-28-54\Sequence - method 3.S
Method : C:\Chem32\1\Data\Sequence - method 3 2017-05-08 16-28-54\Optimizing method 3.M (Sequence Method)
Last changed : 5/8/2017 4:28:54 PM by SYSTEM



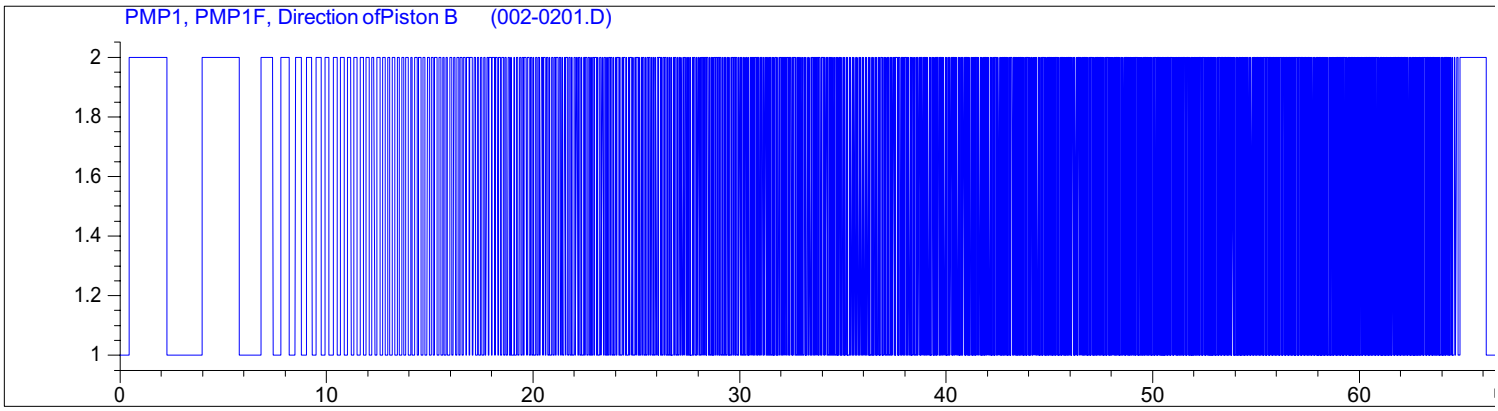
mple Name: D3T50



mple Name: D3T50



mple Name: D3T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.058	BV	0.1069	32.71580	4.29288	0.8046
2	5.419	VB	0.2745	362.85400	16.90007	8.9243
3	6.922	BB	0.2223	64.64195	4.18210	1.5899
4	8.079	BB	0.1963	53.78265	3.55864	1.3228
5	9.256	BB	0.1422	28.17302	2.64708	0.6929
6	11.654	BB	0.3617	104.74708	4.36931	2.5762
7	15.634	BB	1.2018	312.98993	3.09493	7.6979
8	16.106	BV	0.1243	86.89861	10.98980	2.1373
9	16.365	VV	0.1407	68.03450	7.43712	1.6733
10	16.641	VB	0.1364	156.05855	17.44687	3.8382
11	17.714	BV	0.1553	72.94352	7.13530	1.7940
12	18.178	VB	0.1605	278.31705	26.50952	6.8452
13	18.954	BB	0.1989	36.75365	2.96011	0.9039
14	19.573	BB	0.1380	14.32590	1.57614	0.3523
15	20.123	BV	0.1712	44.01504	3.91405	1.0825
16	20.341	VB	0.1670	16.57745	1.31556	0.4077
17	21.165	BV	0.1276	81.06642	9.30798	1.9938
18	21.320	VB	0.1307	54.62227	5.97004	1.3434
19	22.377	BV	0.1496	121.04959	12.43314	2.9772
20	22.794	VV R	0.1650	349.20251	31.58364	8.5886
21	23.244	VB E	0.1649	26.89706	2.43404	0.6615
22	23.624	BB	0.1443	16.64391	1.76105	0.4094
23	24.095	BV	0.1422	34.29020	3.62903	0.8434
24	24.326	VV	0.1160	47.97841	6.21884	1.1800
25	24.622	VB	0.3735	631.65472	23.65465	15.5354
26	25.480	BV	0.1415	55.81989	5.94862	1.3729
27	25.646	VB	0.1237	36.72583	4.57125	0.9033
28	25.909	BV	0.1218	8.68155	1.15319	0.2135
29	26.347	VB	0.1681	86.41737	7.63338	2.1254

mple Name: D3T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	27.467	BB	0.2096	108.69503	7.30459	2.6733
31	28.480	BB	0.1582	403.68491	39.16679	9.9286
32	29.132	BB	0.1959	136.36598	10.61048	3.3539
33	30.064	BB	0.1353	22.32292	2.57148	0.5490
34	30.464	BV	0.1581	14.94763	1.40574	0.3676
35	30.691	VB	0.1491	21.29238	2.15819	0.5237
36	31.157	BB	0.1474	73.70987	7.71703	1.8129
Totals :				4065.89716	305.56264	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.058	BV	0.1021	42.25671	5.86376	0.9874
2	5.414	VB	0.3613	624.71478	21.48305	14.5981
3	6.915	BB	0.2585	60.77385	3.27449	1.4201
4	8.079	BB	0.1855	52.12059	3.63071	1.2179
5	9.255	BB	0.0926	16.44039	2.57017	0.3842
6	11.656	BB	0.3582	117.23144	4.91600	2.7394
7	15.633	BV	1.1708	604.46967	6.13896	14.1250
8	16.105	VV	0.1454	120.36210	12.16503	2.8126
9	16.364	VV	0.1566	88.42995	8.41830	2.0664
10	16.641	VB	0.1457	189.02115	19.39352	4.4170
11	17.327	BV E	0.1717	12.83805	1.05729	0.3000
12	17.714	VV R	0.1613	74.15933	6.90471	1.7329
13	18.179	VB	0.1605	319.49448	30.42858	7.4658
14	18.952	BB	0.1977	44.05670	3.57756	1.0295
15	19.573	BB	0.1405	18.12494	1.98548	0.4235
16	20.124	BV	0.1705	44.91534	4.07691	1.0496
17	20.420	VB	0.1697	16.87908	1.40927	0.3944
18	21.165	BV	0.1314	105.51697	11.90648	2.4657
19	21.317	VB	0.1194	40.22450	4.91741	0.9399
20	22.377	BV	0.1523	135.36189	13.57498	3.1631
21	22.794	VV R	0.1644	213.09389	19.36507	4.9795
22	23.245	VB E	0.1680	23.05478	2.06855	0.5387
23	23.620	BB	0.1442	16.35551	1.76329	0.3822
24	24.096	BV	0.1511	45.08812	4.49303	1.0536
25	24.326	VV	0.1194	35.45218	4.42891	0.8284
26	24.620	VB	0.3961	314.91690	10.99631	7.3588
27	25.480	BV	0.1461	52.66515	5.48019	1.2307
28	25.647	VB	0.1217	30.22252	3.84470	0.7062
29	25.916	BV E	0.1426	14.06715	1.51166	0.3287
30	26.349	VB R	0.1733	96.14464	8.29058	2.2467
31	27.467	BB	0.1975	105.75723	7.54779	2.4713
32	28.480	VB R	0.1629	347.93719	32.50311	8.1304
33	29.125	BB	0.2058	110.71262	8.18425	2.5871
34	29.620	BB	0.1503	11.09982	1.09486	0.2594
35	30.063	BB	0.1364	23.18410	2.69513	0.5418
36	30.463	BV	0.1638	20.18638	1.84333	0.4717
37	30.686	VB	0.1396	13.09742	1.41981	0.3061

mple Name: D3T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
38	31.157	BB	0.1491	79.01101	8.14985	1.8463
Totals :				4279.43852	293.37306	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.397	BB	0.3083	429.30594	18.80688	8.4346
2	7.653	BB	0.1434	33.74828	3.53391	0.6631
3	15.339	BB	0.2199	27.08211	2.02646	0.5321
4	16.638	BB	0.2065	35.69250	2.44164	0.7013
5	17.712	BB	0.1473	772.21912	80.94687	15.1719
6	18.428	BB	0.2556	21.99879	1.20145	0.4322
7	19.846	BB	0.1228	8.86707	1.13932	0.1742
8	20.179	BV E	0.1265	28.21713	3.27583	0.5544
9	20.425	VV E	0.1442	46.30604	4.64733	0.9098
10	20.628	VV E	0.1453	35.47769	3.46857	0.6970
11	21.164	VV R	0.1396	1427.56641	149.27055	28.0476
12	21.850	VB E	0.1132	12.86089	1.80403	0.2527
13	22.431	BV	0.1677	93.65627	8.05478	1.8401
14	22.787	VB	0.1742	86.99619	7.45245	1.7092
15	23.310	BV	0.1618	24.12963	2.20231	0.4741
16	23.643	VB	0.1485	20.06459	2.00939	0.3942
17	24.622	BB	0.4084	1579.06396	56.09838	31.0241
18	26.360	BB	0.1551	332.12509	32.54463	6.5253
19	27.257	BB	0.1659	31.77099	2.81054	0.6242
20	29.280	BB	0.1660	13.89780	1.32884	0.2731
21	30.469	BB	0.1419	15.00154	1.65244	0.2947
22	31.152	BB	0.1569	13.74378	1.34900	0.2700
Totals :				5089.79182	388.06562	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.424	BV	0.1264	30.39898	3.39750	0.7997
2	5.547	VB	0.1134	20.38294	2.54705	0.5362
3	7.654	BB	0.1393	16.41765	1.75248	0.4319
4	15.341	BB	0.2126	15.75788	1.18943	0.4145
5	17.712	BB	0.1511	11.04259	1.11956	0.2905
6	19.815	BB	0.1557	15.82489	1.65337	0.4163
7	20.179	BV	0.1183	20.40848	2.57835	0.5369
8	20.424	VV	0.1365	34.03883	3.59233	0.8954
9	20.627	VB	0.1298	18.55789	2.08499	0.4882
10	21.164	BV R	0.1348	1241.50085	135.64833	32.6582
11	21.848	VB E	0.1140	8.47202	1.17695	0.2229

mple Name: D3T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
12	22.431	BV	0.1614	79.88432	7.20343	2.1014
13	22.791	VB	0.1715	124.91790	10.75697	3.2860
14	23.310	BV	0.1664	17.52458	1.54427	0.4610
15	23.654	VB	0.1428	17.55748	1.84919	0.4619
16	24.622	BB	0.4075	1827.62927	65.09879	48.0766
17	26.362	BB	0.1555	253.24077	24.73162	6.6616
18	27.256	BB	0.1467	33.26941	3.44317	0.8752
19	29.277	BV	0.1917	14.67000	1.14306	0.3859
Totals :				3801.49672	272.51085	

Signal 5: DAD1 E, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.425	BV	0.1121	13.18072	1.70636	8.4973
2	5.551	VB	0.1164	10.91200	1.29408	7.0347
3	21.164	BB	0.1284	45.57562	5.29398	29.3816
4	22.794	BB	0.1660	47.44167	4.25845	30.5846
5	26.332	BB	0.1950	38.00640	2.75444	24.5019
Totals :				155.11642	15.30731	

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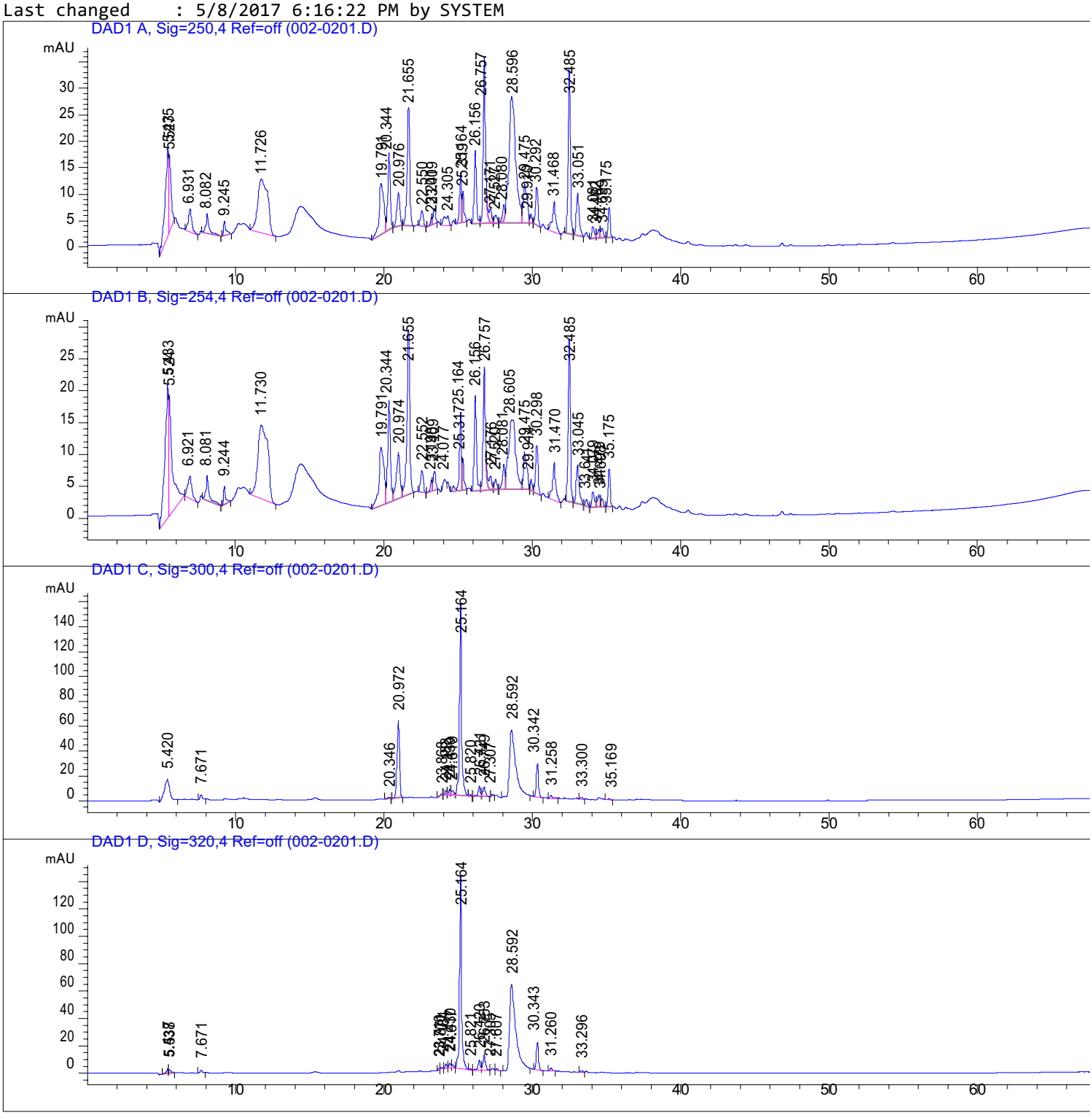
*** End of Report ***

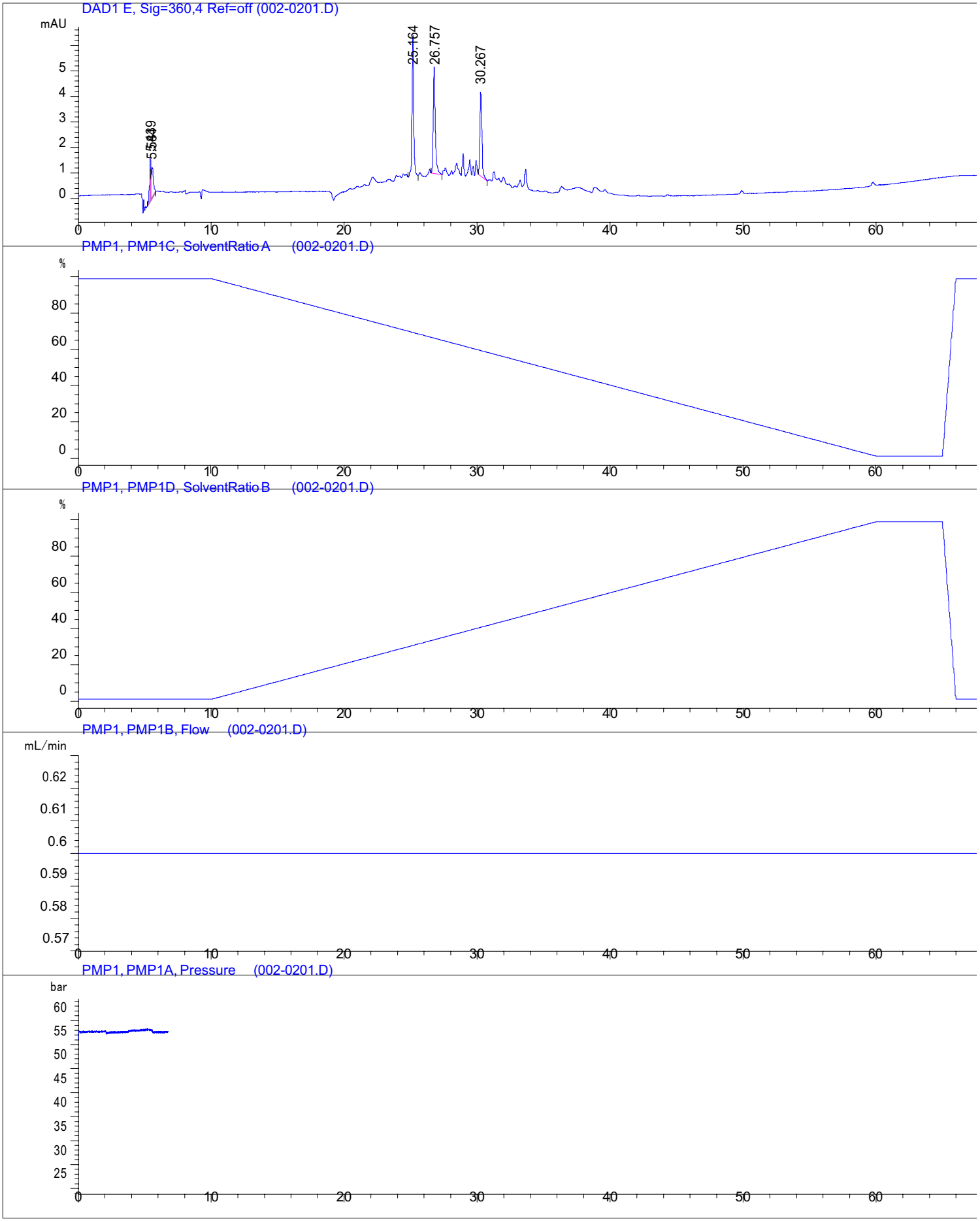
mple Name: D4T50

==========**Method 6**==========

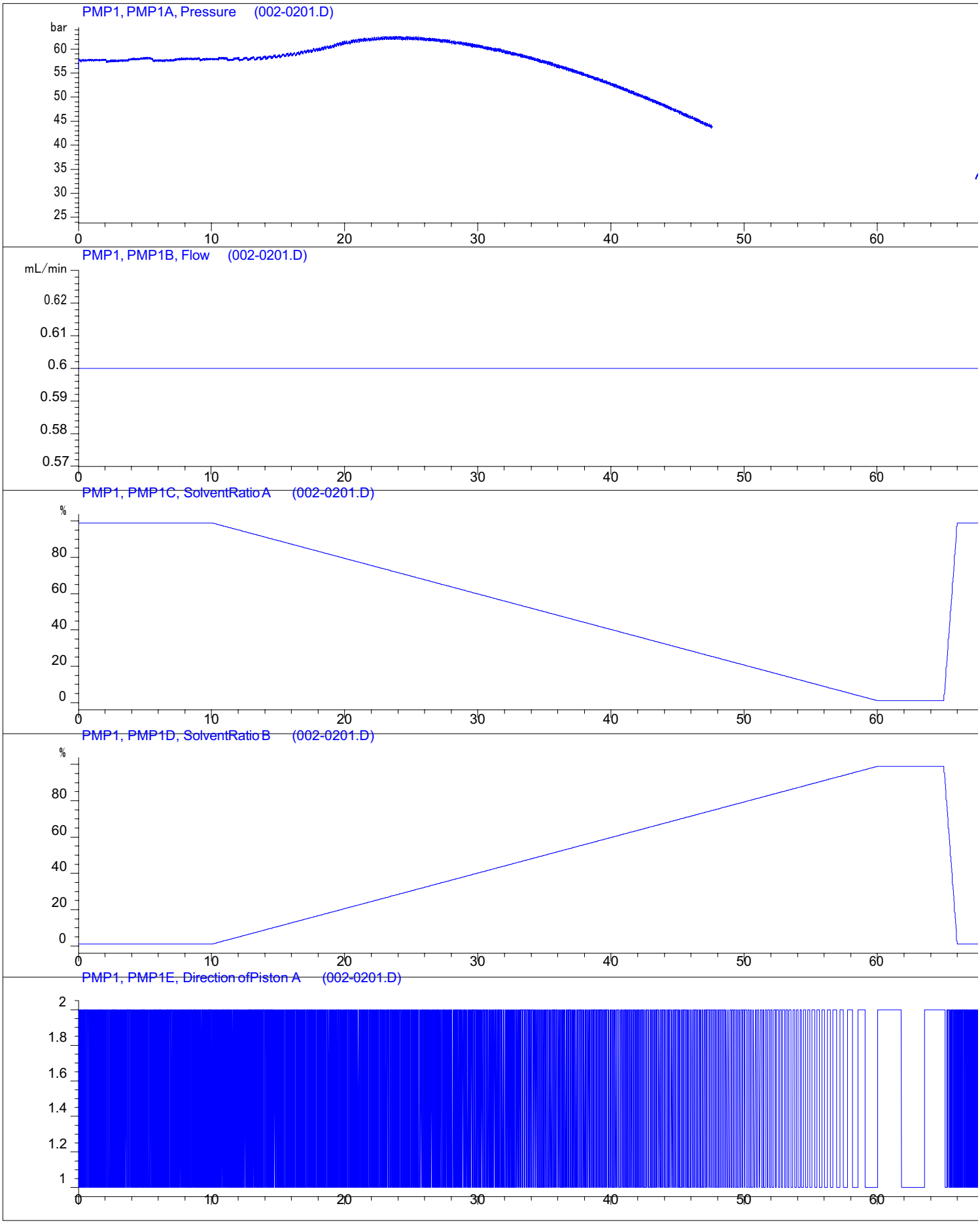
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/8/2017 6:28:23 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 4 2017-05-08 18-16-22\Sequence - method 4.S
Method : C:\Chem32\1\Data\Sequence - method 4 2017-05-08 18-16-22\Optimizing method 4.M (Sequence Method)
Last changed : 5/8/2017 6:16:22 PM by SYSTEM



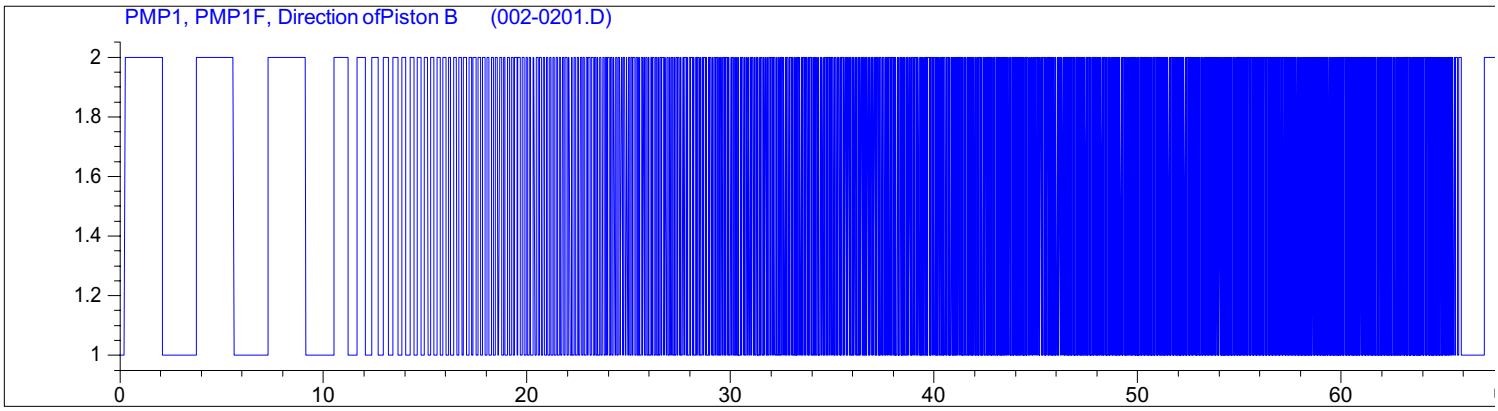
mple Name: D4T50



mple Name: D4T50



mple Name: D4T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.435	VV R	0.1884	248.28096	16.60725	5.8645
2	5.527	VB	0.1608	157.74576	14.51481	3.7260
3	6.931	BB	0.2296	68.36901	4.29673	1.6149
4	8.082	BB	0.1778	51.81980	3.73588	1.2240
5	9.245	BB	0.1432	28.91644	2.69492	0.6830
6	11.726	BB	0.6443	470.61090	10.08784	11.1160
7	19.791	BV	0.2865	214.43166	9.75375	5.0650
8	20.344	VB	0.1755	165.90240	14.49881	3.9187
9	20.976	BB	0.2120	91.02108	6.47329	2.1500
10	21.655	BB	0.1909	277.80569	22.37248	6.5619
11	22.550	BB	0.2115	36.93218	2.88126	0.8724
12	23.201	BV	0.1270	11.64385	1.40067	0.2750
13	23.409	VB	0.1781	33.19483	2.88712	0.7841
14	24.305	BB	0.2843	39.52913	1.78521	0.9337
15	25.164	BV	0.1317	84.31984	9.49084	1.9917
16	25.319	VB	0.1237	50.19489	5.87455	1.1856
17	26.156	BV	0.1574	143.05157	13.75058	3.3789
18	26.757	VV R	0.1671	352.27499	31.34444	8.3209
19	27.171	VV E	0.1819	29.43133	2.32108	0.6952
20	27.527	VB E	0.2007	18.03941	1.47433	0.4261
21	28.080	BV E	0.1580	30.89508	2.90770	0.7298
22	28.596	VV R	0.4832	770.03845	23.81417	18.1886
23	29.475	VV	0.2363	131.93024	7.53365	3.1162
24	29.910	VB	0.1347	13.68296	1.61668	0.3232
25	30.292	BB	0.1838	81.74721	7.02051	1.9309
26	31.468	BB	0.2198	89.11552	5.65767	2.1049
27	32.485	BV	0.1587	319.28696	30.87266	7.5417
28	33.051	VB	0.1960	103.25046	7.92531	2.4388
29	34.081	BV	0.1798	27.55014	2.23593	0.6507

mple Name: D4T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	34.482	VV	0.1732	19.40798	1.64982	0.4584
31	34.699	VB	0.1632	19.86902	1.79482	0.4693
32	35.175	BB	0.1470	53.33405	5.60728	1.2598
Totals :				4233.62381	276.88203	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.433	BV	0.2029	338.21707	20.89265	8.3084
2	5.524	VB	0.2352	324.59650	18.82169	7.9738
3	6.921	BB	0.2636	64.84127	3.44529	1.5928
4	8.081	BB	0.1674	49.31326	3.80026	1.2114
5	9.244	BB	0.1187	23.92688	2.77140	0.5878
6	11.730	BB	0.6389	536.13824	11.52230	13.1704
7	19.791	BV	0.3066	216.46704	9.12939	5.3176
8	20.344	VV	0.1875	198.25279	15.89559	4.8701
9	20.974	VV	0.2554	128.15114	7.20728	3.1481
10	21.655	VB	0.1997	340.09253	25.82176	8.3545
11	22.552	BB	0.2078	43.92757	3.42215	1.0791
12	23.198	BV	0.1340	15.45354	1.73372	0.3796
13	23.409	VB	0.1797	34.94437	3.00460	0.8584
14	24.077	BB	0.1791	12.79959	1.10511	0.3144
15	25.164	VV R	0.1402	117.70308	12.23634	2.8914
16	25.317	VB	0.1129	37.72116	4.84329	0.9266
17	26.156	BV	0.1596	158.33449	14.94506	3.8895
18	26.757	VV R	0.1648	212.75951	19.27905	5.2265
19	27.176	VV E	0.1979	28.95630	2.06122	0.7113
20	27.520	VB E	0.1750	17.16523	1.52908	0.4217
21	28.081	BV	0.1669	43.12112	3.84447	1.0593
22	28.605	VV	0.4518	370.49402	10.81194	9.1013
23	29.475	VB	0.2161	90.76279	5.81597	2.2296
24	29.914	BV	0.1316	13.78633	1.68232	0.3387
25	30.298	VB	0.1898	88.97794	7.42576	2.1858
26	31.470	BB	0.2076	86.44839	5.87704	2.1236
27	32.485	BB	0.1582	261.67975	25.40527	6.4282
28	33.045	BB	0.2015	82.42722	6.10536	2.0248
29	33.641	BB	0.1518	10.80122	1.03471	0.2653
30	34.079	BV	0.1824	29.69938	2.36731	0.7296
31	34.479	VV	0.1751	22.72481	1.90535	0.5582
32	34.698	VB	0.1526	12.94446	1.25212	0.3180
33	35.175	BB	0.1486	57.15327	5.92158	1.4040
Totals :				4070.78223	262.91644	

mple Name: D4T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.420	BB	0.3030	435.08524	17.68872	8.7730
2	7.671	BB	0.1421	33.92415	3.59550	0.6840
3	20.346	BB	0.1685	12.68306	1.18858	0.2557
4	20.972	BB	0.1817	736.54010	62.42223	14.8515
5	23.868	BB	0.1371	13.60930	1.51066	0.2744
6	24.183	BV E	0.1305	32.90233	3.74629	0.6634
7	24.430	VV E	0.1404	47.34184	4.82685	0.9546
8	24.619	VV E	0.1537	43.10170	4.00003	0.8691
9	25.164	VV R	0.1430	1504.94958	155.38983	30.3455
10	25.820	VB E	0.1184	14.09706	1.90455	0.2843
11	26.421	BV	0.1608	92.08785	8.21428	1.8568
12	26.749	VB	0.1747	95.23029	7.89363	1.9202
13	27.307	BB	0.1272	13.11229	1.64101	0.2644
14	28.592	BB	0.4190	1556.82190	53.90322	31.3915
15	30.342	BB	0.1556	278.09103	27.13404	5.6074
16	31.258	BB	0.1693	23.79007	2.08149	0.4797
17	33.300	BV	0.1744	14.37488	1.28584	0.2899
18	35.169	BB	0.1669	11.63349	1.05319	0.2346
Totals :				4959.37616	359.47996	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.437	BV	0.1101	28.51580	3.39292	0.7547
2	5.538	VB	0.1368	27.34022	2.55606	0.7236
3	7.671	BB	0.1402	16.49251	1.77785	0.4365
4	23.710	BV	0.1043	7.77739	1.15885	0.2058
5	23.871	VB	0.1387	13.13301	1.46376	0.3476
6	24.184	BV	0.1251	24.57115	2.95356	0.6503
7	24.430	VV	0.1381	35.42229	3.75310	0.9374
8	24.617	VB	0.1366	22.66647	2.43458	0.5999
9	25.164	BV R	0.1384	1311.22083	141.18439	34.7014
10	25.821	VB E	0.1149	8.58048	1.17949	0.2271
11	26.420	BV	0.1587	81.46798	7.38461	2.1560
12	26.753	VB	0.1701	129.13353	11.06646	3.4175
13	27.305	BB	0.1319	10.51111	1.25235	0.2782
14	27.607	BB	0.1179	8.14781	1.08151	0.2156
15	28.592	BB	0.4191	1806.81116	62.53917	47.8172
16	30.343	BB	0.1560	209.26208	20.35130	5.5381
17	31.260	BB	0.1476	24.91210	2.55884	0.6593
18	33.296	BV	0.1889	12.61670	1.00212	0.3339
Totals :				3778.58261	269.09091	

mple Name: D4T50

Signal 5: DAD1 E, Sig=360,4 Ref=off

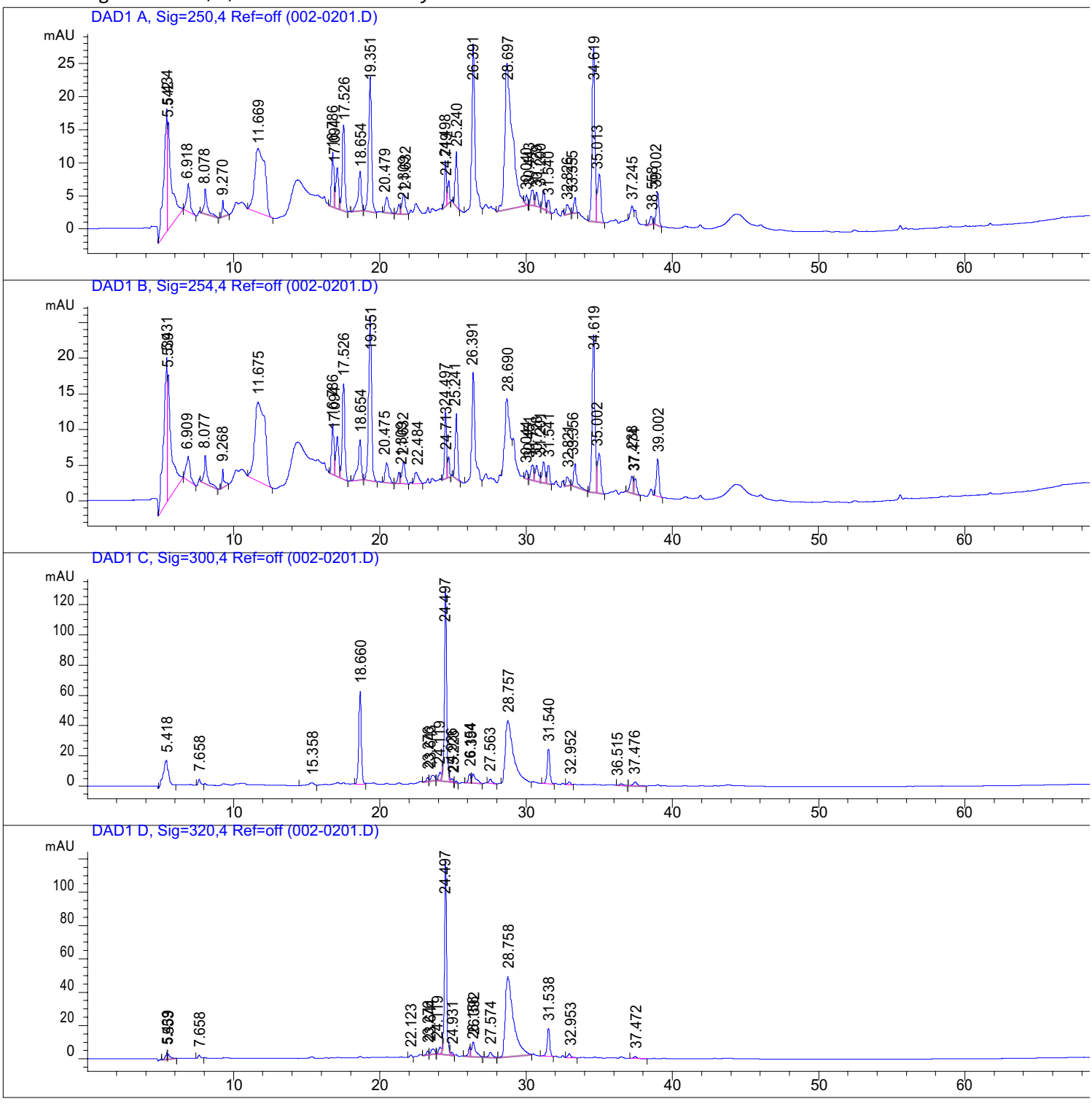
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.439	BV	0.0944	11.52159	1.67262	7.5076
2	5.584	VB	0.1400	13.16818	1.20017	8.5805
3	25.164	BB	0.1315	47.90532	5.50905	31.2156
4	26.757	BB	0.1635	45.58296	4.17184	29.7023
5	30.267	BB	0.1677	35.28786	3.27580	22.9939
Totals :				153.46591	15.82949	

*** End of Report ***

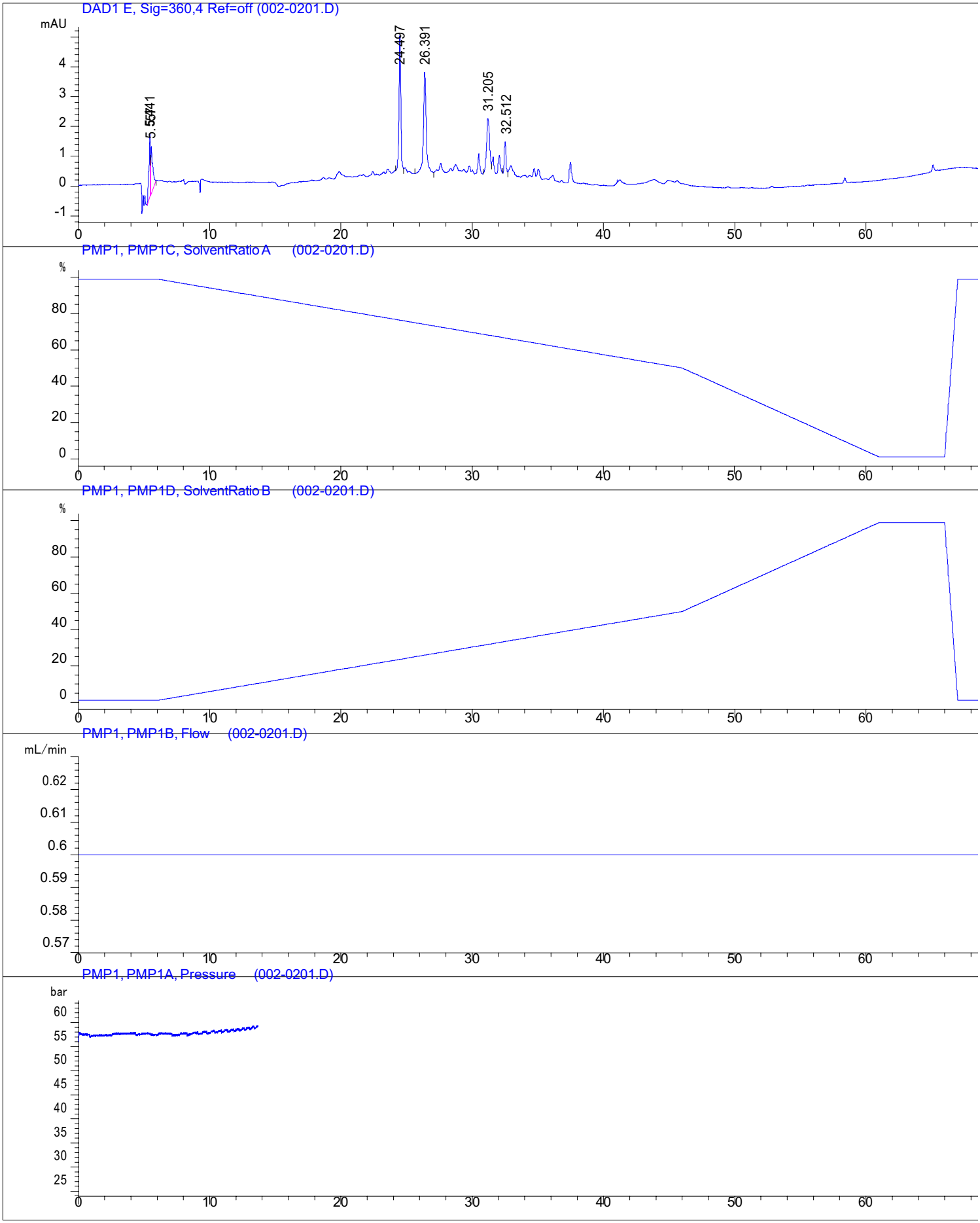
mple Name: D5T50

==========Method 7==========

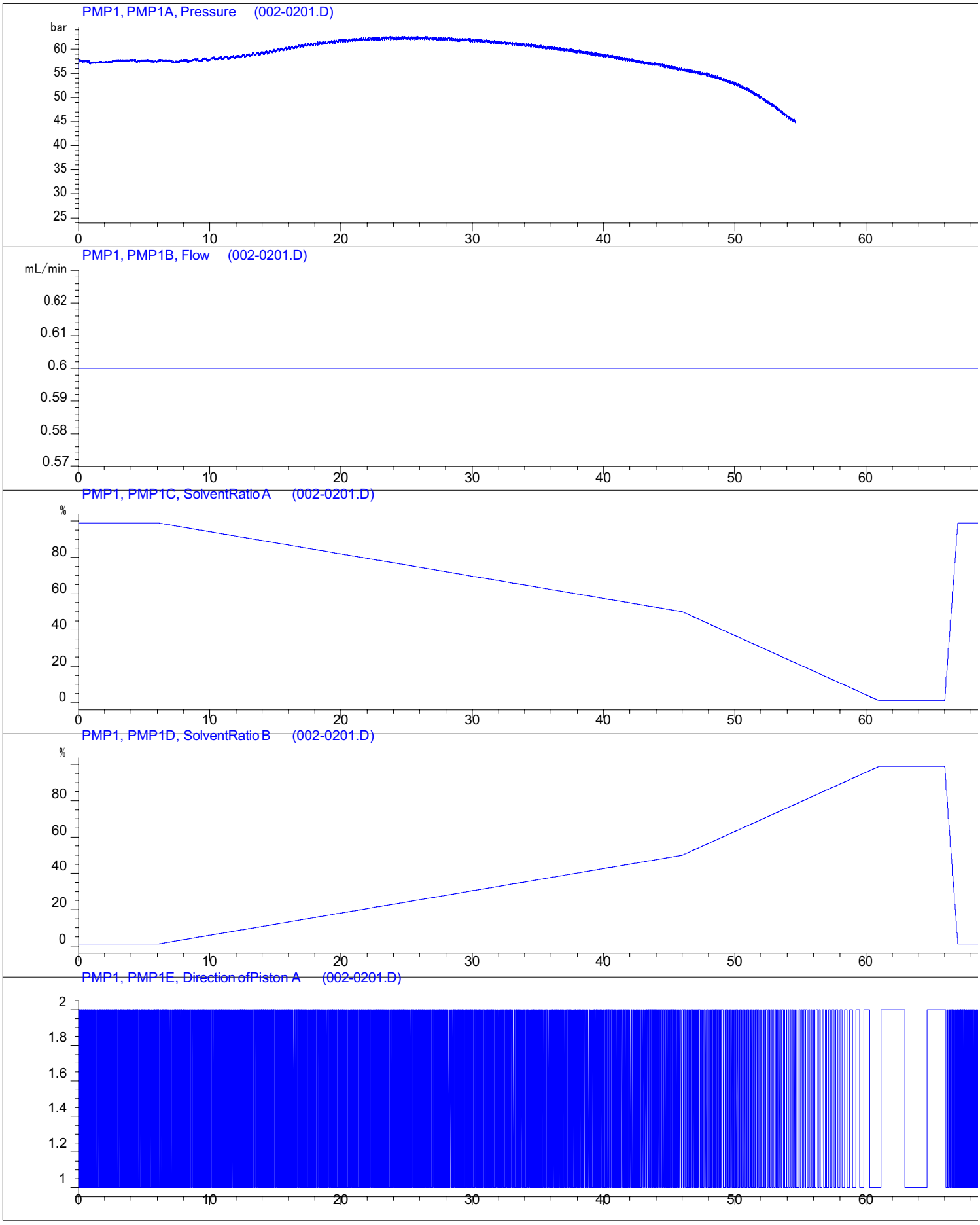
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/8/2017 8:34:26 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Sequence - method 5-6-7-8-9-10-11.S
Method : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Optimizing method 5.M (Sequence Method)
Last changed : 5/8/2017 8:22:26 PM by SYSTEM



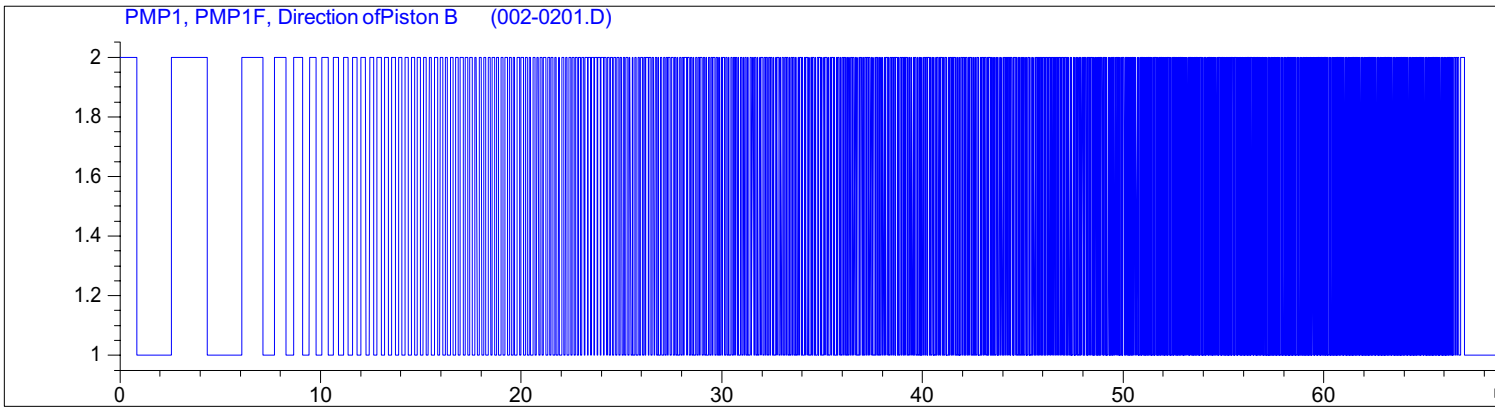
mple Name: D5T50



mple Name: D5T50



mple Name: D5T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.434	BV	0.2101	312.27377	18.58047	7.5935
2	5.542	VB	0.2323	297.82333	16.21113	7.2422
3	6.918	BB	0.2292	68.10989	4.29128	1.6562
4	8.078	BB	0.1851	55.81350	3.80658	1.3572
5	9.270	BB	0.1503	28.76816	2.53725	0.6996
6	11.669	BB	0.6445	470.40292	9.72261	11.4388
7	16.786	BV	0.1885	95.38400	8.15025	2.3194
8	17.094	VV	0.2045	78.56762	6.09111	1.9105
9	17.526	VB	0.1928	157.18947	12.84235	3.8224
10	18.654	BB	0.2226	88.33251	5.90332	2.1480
11	19.351	BB	0.2117	280.84811	20.26333	6.8294
12	20.479	BB	0.2539	38.92822	2.34169	0.9466
13	21.309	BV	0.1706	15.46105	1.36076	0.3760
14	21.632	VB	0.2379	47.61655	3.05344	1.1579
15	24.498	BV	0.1408	63.21405	6.90367	1.5372
16	24.719	VB	0.1299	28.66389	3.41426	0.6970
17	25.240	BB	0.1748	90.75508	8.22143	2.2069
18	26.391	BB	0.2127	364.45883	25.20278	8.8625
19	28.697	BV R	0.4752	792.84796	22.06687	19.2797
20	30.040	VB E	0.1483	12.99894	1.39984	0.3161
21	30.443	BV	0.2387	34.61274	2.39325	0.8417
22	30.729	VB	0.1795	25.12026	2.13092	0.6108
23	31.200	BV	0.1846	33.00902	2.77871	0.8027
24	31.540	VB	0.1746	19.83853	1.79967	0.4824
25	32.826	VB	0.2303	23.65620	1.48153	0.5752
26	33.355	BB	0.1642	24.64390	2.39264	0.5993
27	34.619	BV	0.2086	358.59299	26.38403	8.7199
28	35.013	VB	0.2426	114.83289	7.25922	2.7924
29	37.245	BB	0.1711	13.39863	1.22960	0.3258

mple Name: D5T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	38.558	BB	0.1948	15.31246	1.23351	0.3724
31	39.002	BB	0.1938	60.88009	4.93946	1.4804
Totals :				4112.35558	236.38698	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.431	BV	0.2167	354.15341	20.38867	10.0322
2	5.539	VB	0.2206	304.92535	17.56145	8.6377
3	6.909	BB	0.2641	64.63757	3.42683	1.8310
4	8.077	BB	0.1760	53.89652	3.88070	1.5267
5	9.268	BB	0.1275	24.34368	2.59349	0.6896
6	11.675	BB	0.6471	536.23541	11.07073	15.1901
7	16.786	BV	0.1879	82.89783	7.11526	2.3483
8	17.094	VV	0.2051	72.32301	5.58598	2.0487
9	17.526	VB	0.1929	163.28864	13.32809	4.6255
10	18.654	BB	0.2306	87.14778	5.56954	2.4687
11	19.351	BB	0.2155	322.11115	22.98597	9.1246
12	20.475	BB	0.2547	46.48647	2.78557	1.3168
13	21.309	BV	0.1714	18.37791	1.60675	0.5206
14	21.632	VB	0.2366	48.05743	3.07018	1.3613
15	22.484	BB	0.2991	30.36461	1.54616	0.8601
16	24.497	BV	0.1469	89.27890	9.38820	2.5290
17	24.713	VB	0.1269	22.50881	2.76786	0.6376
18	25.241	BB	0.1777	101.84174	9.02068	2.8849
19	26.391	BB	0.2179	228.26590	15.31629	6.4662
20	28.690	BB	0.2686	153.93213	8.28966	4.3605
21	30.044	BB	0.1484	11.18507	1.22673	0.3168
22	30.451	BV	0.2418	31.32683	2.12683	0.8874
23	30.728	VV	0.2157	33.03411	2.19333	0.9358
24	31.201	VV	0.2033	39.11619	2.93765	1.1081
25	31.541	VB	0.1825	30.30079	2.59001	0.8583
26	32.821	VB	0.2155	18.23094	1.23997	0.5164
27	33.356	BB	0.2076	44.47746	3.17206	1.2599
28	34.619	BV	0.2073	300.86948	22.03489	8.5228
29	35.002	VB	0.2473	90.27164	5.56431	2.5572
30	37.238	BV	0.2175	34.57365	2.32485	0.9794
31	37.474	VB	0.1833	26.32189	2.14322	0.7456
32	39.002	BB	0.1938	65.37084	5.23188	1.8518
Totals :				3530.15314	220.08379	

mple Name: D5T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.418	BB	0.3087	440.03891	17.65858	8.9183
2	7.658	BB	0.1437	34.15795	3.56750	0.6923
3	15.358	BB	0.3326	36.83596	1.62395	0.7466
4	18.660	BB	0.1887	745.56958	60.95886	15.1104
5	23.276	BV	0.1448	24.70279	2.60138	0.5007
6	23.643	VB	0.3004	63.43146	3.48374	1.2856
7	24.119	BV E	0.1922	71.97066	5.37625	1.4586
8	24.497	VV R	0.1583	1327.82666	126.63089	26.9110
9	24.936	VB E	0.1447	16.07754	1.72498	0.3258
10	25.223	BB	0.1293	8.03949	1.00563	0.1629
11	26.154	BV	0.1646	66.64292	6.04547	1.3506
12	26.394	VB	0.2637	114.19986	5.90258	2.3145
13	27.563	BB	0.2192	37.24804	2.63101	0.7549
14	28.757	BB	0.5518	1575.90942	41.24326	31.9389
15	31.540	BB	0.1924	283.82053	22.61984	5.7522
16	32.952	BB	0.1842	21.04158	1.75068	0.4264
17	36.515	BB	0.2430	20.16373	1.25797	0.4087
18	37.476	BB	0.2641	46.46111	2.53200	0.9416
Totals :				4934.13819	308.61457	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.439	BV	0.1138	32.34818	3.78280	0.8515
2	5.553	VB	0.1464	31.53908	2.82271	0.8302
3	7.658	BB	0.1419	16.68053	1.77120	0.4391
4	22.123	BB	0.1490	12.03523	1.24263	0.3168
5	23.276	BV	0.1454	22.13080	2.31859	0.5826
6	23.644	VB	0.2996	55.06593	3.00739	1.4496
7	24.119	BV E	0.1932	56.17645	4.16900	1.4788
8	24.497	VV R	0.1577	1208.83179	115.85051	31.8216
9	24.931	VB E	0.1434	14.11806	1.53346	0.3716
10	26.156	BV	0.1605	60.68956	5.59792	1.5976
11	26.392	VB	0.2389	147.88435	8.76101	3.8929
12	27.574	BB	0.2356	42.32769	2.74931	1.1142
13	28.758	BB	0.5535	1835.28601	47.84226	48.3125
14	31.538	BB	0.1981	214.52866	16.67849	5.6473
15	32.953	BB	0.1983	29.35753	2.21938	0.7728
16	37.472	BB	0.2378	19.78292	1.19105	0.5208
Totals :				3798.78277	221.53773	

mple Name: D5T50

Signal 5: DAD1 E, Sig=360,4 Ref=off

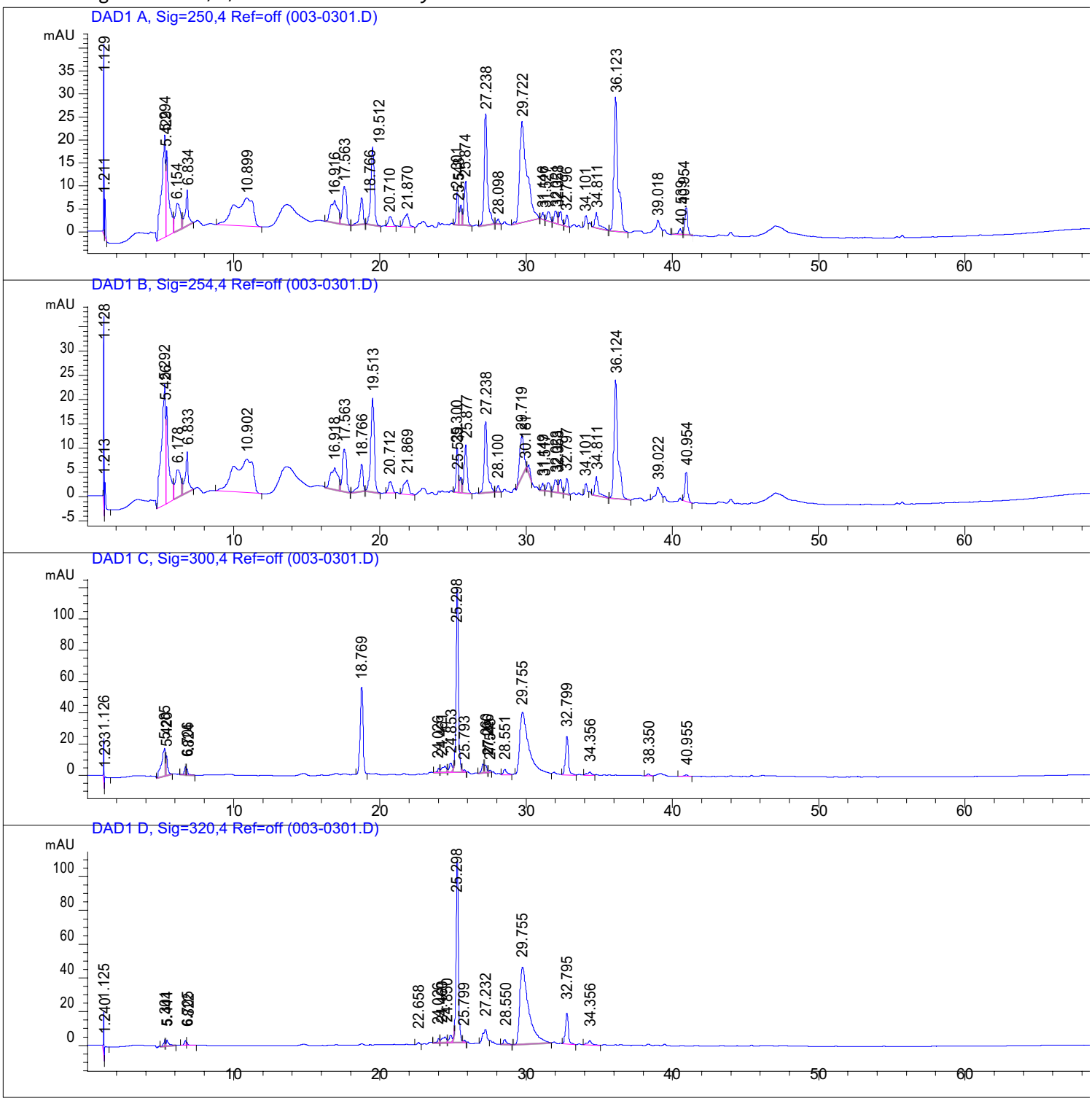
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.441	BV	0.1041	16.53464	2.14085	10.2915
2	5.557	VB	0.1369	16.10343	1.57979	10.0231
3	24.497	BB	0.1502	43.93578	4.48912	27.3464
4	26.391	BB	0.2202	50.37823	3.37465	31.3563
5	31.205	BB	0.2428	24.54269	1.65701	15.2758
6	32.512	BB	0.1387	9.16882	1.04200	5.7068
Totals :				160.66359	14.28342	

*** End of Report ***

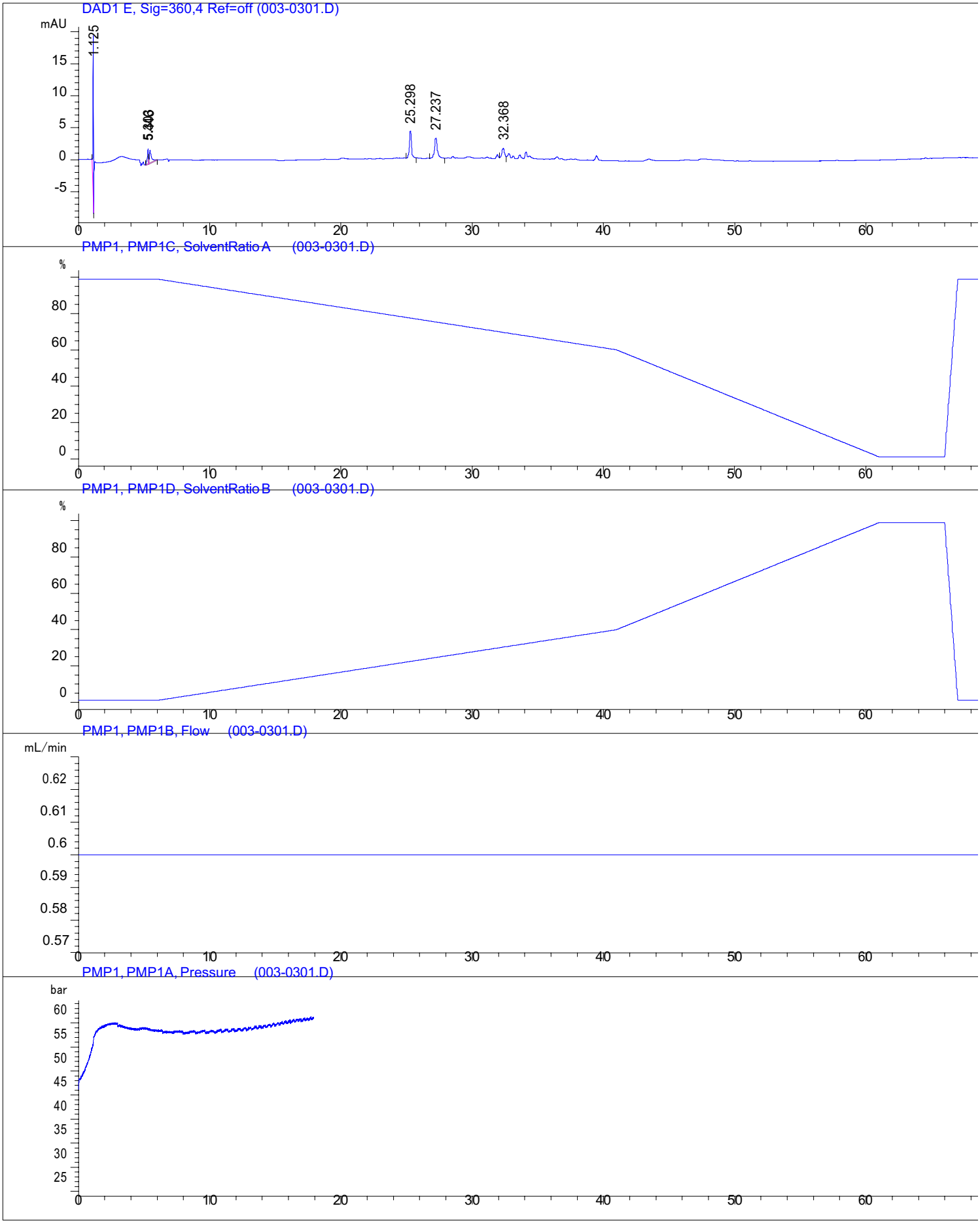
mple Name: D6T50

==========Method 8==========

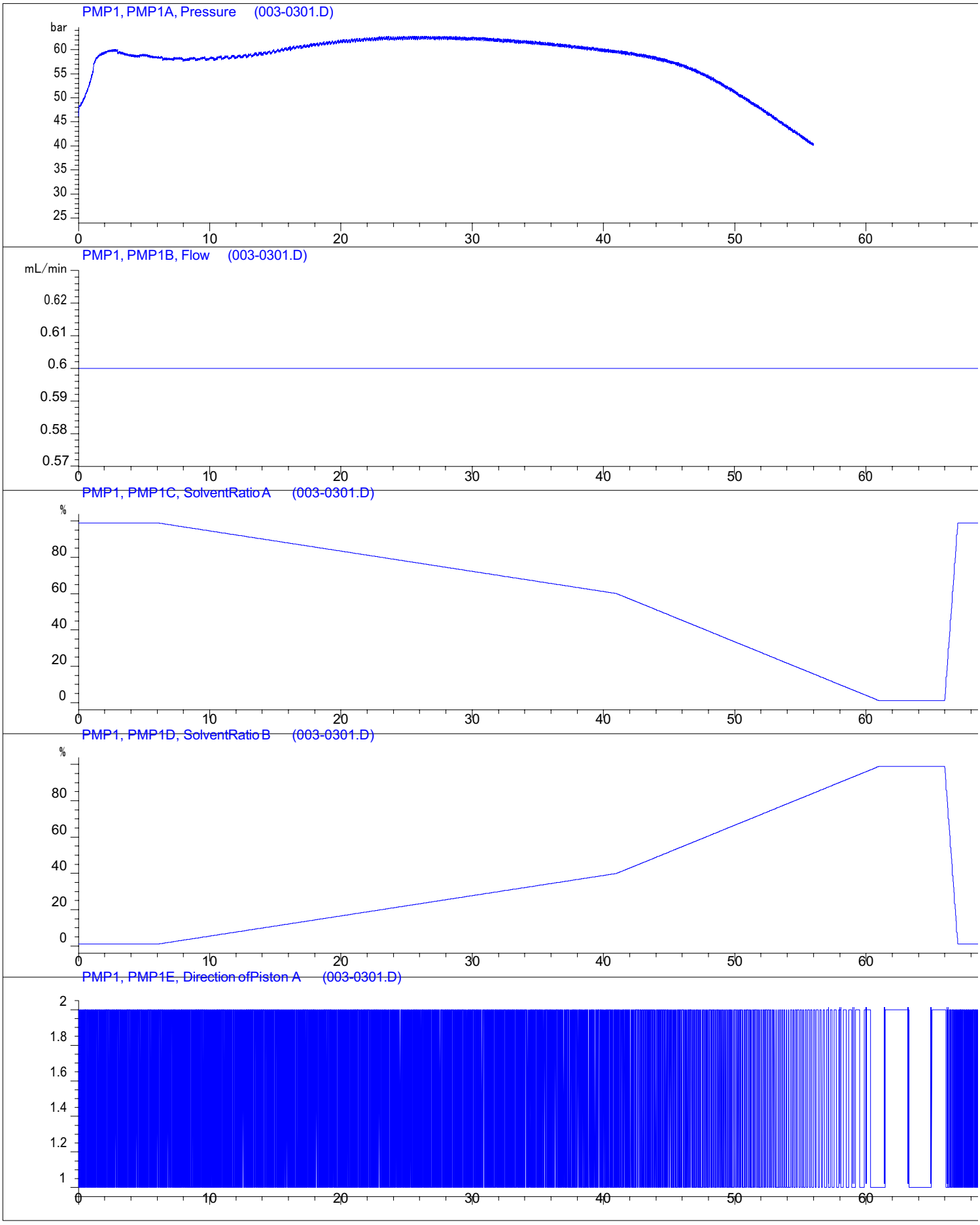
Acq. Operator	: SYSTEM	Seq. Line	: 3
Acq. Instrument	: HPLC 4	Location	: 3
Injection Date	: 5/8/2017 9:45:33 PM	Inj	: 1
		Inj Volume	: 10.000 µl
Sequence File	: C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25 \Sequence - method 5-6-7-8-9-10-11.S		
Method	: C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25 \Optimizing method 6.M (Sequence Method)		
Last changed	: 5/8/2017 8:22:26 PM by SYSTEM		



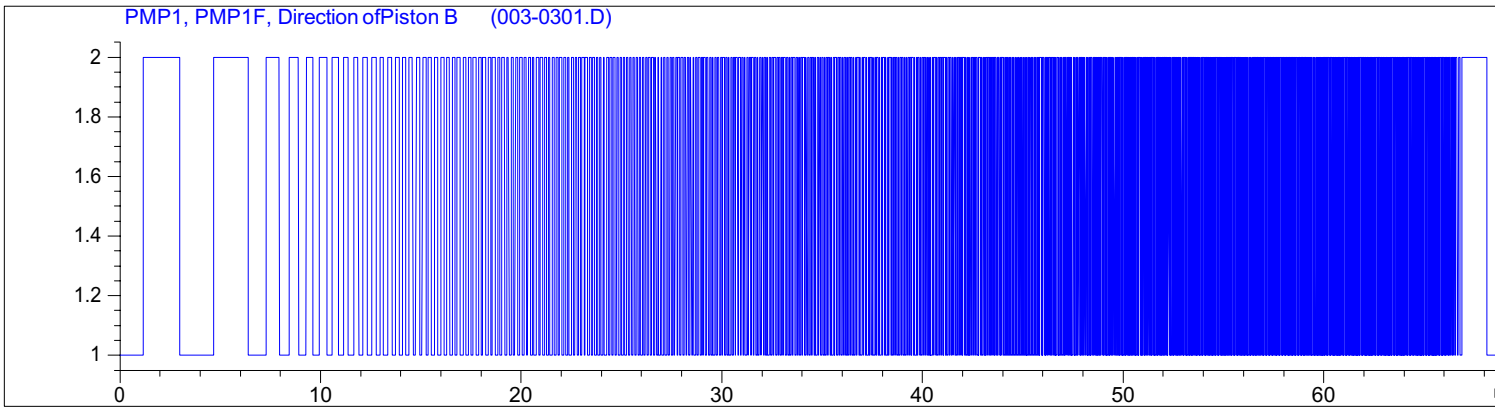
mple Name: D6T50



mple Name: D6T50



mple Name: D6T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.129	BB	0.0404	101.19228	41.55322	2.1242
2	1.211	BB	0.0648	31.91568	8.31151	0.6700
3	5.294	BV	0.2262	406.13525	22.32449	8.5257
4	5.429	VV	0.1999	277.62460	18.62116	5.8279
5	6.154	VV	0.3366	151.05968	5.94182	3.1711
6	6.834	VB	0.1733	105.78646	7.84361	2.2207
7	10.899	BB	1.1016	537.89520	6.11871	11.2916
8	16.916	BV	0.4333	169.82062	4.91304	3.5649
9	17.563	VB	0.2623	157.44055	8.33933	3.3050
10	18.766	BB	0.2412	93.97826	5.79549	1.9728
11	19.512	BB	0.2582	282.15143	16.94698	5.9230
12	20.710	BB	0.2790	36.36254	2.01087	0.7633
13	21.870	BB	0.3181	66.50448	2.91523	1.3961
14	25.301	BV	0.1590	71.78072	6.91999	1.5068
15	25.543	VV	0.1595	47.36351	4.40148	0.9943
16	25.874	VB	0.2164	136.48369	9.57124	2.8651
17	27.238	BB	0.2273	370.71014	24.11886	7.7820
18	28.098	BB	0.1794	12.12292	1.05985	0.2545
19	29.722	BB	0.4753	767.09790	21.97335	16.1030
20	31.146	BB	0.1649	15.31296	1.50278	0.3215
21	31.527	BB	0.2645	31.21598	2.01762	0.6553
22	32.021	BV	0.2334	39.54967	2.66033	0.8302
23	32.358	VV	0.2071	38.53978	2.86297	0.8090
24	32.796	VB	0.1875	28.83018	2.41171	0.6052
25	34.101	BB	0.1592	20.81660	2.07159	0.4370
26	34.811	BB	0.2475	55.54619	3.28204	1.1660
27	36.123	BB	0.2761	559.48981	29.12879	11.7449
28	39.018	BB	0.2627	44.74334	2.40896	0.9393
29	40.539	BV	0.2251	21.09546	1.34432	0.4428

mple Name: D6T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	40.954	VB	0.2159	85.11811	6.05716	1.7868
Totals :				4763.68400	275.42850	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.128	BB	0.0411	99.24603	39.74833	2.3862
2	1.213	BB	0.0804	36.86582	6.68110	0.8864
3	5.292	BV	0.2365	465.32632	24.61998	11.1878
4	5.426	VV	0.1933	282.54929	19.93675	6.7933
5	6.178	VV	0.4073	153.64528	5.72209	3.6941
6	6.833	VB	0.1761	115.29572	8.40048	2.7720
7	10.902	BB	1.1239	620.94080	6.88677	14.9292
8	16.918	BV	0.4324	151.98927	4.40674	3.6543
9	17.563	VB	0.2626	163.84401	8.66505	3.9393
10	18.766	BB	0.2469	92.87443	5.50201	2.2330
11	19.513	BB	0.2604	323.80945	19.23128	7.7853
12	20.712	BB	0.2886	41.82205	2.33919	1.0055
13	21.869	BB	0.3252	70.11490	2.95094	1.6858
14	25.300	BV	0.1615	95.06404	8.98130	2.2856
15	25.539	VV	0.1469	31.79988	3.17379	0.7646
16	25.877	VB	0.2129	138.33383	9.90594	3.3259
17	27.238	BB	0.2342	234.16936	14.67011	5.6301
18	28.100	BB	0.1844	15.85388	1.35570	0.3812
19	29.719	BB	0.2969	182.03099	8.89111	4.3765
20	30.181	BB	0.1746	16.39290	1.48762	0.3941
21	31.149	BV	0.1720	16.18195	1.49774	0.3891
22	31.517	VB	0.2610	26.45461	1.72428	0.6360
23	32.023	BV	0.2509	40.88428	2.55242	0.9830
24	32.358	VV	0.2102	37.93266	2.76205	0.9120
25	32.797	VB	0.1942	39.07595	3.16210	0.9395
26	34.101	BB	0.1605	20.16227	2.01860	0.4848
27	34.811	BB	0.2359	61.62901	3.86828	1.4817
28	36.124	BB	0.2724	460.69757	24.38439	11.0765
29	39.022	BB	0.2743	43.96892	2.24718	1.0571
30	40.954	BB	0.2070	80.29012	6.04295	1.9304
Totals :				4159.24558	253.81628	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.126	BB	0.0421	73.81449	28.65618	1.4176
2	1.233	BB	0.1634	78.43079	6.38077	1.5062
3	5.285	BV	0.2292	324.55048	17.76164	6.2329

mple Name: D6T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	5.420	VB	0.1307	112.97156	12.82736	2.1696
5	6.706	BV	0.1112	38.63255	5.16580	0.7419
6	6.824	VB	0.0831	23.09574	3.89673	0.4435
7	18.769	BB	0.2126	766.14276	55.64664	14.7136
8	24.026	BV E	0.1583	28.40474	2.66513	0.5455
9	24.461	VV E	0.2820	87.18053	4.10049	1.6743
10	24.853	VV E	0.2125	86.81551	5.87168	1.6673
11	25.298	VV R	0.1703	1329.41992	117.22249	25.5311
12	25.793	VB E	0.1409	12.10955	1.34668	0.2326
13	27.060	BV	0.1699	66.68756	5.81213	1.2807
14	27.226	VV	0.1654	55.88568	5.03773	1.0733
15	27.548	VB	0.1478	12.47742	1.30157	0.2396
16	28.551	BB	0.2229	47.84222	3.15471	0.9188
17	29.755	BB	0.6048	1655.86877	39.35975	31.8005
18	32.799	BB	0.2123	338.53055	24.33698	6.5014
19	34.356	BB	0.2476	33.37659	1.91222	0.6410
20	38.350	BB	0.2186	16.18199	1.16110	0.3108
21	40.955	BB	0.2451	18.63375	1.08124	0.3579
Totals :				5207.05316	344.69901	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.125	BB	0.0441	73.46191	26.67985	1.8123
2	1.240	BB	0.1469	72.69616	6.90001	1.7934
3	5.301	BV	0.0982	27.88909	3.95965	0.6880
4	5.444	VB	0.1525	42.22634	3.55597	1.0417
5	6.705	BV	0.1147	21.40141	2.75227	0.5280
6	6.822	VB	0.0834	9.74331	1.63754	0.2404
7	22.658	BB	0.1648	12.94122	1.20937	0.3193
8	24.026	BV E	0.1600	25.02470	2.31743	0.6174
9	24.460	VV E	0.2838	72.92426	3.40627	1.7990
10	24.850	VV E	0.2148	64.66605	4.31629	1.5953
11	25.298	VV R	0.1694	1207.58716	107.18040	29.7909
12	25.799	VB E	0.1328	9.84396	1.16291	0.2428
13	27.232	BB	0.2617	134.75813	7.09265	3.3245
14	28.550	BB	0.2213	43.83268	2.88462	1.0813
15	29.755	BB	0.6080	1935.30029	45.70347	47.7434
16	32.795	BB	0.2128	250.09422	18.14195	6.1698
17	34.356	BB	0.2675	49.15161	2.58907	1.2126
Totals :				4053.54251	241.48973	

mple Name: D6T50

Signal 5: DAD1 E, Sig=360,4 Ref=off

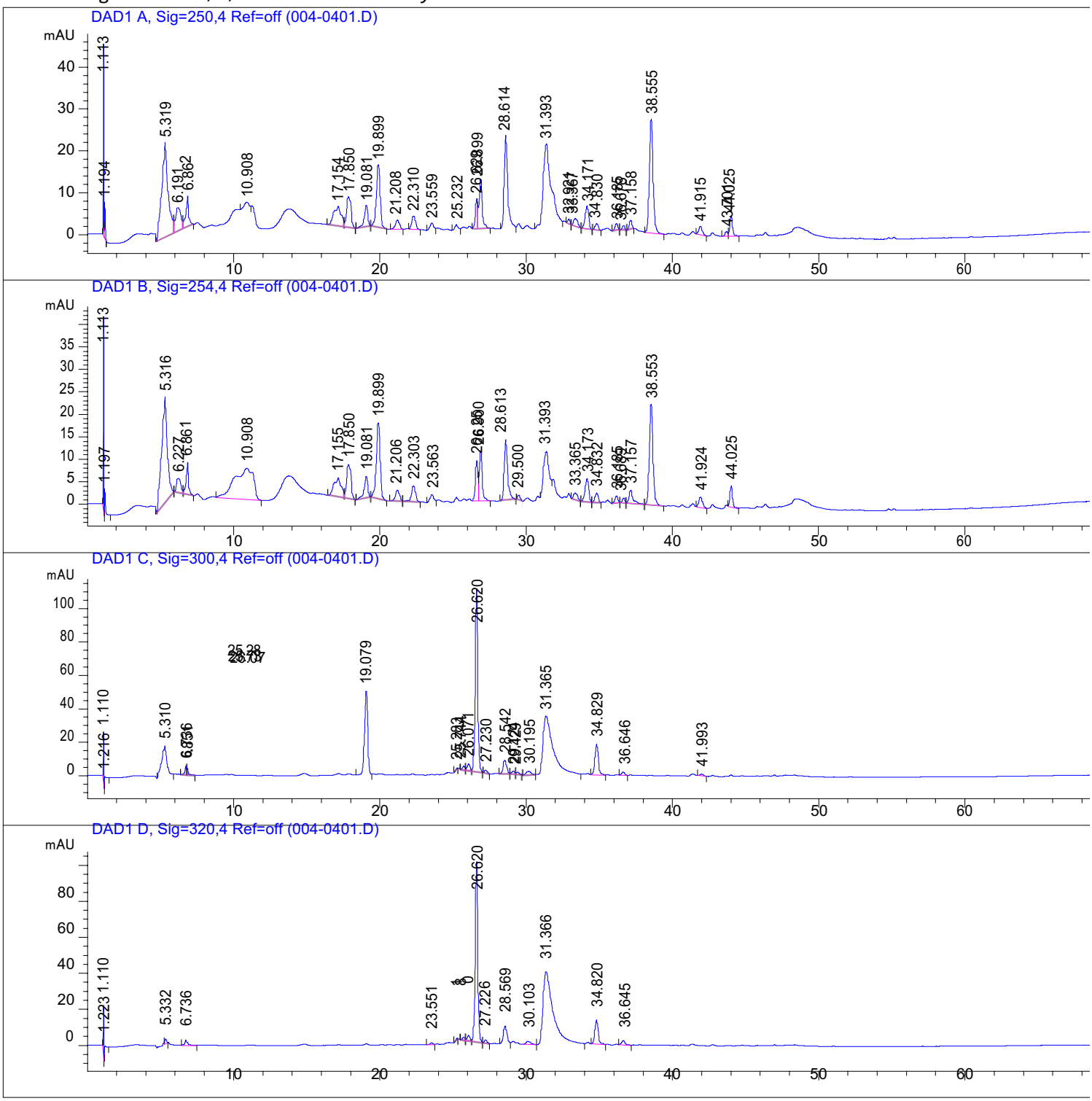
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.125	BB	0.0443	69.05200	24.93267	30.9597
2	5.303	BV	0.0866	15.04939	2.41845	6.7474
3	5.446	VB	0.1508	24.49267	2.08891	10.9814
4	25.298	BB	0.1666	46.51356	4.21980	20.8545
5	27.237	BB	0.2292	49.60074	3.19381	22.2387
6	32.368	BB	0.2037	18.33003	1.40927	8.2183
Totals :				223.03839	38.26290	

*** End of Report ***

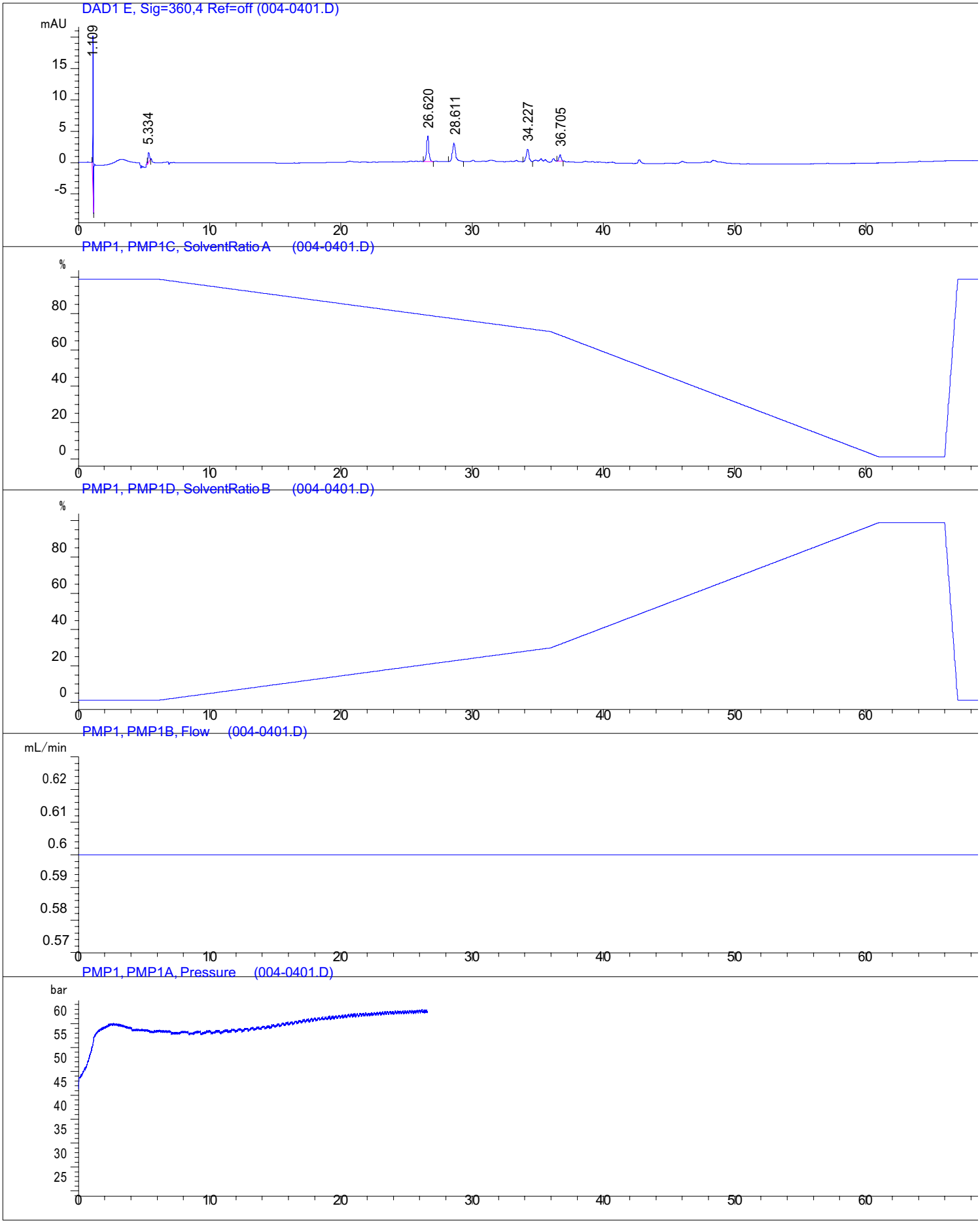
mple Name: D7T50

==========Method 9==========

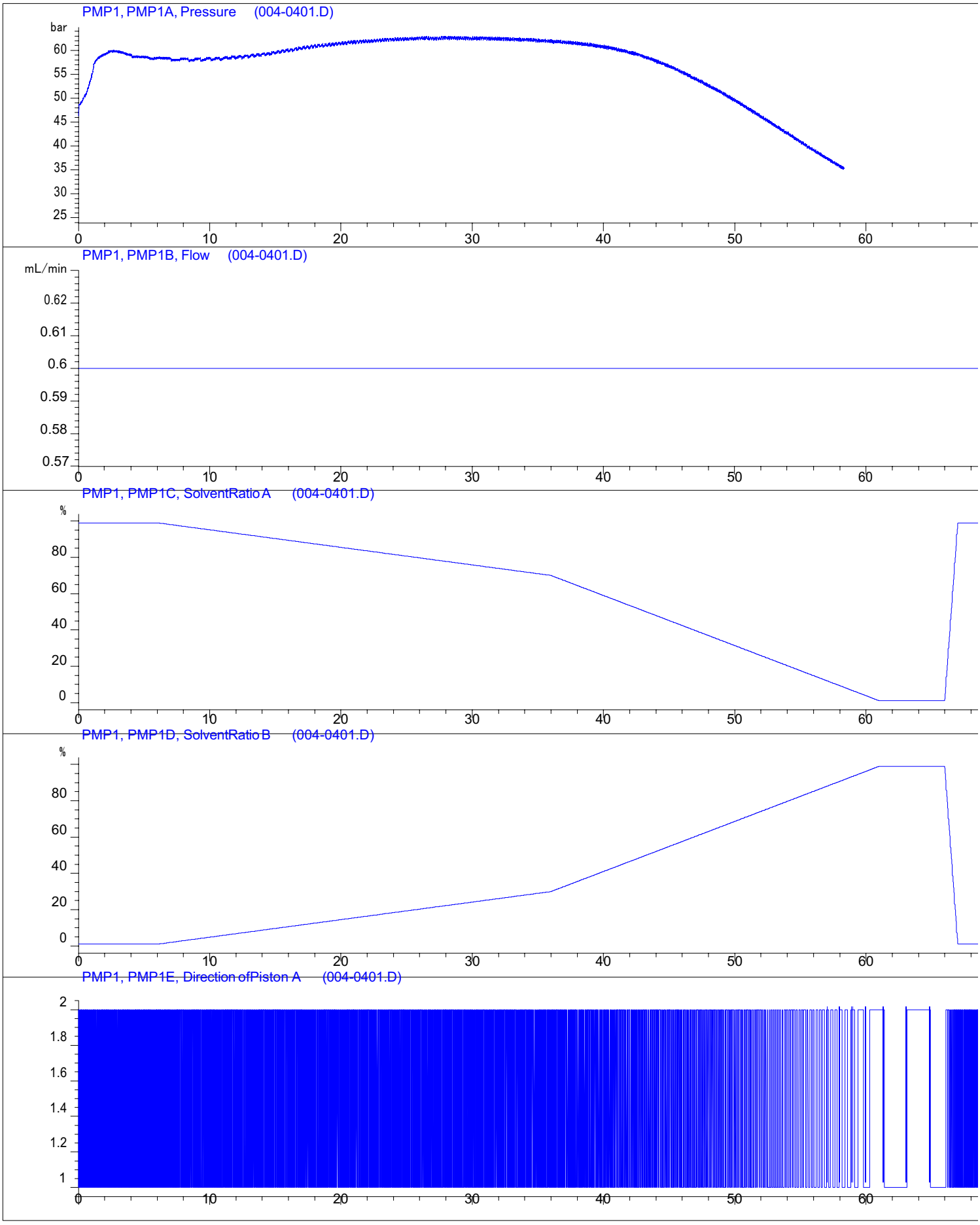
Acq. Operator : SYSTEM Seq. Line : 4
Acq. Instrument : HPLC 4 Location : 4
Injection Date : 5/8/2017 10:56:40 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Sequence - method 5-6-7-8-9-10-11.S
Method : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Optimizing method 7.M (Sequence Method)
Last changed : 5/8/2017 8:22:26 PM by SYSTEM



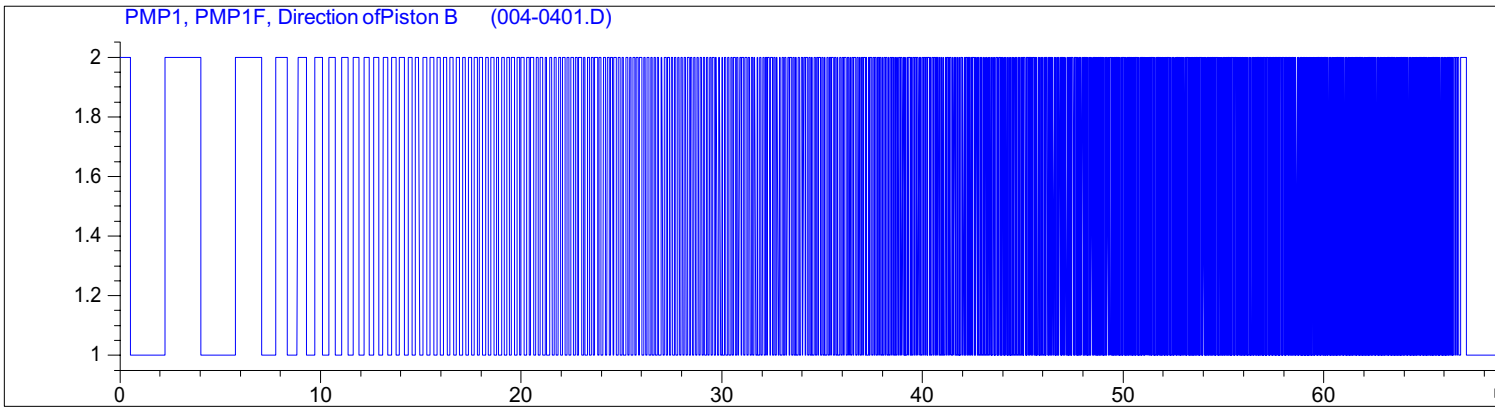
mple Name: D7T50



mple Name: D7T50



mple Name: D7T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.113	BV R	0.0410	115.44231	46.47787	2.8313
2	1.194	VB E	0.0638	33.86799	8.62515	0.8306
3	5.319	BV	0.3863	684.04260	22.77948	16.7767
4	6.191	VV	0.3907	149.33690	5.79321	3.6626
5	6.862	VB	0.1779	103.02116	7.51439	2.5267
6	10.908	BB	0.3043	25.85459	1.27659	0.6341
7	17.154	BV	0.4447	165.29555	4.69534	4.0540
8	17.850	VB	0.2894	152.85182	7.32656	3.7488
9	19.081	BB	0.2533	84.33491	5.03726	2.0684
10	19.899	BB	0.2854	269.86026	14.89191	6.6186
11	21.208	BB	0.2862	43.26534	2.19254	1.0611
12	22.310	BB	0.2940	57.62272	3.14128	1.4132
13	23.559	BB	0.2361	23.58136	1.51042	0.5784
14	25.232	BB	0.1866	14.38174	1.16027	0.3527
15	26.629	BV	0.1729	83.44158	7.21866	2.0465
16	26.899	VB	0.2168	170.55661	11.64778	4.1830
17	28.614	BB	0.2421	361.01288	22.15375	8.8541
18	31.393	BB	0.5258	713.71655	19.55396	17.5045
19	32.921	BB	0.1683	11.23142	1.08904	0.2755
20	33.367	BB	0.3237	36.56874	1.85724	0.8969
21	34.171	BB	0.2673	95.55550	5.53928	2.3436
22	34.830	BB	0.2428	24.55120	1.56711	0.6021
23	36.185	BB	0.2139	23.73012	1.71024	0.5820
24	36.678	BB	0.2002	14.60533	1.16567	0.3582
25	37.158	BB	0.2104	30.30703	2.26064	0.7433
26	38.555	BB	0.2679	479.77466	27.17865	11.7669
27	41.915	BB	0.2577	33.22220	1.96088	0.8148
28	43.701	BV	0.1756	13.37293	1.13426	0.3280
29	44.025	VB	0.1991	62.92579	4.79595	1.5433

mple Name: D7T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				4077.33177	243.25537	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.113	BB	0.0411	108.95100	43.74482	2.8787
2	1.197	BB	0.0692	26.29907	5.99267	0.6949
3	5.316	BB	0.3520	639.66980	23.76351	16.9015
4	6.227	BV	0.3350	66.48081	3.19146	1.7566
5	6.861	VB	0.1451	77.49226	7.11043	2.0475
6	10.908	BB	1.1011	606.69537	6.90499	16.0302
7	17.155	BV	0.4429	147.23402	4.22139	3.8903
8	17.850	VB	0.2878	158.99022	7.60668	4.2009
9	19.081	BB	0.2601	81.26553	4.69047	2.1472
10	19.899	BB	0.2880	309.29144	16.86488	8.1722
11	21.206	BB	0.2909	48.33364	2.46345	1.2771
12	22.303	BB	0.3105	69.44447	3.48413	1.8349
13	23.563	BB	0.2538	24.71935	1.50325	0.6531
14	26.625	BV	0.1751	105.22340	8.95314	2.7802
15	26.900	VB	0.2143	159.23717	11.17264	4.2074
16	28.613	BB	0.2479	224.35725	13.49757	5.9280
17	29.500	BB	0.2071	15.02285	1.15986	0.3969
18	31.393	BB	0.3682	187.32425	8.14964	4.9495
19	33.365	BB	0.3006	28.72171	1.51898	0.7589
20	34.173	BB	0.2712	90.84435	5.21610	2.4003
21	34.832	BB	0.2447	33.01659	2.10828	0.8724
22	36.185	BB	0.2159	21.93724	1.58082	0.5796
23	36.689	BB	0.1813	13.08632	1.11186	0.3458
24	37.157	BB	0.2751	56.05869	2.95845	1.4812
25	38.553	BB	0.2656	391.92801	22.45872	10.3556
26	41.924	BB	0.2778	39.72597	2.25152	1.0496
27	44.025	BB	0.1798	53.34024	4.58364	1.4094
Totals :				3784.69103	218.26339	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.110	BB	0.0422	79.77888	30.80406	1.5905
2	1.216	BB	0.1374	59.98807	5.86035	1.1960
3	5.310	BB	0.3121	438.68979	18.27311	8.7460
4	6.736	BV	0.1158	38.87441	5.04954	0.7750
5	6.851	VB	0.0861	22.47298	3.74156	0.4480
6	19.079	BB	0.2322	741.59265	49.66829	14.7849
7	25.283	BB	0.1326	9.15666	1.10621	0.1826
8	25.744	BV E	0.1958	27.80000	2.25585	0.5542

mple Name: D7T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	26.071	VV E	0.1958	53.60913	4.06744	1.0688
10	26.620	VB R	0.1808	1320.22546	109.39784	26.3209
11	27.230	BB	0.1986	23.64077	1.85549	0.4713
12	28.542	BB	0.2607	128.31129	7.84797	2.5581
13	29.124	BV	0.1993	19.30993	1.50929	0.3850
14	29.429	VB	0.1940	15.90659	1.25353	0.3171
15	30.195	BB	0.3406	43.86449	2.02591	0.8745
16	31.365	BB	0.6717	1650.91370	35.03550	32.9137
17	34.829	BB	0.2522	298.20746	18.28899	5.9453
18	36.646	BB	0.2304	25.83323	1.76912	0.5150
19	41.993	BB	0.2506	17.71374	1.10765	0.3532
Totals :				5015.88922	300.91769	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.110	BB	0.0417	69.61136	27.30289	1.7952
2	1.223	BB	0.1356	61.23304	6.40193	1.5791
3	5.332	VB	0.1079	22.27217	2.76605	0.5744
4	6.736	BB	0.1576	30.16558	2.67389	0.7779
5	23.551	BB	0.1816	11.89332	1.00823	0.3067
6	25.281	BB	0.1341	9.18298	1.09243	0.2368
7	25.738	BV E	0.1886	21.67202	1.82448	0.5589
8	26.070	VV E	0.1904	38.69490	3.04254	0.9979
9	26.620	VB R	0.1800	1201.92493	100.14787	30.9962
10	27.226	BB	0.1900	21.14428	1.69015	0.5453
11	28.569	BB	0.2662	158.56738	9.42957	4.0893
12	30.103	BB	0.3248	40.32917	1.66402	1.0400
13	31.366	BB	0.6848	1936.65027	40.43491	49.9439
14	34.820	BB	0.2517	219.59399	13.50601	5.6631
15	36.645	BB	0.2428	34.71451	2.23961	0.8952
Totals :				3877.64989	215.22457	

Signal 5: DAD1 E, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.109	BB	0.0430	67.17854	25.30421	30.7397
2	5.334	VB	0.1024	13.03935	1.68365	5.9666
3	26.620	BB	0.1815	49.22476	4.05798	22.5244
4	28.611	BB	0.2498	49.02792	2.95212	22.4343
5	34.227	BB	0.2209	28.64509	1.97830	13.1075
6	36.705	BB	0.1716	11.42445	1.02882	5.2276
Totals :				218.54010	37.00508	

mple Name: D7T50

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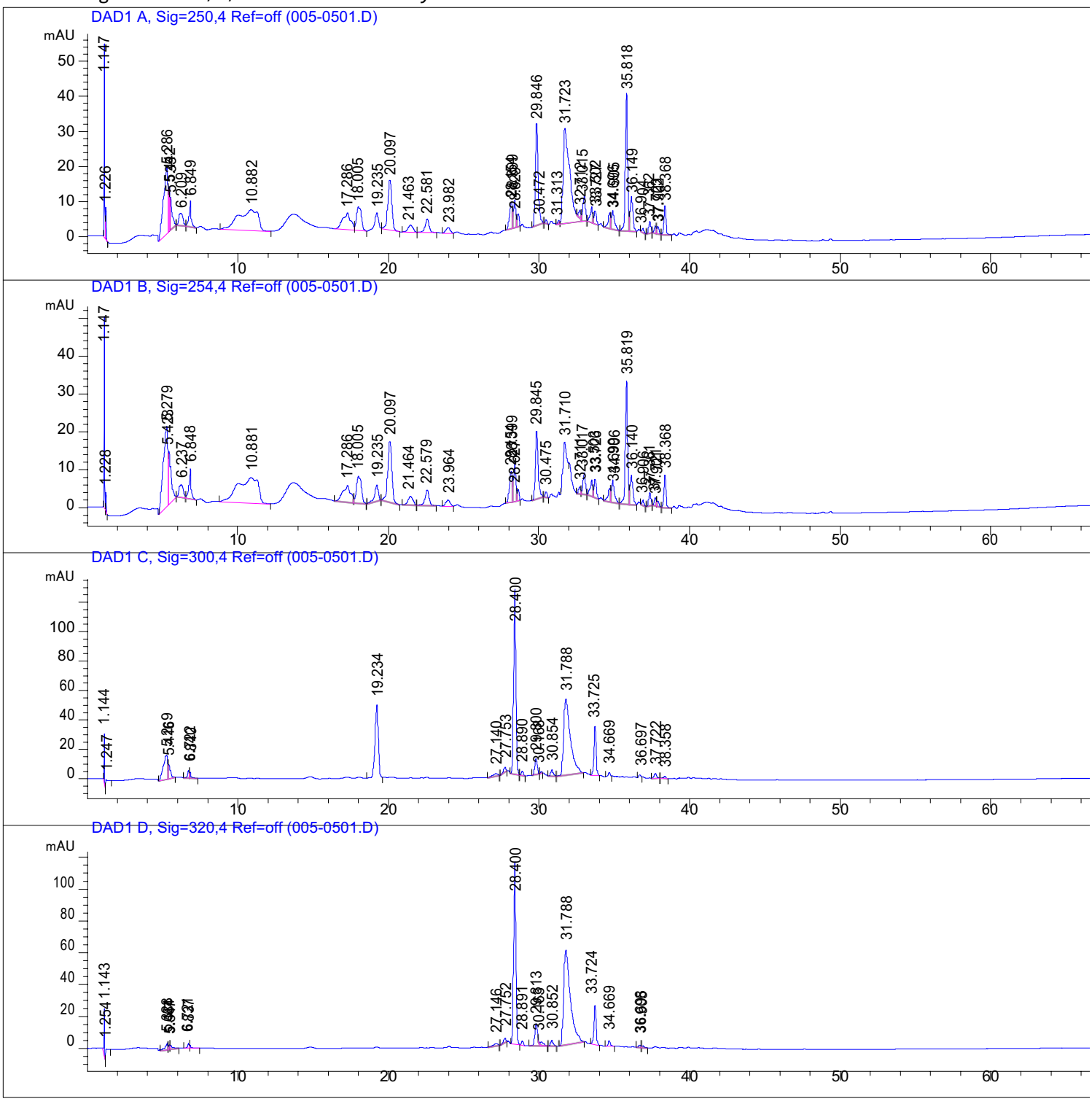
*** End of Report ***

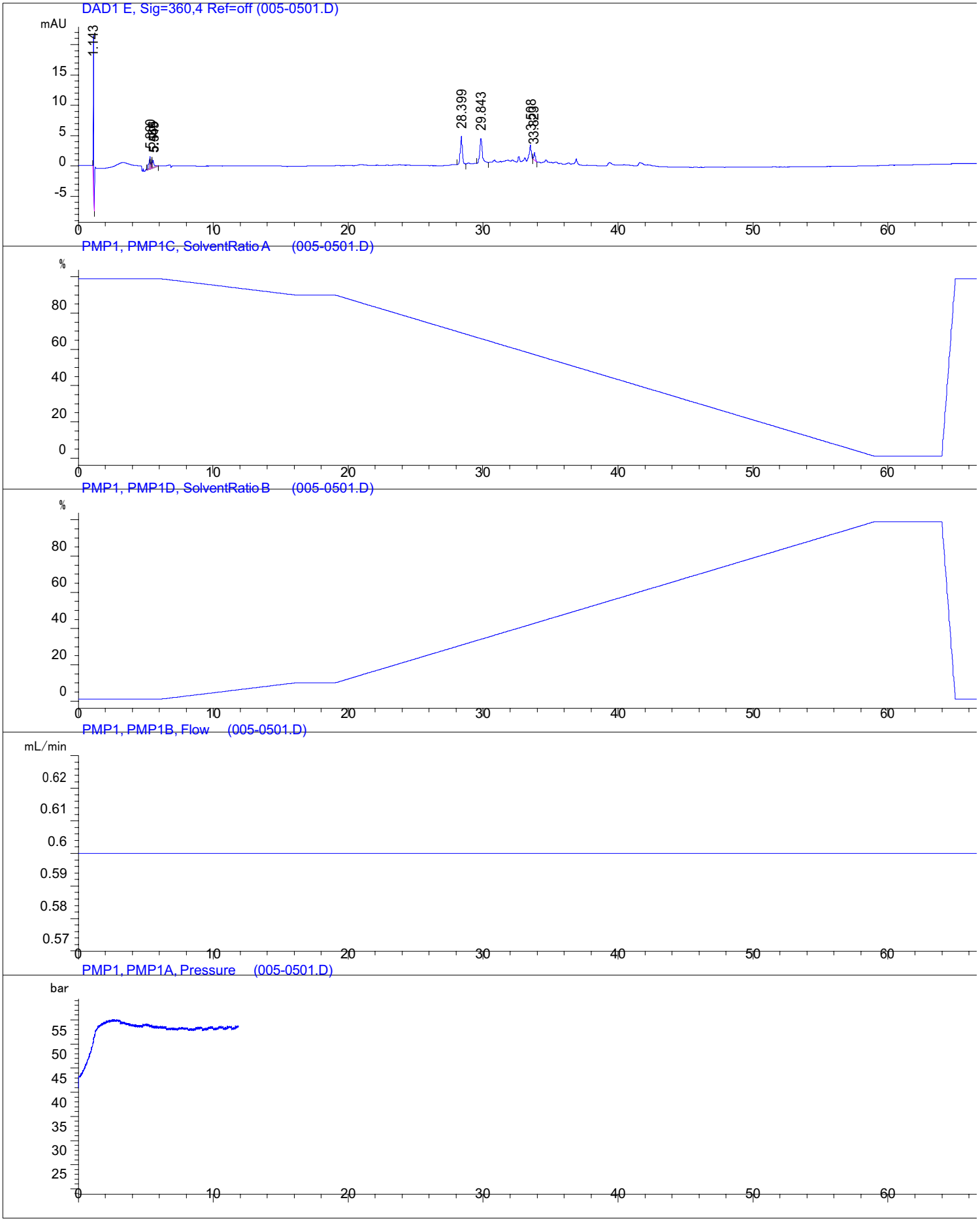
mple Name: D8T50

==========Method 10==========

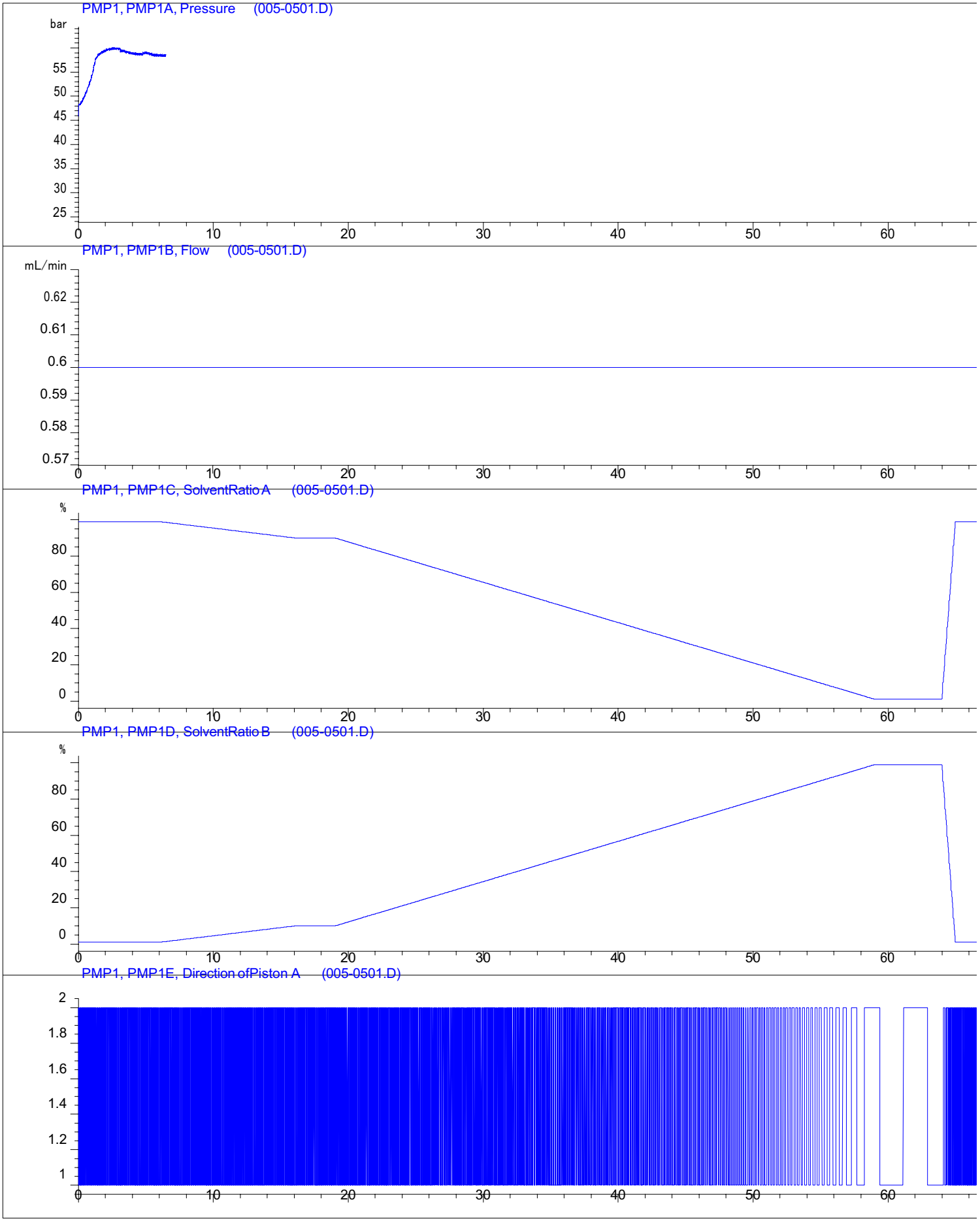
Acq. Operator : SYSTEM Seq. Line : 5
Acq. Instrument : HPLC 4 Location : 5
Injection Date : 5/9/2017 12:07:46 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Sequence - method 5-6-7-8-9-10-11.S
Method : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Optimizing method 8.M (Sequence Method)
Last changed : 5/8/2017 8:22:26 PM by SYSTEM



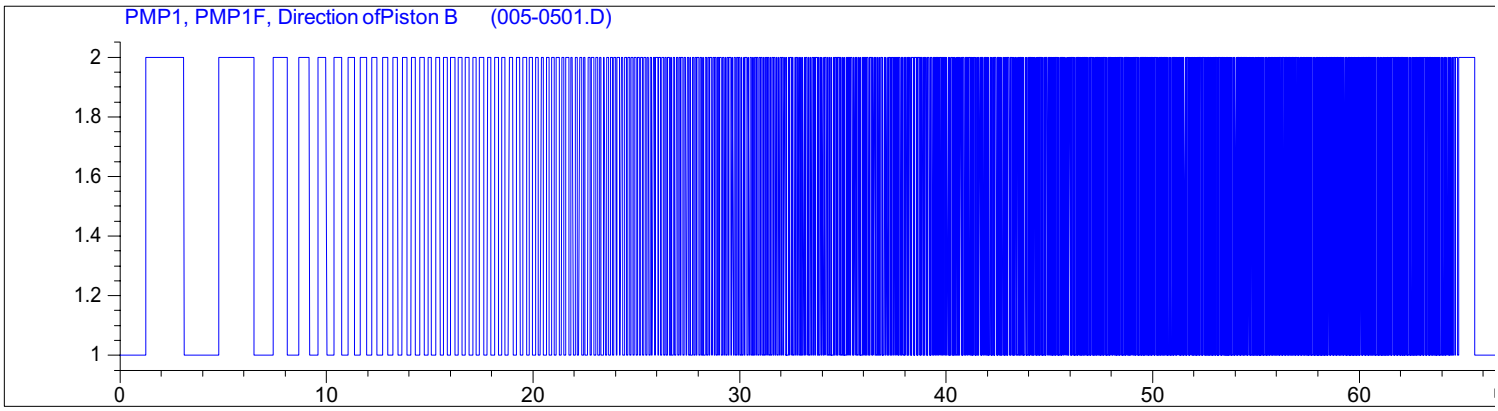
mple Name: D8T50



mple Name: D8T50



mple Name: D8T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.147	BV R	0.0422	144.38351	55.80112	3.0789
2	1.226	VB E	0.0640	35.67554	9.04324	0.7608
3	5.286	BV	0.2580	401.01999	19.01490	8.5516
4	5.442	VV	0.1092	91.81970	12.87354	1.9580
5	5.535	VB	0.1112	80.61378	9.48247	1.7191
6	6.209	BV	0.3082	66.00652	3.49774	1.4076
7	6.849	VB	0.1274	70.96476	7.43163	1.5133
8	10.882	BB	1.1379	538.37415	5.93716	11.4806
9	17.286	BV	0.4687	177.03693	4.81739	3.7753
10	18.005	VB	0.3125	154.97639	6.78512	3.3048
11	19.235	BB	0.2513	76.88805	4.64049	1.6396
12	20.097	BB	0.2943	262.29230	14.14582	5.5933
13	21.463	BB	0.3357	46.32378	2.01869	0.9878
14	22.581	BB	0.2831	71.51308	3.80731	1.5250
15	23.982	BB	0.3035	29.53167	1.50142	0.6298
16	28.154	BV	0.2151	107.11742	7.56753	2.2842
17	28.399	VV	0.1542	81.55006	7.91623	1.7390
18	28.629	VB	0.1312	31.45896	3.70088	0.6709
19	29.846	BB	0.1884	365.45914	29.12909	7.7933
20	30.472	BB	0.1372	9.37767	1.10271	0.2000
21	31.313	BV E	0.1245	8.07525	1.04068	0.1722
22	31.723	VV R	0.3978	829.81049	27.05620	17.6954
23	32.712	VV E	0.1678	22.56176	2.16040	0.4811
24	33.015	VB	0.1843	84.76880	7.15241	1.8077
25	33.502	BV	0.1634	49.32579	4.44881	1.0519
26	33.727	VB	0.1780	39.42395	3.65141	0.8407
27	34.696	BV	0.1798	53.76787	4.36369	1.1466
28	34.905	VB	0.1799	68.05849	5.29745	1.4513
29	35.818	BV	0.1592	407.72086	39.23690	8.6945

mple Name: D8T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	36.149	VB	0.1926	126.55540	9.93781	2.6988
31	36.904	BB	0.1200	8.77800	1.13765	0.1872
32	37.362	BB	0.1571	33.80362	3.36895	0.7209
33	37.723	BV	0.1467	19.03842	2.00721	0.4060
34	37.902	VB	0.1328	17.70237	1.97006	0.3775
35	38.368	BB	0.1467	77.63253	8.17985	1.6555
Totals :				4689.40698	331.22395	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.147	BB	0.0411	128.36093	51.37613	3.2793
2	1.228	BB	0.0569	16.58553	4.73625	0.4237
3	5.279	BV	0.2648	461.38858	21.29419	11.7874
4	5.423	VB	0.1710	173.58409	13.96598	4.4347
5	6.237	BV	0.3158	66.28941	3.36590	1.6935
6	6.848	VB	0.1333	80.26363	7.98051	2.0505
7	10.881	BB	1.1601	622.38788	6.69793	15.9005
8	17.286	BV	0.4636	158.20348	4.33688	4.0417
9	18.005	VB	0.3129	160.87201	7.03298	4.1099
10	19.235	BB	0.2584	72.83268	4.28188	1.8607
11	20.097	BB	0.2979	302.37494	16.04389	7.7249
12	21.464	BB	0.3210	51.11685	2.28517	1.3059
13	22.579	BB	0.2752	76.62477	4.07861	1.9576
14	23.964	BB	0.3198	30.61008	1.54063	0.7820
15	28.154	BV	0.2109	112.09433	8.02924	2.8637
16	28.399	VV	0.1559	105.92236	10.13761	2.7061
17	28.627	VB	0.1256	22.93140	2.79858	0.5858
18	29.845	BB	0.1882	227.20396	17.88187	5.8045
19	30.475	BB	0.1439	12.42275	1.39517	0.3174
20	31.710	BB	0.2029	133.97194	9.36501	3.4227
21	32.711	BV	0.1639	17.85771	1.76726	0.4562
22	33.017	VB	0.1758	61.56629	5.53512	1.5729
23	33.503	BV	0.1640	49.40537	4.30261	1.2622
24	33.726	VB	0.1593	51.97543	4.76294	1.3278
25	34.699	BV	0.1796	44.41822	3.56023	1.1348
26	34.906	VB	0.1782	73.81126	5.88851	1.8857
27	35.819	BV	0.1602	339.74759	32.42357	8.6797
28	36.140	VB	0.1990	99.60046	7.59324	2.5446
29	36.906	BB	0.1211	10.03132	1.28483	0.2563
30	37.361	BB	0.1572	35.39199	3.52329	0.9042
31	37.721	BV	0.1487	21.33195	2.20823	0.5450
32	37.901	VB	0.1265	11.98202	1.41959	0.3061
33	38.368	BB	0.1466	81.10294	8.55276	2.0720
Totals :				3914.26417	281.44659	

mple Name: D8T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.144	BB	0.0419	87.75072	34.23376	1.7472
2	1.247	BB	0.1557	62.59734	5.30224	1.2464
3	5.269	BV	0.2748	343.89862	16.39229	6.8475
4	5.416	VB	0.1379	92.59641	9.48100	1.8437
5	6.722	BV	0.1134	39.37620	5.25565	0.7840
6	6.840	VB	0.0837	23.39522	4.03313	0.4658
7	19.234	BB	0.2404	763.01129	49.33511	15.1926
8	27.140	BB	0.3273	40.00953	1.68330	0.7966
9	27.753	BB	0.1778	41.47665	3.51221	0.8259
10	28.400	BB	0.1563	1298.39307	125.90517	25.8528
11	28.890	BB	0.1431	26.17626	2.90694	0.5212
12	29.800	BB	0.1777	121.00666	10.71645	2.4094
13	30.168	BB	0.1047	8.49425	1.32667	0.1691
14	30.854	BB	0.1718	49.24739	4.17088	0.9806
15	31.788	BB	0.4455	1600.94055	51.67227	31.8769
16	33.725	BB	0.1517	330.74808	33.34793	6.5856
17	34.669	BB	0.1383	21.31160	2.38490	0.4243
18	36.697	BB	0.1392	13.76524	1.58652	0.2741
19	37.722	BB	0.1794	41.01522	3.43308	0.8167
20	38.358	BB	0.1641	17.04826	1.55260	0.3395
Totals :				5022.25857	368.23210	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.143	BB	0.0459	88.85555	30.58884	2.2390
2	1.254	BB	0.1455	66.11127	6.24527	1.6659
3	5.328	BV	0.1483	45.64914	3.90787	1.1503
4	5.464	VV	0.0871	18.02052	2.87547	0.4541
5	5.547	VB	0.1254	24.03065	2.47255	0.6055
6	6.721	BV	0.1174	21.98489	2.80697	0.5540
7	6.837	VB	0.0810	10.03778	1.69681	0.2529
8	27.146	BB	0.2970	31.20314	1.43875	0.7863
9	27.752	BB	0.1808	34.00997	2.85898	0.8570
10	28.400	BB	0.1563	1182.36365	114.71070	29.7937
11	28.891	BB	0.1442	23.83246	2.61921	0.6005
12	29.813	BV R	0.2031	184.57399	13.88333	4.6510
13	30.163	VB E	0.2027	34.23886	2.31307	0.8628
14	30.852	BB	0.1715	43.00957	3.70206	1.0838
15	31.788	BB	0.4457	1848.81201	59.64132	46.5872
16	33.724	BB	0.1525	248.53192	24.46183	6.2626
17	34.669	BB	0.1543	35.43660	3.43746	0.8929
18	36.698	BV	0.1649	16.72089	1.56201	0.4213
19	36.905	VB	0.1408	11.07547	1.14575	0.2791
Totals :				3968.49835	282.36826	

mple Name: D8T50

Signal 5: DAD1 E, Sig=360,4 Ref=off

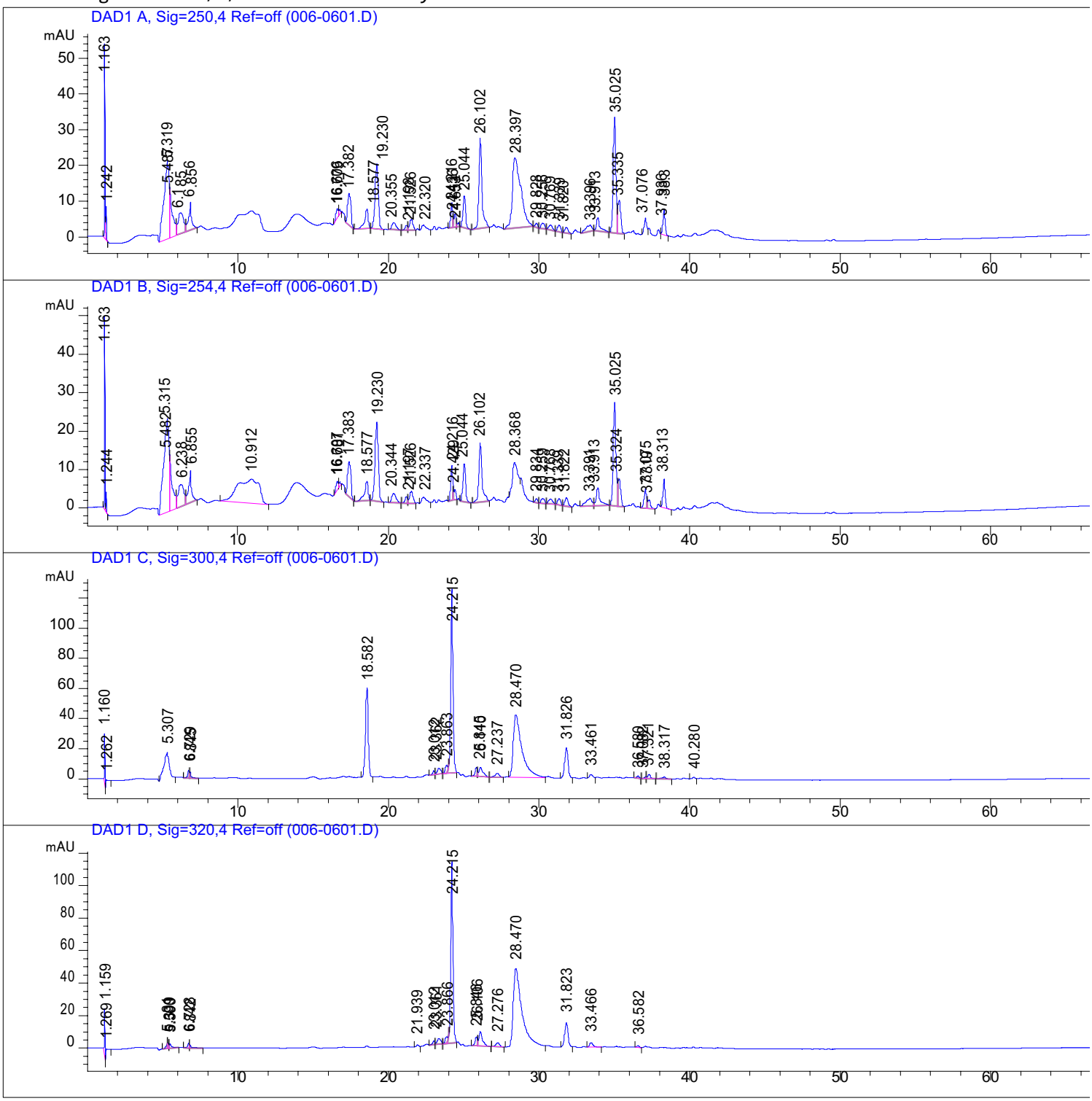
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.143	BB	0.0423	67.92402	26.11078	28.5990
2	5.330	BV	0.1090	16.78777	2.06245	7.0684
3	5.466	VV	0.0938	8.40727	1.44562	3.5398
4	5.549	VB	0.1081	10.19458	1.26397	4.2924
5	28.399	BB	0.1576	48.16151	4.61948	20.2781
6	29.843	BB	0.1897	51.83506	4.03844	21.8248
7	33.508	BB	0.1530	24.76051	2.42828	10.4253
8	33.823	BB	0.1124	9.43422	1.30431	3.9722
Totals :				237.50493	43.27333	

*** End of Report ***

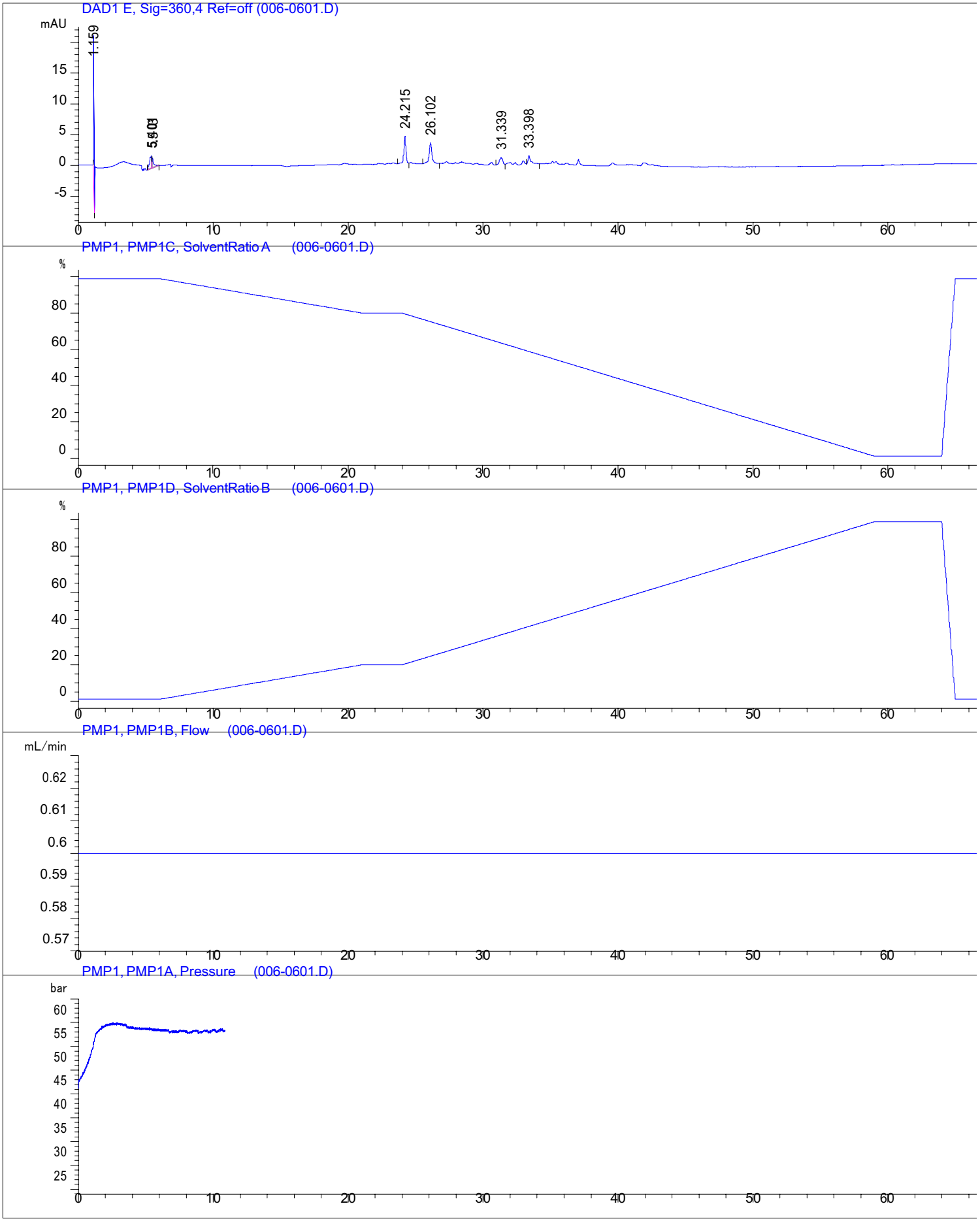
mple Name: D9T50

==========Method 11==========

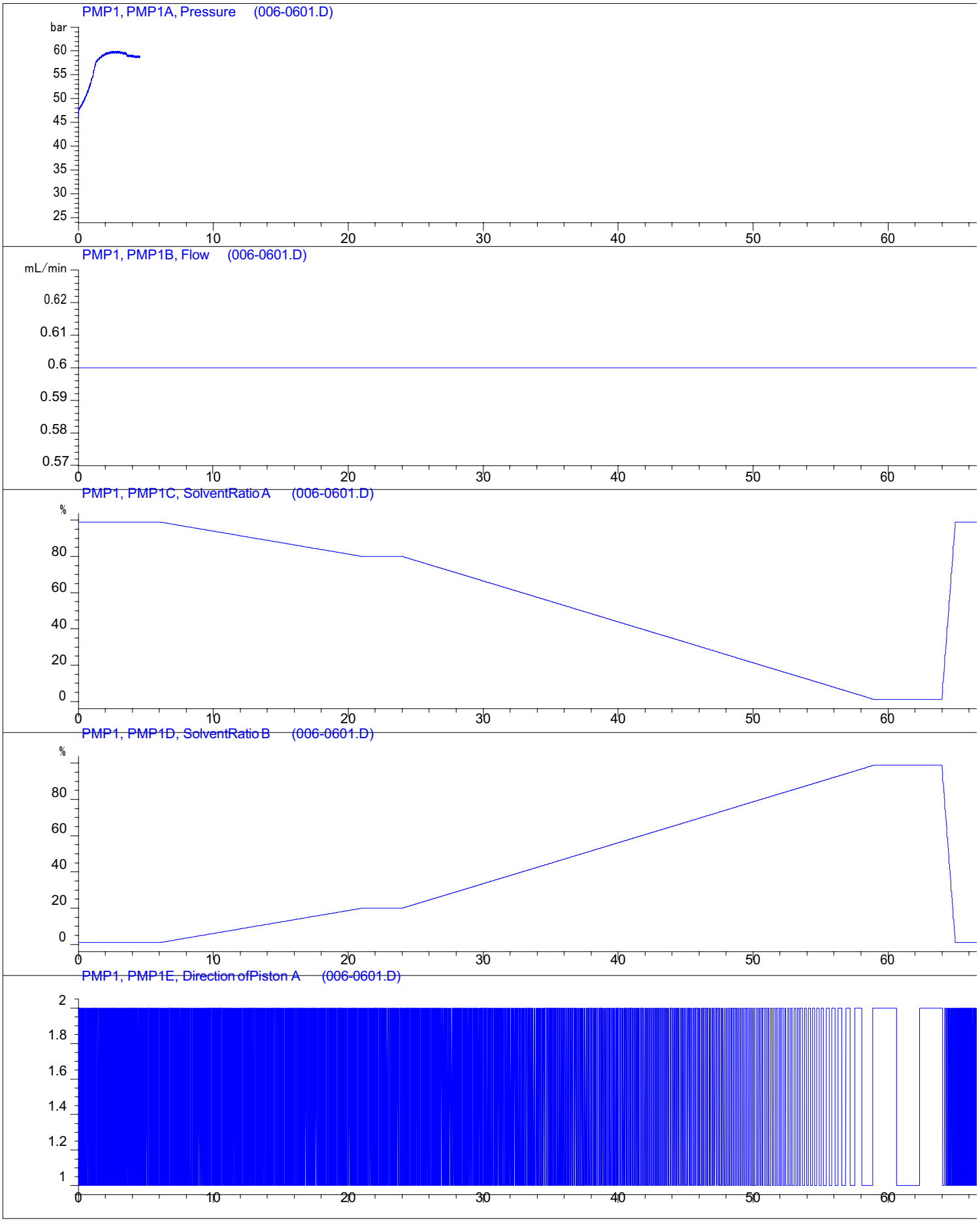
Acq. Operator : SYSTEM Seq. Line : 6
Acq. Instrument : HPLC 4 Location : 6
Injection Date : 5/9/2017 1:16:51 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Sequence - method 5-6-7-8-9-10-11.S
Method : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Optimizing method 9.M (Sequence Method)
Last changed : 5/8/2017 8:22:26 PM by SYSTEM



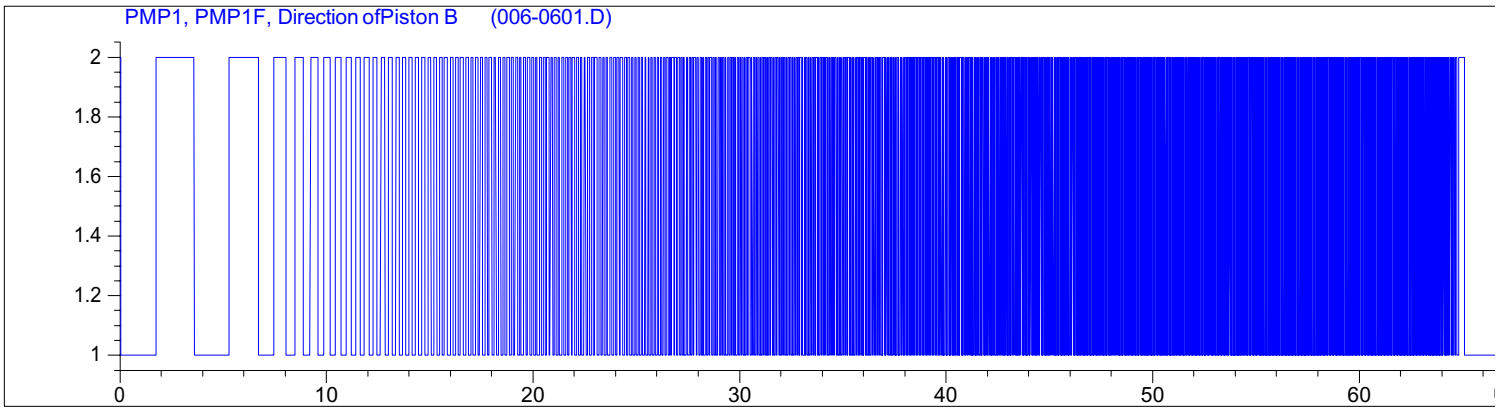
mple Name: D9T50



mple Name: D9T50



mple Name: D9T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.163	BV R	0.0426	142.96086	54.58696	3.5628
2	1.242	VB E	0.0646	36.91279	9.24984	0.9199
3	5.319	BV	0.2844	500.19785	21.90545	12.4658
4	5.487	VV	0.1710	188.28918	14.17124	4.6925
5	6.185	VV	0.3897	153.35365	5.93080	3.8218
6	6.856	VB	0.1762	107.48193	7.82870	2.6786
7	16.606	BV	0.1764	30.66289	2.66216	0.7642
8	16.779	VB	0.1075	9.38439	1.28074	0.2339
9	17.382	BB	0.2490	130.05486	8.67214	3.2412
10	18.577	BB	0.2365	86.18661	5.33352	2.1479
11	19.230	BB	0.2390	278.96729	18.17957	6.9523
12	20.355	BB	0.3020	38.27410	1.97549	0.9539
13	21.198	BV	0.1713	15.25041	1.33505	0.3801
14	21.526	VB	0.2348	46.36582	3.02456	1.1555
15	22.320	BB	0.2889	26.84197	1.39263	0.6689
16	24.216	BV	0.1474	69.13169	7.10969	1.7229
17	24.436	VV	0.1432	36.27221	3.80603	0.9040
18	24.653	VB	0.1289	10.24907	1.23404	0.2554
19	25.044	BB	0.1900	109.53062	9.00209	2.7297
20	26.102	BB	0.2158	367.89517	25.28283	9.1686
21	28.397	BB	0.5600	776.05591	19.68066	19.3407
22	29.828	BB	0.1756	11.74526	1.09087	0.2927
23	30.256	BB	0.2645	25.04110	1.60043	0.6241
24	30.769	BB	0.2633	23.41566	1.39863	0.5836
25	31.339	BB	0.2262	26.72672	1.94691	0.6661
26	31.820	BB	0.2230	23.69432	1.63529	0.5905
27	33.396	BB	0.3450	44.34451	1.63204	1.1051
28	33.913	BB	0.2275	55.47876	3.56649	1.3826
29	35.025	BV	0.1902	404.72913	32.29050	10.0865

mple Name: D9T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	35.335	VB	0.2036	121.61250	9.11902	3.0308
31	37.076	BB	0.1510	35.43713	3.65866	0.8832
32	37.936	BB	0.1269	10.51985	1.29338	0.2622
33	38.313	BB	0.1494	69.49902	7.14784	1.7320
Totals :				4012.56323	290.02426	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.163	BV R	0.0417	128.47452	50.44413	3.2707
2	1.244	VB E	0.0604	19.10277	5.25435	0.4863
3	5.315	BV	0.2910	568.94611	24.31435	14.4840
4	5.482	VV	0.1620	183.81635	14.69282	4.6795
5	6.238	VV	0.4074	156.08485	5.73744	3.9735
6	6.855	VB	0.1791	117.36693	8.39754	2.9879
7	10.912	BB	1.2301	612.96808	6.22900	15.6047
8	16.607	BV	0.1749	25.39514	2.22851	0.6465
9	16.781	VB	0.1075	7.49506	1.02231	0.1908
10	17.383	BB	0.2480	136.69530	9.05906	3.4799
11	18.577	BB	0.2451	84.58356	5.00646	2.1533
12	19.230	BB	0.2410	320.09851	20.63415	8.1489
13	20.344	BB	0.3033	45.86306	2.33340	1.1676
14	21.197	BV	0.1774	18.59925	1.60218	0.4735
15	21.526	VB	0.2391	47.86844	3.08395	1.2186
16	22.337	BB	0.2984	31.20213	1.57984	0.7943
17	24.216	BV	0.1475	89.04672	9.15541	2.2669
18	24.429	VB	0.1240	20.96292	2.60267	0.5337
19	25.044	BB	0.1914	119.91825	9.75508	3.0528
20	26.102	BB	0.2204	229.58727	15.35868	5.8447
21	28.368	BB	0.3136	129.35599	6.57178	3.2931
22	29.834	BB	0.1784	11.45554	1.04110	0.2916
23	30.259	BB	0.2575	19.62528	1.28976	0.4996
24	30.768	BB	0.2845	23.08569	1.25562	0.5877
25	31.339	BB	0.2165	23.21281	1.72998	0.5909
26	31.822	BB	0.2291	32.90325	2.21720	0.8376
27	33.391	BV	0.3492	55.26539	2.01999	1.4069
28	33.913	VB	0.2418	77.87421	4.59317	1.9825
29	35.025	BV	0.1919	340.47961	26.85622	8.6678
30	35.324	VB	0.2055	93.82271	7.03843	2.3885
31	37.075	BV	0.1734	59.09163	5.01823	1.5043
32	37.319	VB	0.1695	25.24036	2.23887	0.6426
33	38.313	BB	0.1502	72.61333	7.41638	1.8486
Totals :				3928.10101	267.77806	

mple Name: D9T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.160	BB	0.0424	87.88046	33.77106	1.7098
2	1.262	BB	0.1465	56.56433	5.21840	1.1005
3	5.307	BB	0.3079	438.20676	17.63212	8.5259
4	6.729	BV	0.1160	39.26698	5.09141	0.7640
5	6.845	VB	0.0874	23.65302	3.86652	0.4602
6	18.582	BB	0.2016	767.85938	59.07787	14.9398
7	23.012	BV	0.1465	25.37681	2.63277	0.4937
8	23.362	VB	0.2452	65.06551	3.97029	1.2659
9	23.863	BV E	0.1798	62.80952	5.24181	1.2220
10	24.215	VB R	0.1602	1279.77161	122.15627	24.8998
11	25.845	BV	0.1678	66.26023	5.95605	1.2892
12	26.110	VB	0.2566	113.78013	6.18702	2.2138
13	27.237	BB	0.3101	51.41449	2.49849	1.0003
14	28.470	BB	0.5723	1621.28772	41.09035	31.5445
15	31.826	BB	0.2414	310.99753	20.00445	6.0509
16	33.461	BB	0.2244	29.22637	2.07394	0.5686
17	36.580	BB	0.1644	16.07418	1.50786	0.3127
18	37.082	BV	0.1373	12.20281	1.30255	0.2374
19	37.321	VB	0.1952	35.74164	2.75705	0.6954
20	38.317	BB	0.2338	24.80726	1.43384	0.4827
21	40.280	BB	0.1513	11.43885	1.15767	0.2226
Totals :				5139.68556	344.62779	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.159	BB	0.0410	78.57504	29.55817	1.9679
2	1.269	BB	0.1485	67.12920	6.19260	1.6812
3	5.331	BV	0.1016	25.00080	3.40921	0.6261
4	5.399	VV	0.0700	17.12764	3.57040	0.4290
5	5.500	VB	0.1266	26.97408	2.84663	0.6756
6	6.728	BV	0.1204	22.02570	2.72119	0.5516
7	6.842	VB	0.0863	10.13525	1.63480	0.2538
8	21.939	BB	0.1512	11.44540	1.15887	0.2866
9	23.012	BV	0.1463	22.41990	2.32823	0.5615
10	23.361	VB	0.2509	56.42039	3.41231	1.4130
11	23.866	BV E	0.1792	49.83234	4.06056	1.2480
12	24.215	VB R	0.1572	1158.24829	111.50757	29.0077
13	25.846	BV	0.1663	61.84625	5.53839	1.5489
14	26.106	VB	0.2367	149.00066	9.02006	3.7316
15	27.276	BB	0.2722	42.88616	2.47464	1.0741
16	28.470	BB	0.5723	1909.06958	47.96528	47.8117
17	31.823	BB	0.2383	227.84691	14.74406	5.7063
18	33.466	BB	0.2518	45.57533	2.77159	1.1414
19	36.582	BB	0.1579	11.33671	1.08541	0.2839

mple Name: D9T50

Totals : 3992.89563 255.99995

Signal 5: DAD1 E, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.159	BB	0.0419	65.90710	25.71043	27.8658
2	5.401	BV	0.1375	21.89582	2.06796	9.2576
3	5.503	VB	0.1228	15.37186	1.67859	6.4993
4	24.215	BB	0.1565	45.50212	4.40478	19.2385
5	26.102	BB	0.2181	50.04077	3.39247	21.1574
6	31.339	BB	0.2466	18.74784	1.13591	7.9267
7	33.398	BB	0.1973	19.05060	1.34485	8.0547

Totals : 236.51611 39.73499

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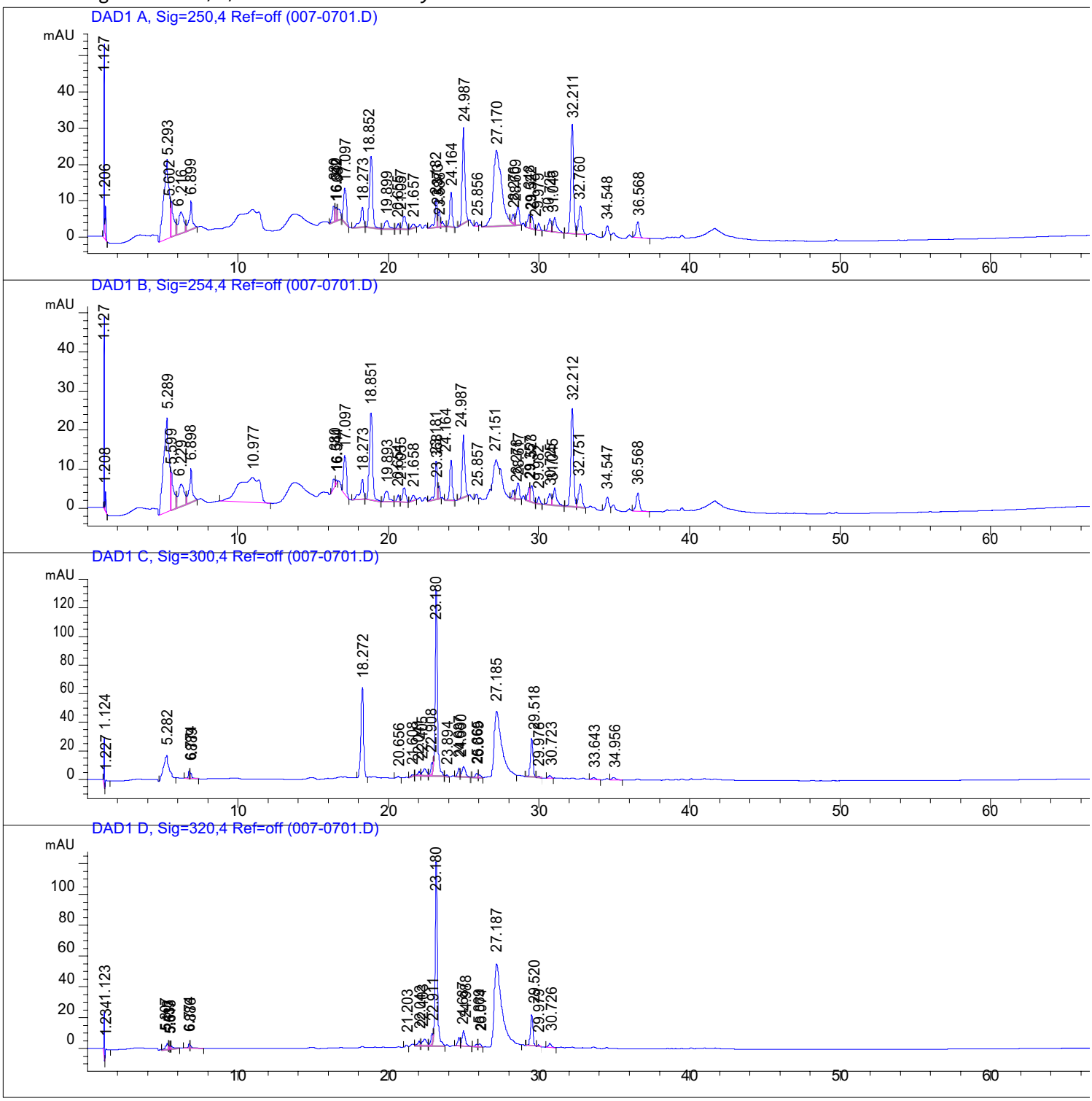
*** End of Report ***

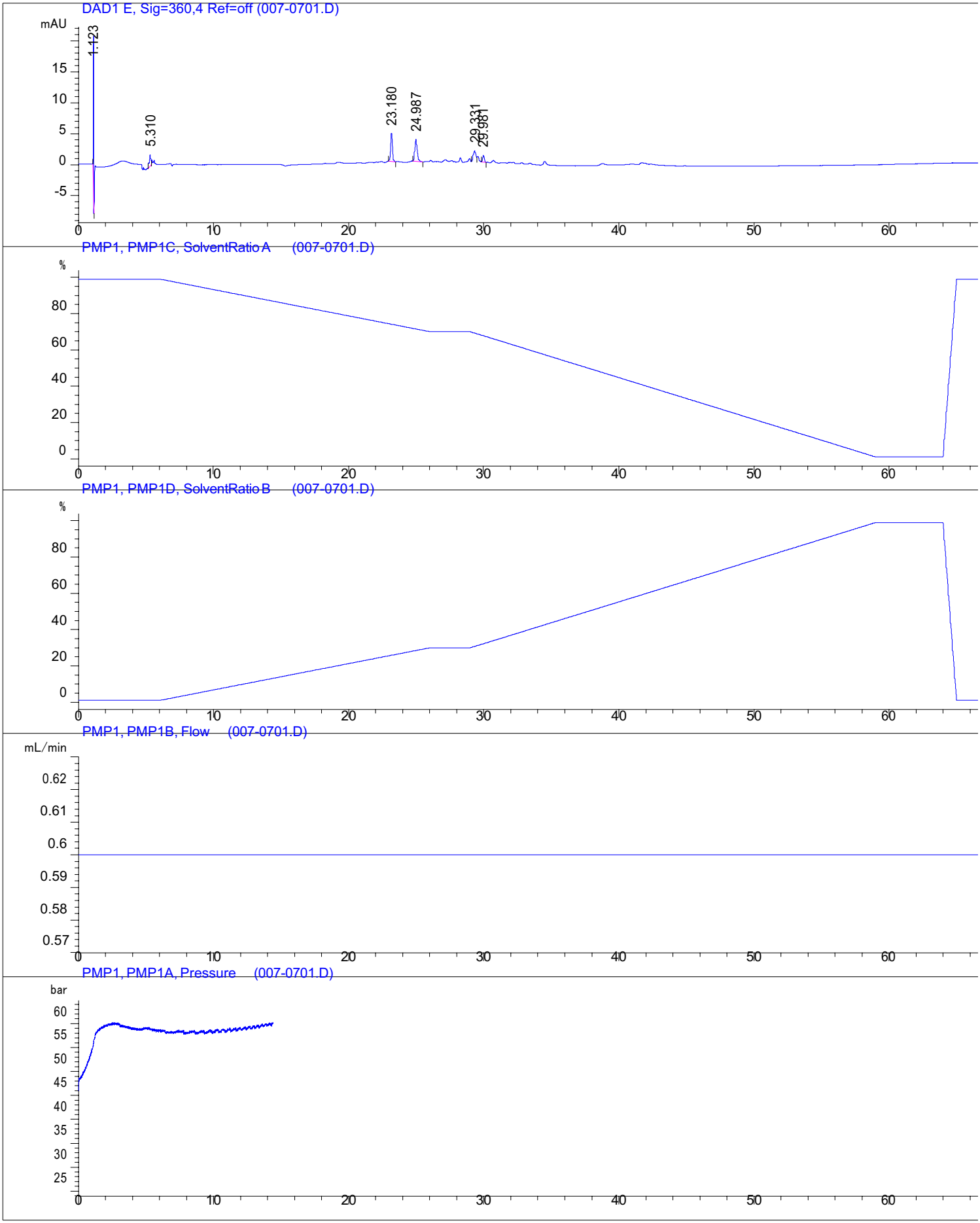
mple Name: D10T50

==========Method 12==========

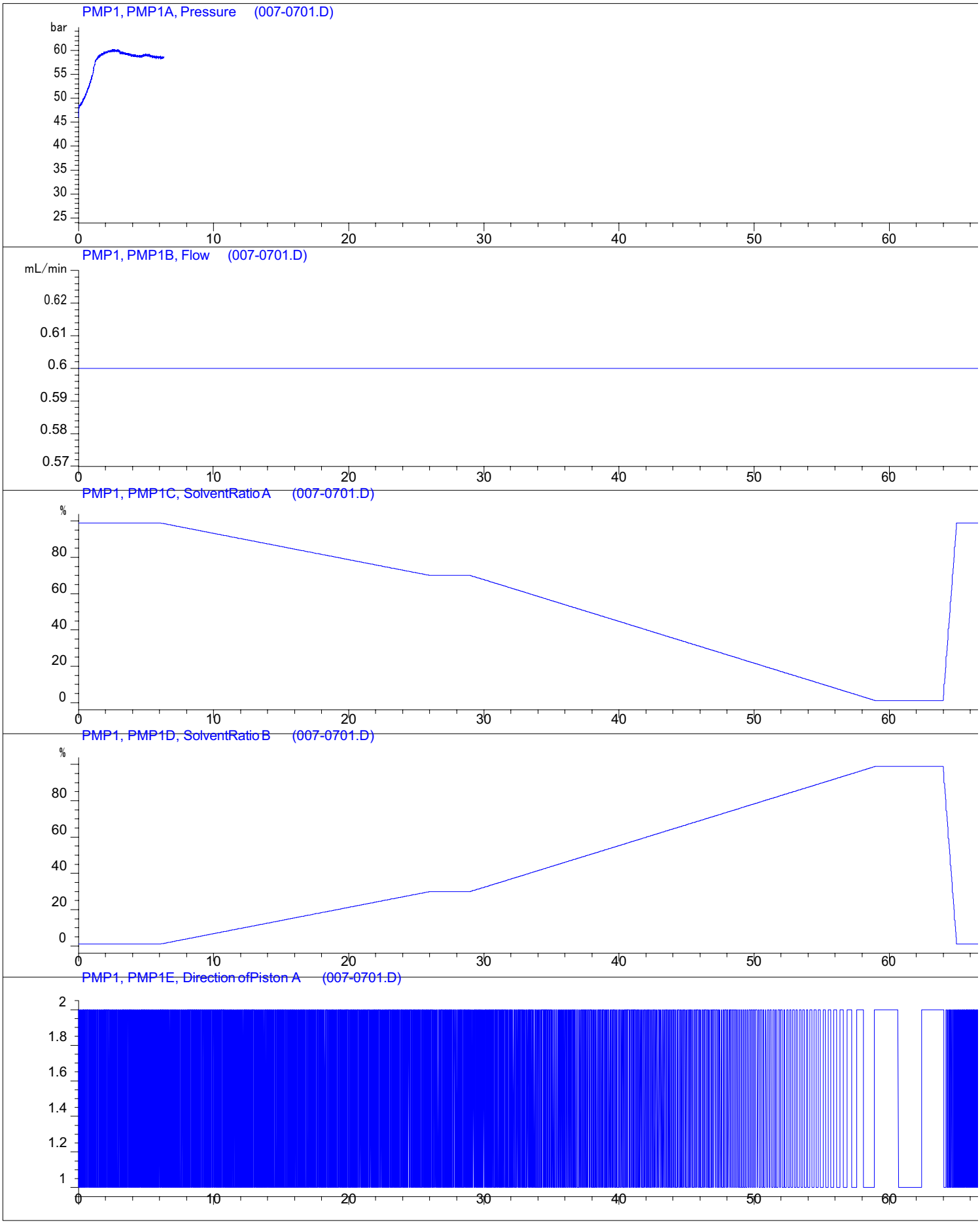
Acq. Operator : SYSTEM Seq. Line : 7
Acq. Instrument : HPLC 4 Location : 7
Injection Date : 5/9/2017 2:25:58 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Sequence - method 5-6-7-8-9-10-11.S
Method : C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25
\Optimizing method 10.M (Sequence Method)
Last changed : 5/8/2017 8:22:26 PM by SYSTEM



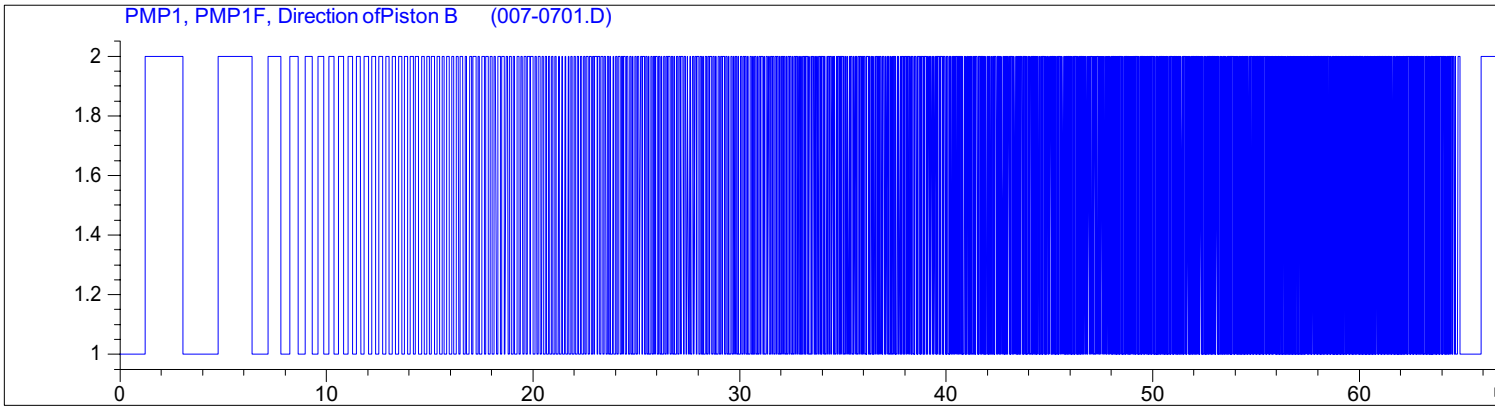
mple Name: D10T50



mple Name: D10T50



mple Name: D10T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.127	BV R	0.0428	141.72890	53.64550	3.3907
2	1.206	VB E	0.0619	36.27404	9.22410	0.8678
3	5.293	BV	0.3261	564.19824	21.93709	13.4976
4	5.602	VV	0.1590	123.41212	9.79448	2.9525
5	6.216	VV	0.3770	145.50993	5.91489	3.4811
6	6.899	VB	0.1659	103.70004	7.96301	2.4809
7	16.380	BV	0.1900	53.18587	4.30910	1.2724
8	16.542	VV	0.1142	30.19920	3.73894	0.7225
9	16.682	VB	0.1642	38.46948	3.11258	0.9203
10	17.097	BB	0.2242	127.47929	9.40029	3.0498
11	18.273	BB	0.2246	85.20817	5.50431	2.0385
12	18.852	BB	0.2291	283.95032	19.59536	6.7931
13	19.899	BB	0.2987	42.94062	2.26974	1.0273
14	20.655	BV	0.1540	14.28699	1.43649	0.3418
15	21.057	VB	0.2259	53.19987	3.56836	1.2727
16	21.657	BB	0.2357	17.33236	1.12557	0.4147
17	23.182	BV	0.1585	85.86010	7.91892	2.0541
18	23.373	VV	0.1312	39.47544	4.55220	0.9444
19	23.588	VB	0.1431	14.40826	1.48600	0.3447
20	24.164	BB	0.1732	105.54714	9.53296	2.5251
21	24.987	BB	0.1828	320.12726	26.52035	7.6586
22	25.856	BV	0.1566	10.68401	1.06887	0.2556
23	27.170	BV R	0.5193	819.33844	20.90305	19.6015
24	28.276	VV E	0.1610	26.89855	2.55032	0.6435
25	28.609	VB	0.2317	75.40361	5.12207	1.8039
26	29.348	BV	0.1844	44.79596	3.67051	1.0717
27	29.522	VB	0.1560	35.00357	3.34799	0.8374
28	29.979	BB	0.1628	20.14989	1.88382	0.4821
29	30.725	BV	0.2081	48.83274	3.51420	1.1683

mple Name: D10T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	31.046	VB	0.2309	65.68456	4.01482	1.5714
31	32.211	BV	0.1963	383.49954	30.17596	9.1747
32	32.760	VB	0.2366	119.93665	7.83301	2.8693
33	34.548	BB	0.2085	36.76833	2.77566	0.8796
34	36.568	BB	0.2345	66.49094	4.39417	1.5907
Totals :				4179.98043	303.80467	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.127	BV R	0.0414	124.25043	49.37661	3.2353
2	1.208	VB E	0.0583	18.71298	5.16007	0.4873
3	5.289	BV	0.3308	632.87921	24.22289	16.4794
4	5.599	VV	0.1557	119.78578	9.72404	3.1191
5	6.229	VV	0.3969	148.67279	5.68873	3.8713
6	6.898	VB	0.1712	113.98140	8.56939	2.9679
7	10.977	BB	1.2014	624.11023	6.44728	16.2511
8	16.380	BV	0.1779	26.10981	2.34451	0.6799
9	16.544	VB	0.0965	6.57287	1.00251	0.1711
10	17.097	BB	0.2249	133.68016	9.81365	3.4809
11	18.273	BB	0.2340	84.28489	5.17414	2.1947
12	18.851	BB	0.2305	325.70114	22.28039	8.4809
13	19.893	BB	0.2931	50.41370	2.68399	1.3127
14	20.654	BV	0.1552	17.13703	1.70564	0.4462
15	21.055	VB	0.2226	52.85370	3.65767	1.3762
16	21.658	BB	0.2262	19.45472	1.33391	0.5066
17	23.181	BV	0.1497	98.40923	9.75541	2.5625
18	23.368	VB	0.1139	23.91957	3.24786	0.6228
19	24.164	BB	0.1754	115.68031	10.27484	3.0122
20	24.987	BB	0.1827	193.69838	16.05626	5.0437
21	25.857	BB	0.1505	11.46723	1.23250	0.2986
22	27.151	BB	0.2641	85.37376	4.65222	2.2230
23	28.278	BV	0.1527	20.87255	2.12397	0.5435
24	28.617	VB	0.2224	55.07006	3.95550	1.4340
25	29.357	BV	0.1642	37.97849	3.51117	0.9889
26	29.523	VB	0.1722	48.48691	4.21420	1.2625
27	29.982	BB	0.1576	17.90404	1.71782	0.4662
28	30.725	BV	0.2088	40.99303	2.93838	1.0674
29	31.045	VB	0.2197	67.65002	4.39117	1.7615
30	32.212	BV	0.1971	321.18088	25.13891	8.3632
31	32.751	VB	0.2486	94.63867	5.91761	2.4643
32	34.547	BB	0.2047	38.62906	2.95096	1.0059
33	36.568	BB	0.2338	69.87305	4.58528	1.8194
Totals :				3840.42608	265.84947	

mple Name: D10T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.124	BB	0.0437	91.97941	33.80552	1.7697
2	1.227	BB	0.1317	48.68882	4.99476	0.9368
3	5.282	BB	0.3208	437.50745	17.31336	8.4177
4	6.774	BV	0.1115	40.06002	5.34191	0.7708
5	6.889	VB	0.0851	24.03585	3.94266	0.4625
6	18.272	BB	0.1865	769.75989	63.02219	14.8103
7	20.656	BB	0.1674	11.93848	1.09367	0.2297
8	21.608	BB	0.2070	14.21146	1.11249	0.2734
9	22.041	BV E	0.1454	29.67789	3.00003	0.5710
10	22.405	VV E	0.2454	87.15195	4.85462	1.6768
11	22.908	VV E	0.1492	86.15742	8.57622	1.6577
12	23.180	VB R	0.1547	1351.07397	130.58873	25.9949
13	23.894	BB	0.1452	10.22148	1.09215	0.1967
14	24.687	BV	0.1557	62.76538	6.11789	1.2076
15	24.990	VB	0.2430	112.45721	7.33120	2.1637
16	25.865	BV	0.1762	32.16042	2.71481	0.6188
17	26.069	VB	0.1485	18.67709	1.90255	0.3594
18	27.185	BB	0.5018	1574.36865	45.70868	30.2911
19	29.518	BV R	0.1767	316.15192	27.38770	6.0828
20	29.978	VB E	0.1415	10.68163	1.15978	0.2055
21	30.723	BB	0.1708	21.53339	1.94988	0.4143
22	33.643	BB	0.2308	19.37685	1.25023	0.3728
23	34.956	BB	0.2583	26.82209	1.51705	0.5161
Totals :				5197.45874	375.77805	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.123	BB	0.0438	80.15947	29.36463	1.9567
2	1.234	BB	0.1518	71.96731	6.36816	1.7567
3	5.307	BV	0.1521	42.77594	3.95642	1.0442
4	5.433	VV	0.0703	11.64066	2.33039	0.2841
5	5.537	VV	0.0620	7.44296	1.73513	0.1817
6	5.613	VB	0.1304	19.02518	1.90759	0.4644
7	6.774	BV	0.1145	21.94034	2.82963	0.5356
8	6.886	VB	0.0854	10.02224	1.63723	0.2446
9	21.203	BB	0.1357	9.67479	1.13203	0.2362
10	22.042	BV E	0.1488	26.16424	2.61361	0.6387
11	22.403	VV E	0.2482	72.43832	4.02145	1.7682
12	22.911	VV E	0.1482	67.61463	6.78798	1.6505
13	23.180	VB R	0.1538	1224.83789	119.23114	29.8981
14	24.687	BV	0.1580	58.86370	5.62898	1.4369
15	24.988	VB	0.2121	148.45135	10.18140	3.6237
16	25.869	BV	0.1609	17.12531	1.59947	0.4180
17	26.074	VB	0.1651	23.10754	2.08802	0.5641
18	27.187	BB	0.5133	1904.31763	53.49008	46.4841

mple Name: D10T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
19	29.520	BV R	0.1778	236.19778	20.29026	5.7656
20	29.979	VB E	0.1365	10.95416	1.24693	0.2674
21	30.726	BB	0.1821	31.98395	2.62560	0.7807
Totals :				4096.70541	281.06613	

Signal 5: DAD1 E, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.123	BB	0.0417	64.48689	25.32514	34.1174
2	5.310	BV	0.1164	13.91007	1.79567	7.3593
3	23.180	BB	0.1416	42.00897	4.55342	22.2252
4	24.987	BB	0.1850	43.91623	3.58325	23.2343
5	29.331	BB	0.1935	15.48007	1.33317	8.1899
6	29.981	BB	0.1314	9.21249	1.10351	4.8740
Totals :				189.01472	37.69417	

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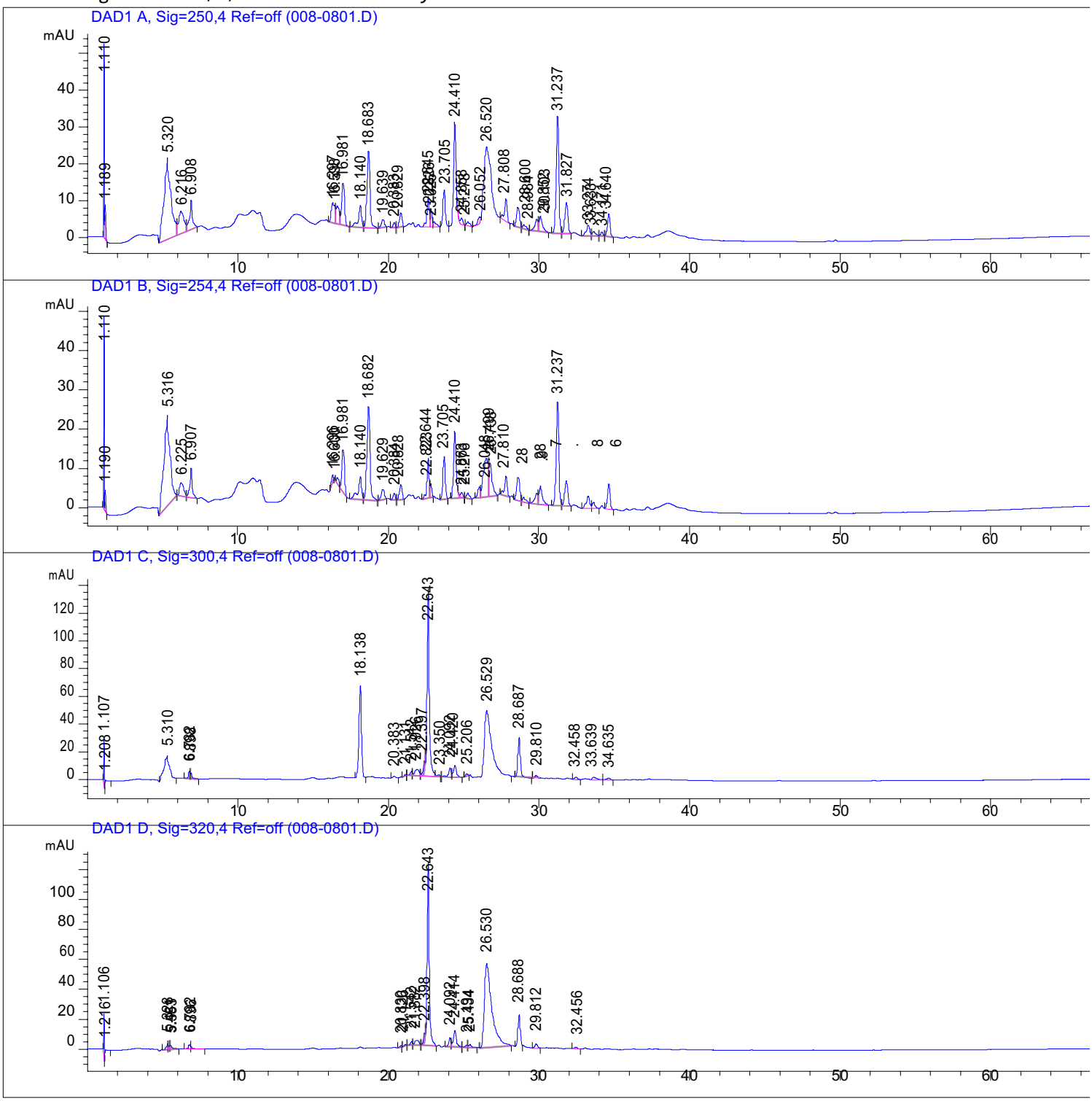
*** End of Report ***

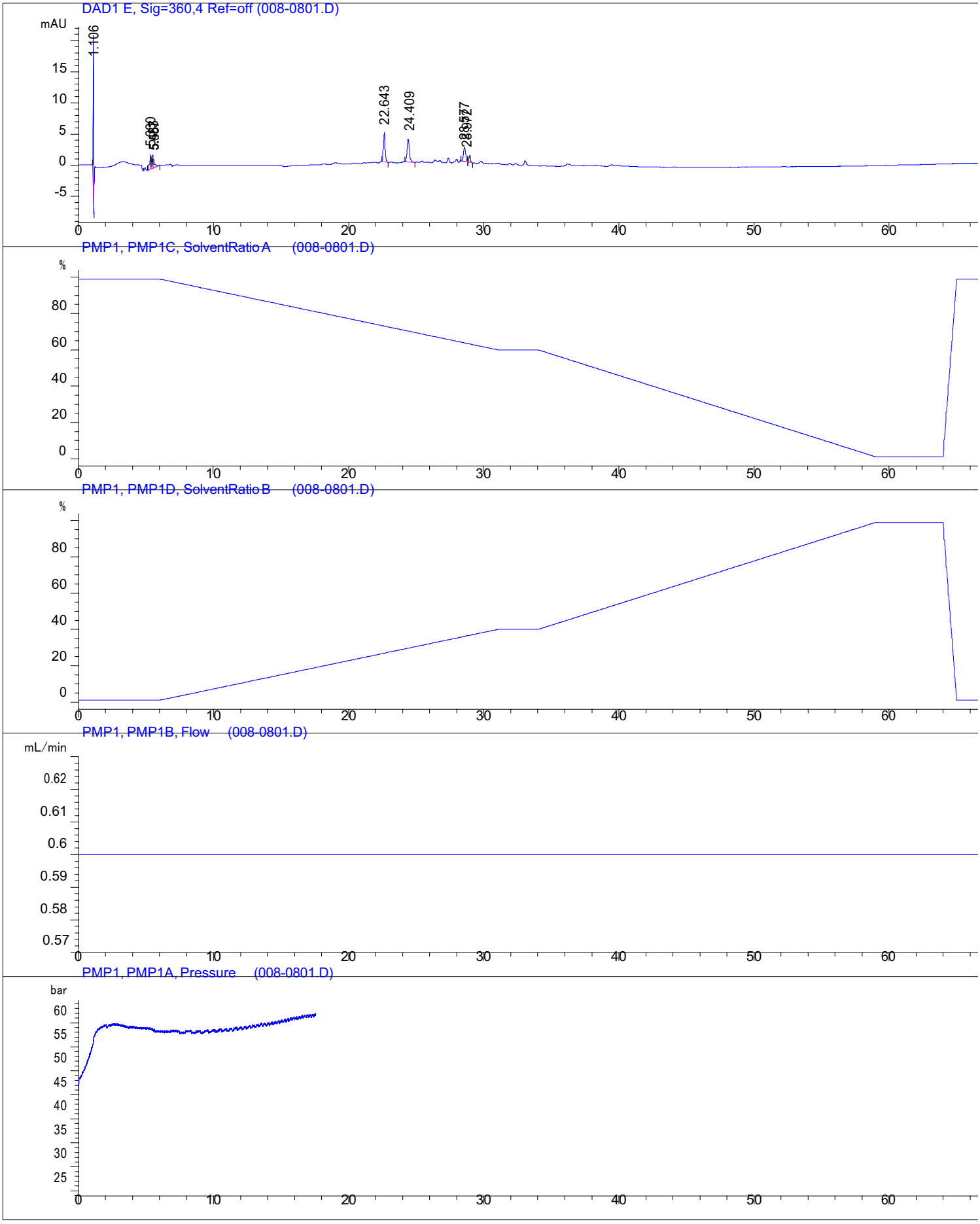
mple Name: D11T50

==========Method 13==========

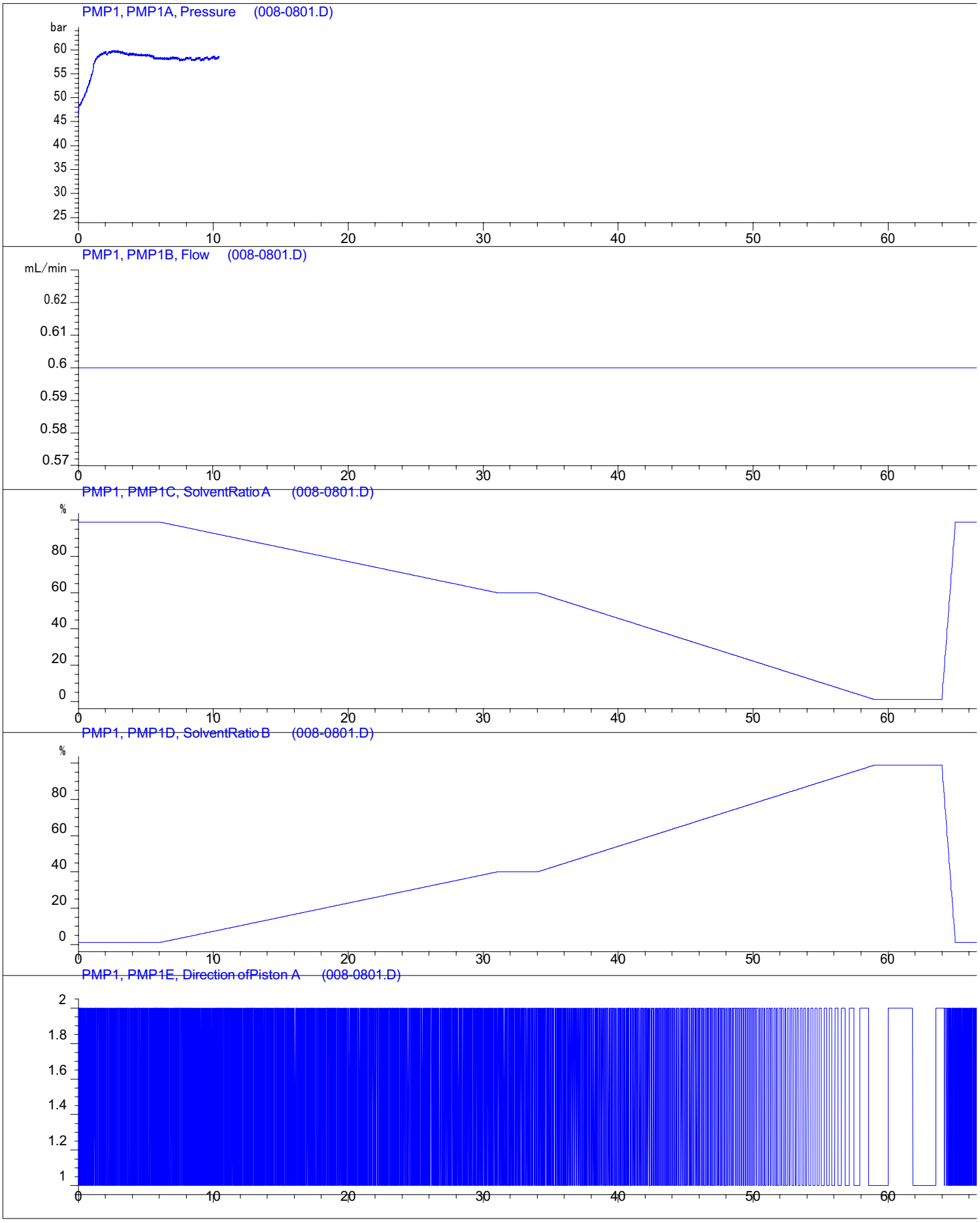
Acq. Operator	: SYSTEM	Seq. Line	: 8
Acq. Instrument	: HPLC 4	Location	: 8
Injection Date	: 5/9/2017 3:35:07 AM	Inj	: 1
		Inj Volume	: 10.000 µl
Sequence File	: C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25 \Sequence - method 5-6-7-8-9-10-11.S		
Method	: C:\Chem32\1\Data\Sequence - method 5-6-7-8-9-10-11 2017-05-08 20-22-25 \Optimizing method 11.M (Sequence Method)		
Last changed	: 5/8/2017 8:22:26 PM by SYSTEM		



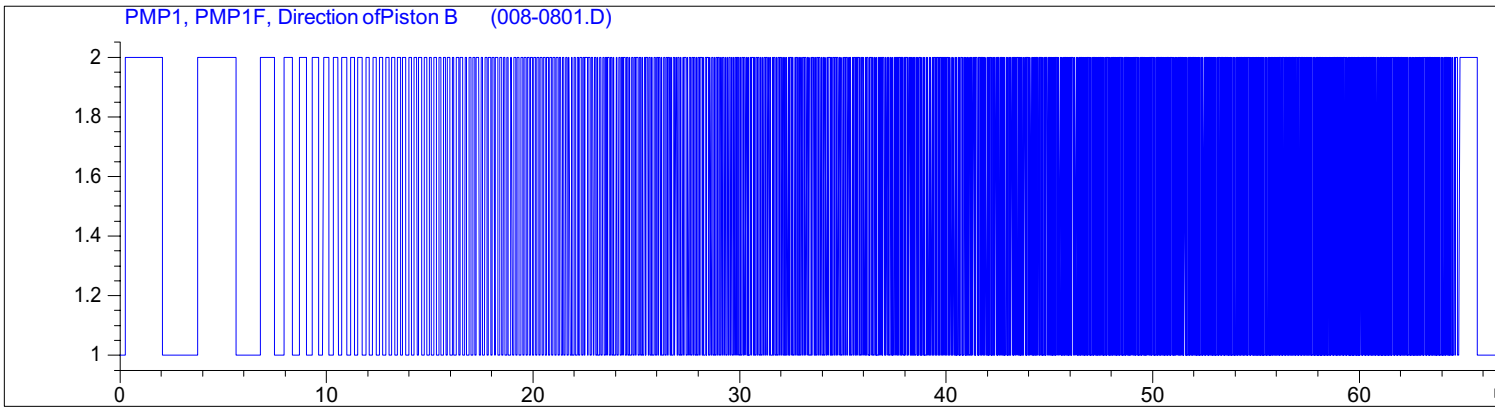
mple Name: D11T50



mple Name: D11T50



mple Name: D11T50



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.110	BV R	0.0424	138.80104	53.33618	3.2868
2	1.189	VB E	0.0642	37.04944	9.35069	0.8773
3	5.320	BV	0.3844	702.94830	22.26144	16.6459
4	6.216	VV	0.3767	150.57487	6.16995	3.5656
5	6.908	VB	0.1714	105.44033	7.91519	2.4968
6	16.297	BV	0.2431	97.64965	5.55481	2.3124
7	16.598	VV	0.1930	69.67026	4.86621	1.6498
8	16.981	VB	0.2184	155.38231	11.44217	3.6795
9	18.140	BV	0.2203	92.51630	6.05427	2.1908
10	18.683	VB	0.2189	294.00647	20.79646	6.9621
11	19.639	BB	0.2401	30.85728	2.09200	0.7307
12	20.383	BB	0.1535	14.04905	1.44470	0.3327
13	20.829	BB	0.1860	47.47735	3.95707	1.1243
14	22.645	BV	0.1514	85.38511	8.20229	2.0219
15	22.826	VV	0.1279	42.23007	4.92989	1.0000
16	23.025	VB	0.1882	19.39629	1.37837	0.4593
17	23.705	BB	0.1678	109.43391	9.99211	2.5914
18	24.410	BV R	0.1867	348.58572	28.09756	8.2546
19	24.858	VB E	0.1802	21.92250	1.79798	0.5191
20	25.278	BB	0.1992	15.69424	1.10854	0.3716
21	26.052	BV E	0.1987	22.03417	1.72906	0.5218
22	26.520	VB R	0.4553	685.89960	20.45130	16.2422
23	27.808	VB R	0.1849	73.16704	6.06023	1.7326
24	28.600	BV	0.2442	79.54209	5.38829	1.8836
25	28.984	VB	0.2118	18.09671	1.14868	0.4285
26	29.852	BV	0.2238	49.68140	3.22382	1.1765
27	30.103	VB	0.2176	66.16742	4.29837	1.5669
28	31.237	BV	0.1861	388.18451	31.86996	9.1923
29	31.827	VB	0.2185	118.48683	8.40176	2.8058

mple Name: D11T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
30	33.274	BV	0.2292	45.11332	2.84253	1.0683
31	33.633	VB	0.2116	16.89289	1.14835	0.4000
32	34.171	BB	0.1385	9.00109	1.02426	0.2131
33	34.640	BB	0.1775	71.61140	6.16480	1.6958
Totals :				4222.94896	304.49925	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.110	BV R	0.0416	125.53039	49.45898	3.7768
2	1.190	VB E	0.0583	19.56697	5.39718	0.5887
3	5.316	BB	0.3473	654.76563	23.19606	19.6996
4	6.225	BV	0.3128	64.69962	3.35733	1.9466
5	6.907	VB	0.1382	78.81882	7.52514	2.3714
6	16.296	BB	0.1404	17.31414	1.97208	0.5209
7	16.600	BB	0.1656	15.51638	1.31530	0.4668
8	16.981	BB	0.2051	131.68672	10.58891	3.9620
9	18.140	BV	0.2302	92.94289	5.76282	2.7963
10	18.682	VB	0.2199	337.32281	23.72384	10.1488
11	19.629	BB	0.2380	37.03988	2.51283	1.1144
12	20.384	BB	0.1516	16.38432	1.68334	0.4929
13	20.828	BB	0.1906	46.29041	3.78643	1.3927
14	22.644	BV	0.1454	97.78044	10.06405	2.9419
15	22.823	VB	0.1092	24.52333	3.43896	0.7378
16	23.705	BB	0.1674	117.74279	10.78232	3.5425
17	24.410	BV R	0.1847	207.58514	16.97159	6.2455
18	24.863	VB E	0.1639	14.13707	1.31020	0.4253
19	25.276	BB	0.1869	17.43603	1.34783	0.5246
20	26.048	BV	0.1983	32.07368	2.62966	0.9650
21	26.499	VV	0.3187	216.03310	9.66745	6.4997
22	26.738	VB	0.2140	125.50874	8.41494	3.7761
23	27.810	VB R	0.1864	60.94265	4.99151	1.8335
24	28.619	BV	0.2420	86.50072	5.80017	2.6025
25	28.978	VB	0.1897	15.88500	1.16074	0.4779
26	29.855	BV	0.2218	40.60415	2.66371	1.2216
27	30.105	VB	0.2099	69.48877	4.71689	2.0907
28	31.237	BV	0.1867	323.45749	26.43871	9.7317
29	31.819	VB	0.2270	91.83916	6.34183	2.7631
30	33.273	BV	0.2357	50.10039	3.04857	1.5073
31	33.628	VB	0.1961	20.81388	1.57535	0.6262
32	34.640	BB	0.1766	73.42367	6.36398	2.2091
Totals :				3323.75517	268.00871	

mple Name: D11T50

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.107	BB	0.0426	87.19864	33.29745	1.6339
2	1.208	BB	0.1526	61.90928	5.36657	1.1600
3	5.310	BB	0.3119	440.99966	17.38648	8.2633
4	6.782	BV	0.1101	39.69087	5.37645	0.7437
5	6.898	VB	0.0871	24.60223	3.92528	0.4610
6	18.138	BB	0.1805	774.27942	66.18314	14.5082
7	20.383	BB	0.1679	12.58643	1.14807	0.2358
8	21.131	BB	0.1463	11.74835	1.26538	0.2201
9	21.542	BV E	0.1471	32.26026	3.21457	0.6045
10	21.926	VV E	0.3192	91.52882	4.50123	1.7150
11	22.397	VV E	0.1350	86.67535	9.45106	1.6241
12	22.643	VV R	0.1517	1385.50720	135.08028	25.9612
13	23.350	VB E	0.1519	14.43384	1.45339	0.2705
14	24.092	VV R	0.1768	82.61123	6.74775	1.5479
15	24.420	VB	0.2068	114.86249	8.23369	2.1523
16	25.206	BB	0.1538	14.51874	1.51496	0.2720
17	26.529	BB	0.4915	1639.83350	47.89371	30.7266
18	28.687	BB	0.1768	331.38895	28.67580	6.2095
19	29.810	BB	0.1635	22.29958	2.10657	0.4178
20	32.458	BB	0.1925	17.54379	1.41666	0.3287
21	33.639	BB	0.2469	31.02684	1.74969	0.5814
22	34.635	BB	0.2136	19.34223	1.34632	0.3624

Totals :				5336.84770	387.33452	

Signal 4: DAD1 D, Sig=320,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.106	BB	0.0426	75.57449	28.77826	1.8527
2	1.216	BB	0.1590	75.59042	6.43693	1.8531
3	5.328	BV	0.1163	32.46049	3.85178	0.7958
4	5.481	VV	0.0846	14.80246	2.51802	0.3629
5	5.553	VB	0.1239	22.89787	2.43065	0.5613
6	6.782	BV	0.1131	21.72812	2.84362	0.5327
7	6.896	VB	0.0872	10.28644	1.63980	0.2522
8	20.830	BB	0.1251	9.18818	1.15089	0.2252
9	21.126	BB	0.1424	10.97407	1.20338	0.2690
10	21.542	BV	0.1395	24.59018	2.61989	0.6028
11	21.882	VB	0.2687	60.98244	3.19446	1.4950
12	22.398	BV E	0.1219	57.59555	7.00484	1.4119
13	22.643	VB R	0.1483	1224.19702	122.82600	30.0105
14	24.092	BV	0.1527	59.04050	5.80147	1.4473
15	24.414	VB	0.1981	143.91359	10.75476	3.5280
16	25.194	BV	0.1651	17.26646	1.53609	0.4233
17	25.434	VB	0.1629	21.86184	1.97930	0.5359
18	26.530	BB	0.4951	1929.72119	55.85699	47.3061
19	28.688	BB	0.1670	221.96071	20.71105	5.4412

mple Name: D11T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
20	29.812	BB	0.1696	30.51707	2.74808	0.7481
21	32.456	BB	0.1950	14.07490	1.08732	0.3450
Totals :				4079.22399	286.97360	

Signal 5: DAD1 E, Sig=360,4 Ref=off

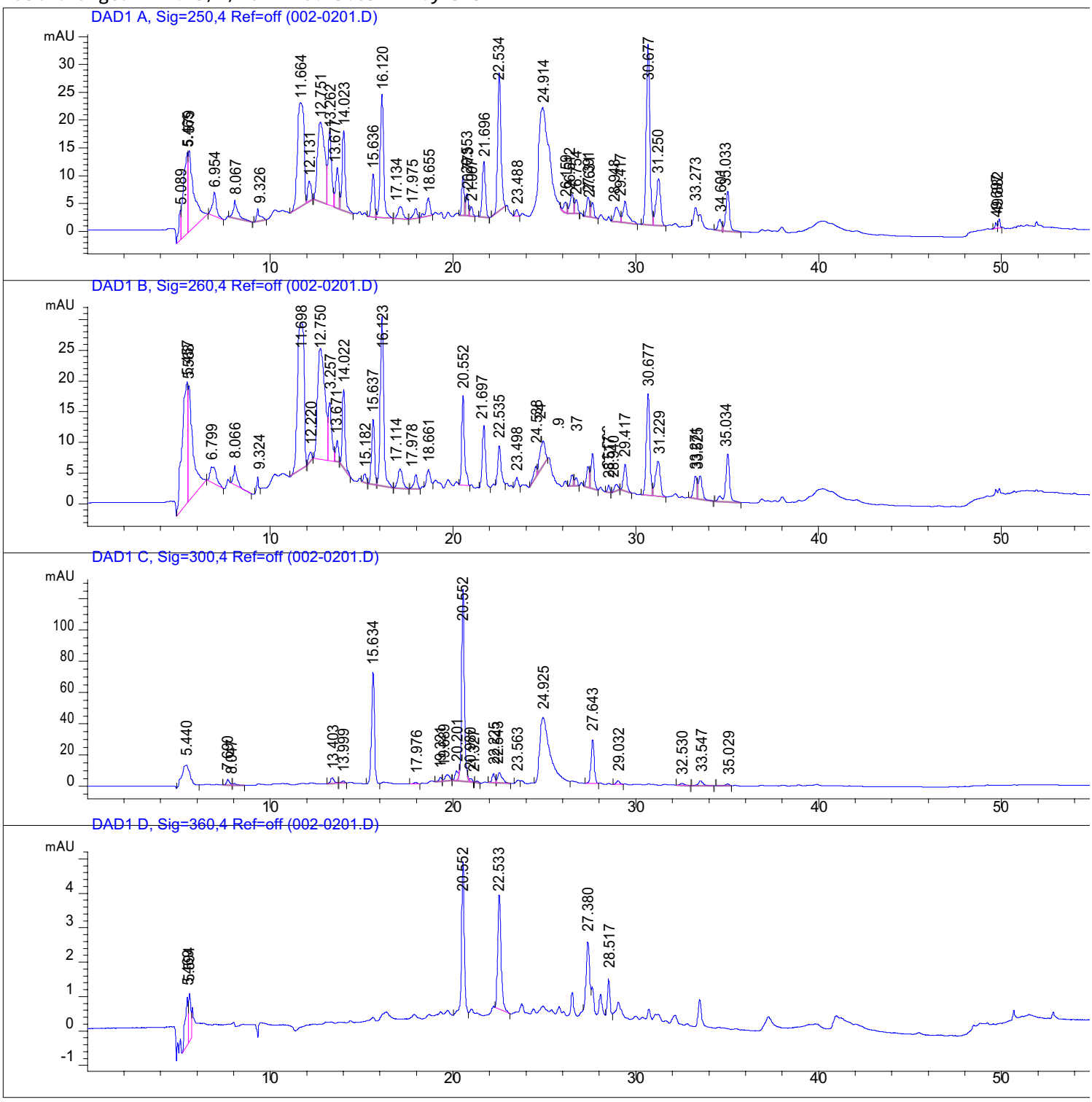
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.106	BB	0.0420	65.04593	25.31583	28.2185
2	5.330	BV	0.1069	18.59410	2.38746	8.0666
3	5.483	VV	0.0833	8.56800	1.48536	3.7170
4	5.557	VB	0.1236	13.85600	1.47458	6.0111
5	22.643	BB	0.1365	41.99018	4.68960	18.2164
6	24.409	BB	0.1806	44.16170	3.71600	19.1584
7	28.577	BV	0.1830	28.62311	2.36814	12.4174
8	28.972	VB	0.1275	9.66901	1.15689	4.1947
Totals :				230.50804	42.59385	

*** End of Report ***

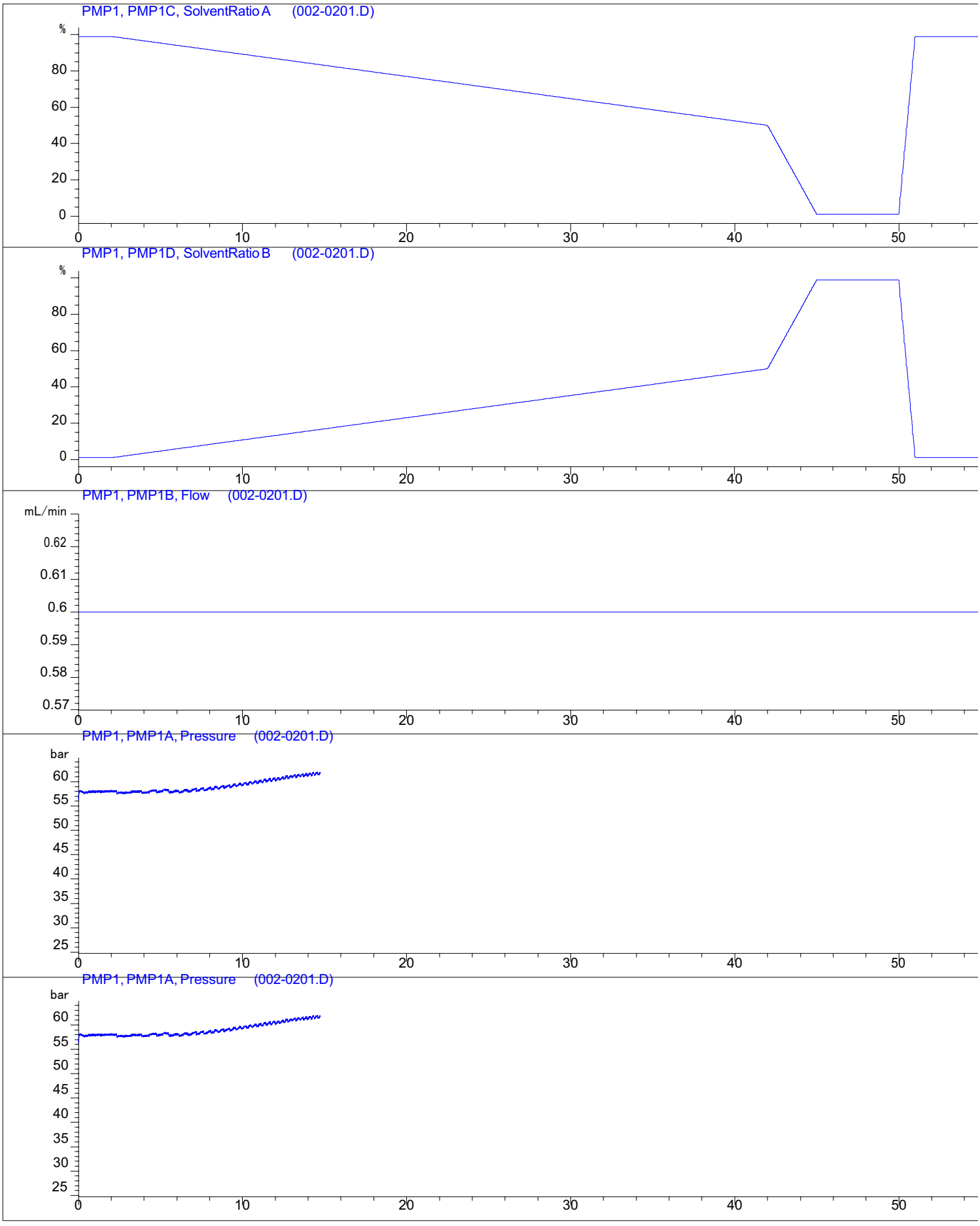
mple Name: D12T50

==========Method 14==========

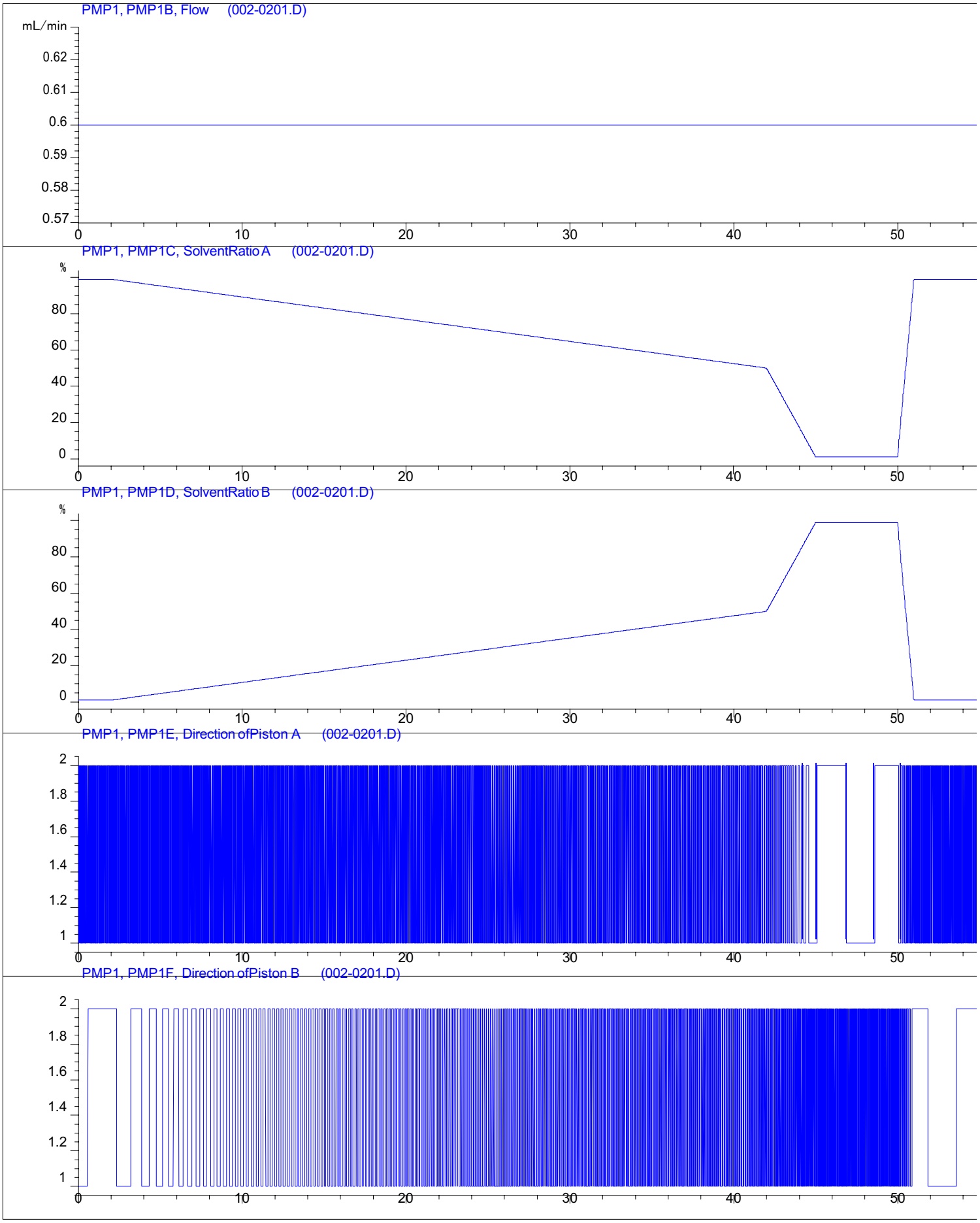
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/9/2017 10:37:08 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 12-13 2017-05-09 10-25-05\Sequence - method 12-13.S
Method : C:\Chem32\1\Data\Sequence - method 12-13 2017-05-09 10-25-05\Optimizing method 12.M (Sequence Method)
Last changed : 5/9/2017 10:25:05 AM by SYSTEM



mple Name: D12T50



mple Name: D12T50



mple Name: D12T50

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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.089	BV	0.1178	41.65091	5.41104	0.8331
2	5.465	VV	0.2112	246.53911	14.58498	4.9312
3	5.579	VB	0.2717	313.23572	14.51711	6.2652
4	6.954	BB	0.2220	65.96947	4.27695	1.3195
5	8.067	BB	0.2105	53.72276	3.22166	1.0745
6	9.326	BB	0.1579	28.68771	2.35816	0.5738
7	11.664	BV R	0.3611	461.11093	18.73902	9.2229
8	12.131	VB E	0.2032	45.52089	3.70963	0.9105
9	12.751	BV	0.4168	393.75961	14.32003	7.8758
10	13.262	VV	0.1991	182.38943	13.37832	3.6481
11	13.677	VV	0.1895	90.54594	7.25945	1.8111
12	14.023	VB	0.1779	165.99460	14.25030	3.3201
13	15.636	BV	0.1794	90.78725	7.71110	1.8159
14	16.120	VB	0.1989	287.45563	22.22321	5.7495
15	17.134	BB	0.2916	41.08925	2.18306	0.8218
16	17.975	BB	0.1950	24.04731	1.80950	0.4810
17	18.655	BB	0.2235	48.07318	3.27068	0.9615
18	20.553	BV	0.1436	67.17692	7.15167	1.3436
19	20.773	VV	0.1423	37.89798	4.00753	0.7580
20	21.007	VB	0.1500	16.25737	1.66433	0.3252
21	21.696	BB	0.1801	116.60595	9.99515	2.3323
22	22.534	BB	0.1908	312.28412	24.81320	6.2462
23	23.488	BB	0.1609	11.64894	1.16285	0.2330
24	24.914	BV R	0.6062	830.26367	19.44379	16.6065
25	26.159	VV E	0.1859	22.64935	1.86280	0.4530
26	26.512	VV E	0.2163	48.01577	3.40900	0.9604
27	26.754	VB E	0.1596	25.13066	2.41152	0.5027
28	27.391	BV	0.1892	43.76684	3.56502	0.8754
29	27.631	VB	0.1616	29.12401	2.70486	0.5825
30	28.948	BV	0.2846	48.10369	2.59123	0.9621
31	29.417	VB	0.2545	69.17107	3.83426	1.3835
32	30.677	BV	0.2060	434.49976	32.49297	8.6906
33	31.250	VB	0.2572	142.32271	8.42186	2.8467
34	33.273	BB	0.1733	23.08846	2.14991	0.4618
35	34.601	BV	0.1982	25.67694	1.94212	0.5136
36	35.033	VB	0.2093	98.15529	7.10138	1.9633
37	49.697	BV	0.0983	7.05244	1.07869	0.1411
38	49.882	VB	0.0946	10.15376	1.63250	0.2031
Totals :				4999.62538	296.66086	

mple Name: D12T50

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.457	BV	0.2646	428.77600	19.80117	10.3840
2	5.568	VB	0.2308	338.51520	18.73675	8.1981
3	6.799	BB	0.3264	64.21709	2.54479	1.5552
4	8.066	BB	0.1691	41.42132	3.15578	1.0031
5	9.324	BB	0.0897	14.64179	2.31791	0.3546
6	11.698	BB	0.4017	589.92023	23.92065	14.2865
7	12.220	BB	0.1705	17.65718	1.42604	0.4276
8	12.750	BV	0.4170	496.28479	18.03555	12.0189
9	13.257	VV	0.2019	129.37494	9.43785	3.1332
10	13.671	VB	0.1618	35.68653	3.47478	0.8642
11	14.022	BB	0.1628	130.71577	12.62033	3.1656
12	15.182	BB	0.1726	14.17465	1.34909	0.3433
13	15.637	BV	0.1734	119.01821	10.56598	2.8824
14	16.123	VB	0.1996	357.39462	27.50526	8.6553
15	17.114	BB	0.2800	56.87065	3.13011	1.3773
16	17.978	BB	0.1826	28.05576	2.26371	0.6794
17	18.661	BB	0.1993	32.71124	2.55696	0.7922
18	20.552	BB	0.1599	154.80495	14.58082	3.7490
19	21.697	BB	0.1828	118.81657	10.13455	2.8775
20	22.535	BB	0.1876	77.09959	6.26400	1.8672
21	23.498	BB	0.1674	15.92469	1.53041	0.3857
22	24.538	BV	0.1902	23.61061	1.76404	0.5718
23	24.937	VB	0.2904	79.82935	3.93948	1.9333
24	26.523	BV	0.1734	20.99044	1.83588	0.5083
25	26.743	VB	0.1513	12.82176	1.32078	0.3105
26	27.390	BV	0.1742	37.74248	3.33040	0.9140
27	27.637	VB	0.1798	68.19432	5.69064	1.6515
28	28.517	BB	0.1299	9.34811	1.13742	0.2264
29	28.940	BB	0.2151	14.88833	1.05194	0.3606
30	29.417	BB	0.1888	53.44000	4.36564	1.2942
31	30.677	BV	0.2048	219.61084	16.54683	5.3185
32	31.229	VB	0.2834	103.47438	5.65674	2.5059
33	33.271	BV	0.1991	48.21877	3.62672	1.1677
34	33.525	VB	0.2196	55.01018	3.78422	1.3322
35	35.034	VB R	0.2278	119.94324	7.78372	2.9048
Totals :				4129.20458	257.18693	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.440	BB	0.3835	435.41541	13.82338	8.5580
2	7.690	BV	0.1715	44.33926	3.76325	0.8715
3	8.047	VB	0.1588	13.59368	1.12638	0.2672
4	13.403	BB	0.1892	44.35696	3.56287	0.8718
5	13.999	BB	0.1838	13.61485	1.17007	0.2676
6	15.634	BB	0.1715	791.48016	71.32595	15.5564

mple Name: D12T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
7	17.976	BB	0.1920	13.66375	1.03539	0.2686
8	19.321	BV	0.1437	24.68010	2.62423	0.4851
9	19.689	VB	0.2466	65.91974	3.83390	1.2956
10	20.201	BV E	0.1825	75.58730	6.18918	1.4857
11	20.552	VV R	0.1599	1304.07910	122.77167	25.6315
12	20.980	VB E	0.1470	16.51353	1.70500	0.3246
13	21.327	BB	0.1423	8.95081	1.00151	0.1759
14	22.225	BV	0.1654	65.24754	5.97511	1.2824
15	22.543	VB	0.2452	114.38102	6.63313	2.2481
16	23.563	BB	0.1550	10.39575	1.07344	0.2043
17	24.925	BB	0.5511	1584.53296	41.91177	31.1438
18	27.643	BB	0.1859	332.76300	27.75021	6.5404
19	29.032	BB	0.1897	27.41017	2.19440	0.5387
20	32.530	BB	0.2362	22.03541	1.38064	0.4331
21	33.547	BB	0.2829	60.70461	3.14841	1.1931
22	35.029	BB	0.2210	18.13527	1.19561	0.3564
Totals :				5087.80040	325.19551	

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.469	BV	0.1412	15.19820	1.39408	10.5709
2	5.594	VV	0.1171	11.98725	1.38350	8.3376
3	20.552	BB	0.1578	46.15121	4.42032	32.0999
4	22.533	BB	0.1941	42.73319	3.32072	29.7226
5	27.380	BB	0.1795	18.36590	1.62963	12.7742
6	28.517	BB	0.1408	9.33776	1.03995	6.4948
Totals :				143.77351	13.18820	

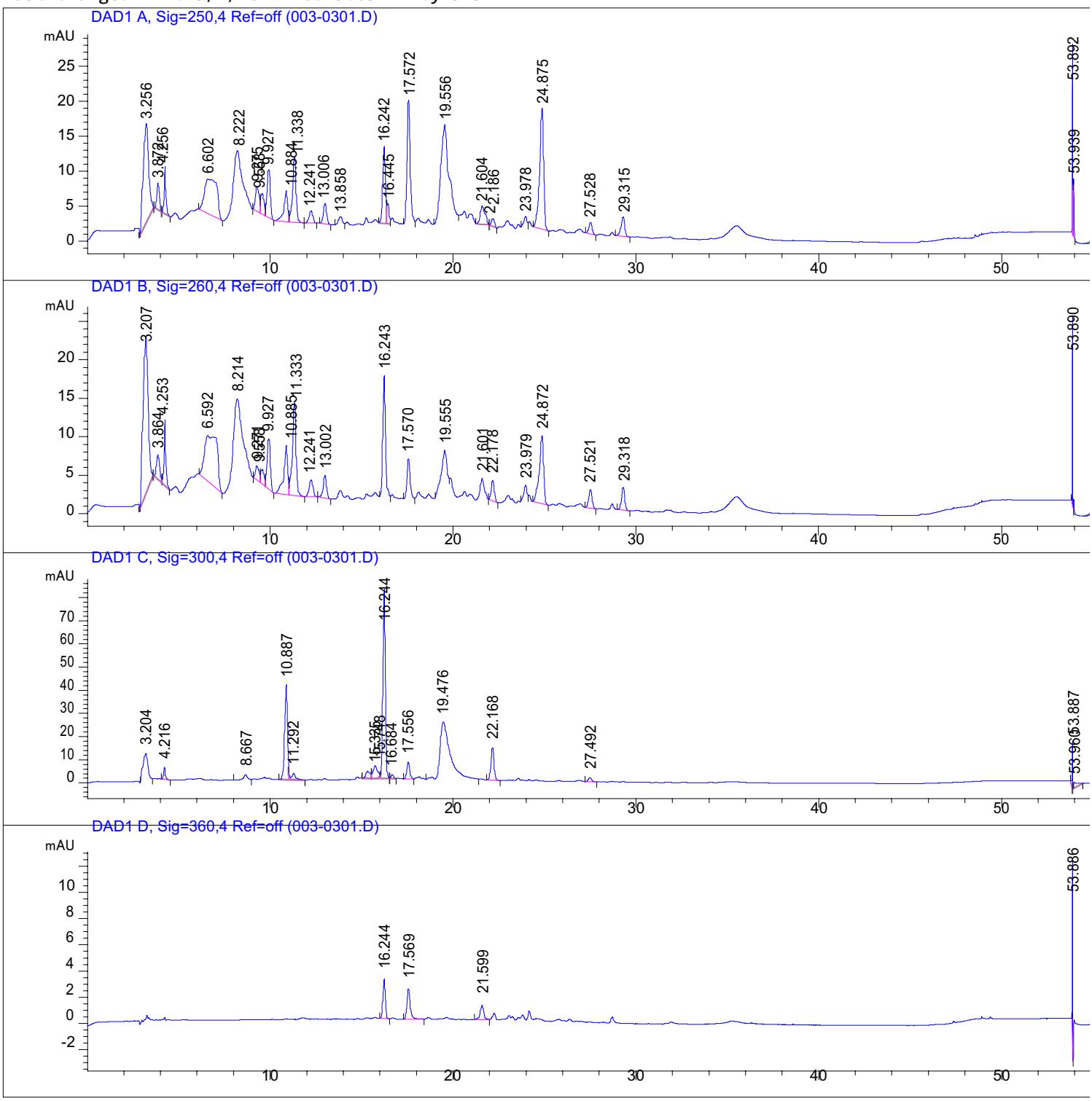
==========

*** End of Report ***

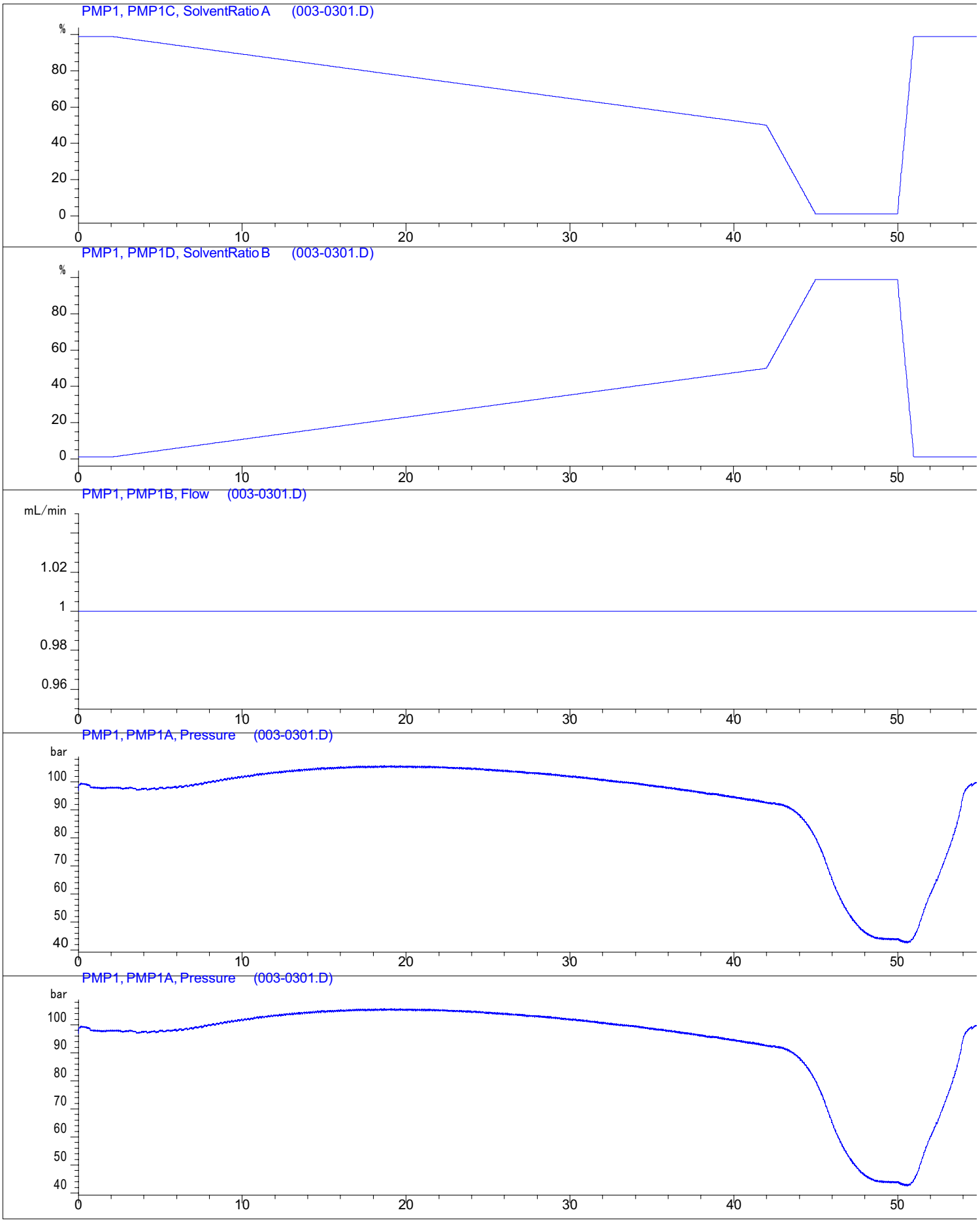
mple Name: D13T50

==========Method 15==========

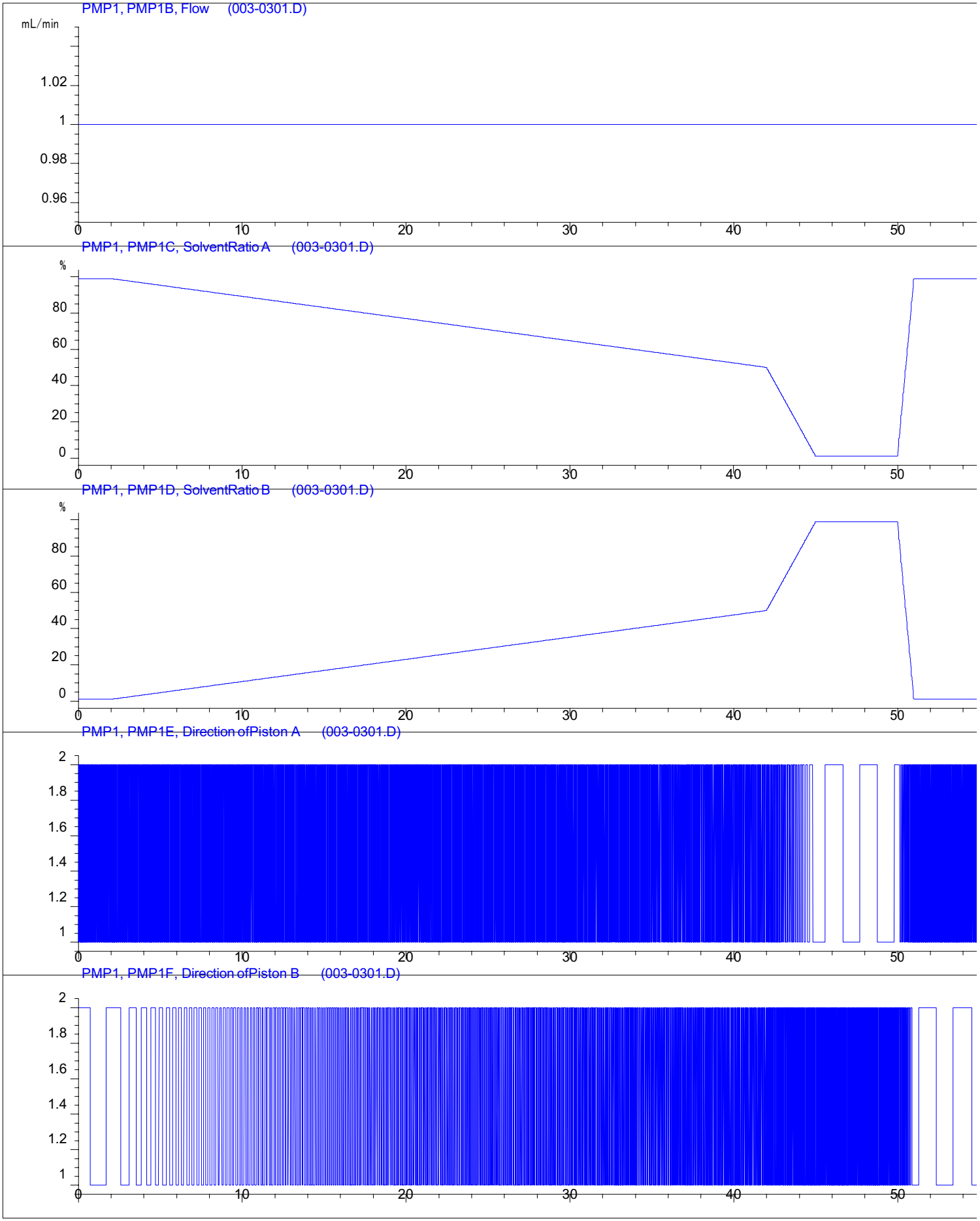
Acq. Operator : SYSTEM Seq. Line : 3
Acq. Instrument : HPLC 4 Location : 3
Injection Date : 5/9/2017 11:34:16 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - method 12-13 2017-05-09 10-25-05\Sequence - method 12-13.S
Method : C:\Chem32\1\Data\Sequence - method 12-13 2017-05-09 10-25-05\Optimizing method 13.M (Sequence Method)
Last changed : 5/9/2017 10:25:05 AM by SYSTEM



mple Name: D13T50



mple Name: D13T50



mple Name: D13T50

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.256	BB	0.2623	305.38254	14.23655	11.6426
2	3.872	BV	0.1507	36.64688	3.86221	1.3971
3	4.256	VB	0.0900	44.20792	6.78688	1.6854
4	6.602	BB	0.6332	240.75403	4.91751	9.1786
5	8.222	BB	0.4969	323.26459	9.22380	12.3243
6	9.275	BV	0.2013	50.31596	3.50800	1.9183
7	9.558	VV	0.2008	37.55869	2.94412	1.4319
8	9.927	VB	0.2008	86.54161	6.87772	3.2994
9	10.884	BV	0.2024	61.76040	4.38171	2.3546
10	11.338	VB	0.2053	125.69935	9.32526	4.7922
11	12.241	BB	0.2319	25.86079	1.75476	0.9859
12	13.006	BB	0.1874	36.34567	2.95711	1.3857
13	13.858	BB	0.2266	14.70280	1.05471	0.5605
14	16.242	BV	0.1584	114.64514	10.92622	4.3708
15	16.445	VV	0.1206	22.44789	2.82853	0.8558
16	17.572	BB	0.1803	207.88347	17.53913	7.9255
17	19.556	BB	0.4019	405.14093	13.66308	15.4458
18	21.604	BB	0.2590	48.23875	2.66708	1.8391
19	22.186	BB	0.1862	12.88519	1.10401	0.4912
20	23.978	BB	0.1590	12.22258	1.17897	0.4660
21	24.875	BB	0.2277	263.37994	17.28719	10.0412
22	27.528	BB	0.1943	20.22095	1.63497	0.7709
23	29.315	BB	0.2049	38.16957	2.76822	1.4552
24	53.892	BV	0.0426	71.37484	27.25580	2.7211
25	53.939	VB	0.0351	17.33156	8.22886	0.6608
Totals :				2622.98203	178.91241	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.207	BB	0.2478	411.55060	20.71586	17.4466
2	3.864	BV	0.1890	38.86637	3.17124	1.6476
3	4.253	VB	0.0933	56.89789	8.58789	2.4120
4	6.592	BB	0.6566	298.33707	5.93307	12.6472
5	8.214	BB	0.5167	413.03943	11.34767	17.5097
6	9.271	BV	0.1945	25.86554	1.83419	1.0965
7	9.558	VV	0.2062	24.61465	1.88732	1.0435

mple Name: D13T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
8	9.927	VB	0.2021	83.05672	6.54340	3.5210
9	10.885	BV	0.2076	93.64499	6.36657	3.9698
10	11.333	VB	0.2145	172.25334	12.06673	7.3022
11	12.241	BB	0.2378	31.97570	2.17133	1.3555
12	13.002	BB	0.1872	36.67774	2.94601	1.5549
13	16.243	BB	0.1542	159.62906	15.75483	6.7670
14	17.570	BB	0.1779	60.14194	5.16239	2.5496
15	19.555	BB	0.2778	86.82156	4.48685	3.6806
16	21.601	BB	0.2345	44.32465	2.77226	1.8790
17	22.178	BB	0.1863	31.59075	2.62714	1.3392
18	23.979	BB	0.1640	17.69298	1.63786	0.7500
19	24.872	BB	0.2435	146.81129	8.85069	6.2237
20	27.521	BB	0.1995	30.35946	2.36993	1.2870
21	29.318	BB	0.1845	35.63728	2.96018	1.5107
22	53.890	BB	0.0399	59.12991	24.72382	2.5067
Totals :				2358.91891	154.91723	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.204	BB	0.2370	224.97955	11.87637	7.5651
2	4.216	BB	0.1122	37.36057	5.30003	1.2563
3	8.667	BB	0.1921	27.01445	2.12847	0.9084
4	10.887	BV R	0.1641	435.31122	40.92956	14.6376
5	11.292	VB E	0.1969	37.31395	2.70575	1.2547
6	15.335	BV E	0.2277	47.77650	2.96882	1.6065
7	15.748	VV E	0.2276	89.00562	5.41902	2.9929
8	16.244	VB R	0.1440	769.00116	81.59437	25.8582
9	16.684	BB	0.1518	14.31162	1.49308	0.4812
10	17.556	BB	0.1665	79.68649	7.23672	2.6795
11	19.476	VB R	0.5446	943.09314	24.74846	31.7121
12	22.168	BB	0.1754	162.86774	14.03758	5.4765
13	27.492	BB	0.2018	23.04668	1.77168	0.7750
14	53.887	BB	0.0405	47.42241	19.42298	1.5946
15	53.960	BB	0.1750	35.72728	2.55656	1.2014
Totals :				2973.91840	224.18944	

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.244	BB	0.1422	28.03898	3.02409	26.7583
2	17.569	BB	0.1877	28.90064	2.31383	27.5806
3	21.599	BB	0.2107	15.28797	1.11016	14.5897
4	53.886	BB	0.0372	32.55863	13.98929	31.0715

mple Name: D13T50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				104.78622	20.43737	

*** End of Report ***

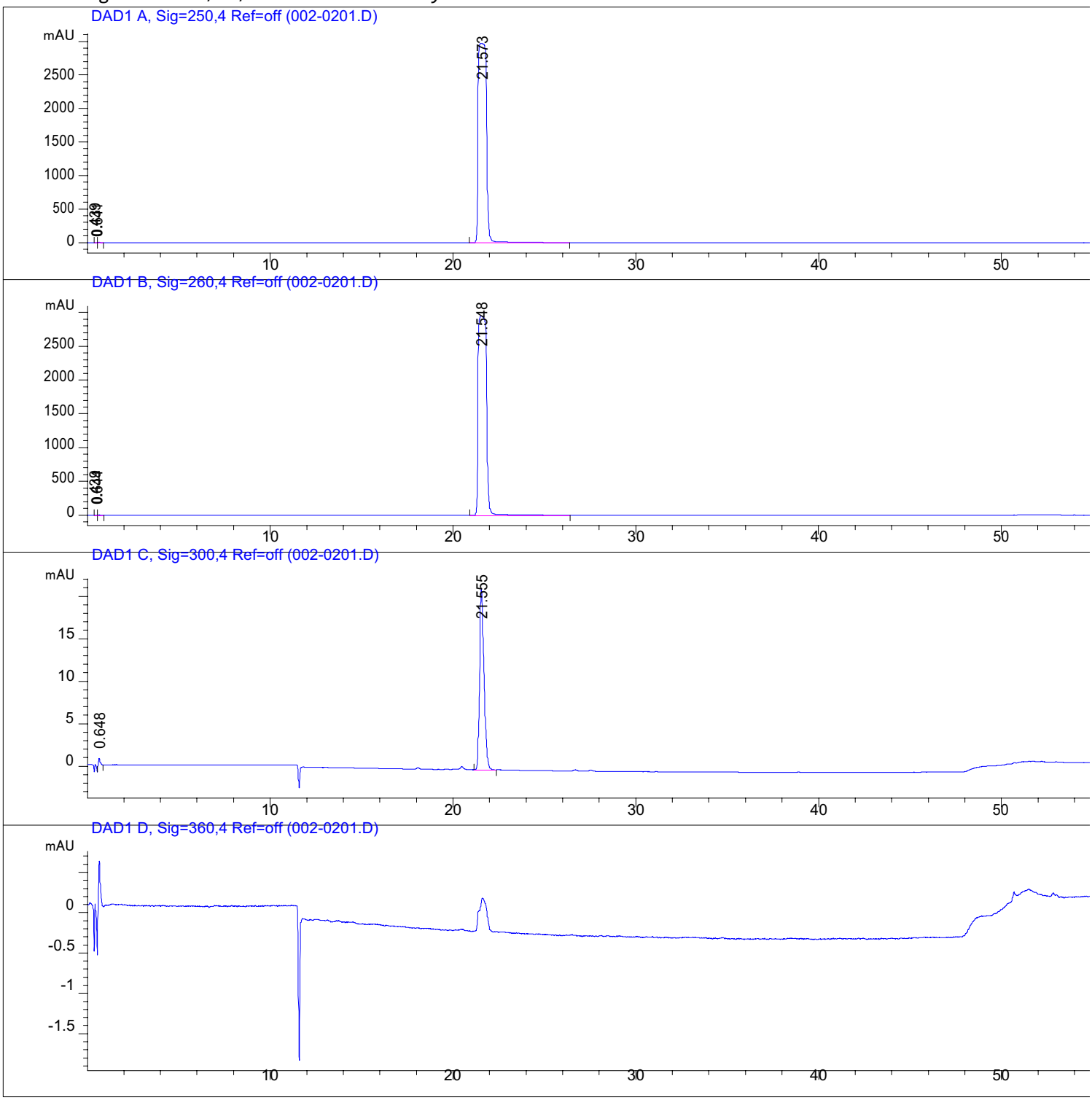
Appendix H

Standards HPLC chromatograms

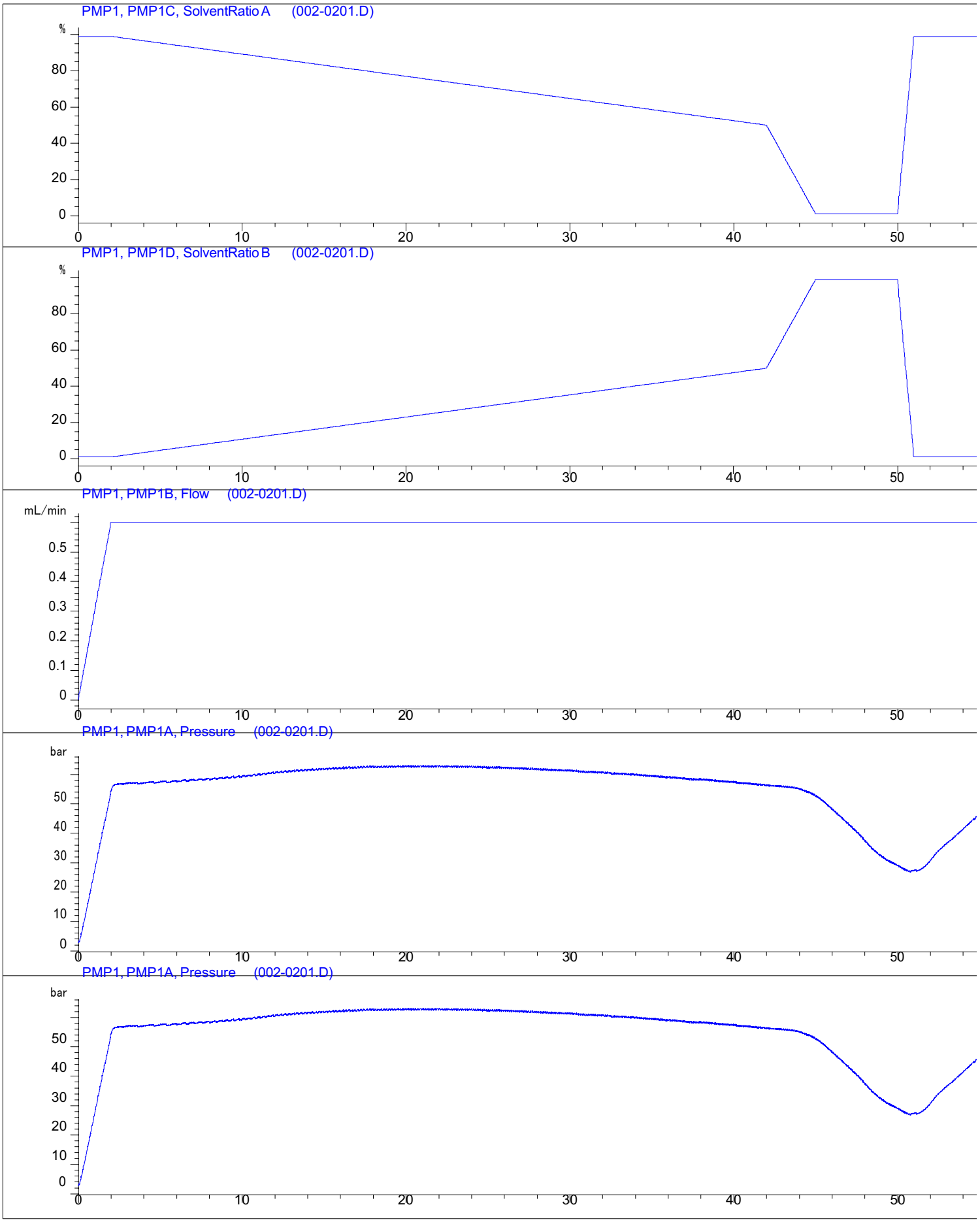
mple Name: S1

==========**S1**==========

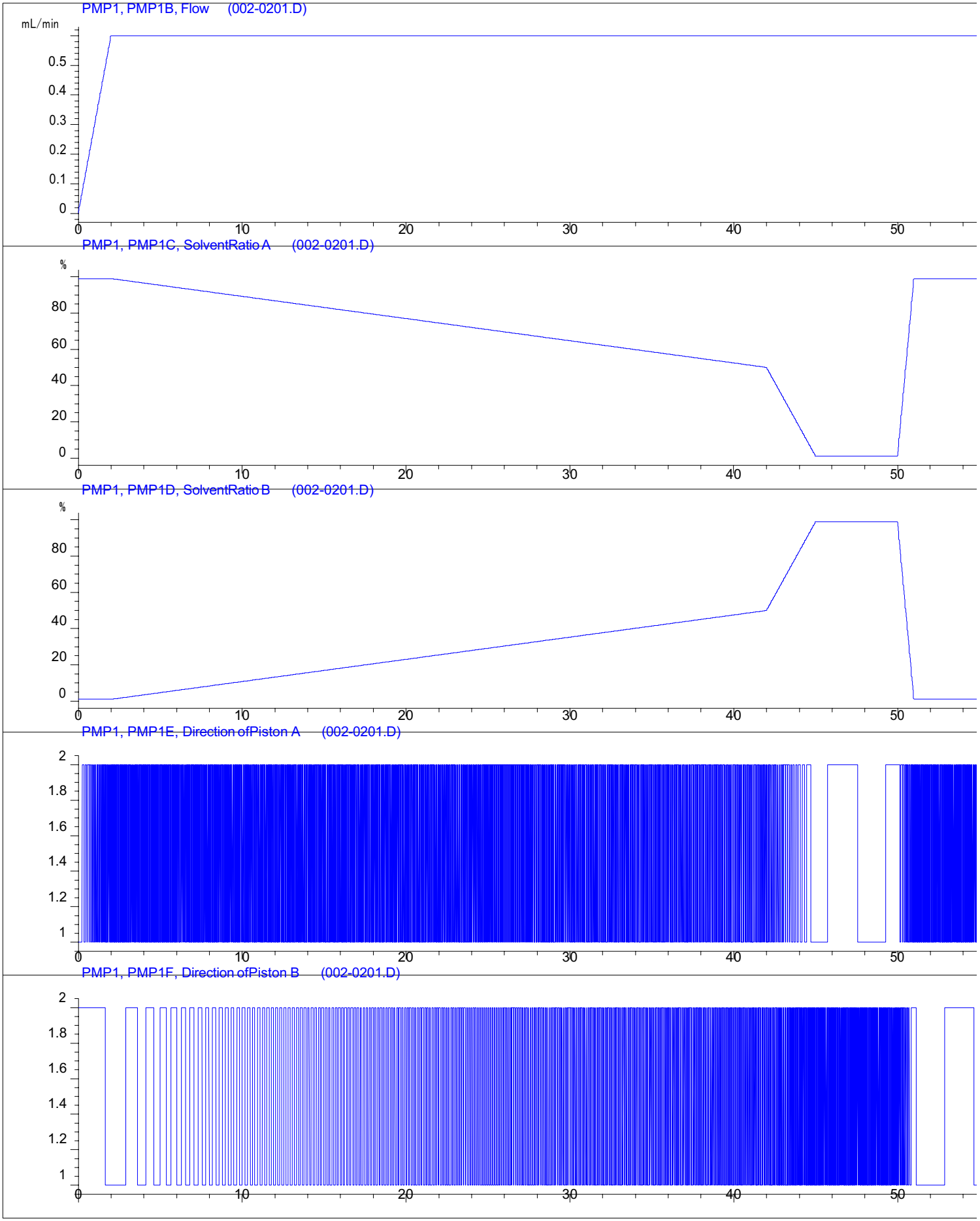
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 2
Injection Date : 5/10/2017 3:33:02 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S1


PMP1, PMP1C, SolventRatio A (002-0201.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (002-0201.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (002-0201.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (002-0201.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (002-0201.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S1


PMP1, PMP1B, Flow (002-0201.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (002-0201.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (002-0201.D)
PMP1, PMP1E, Direction ofPiston A (002-0201.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (002-0201.D)

mple Name: S1

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.439	BB	0.1059	6.93889	1.19270	7.569e-3
2	0.641	BB	0.1252	15.01179	1.80200	0.0164
3	21.573	BB	0.3718	9.16488e4	2971.65186	99.9761
Totals :				9.16707e4	2974.64656	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.439	BB	0.1070	6.61899	1.11953	7.270e-3
2	0.644	BB	0.1308	15.57889	1.76854	0.0171
3	21.548	BB	0.4341	9.10231e4	2948.62695	99.9756
Totals :				9.10453e4	2951.51502	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.648	BB	0.1262	11.23943	1.36317	3.0704
2	21.555	BB	0.2438	354.81854	21.36070	96.9296
Totals :				366.05798	22.72387	

Signal 4: DAD1 D, Sig=360,4 Ref=off

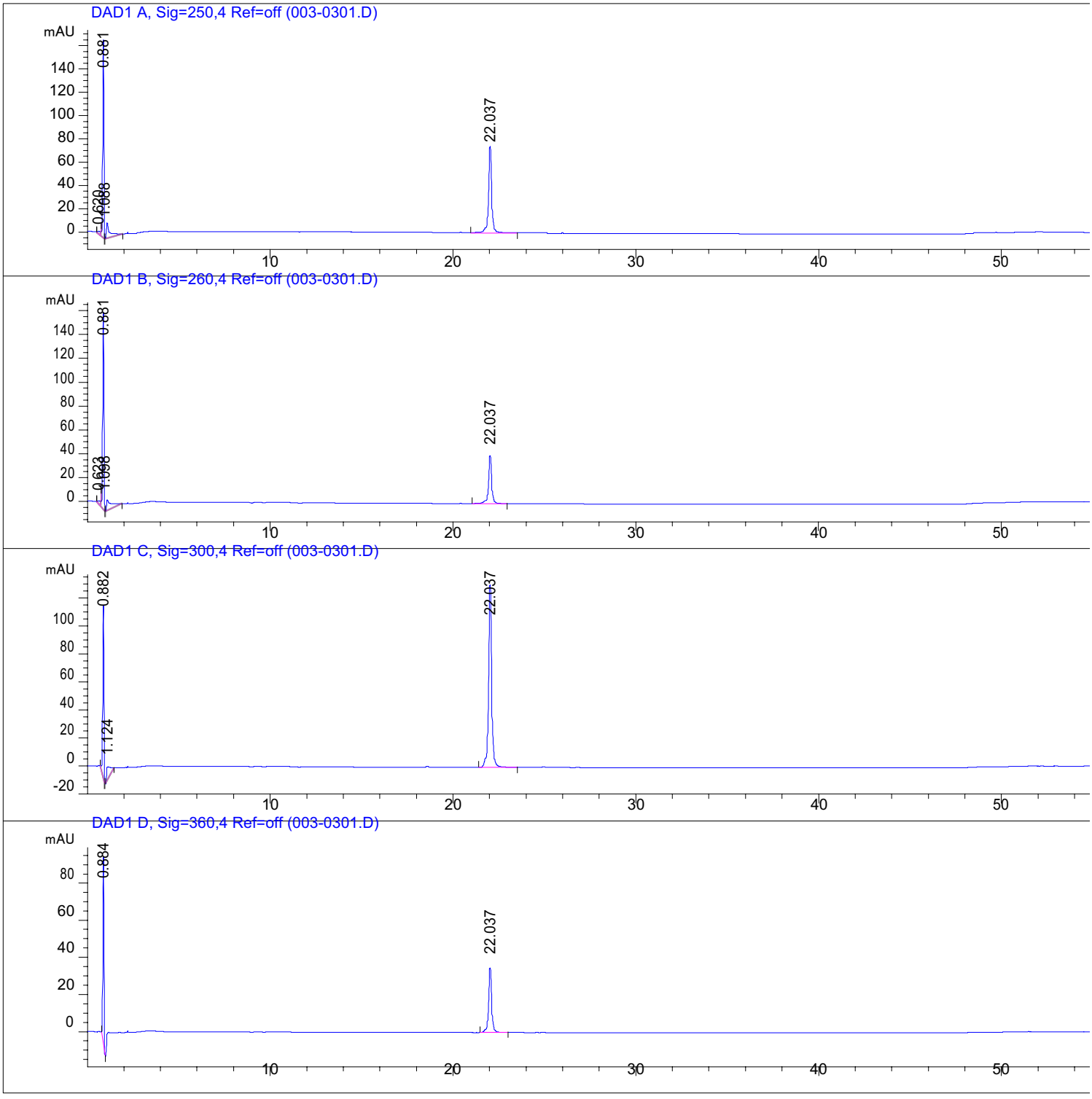
==========

*** End of Report ***

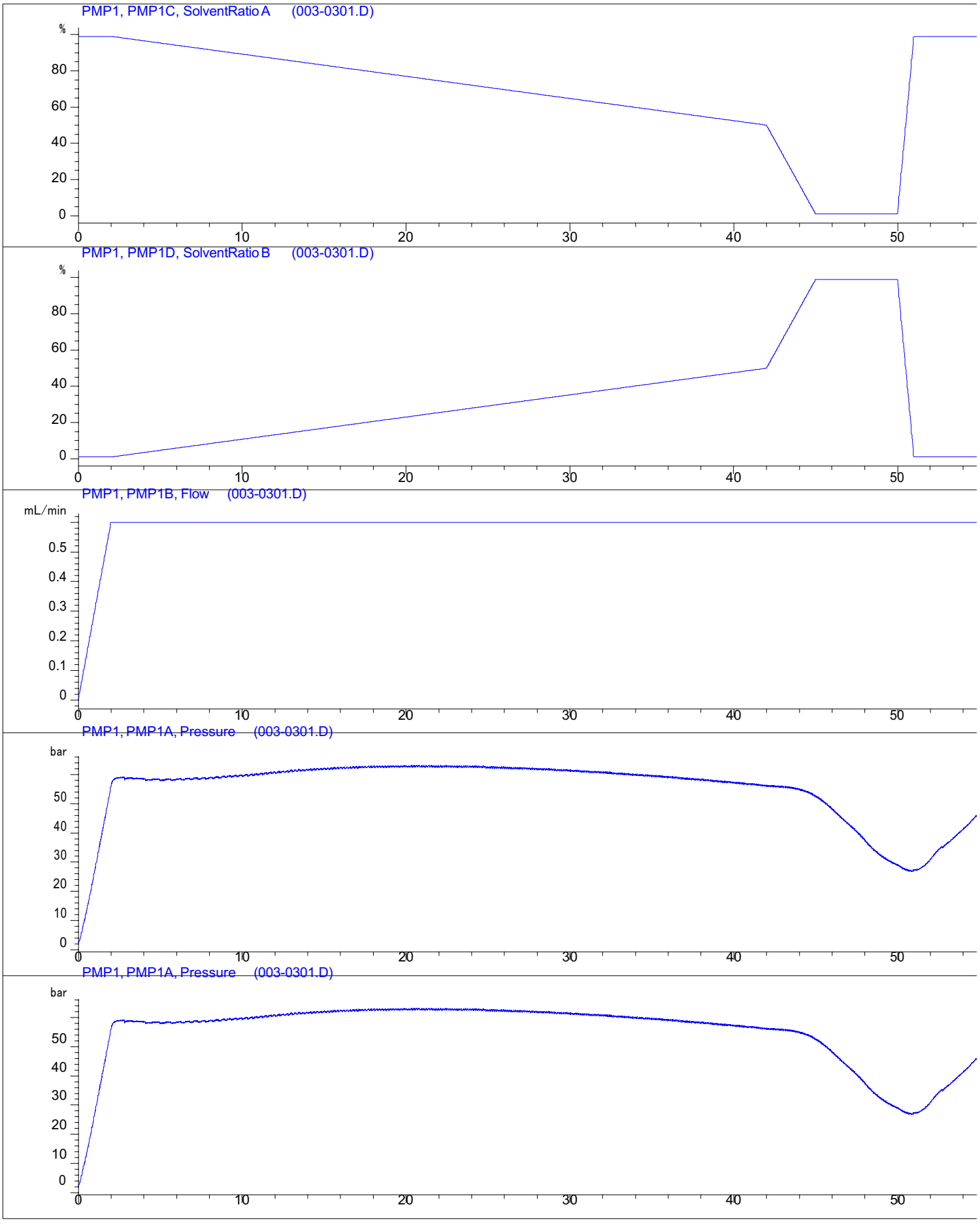
mple Name: S2

==========**S2**==========

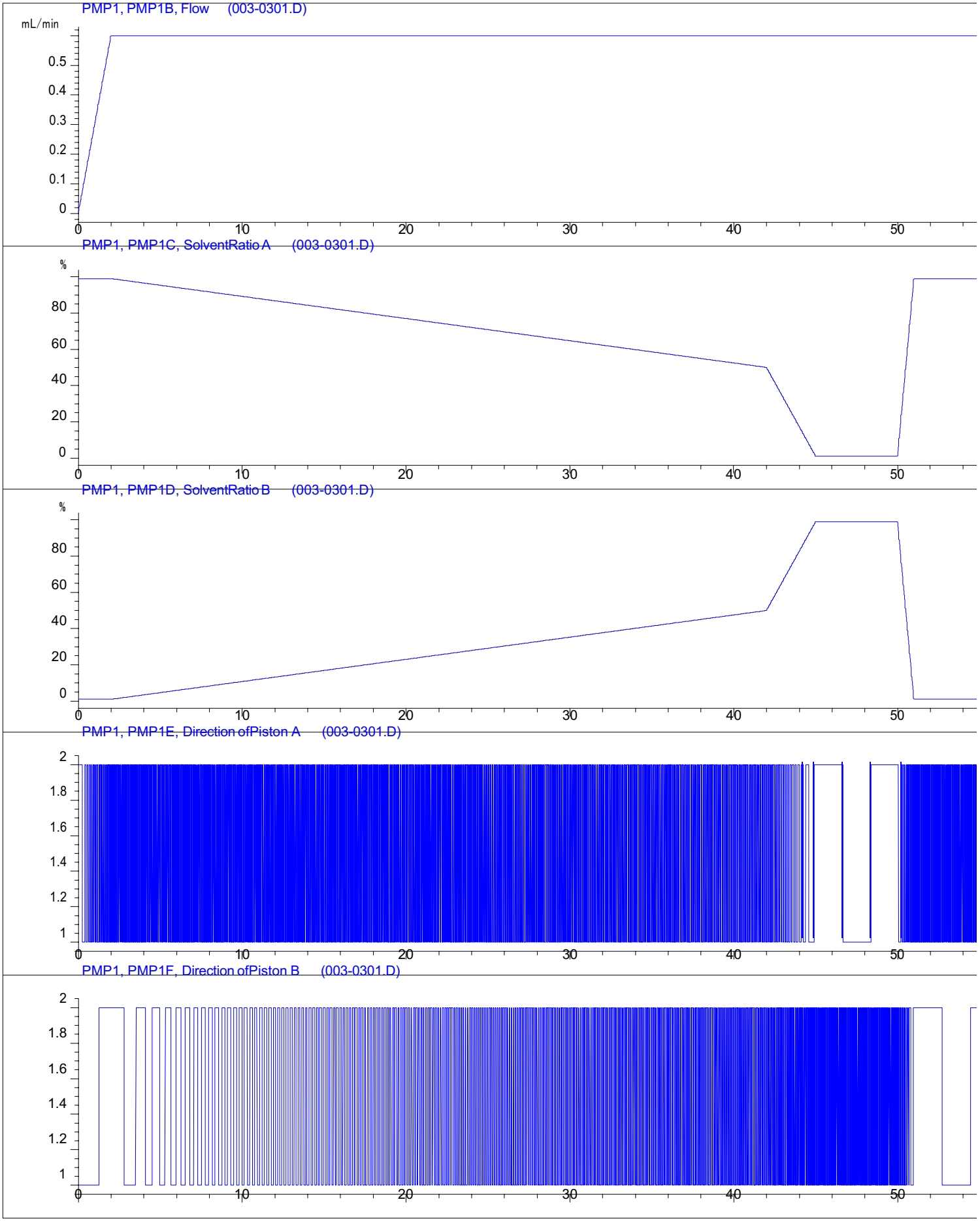
Acq. Operator : SYSTEM Seq. Line : 3
Acq. Instrument : HPLC 4 Location : 3
Injection Date : 5/10/2017 4:30:04 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S2


PMP1, PMP1C, SolventRatio A (003-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (003-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (003-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (003-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (003-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S2


PMP1, PMP1B, Flow (003-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (003-0301.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (003-0301.D)
PMP1, PMP1E, Direction ofPiston A (003-0301.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (003-0301.D)

mple Name: S2

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.620	BV E	0.1980	30.64550	2.07842	1.6644
2	0.881	VB R	0.0632	717.44104	170.06786	38.9641
3	1.088	BB	0.1890	186.98808	13.38037	10.1553
4	22.037	BB	0.1810	906.21442	73.90932	49.2163
Totals :				1841.28904	259.43597	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.623	BV E	0.2073	37.58267	2.41853	2.5515
2	0.881	VB R	0.0684	742.35468	165.39696	50.3991
3	1.098	BB	0.2676	193.03679	9.24359	13.1054
4	22.037	BB	0.1829	499.97751	40.24466	33.9439
Totals :				1472.95164	217.30374	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.882	BB	0.0612	491.05569	121.39565	22.4196
2	1.124	BB	0.2001	137.21022	9.40961	6.2645
3	22.037	BB	0.1779	1562.03235	130.25215	71.3160
Totals :				2190.29826	261.05742	

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S2

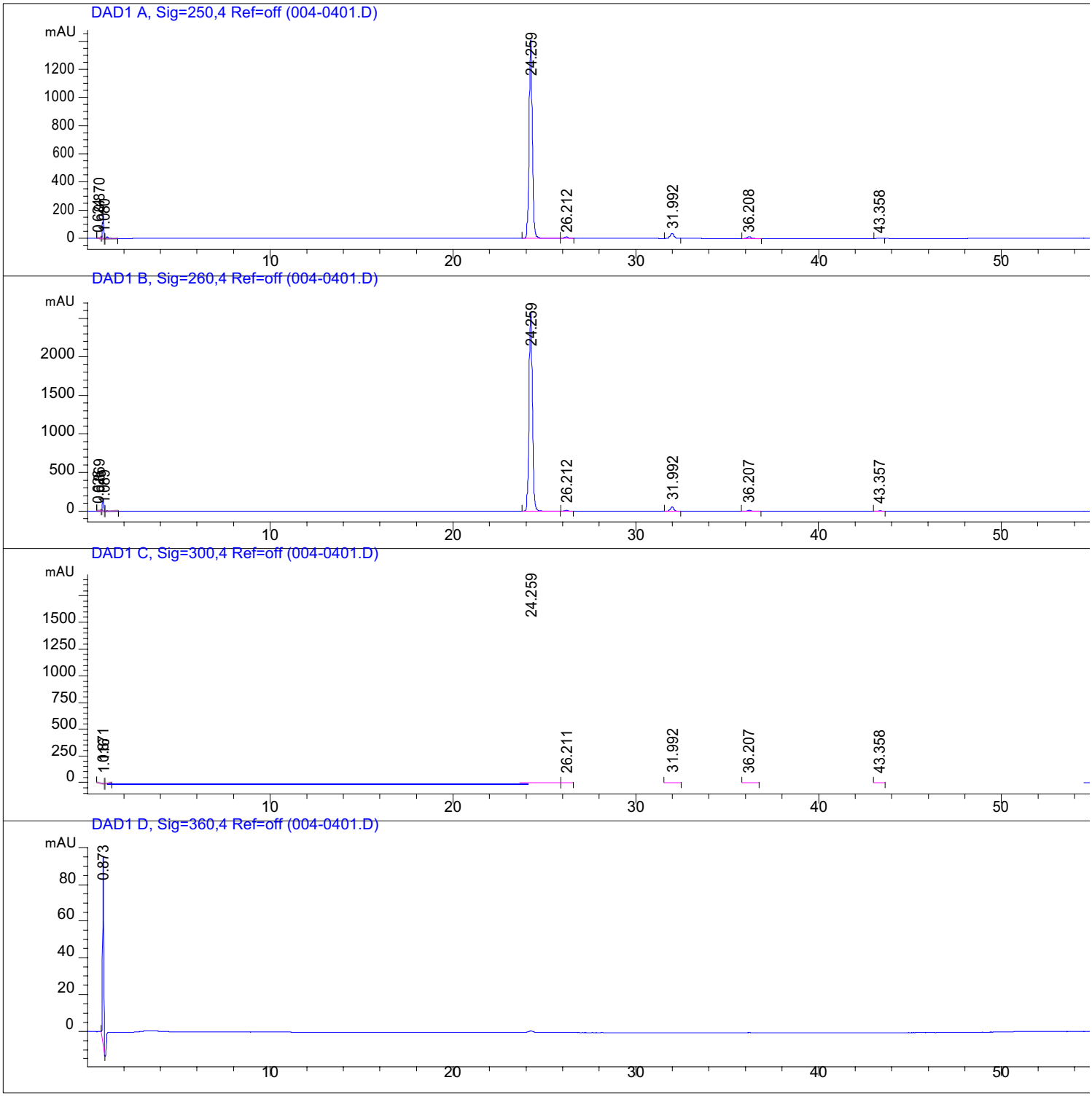
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.884	BB	0.0585	387.12692	101.48766	48.1177
2	22.037	BB	0.1775	417.41467	34.91534	51.8823
Totals :				804.54160	136.40300	

*** End of Report ***

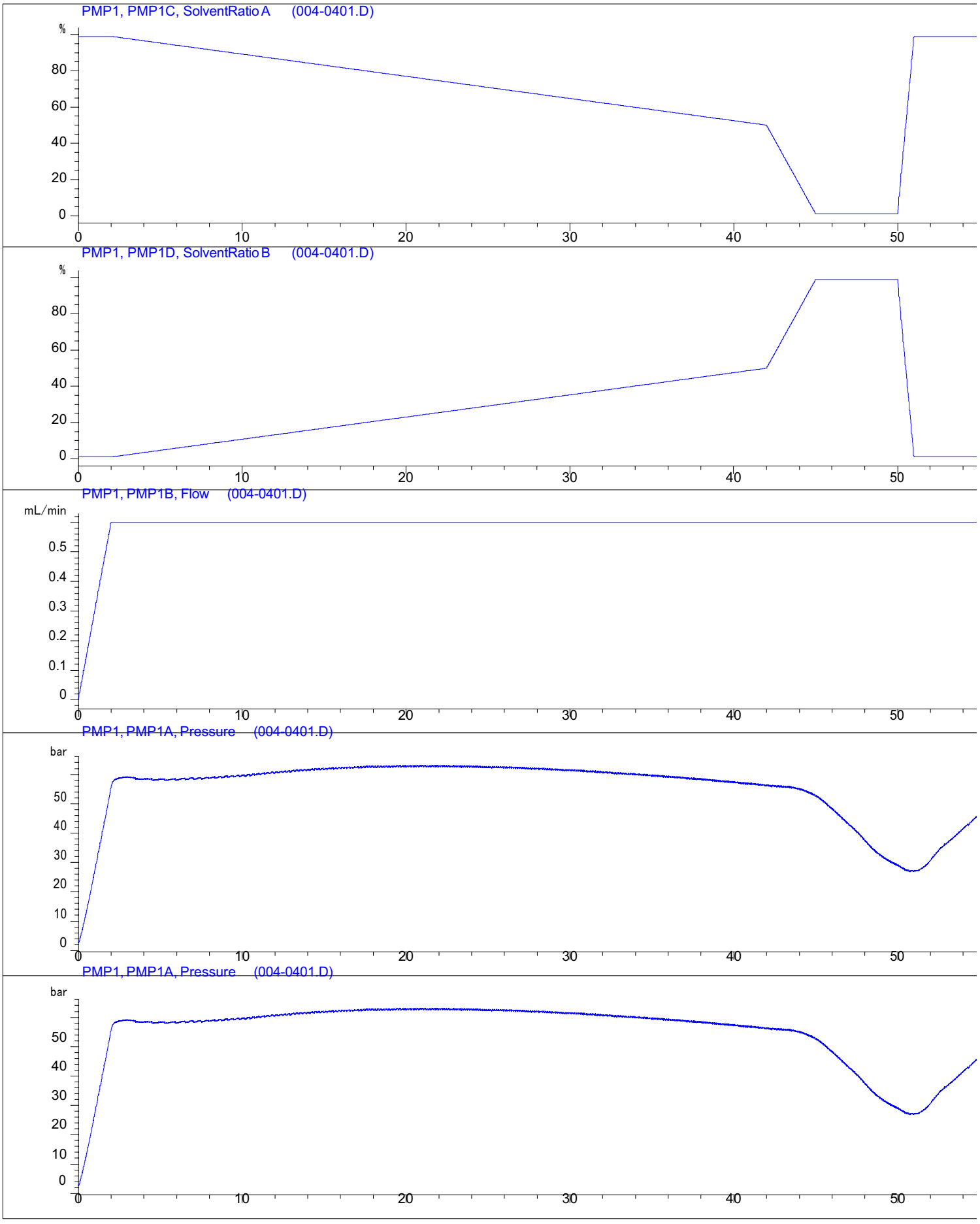
mple Name: S3

==========S3==========

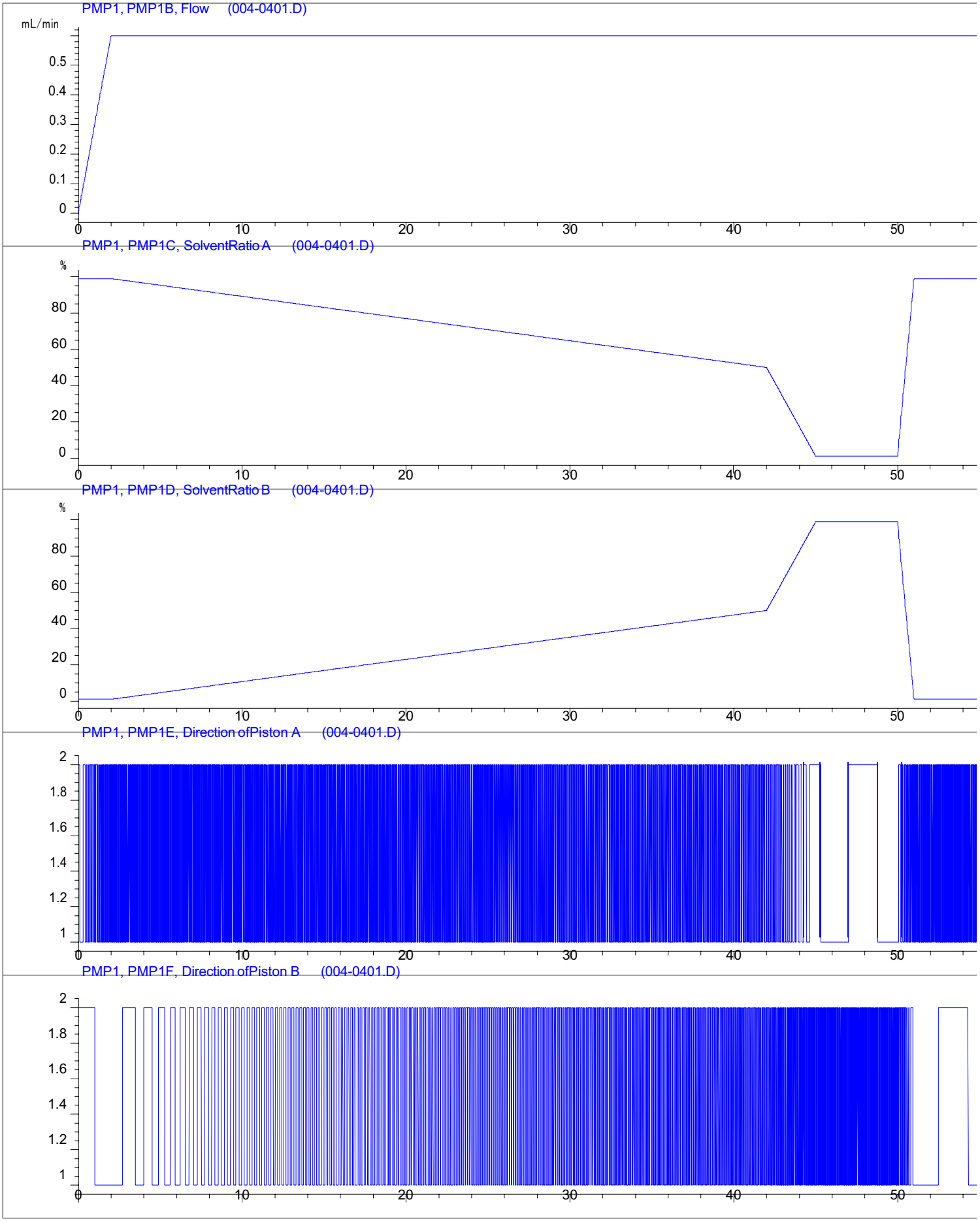
Acq. Operator : SYSTEM Seq. Line : 4
Acq. Instrument : HPLC 4 Location : 4
Injection Date : 5/10/2017 5:27:06 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S3


PMP1, PMP1C, SolventRatio A (004-0401.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (004-0401.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (004-0401.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (004-0401.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (004-0401.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S3


PMP1, PMP1B, Flow (004-0401.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (004-0401.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (004-0401.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1E, Direction ofPiston A (004-0401.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50
PMP1, PMP1F, Direction ofPiston B (004-0401.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50

mple Name: S3

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.624	BV E	0.1956	31.77559	2.18570	0.1701
2	0.870	VB R	0.0636	724.38574	170.51456	3.8788
3	1.080	BB	0.1624	155.93748	13.53944	0.8350
4	24.259	BB	0.1848	1.70314e4	1411.27393	91.1962
5	26.212	BB	0.1864	89.71854	7.35067	0.4804
6	31.992	BB	0.2066	481.61496	35.88268	2.5788
7	36.208	BB	0.1933	124.92771	9.89746	0.6689
8	43.358	BB	0.2081	35.79693	2.64161	0.1917
Totals :				1.86756e4	1653.28605	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.626	BV E	0.1915	35.91905	2.50248	0.1024
2	0.869	VB R	0.0669	750.71063	165.63628	2.1406
3	1.089	BB	0.2151	152.27864	9.29824	0.4342
4	24.259	BB	0.1976	3.30959e4	2580.82080	94.3703
5	26.212	BB	0.1860	121.80634	10.00707	0.3473
6	31.992	BB	0.2066	718.02112	53.48938	2.0474
7	36.207	BB	0.1936	144.05269	11.38267	0.4108
8	43.357	BB	0.2138	51.54855	3.76307	0.1470
Totals :				3.50703e4	2836.89998	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.871	BB	0.0684	583.79590	125.17918	2.4680
2	1.116	BB	0.1654	101.51138	8.62096	0.4291
3	24.259	BB	0.1862	2.26509e4	1858.80542	95.7568
4	26.211	BB	0.1854	34.07602	2.81120	0.1441
5	31.992	BB	0.2067	213.60788	15.90770	0.9030
6	36.207	BB	0.1982	56.79379	4.41219	0.2401

mple Name: S3

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
7	43.358	BB	0.2116	13.93228	1.00611	0.0589
Totals :				2.36546e4	2016.74275	

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.873	BB	0.0609	396.51233	102.91444	100.0000
Totals :				396.51233	102.91444	

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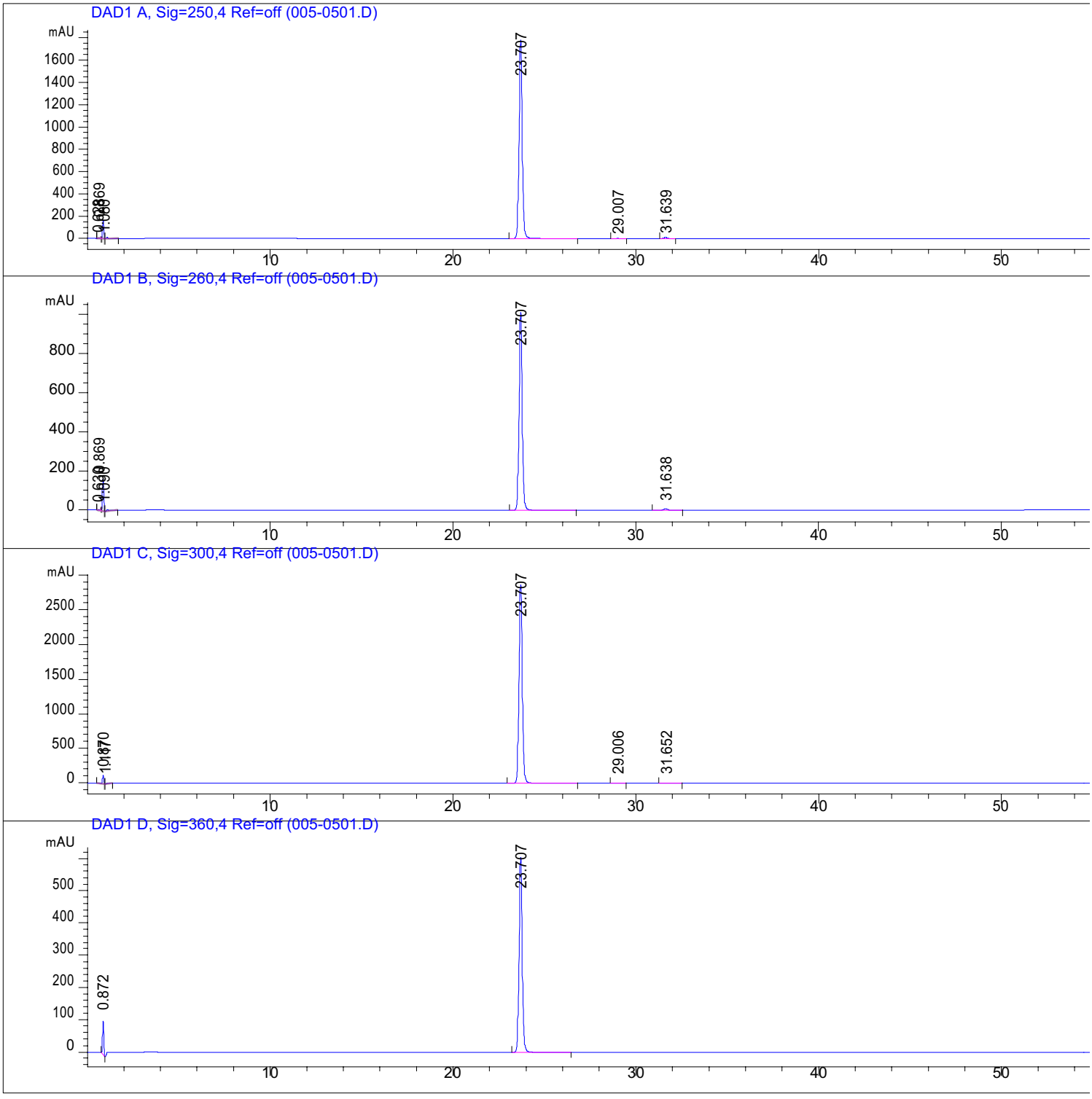
*** End of Report ***

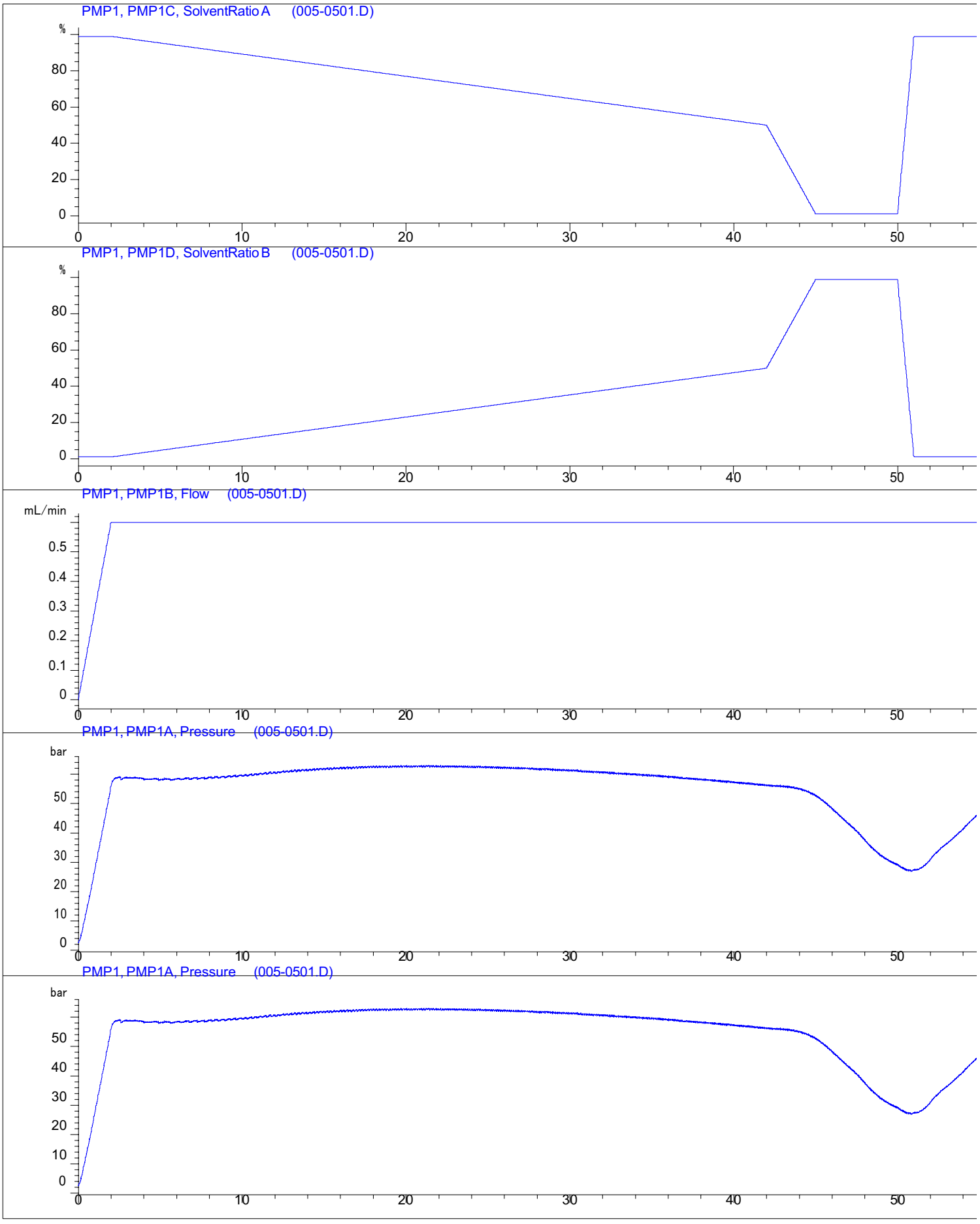
mple Name: S4

==========**S4**==========

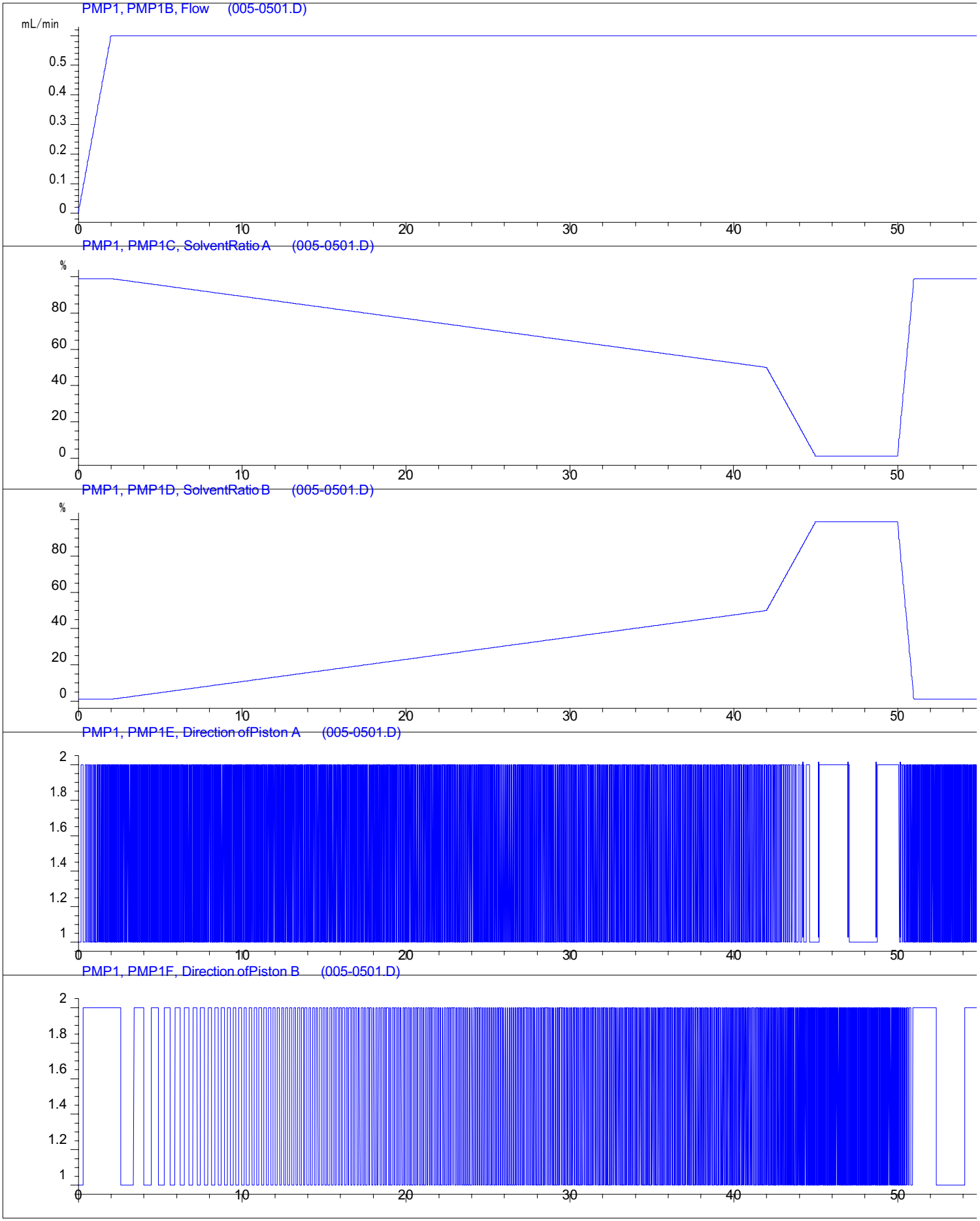
Acq. Operator : SYSTEM Seq. Line : 5
Acq. Instrument : HPLC 4 Location : 5
Injection Date : 5/10/2017 6:24:06 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S4


PMP1, PMP1C, SolventRatio A (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (005-0501.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (005-0501.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (005-0501.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S4


PMP1, PMP1B, Flow (005-0501.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1E, Direction ofPiston A (005-0501.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50
PMP1, PMP1F, Direction ofPiston B (005-0501.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50

mple Name: S4

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Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.628	BV E	0.1841	30.28533	2.23554	0.1356
2	0.869	VB R	0.0637	723.04901	169.64560	3.2377
3	1.080	BB	0.1667	162.32394	13.65316	0.7269
4	23.707	BB	0.1814	2.12986e4	1782.94824	95.3710
5	29.007	BB	0.2026	18.72082	1.37681	0.0838
6	31.639	BB	0.2434	99.38278	6.25303	0.4450

Totals : 2.23324e4 1976.11238

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.630	BV E	0.1831	34.02602	2.52663	0.2599
2	0.869	VB R	0.0690	747.54852	164.60478	5.7104
3	1.090	BB	0.2111	149.38431	9.31497	1.1411
4	23.707	BB	0.1785	1.20207e4	1012.74609	91.8243
5	31.638	BB	0.2566	139.31746	8.18257	1.0642

Totals : 1.30909e4 1197.37503

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.870	BB	0.0684	581.33008	124.74999	1.5619
2	1.117	BB	0.1770	112.62622	8.93653	0.3026
3	23.707	BB	0.1963	3.64366e4	2866.94238	97.8937
4	29.006	BB	0.1974	32.25098	2.45317	0.0866
5	31.652	BB	0.2732	57.77931	3.22290	0.1552

Totals : 3.72206e4 3006.30497

mple Name: S4

Signal 4: DAD1 D, Sig=360,4 Ref=off

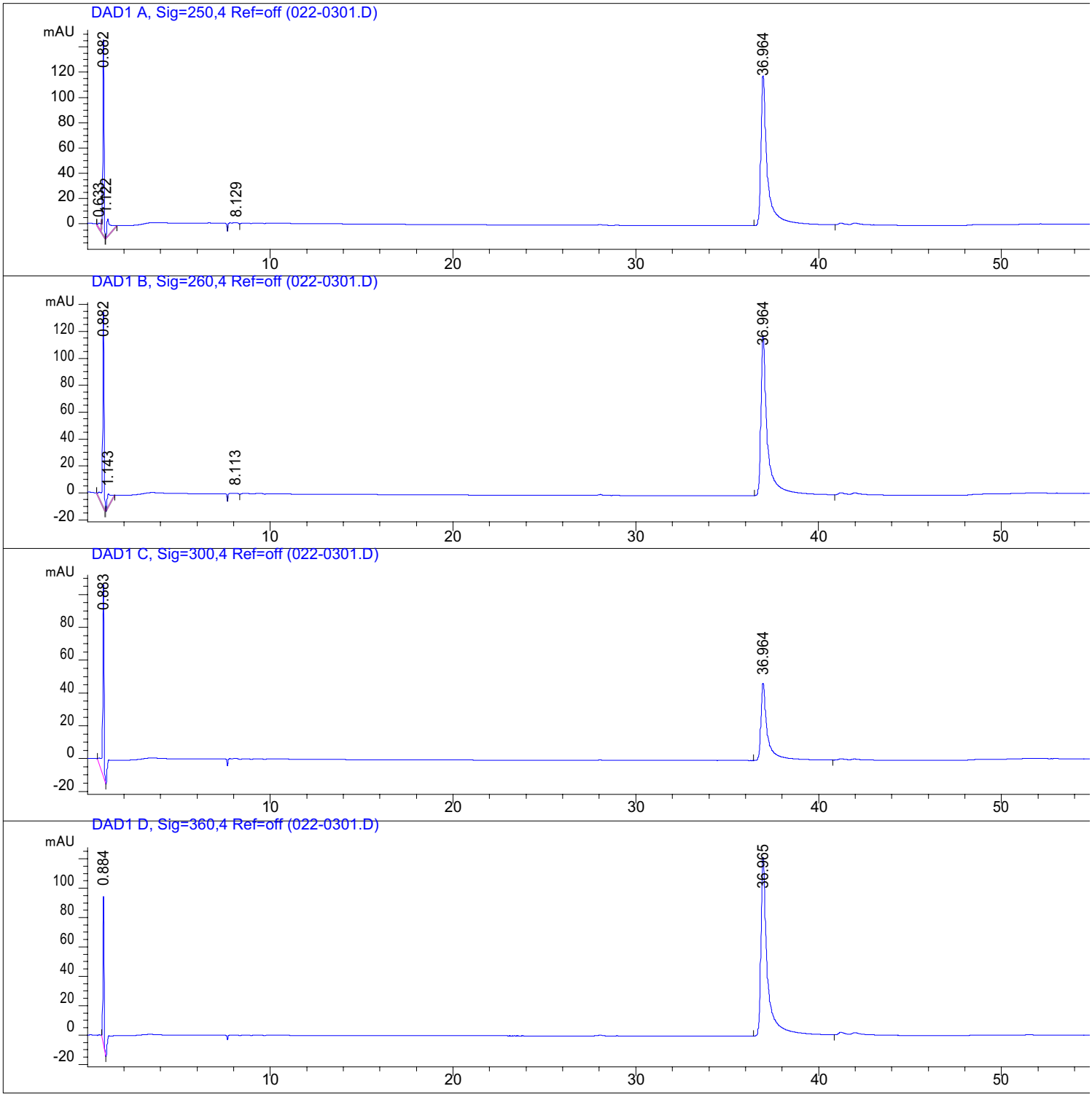
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.872	BB	0.0589	397.16681	103.12437	5.2110
2	23.707	BB	0.1813	7224.53857	605.08350	94.7890
Totals :				7621.70538	708.20786	

*** End of Report ***

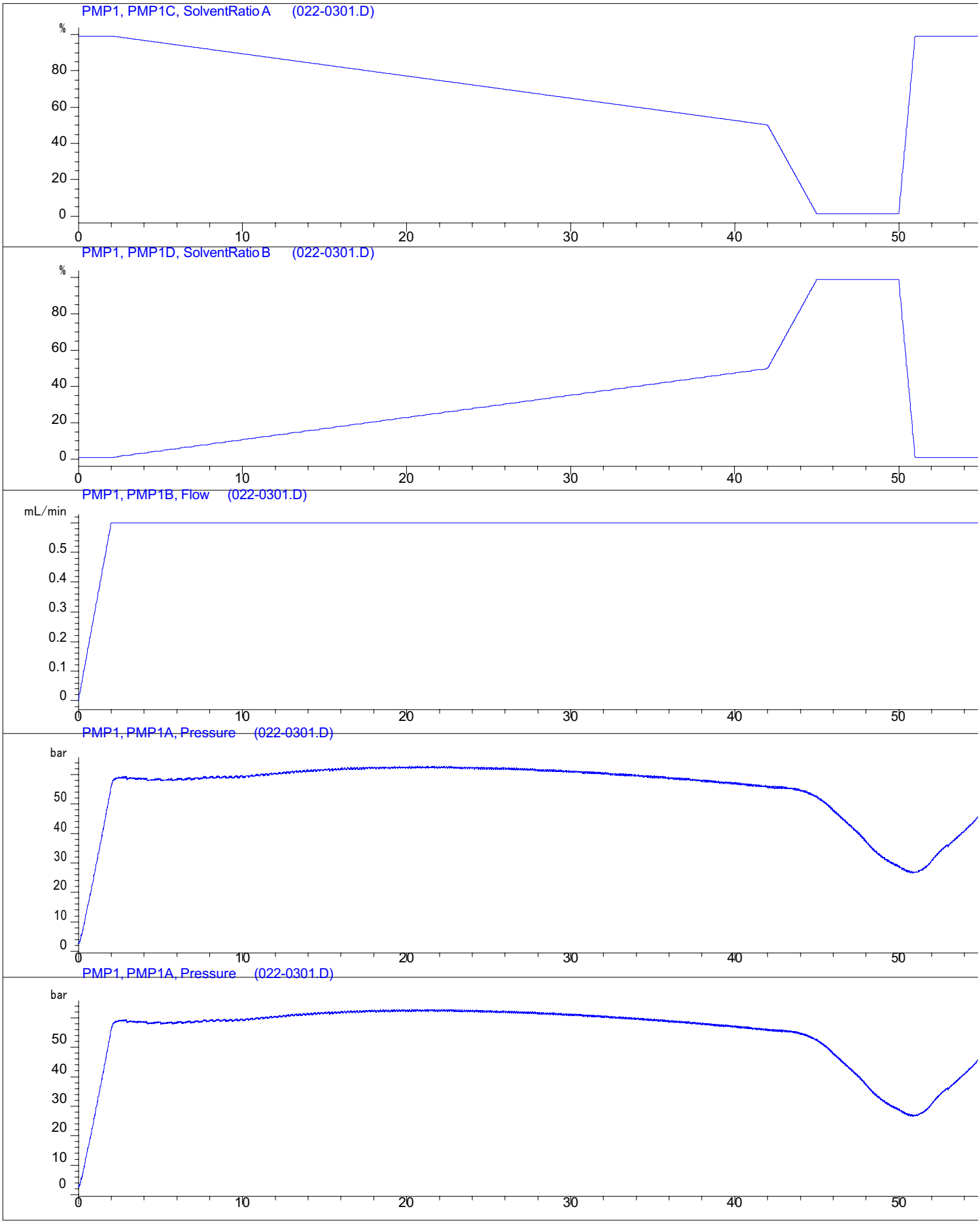
mple Name: S5

==========S5==========

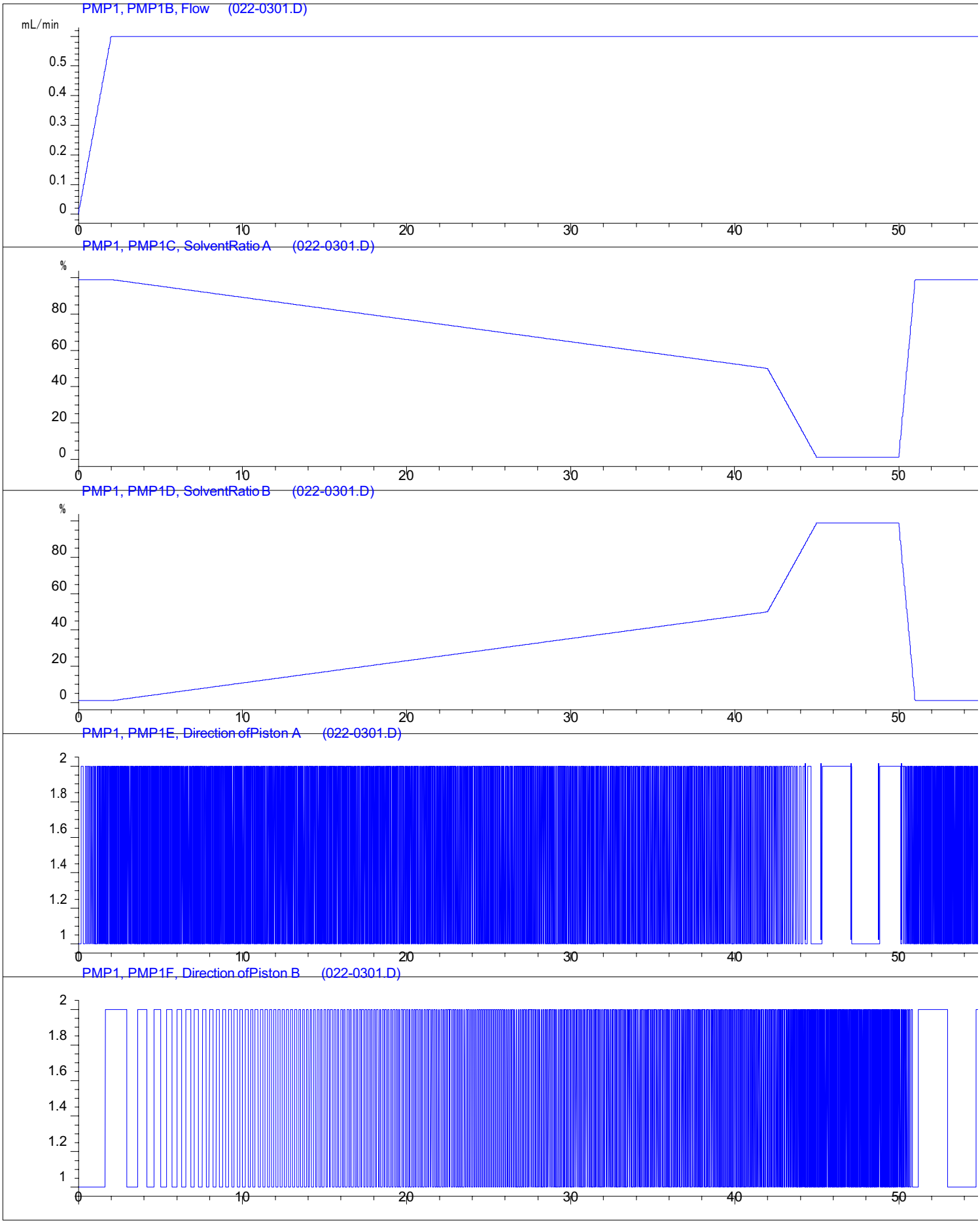
Acq. Operator : SYSTEM Seq. Line : 3
Acq. Instrument : HPLC 4 Location : 22
Injection Date : 5/11/2017 12:02:27 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\DEF_LC.S
Method : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\Optimized method = method 12.M
(Sequence Method)
Last changed : 5/11/2017 10:50:25 AM by SYSTEM



mple Name: S5


PMP1, PMP1C, SolventRatio A (022-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (022-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (022-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (022-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (022-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S5


PMP1, PMP1B, Flow (022-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (022-0301.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (022-0301.D)
PMP1, PMP1E, Direction ofPiston A (022-0301.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (022-0301.D)

mple Name: S5

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.633	BV E	0.2395	56.90032	3.08105	1.4209
2	0.882	VB R	0.0634	656.13300	154.80637	16.3846
3	1.122	BB	0.1912	198.49310	13.84883	4.9567
4	8.129	BB	0.5743	132.26205	2.74403	3.3028
5	36.964	BB	0.3475	2960.79175	118.51952	73.9351

Totals : 4004.58022 292.99980

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.882	BB	0.0704	696.79718	144.18008	17.8026
2	1.143	BB	0.1842	142.93823	10.41226	3.6519
3	8.113	BB	0.5493	119.78544	2.60113	3.0604
4	36.964	BB	0.3475	2954.50830	118.25484	75.4851

Totals : 3914.02915 275.44831

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.883	BB	0.0701	564.76154	117.40015	32.3839
2	36.964	BB	0.3481	1179.19592	47.10185	67.6161

Totals : 1743.95746 164.50201

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S5

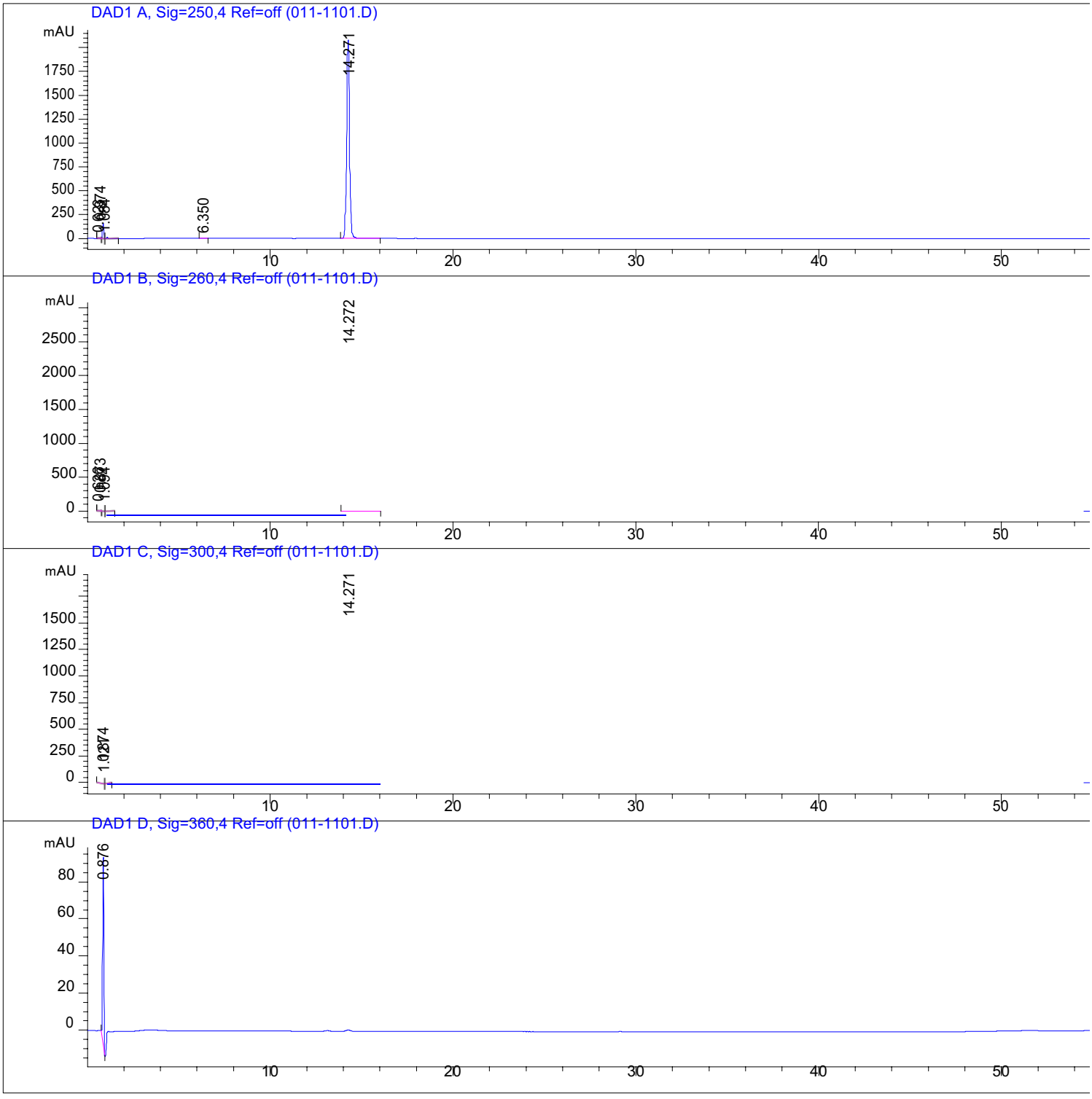
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.884	BB	0.0628	408.84875	102.01109	11.8499
2	36.965	BB	0.3478	3041.38940	121.60992	88.1501
Totals :				3450.23816	223.62101	

*** End of Report ***

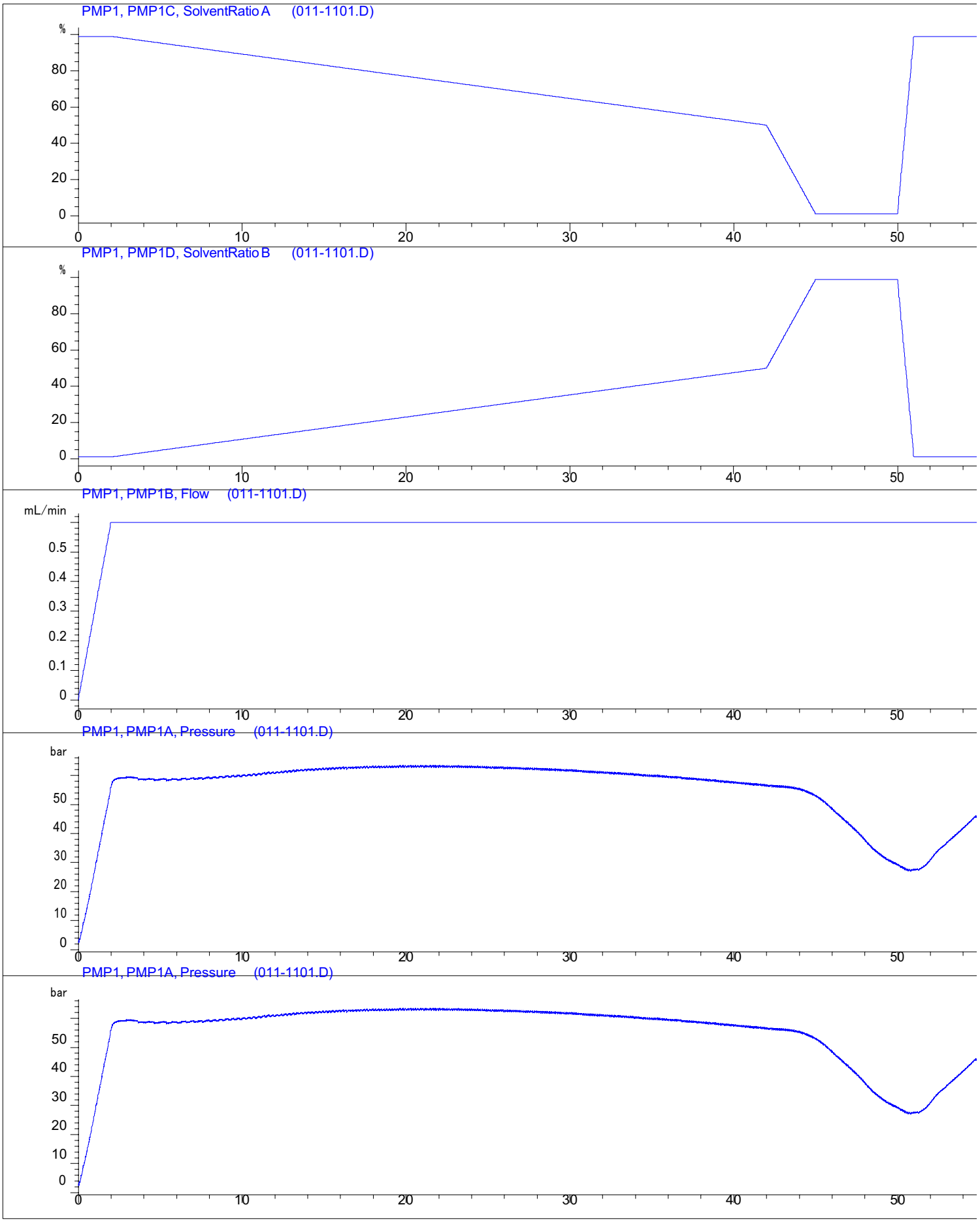
mple Name: S6

==========S6==========

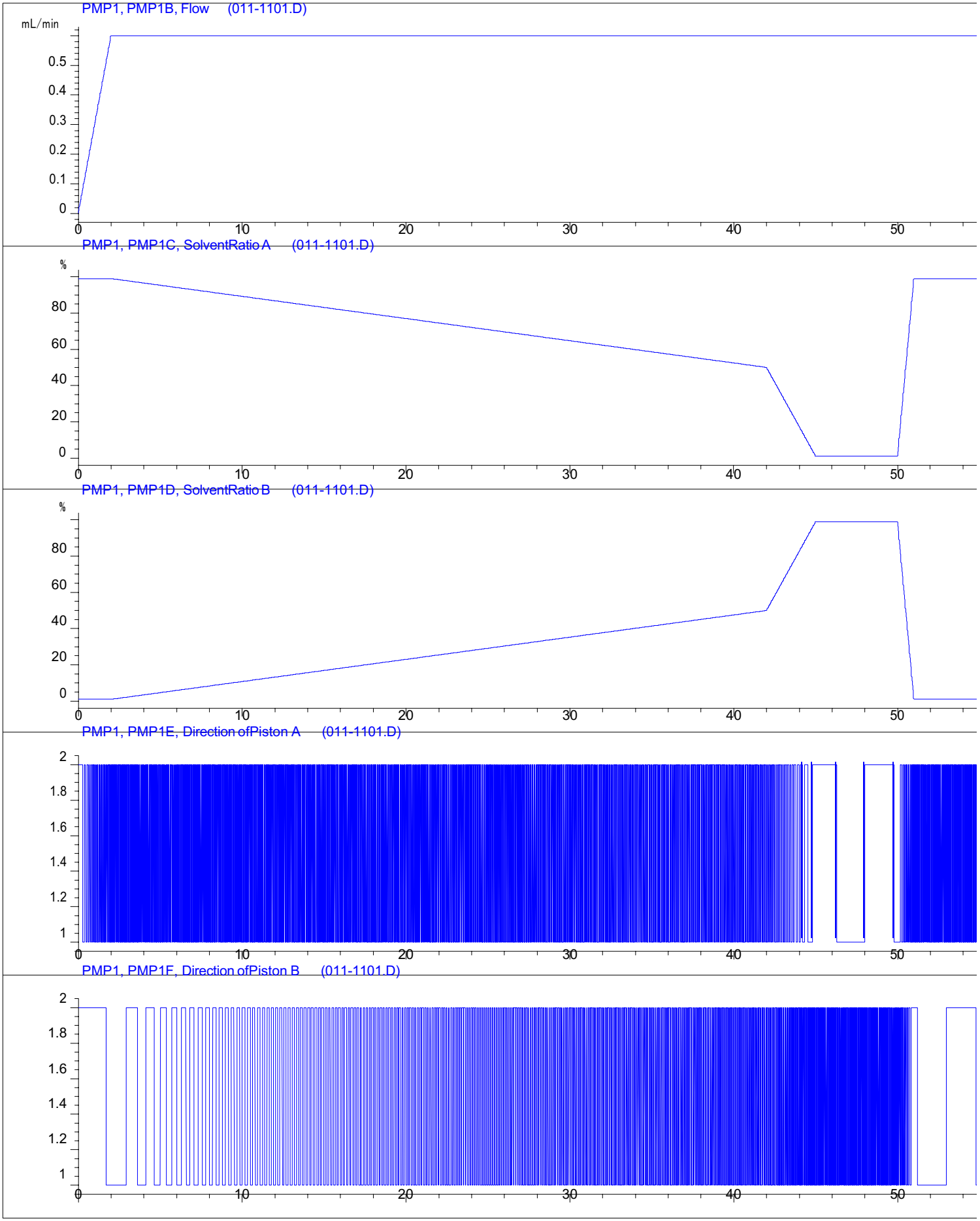
Acq. Operator : SYSTEM Seq. Line : 11
Acq. Instrument : HPLC 4 Location : 11
Injection Date : 5/11/2017 12:06:20 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S6


PMP1, PMP1C, SolventRatio A (011-1101.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (011-1101.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (011-1101.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (011-1101.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (011-1101.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S6


PMP1, PMP1B, Flow (011-1101.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (011-1101.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (011-1101.D)
PMP1, PMP1E, Direction ofPiston A (011-1101.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (011-1101.D)

mple Name: S6

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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.623	BV E	0.2144	39.63067	2.45465	0.1683
2	0.874	VB R	0.0639	657.26990	160.12611	2.7906
3	1.084	BB	0.1766	185.07585	14.52697	0.7858
4	6.350	BB	0.1572	14.34937	1.25636	0.0609
5	14.271	BB	0.1631	2.26569e4	2080.13110	96.1945

Totals : 2.35532e4 2258.49520

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.626	BV E	0.2057	41.89474	2.71993	0.1108
2	0.873	VB R	0.0669	668.02618	153.08780	1.7673
3	1.094	BB	0.1742	121.55637	9.56659	0.3216
4	14.272	BB	0.1949	3.69682e4	2935.59839	97.8003

Totals : 3.77997e4 3100.97270

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.874	BB	0.0700	552.28540	119.39304	2.6581
2	1.121	BB	0.1738	112.10346	9.09733	0.5395
3	14.271	BB	0.1622	2.01131e4	1860.05151	96.8024

Totals : 2.07775e4 1988.54188

mple Name: S6

Signal 4: DAD1 D, Sig=360,4 Ref=off

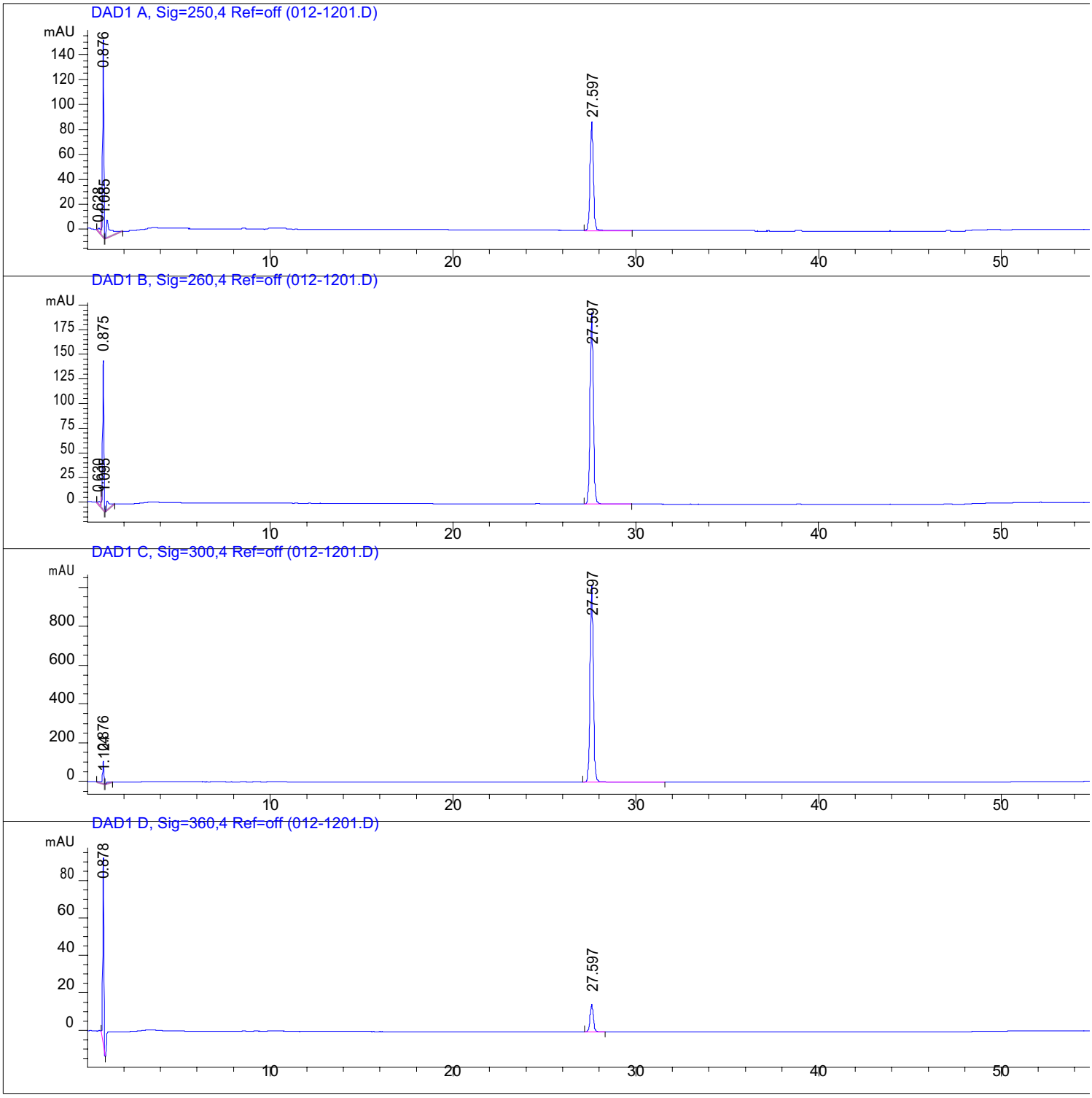
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.876	BB	0.0582	382.11594	100.84788	100.0000
Totals :				382.11594	100.84788	

*** End of Report ***

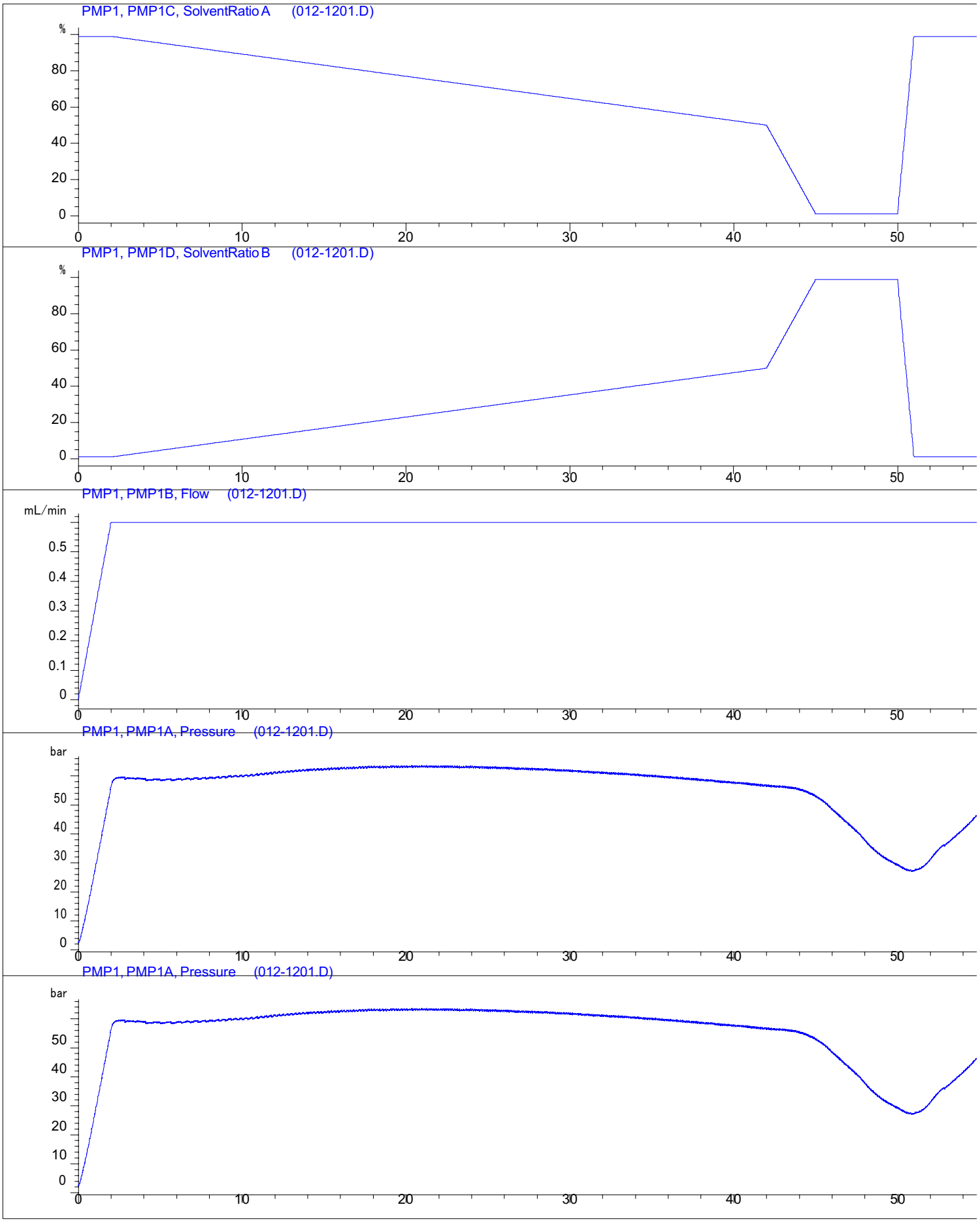
mple Name: S7

==========S7==========

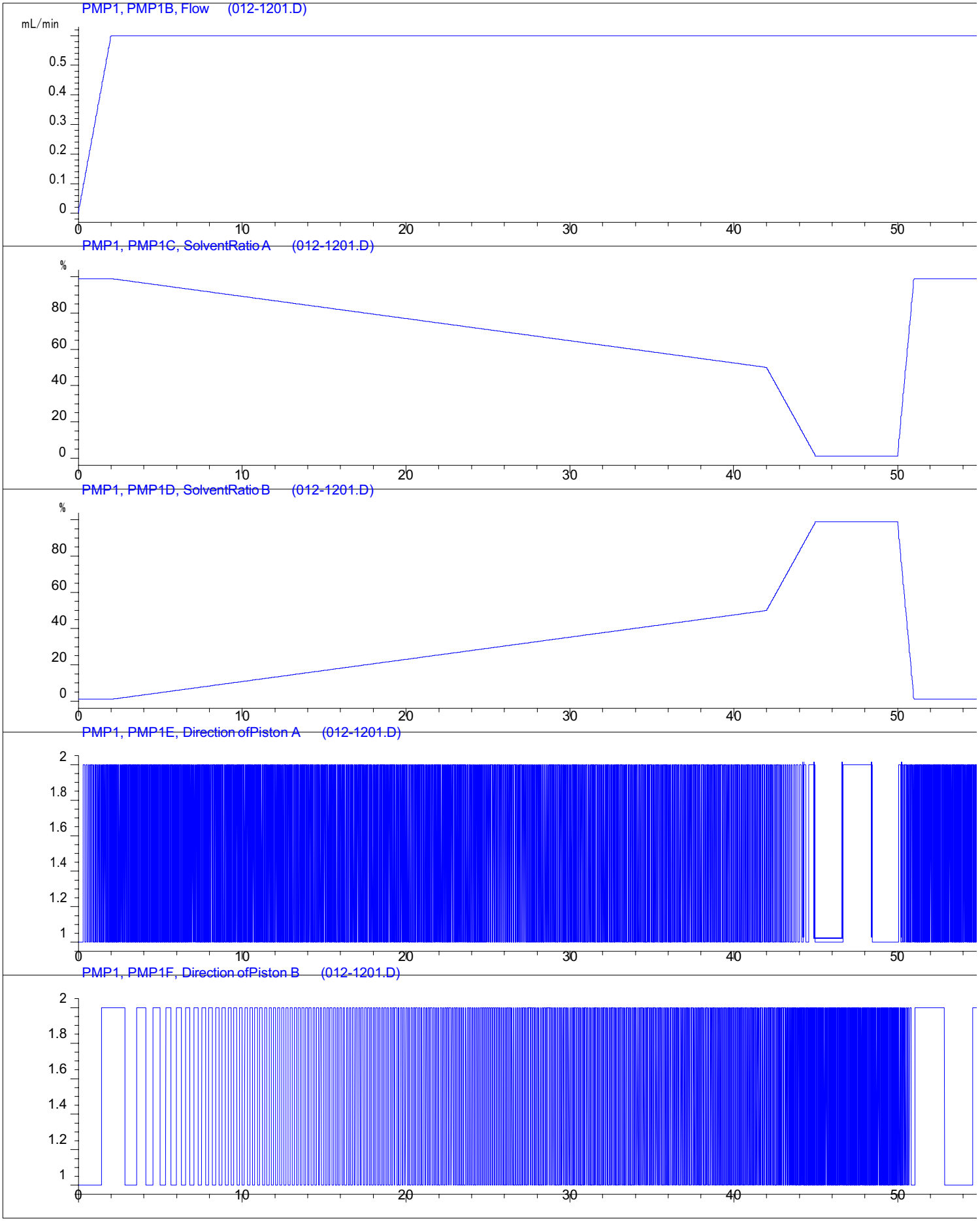
Acq. Operator : SYSTEM Seq. Line : 12
Acq. Instrument : HPLC 4 Location : 12
Injection Date : 5/11/2017 1:03:20 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S7


PMP1, PMP1C, SolventRatio A (012-1201.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (012-1201.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (012-1201.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (012-1201.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (012-1201.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S7


PMP1, PMP1B, Flow (012-1201.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (012-1201.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (012-1201.D)
PMP1, PMP1E, Direction ofPiston A (012-1201.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (012-1201.D)

mple Name: S7

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.628	BV E	0.2222	40.42308	2.42795	2.0129
2	0.876	VB R	0.0615	643.82507	158.07489	32.0591
3	1.085	BB	0.2138	235.61478	14.79562	11.7324
4	27.597	BB	0.1915	1088.38403	87.28738	54.1957
Totals :				2008.24696	262.58584	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.630	BV E	0.2172	43.70900	2.69587	1.3483
2	0.875	VB R	0.0644	652.40747	151.01953	20.1250
3	1.095	BB	0.1801	128.58301	9.60963	3.9664
4	27.597	BB	0.1910	2417.07861	194.48579	74.5603
Totals :				3241.77809	357.81082	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.876	BB	0.0677	544.76001	118.36011	4.1040
2	1.124	BB	0.1818	118.23864	9.20986	0.8908
3	27.597	BB	0.1910	1.26107e4	1014.97723	95.0052
Totals :				1.32737e4	1142.54720	

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S7

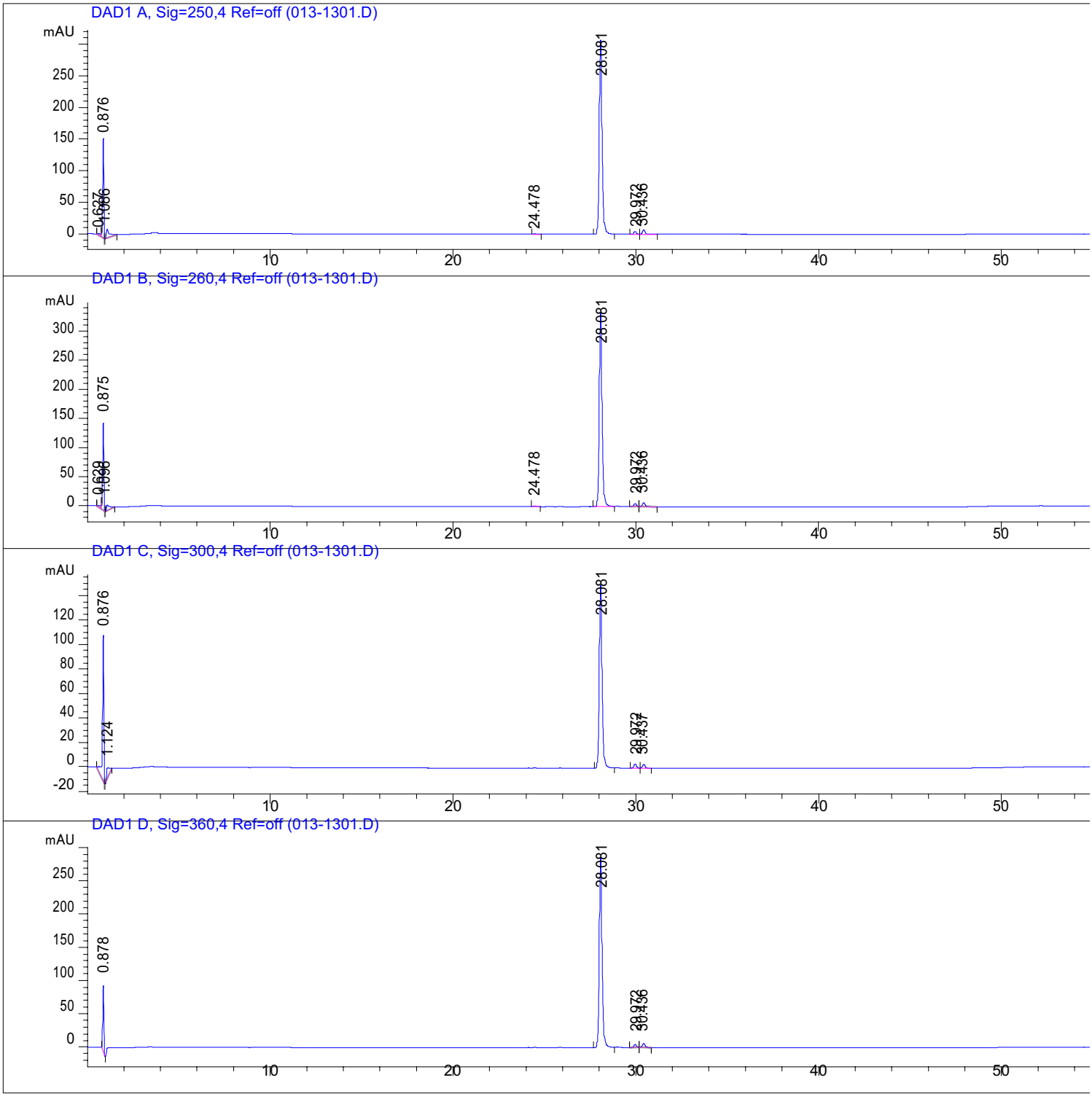
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.878	BB	0.0583	380.67361	100.32857	67.4135
2	27.597	BB	0.1921	184.01060	14.69248	32.5865
Totals :				564.68422	115.02105	

*** End of Report ***

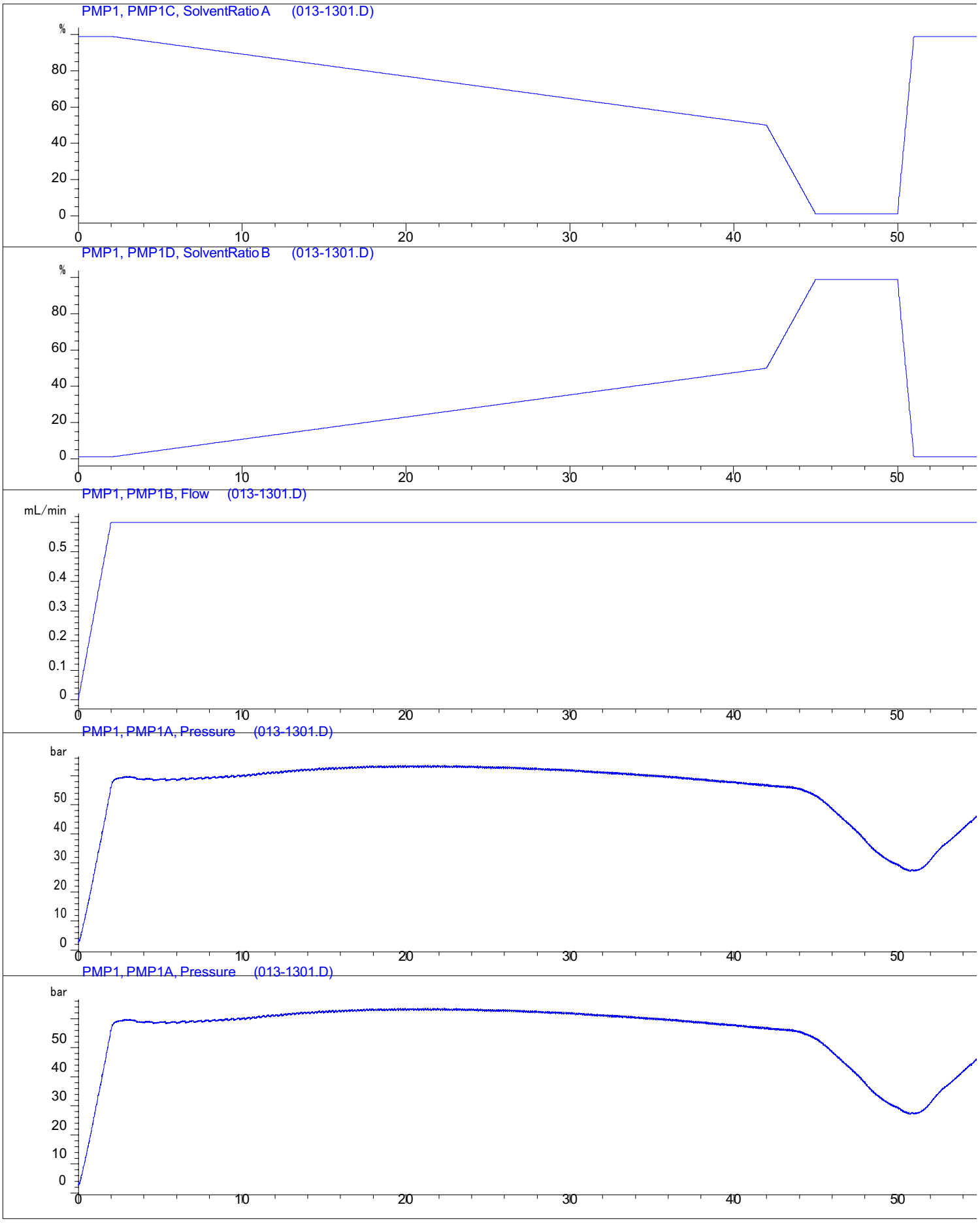
mple Name: S8

==========S8==========

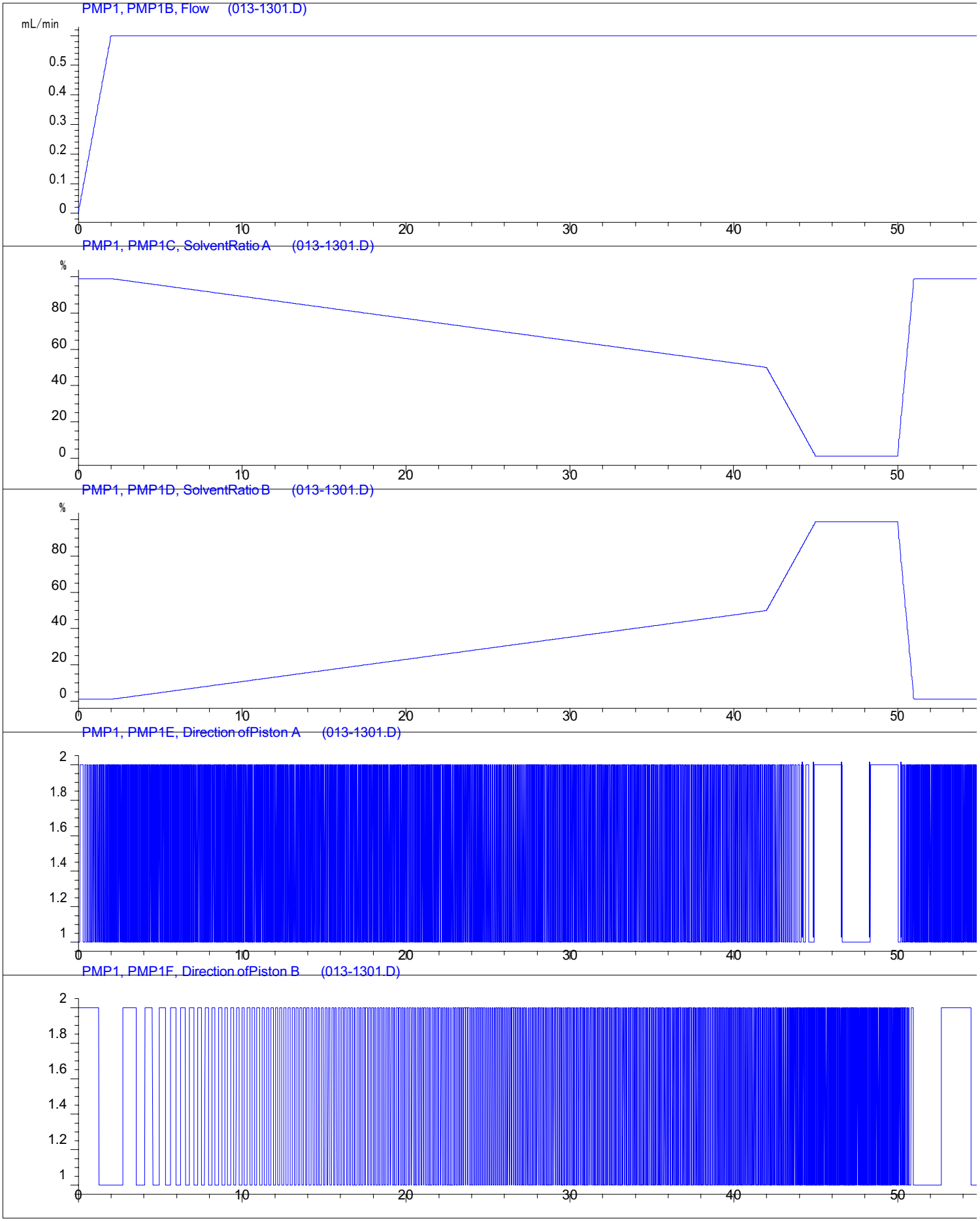
Acq. Operator : SYSTEM Seq. Line : 13
Acq. Instrument : HPLC 4 Location : 13
Injection Date : 5/11/2017 2:00:20 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S8


PMP1, PMP1C, SolventRatio A (013-1301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (013-1301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (013-1301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (013-1301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (013-1301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S8


PMP1, PMP1B, Flow (013-1301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (013-1301.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (013-1301.D)
PMP1, PMP1E, Direction ofPiston A (013-1301.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (013-1301.D)

mple Name: S8

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.627	BV E	0.2237	42.83832	2.50211	1.0094
2	0.876	VB R	0.0614	640.96368	157.75931	15.1036
3	1.086	BB	0.1727	183.10904	14.76226	4.3148
4	24.478	BB	0.1399	9.94272	1.09525	0.2343
5	28.081	BB	0.1592	3248.46655	307.55197	76.5465
6	29.972	BV	0.1584	43.64417	4.15905	1.0284
7	30.436	VB	0.1647	74.81878	6.88896	1.7630

Totals : 4243.78326 494.71892

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.629	BV E	0.2198	45.92606	2.76514	1.0246
2	0.875	VB R	0.0642	646.19141	150.10028	14.4170
3	1.096	BB	0.1710	122.42313	9.71766	2.7314
4	24.478	BB	0.1372	10.59017	1.17423	0.2363
5	28.081	BB	0.1592	3524.41602	333.70734	78.6324
6	29.972	BV	0.1583	57.83500	5.51534	1.2903
7	30.436	VB	0.1648	74.76064	6.88009	1.6680

Totals : 4482.14242 509.86007

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.876	BB	0.0677	544.48273	118.23140	23.6976
2	1.124	BB	0.1691	103.81461	8.83397	4.5183
3	28.081	BB	0.1592	1583.88879	150.00385	68.9358
4	29.972	BB	0.1554	33.25615	3.30363	1.4474
5	30.437	BB	0.1580	32.18727	3.12839	1.4009

Totals : 2297.62955 283.50124

mple Name: S8

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.878	BB	0.0581	379.62265	100.53841	10.8068
2	28.081	BB	0.1593	3024.73218	286.28497	86.1055
3	29.972	BB	0.1531	44.65593	4.45047	1.2712
4	30.436	BB	0.1592	63.80901	6.14309	1.8165
Totals :				3512.81977	397.41695	

*** End of Report ***

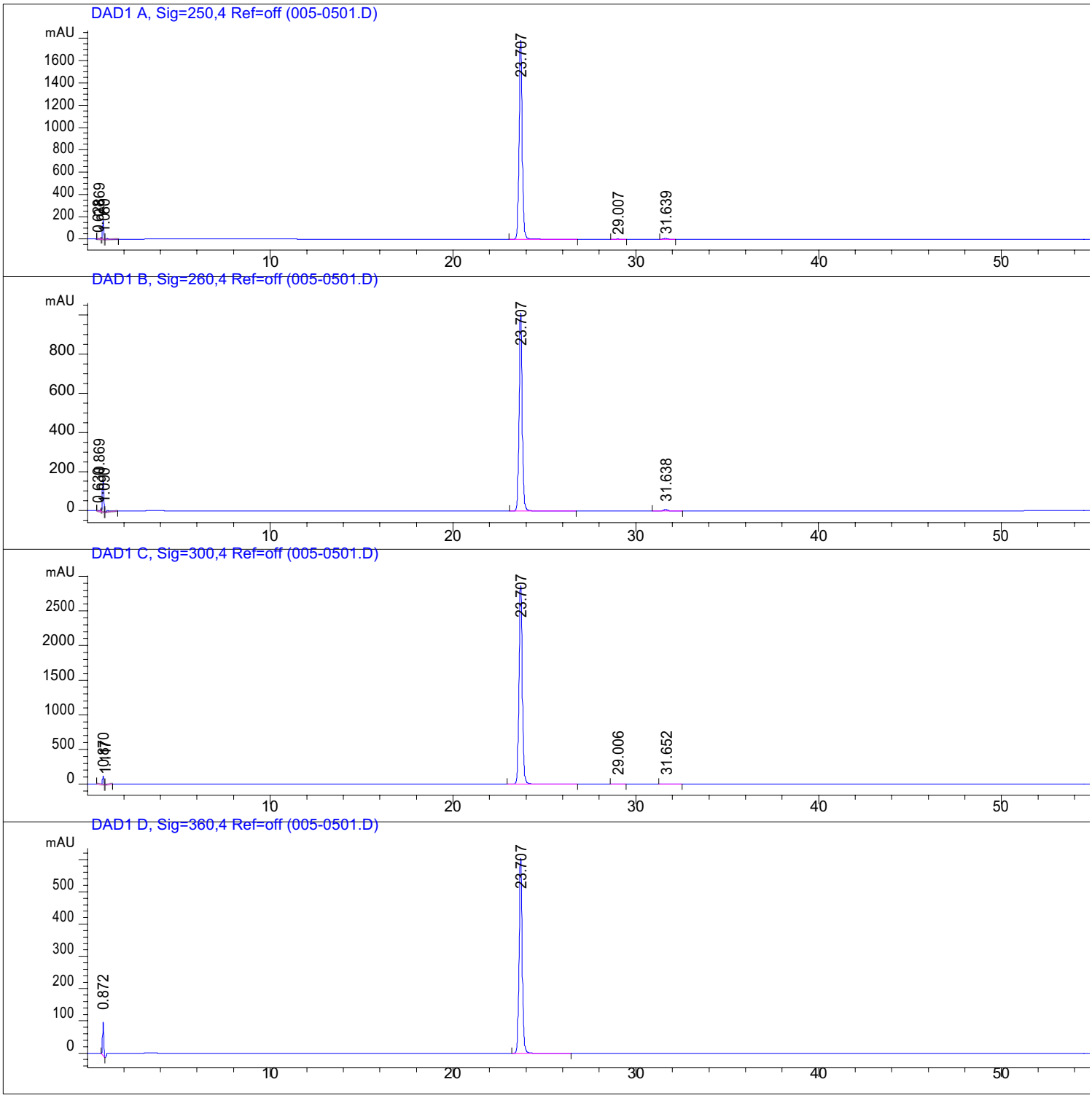
Appendix I

Standard curve caffeic acid HPLC chromatograms

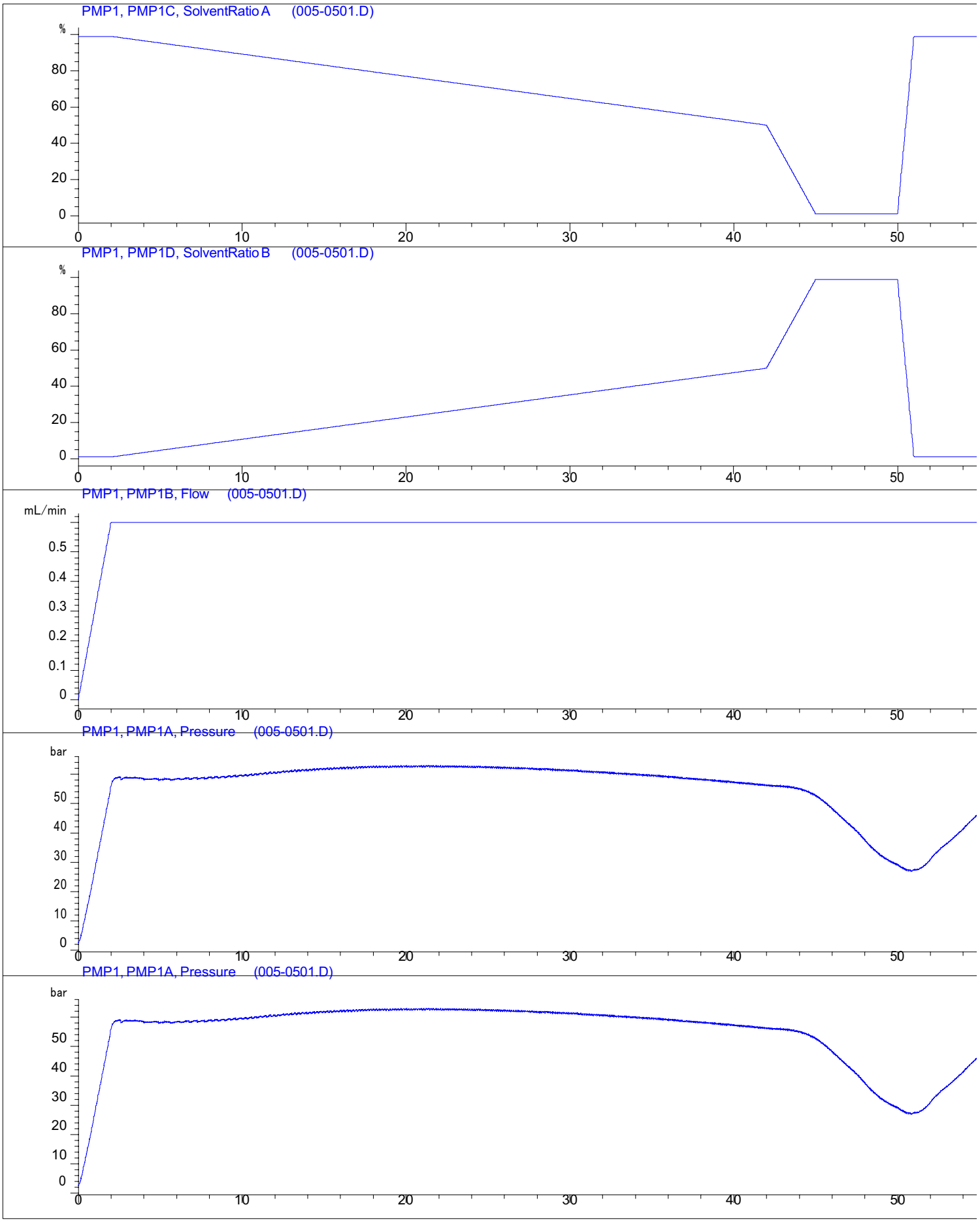
mple Name: S4

==========

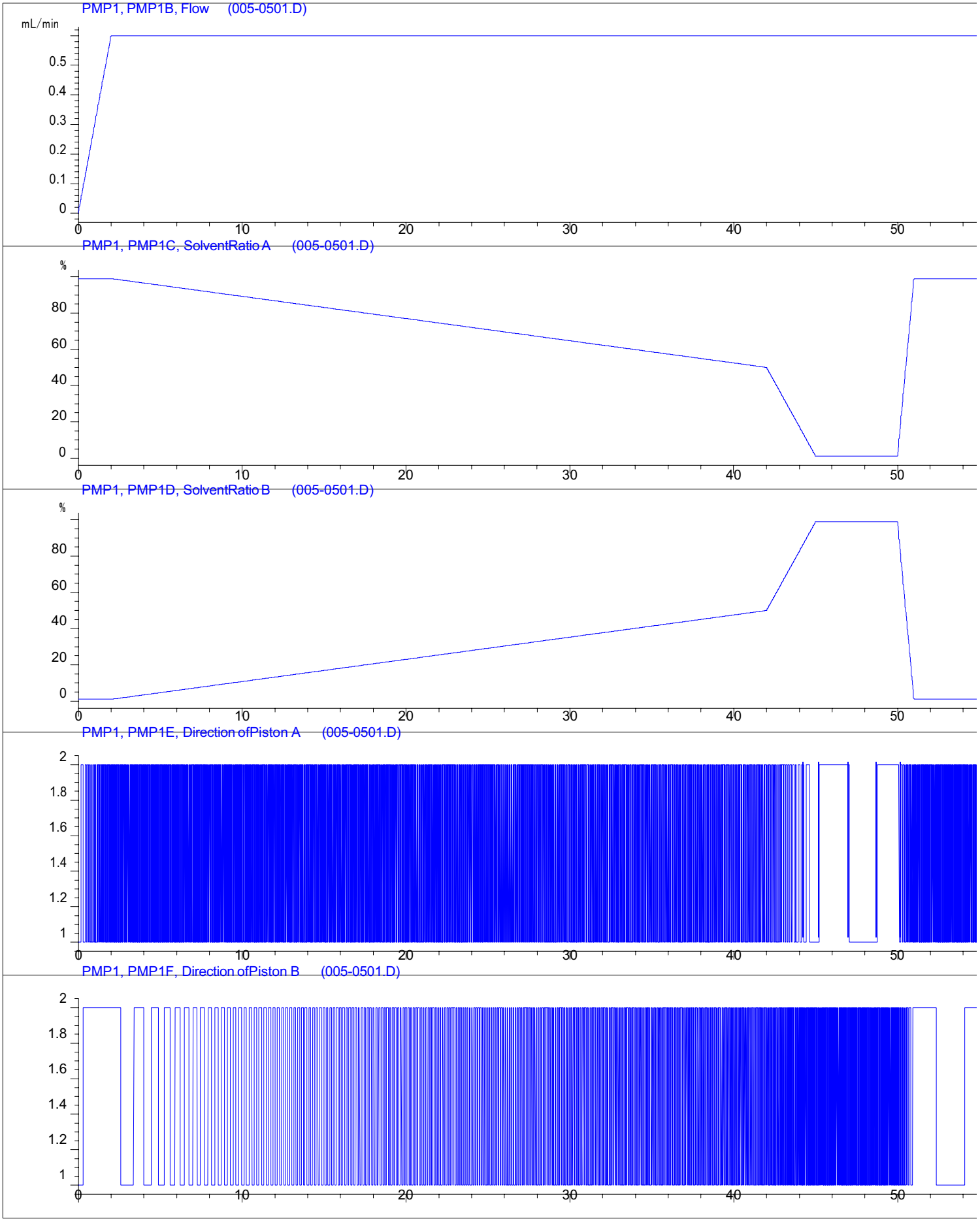
Acq. Operator : SYSTEM Seq. Line : 5
Acq. Instrument : HPLC 4 Location : 5
Injection Date : 5/10/2017 6:24:06 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S4


PMP1, PMP1C, SolventRatio A (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (005-0501.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (005-0501.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (005-0501.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S4


PMP1, PMP1B, Flow (005-0501.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (005-0501.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1E, Direction ofPiston A (005-0501.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50
PMP1, PMP1F, Direction ofPiston B (005-0501.D)
2
1.8
1.6
1.4
1.2
1
0
10
20
30
40
50

mple Name: S4

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.628	BV E	0.1841	30.28533	2.23554	0.1356
2	0.869	VB R	0.0637	723.04901	169.64560	3.2377
3	1.080	BB	0.1667	162.32394	13.65316	0.7269
4	23.707	BB	0.1814	2.12986e4	1782.94824	95.3710
5	29.007	BB	0.2026	18.72082	1.37681	0.0838
6	31.639	BB	0.2434	99.38278	6.25303	0.4450

Totals : 2.23324e4 1976.11238

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.630	BV E	0.1831	34.02602	2.52663	0.2599
2	0.869	VB R	0.0690	747.54852	164.60478	5.7104
3	1.090	BB	0.2111	149.38431	9.31497	1.1411
4	23.707	BB	0.1785	1.20207e4	1012.74609	91.8243
5	31.638	BB	0.2566	139.31746	8.18257	1.0642

Totals : 1.30909e4 1197.37503

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.870	BB	0.0684	581.33008	124.74999	1.5619
2	1.117	BB	0.1770	112.62622	8.93653	0.3026
3	23.707	BB	0.1963	3.64366e4	2866.94238	97.8937
4	29.006	BB	0.1974	32.25098	2.45317	0.0866
5	31.652	BB	0.2732	57.77931	3.22290	0.1552

Totals : 3.72206e4 3006.30497

mple Name: S4

Signal 4: DAD1 D, Sig=360,4 Ref=off

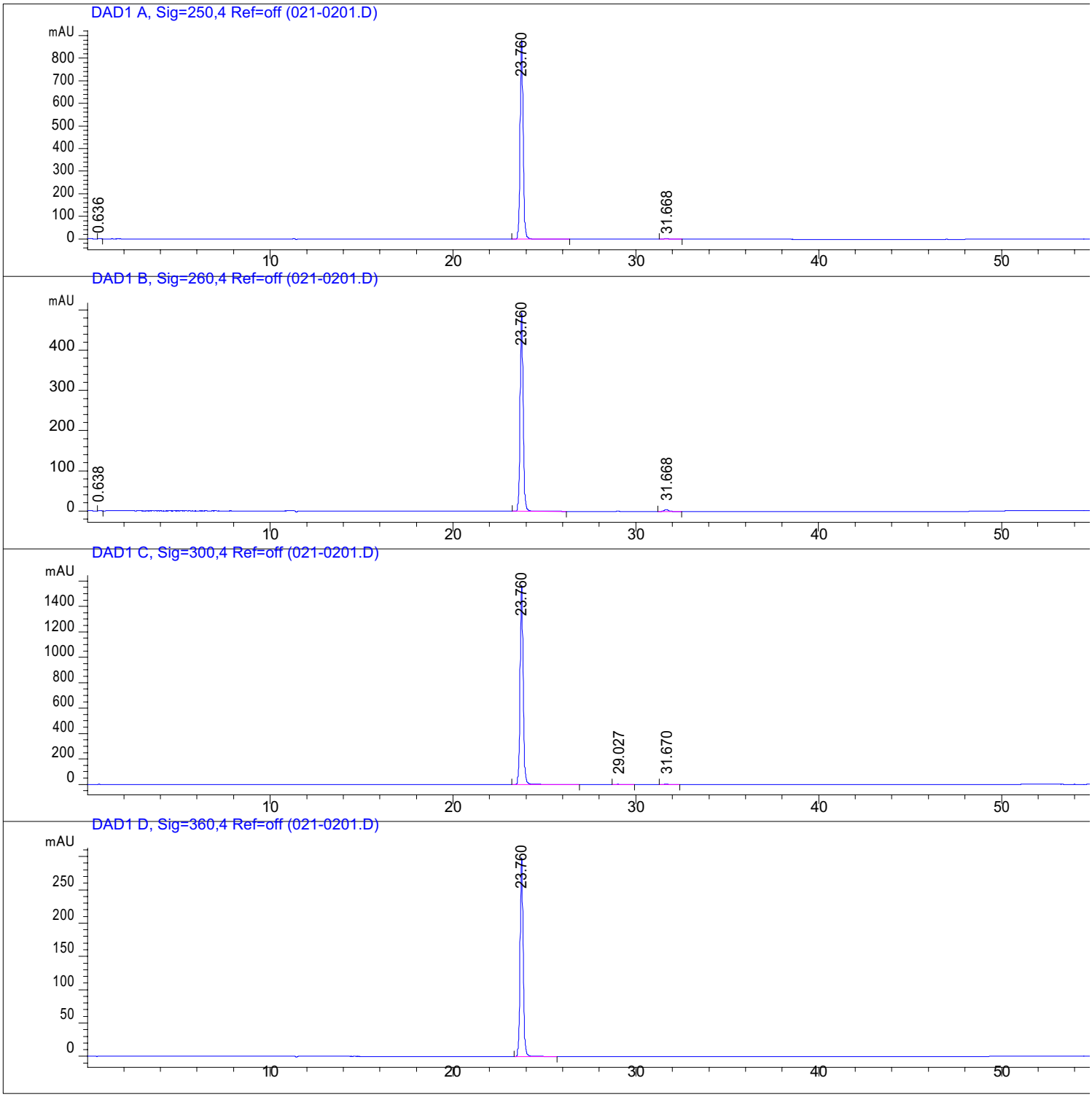
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.872	BB	0.0589	397.16681	103.12437	5.2110
2	23.707	BB	0.1813	7224.53857	605.08350	94.7890
Totals :				7621.70538	708.20786	

*** End of Report ***

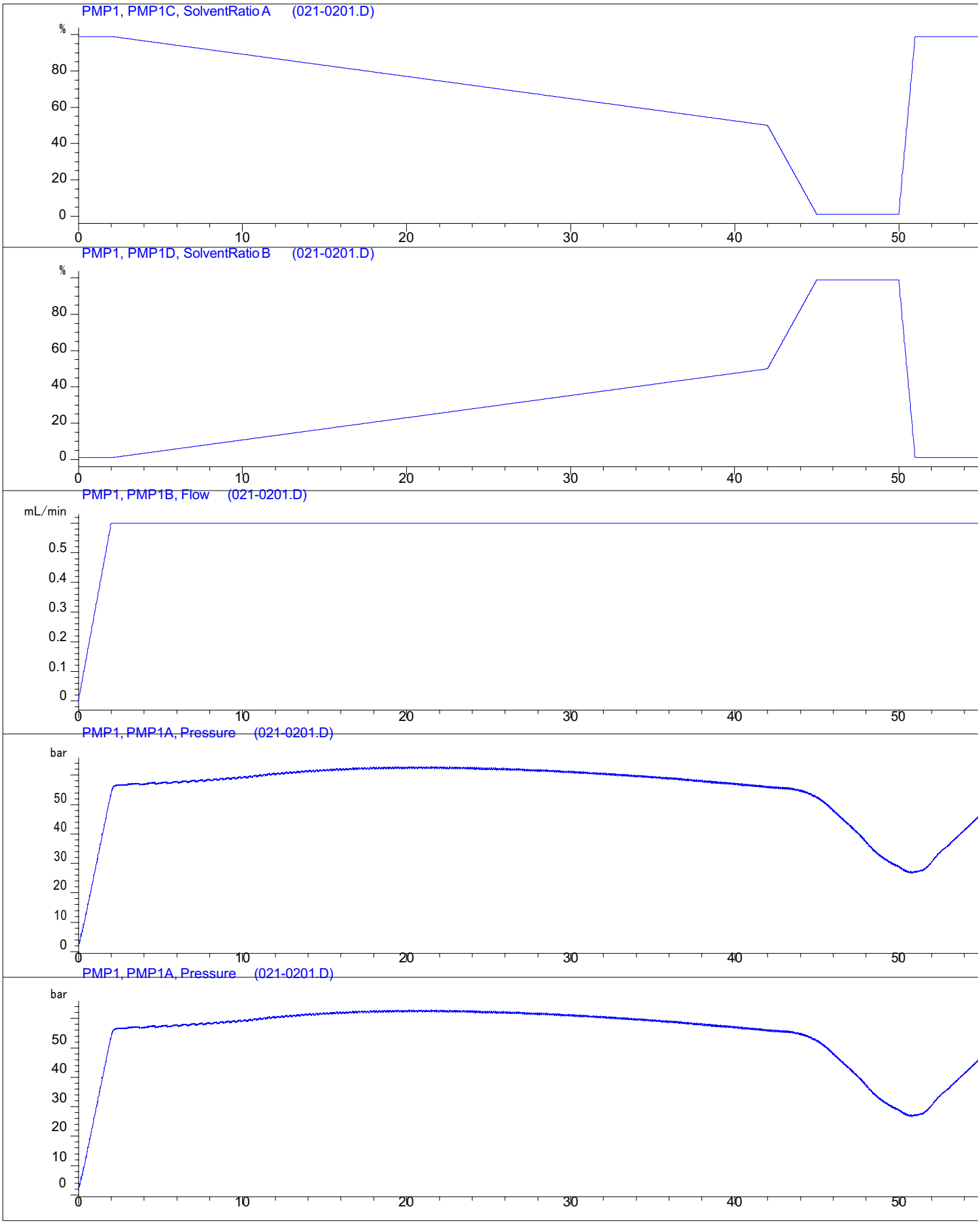
mple Name: S4-0,5

==========

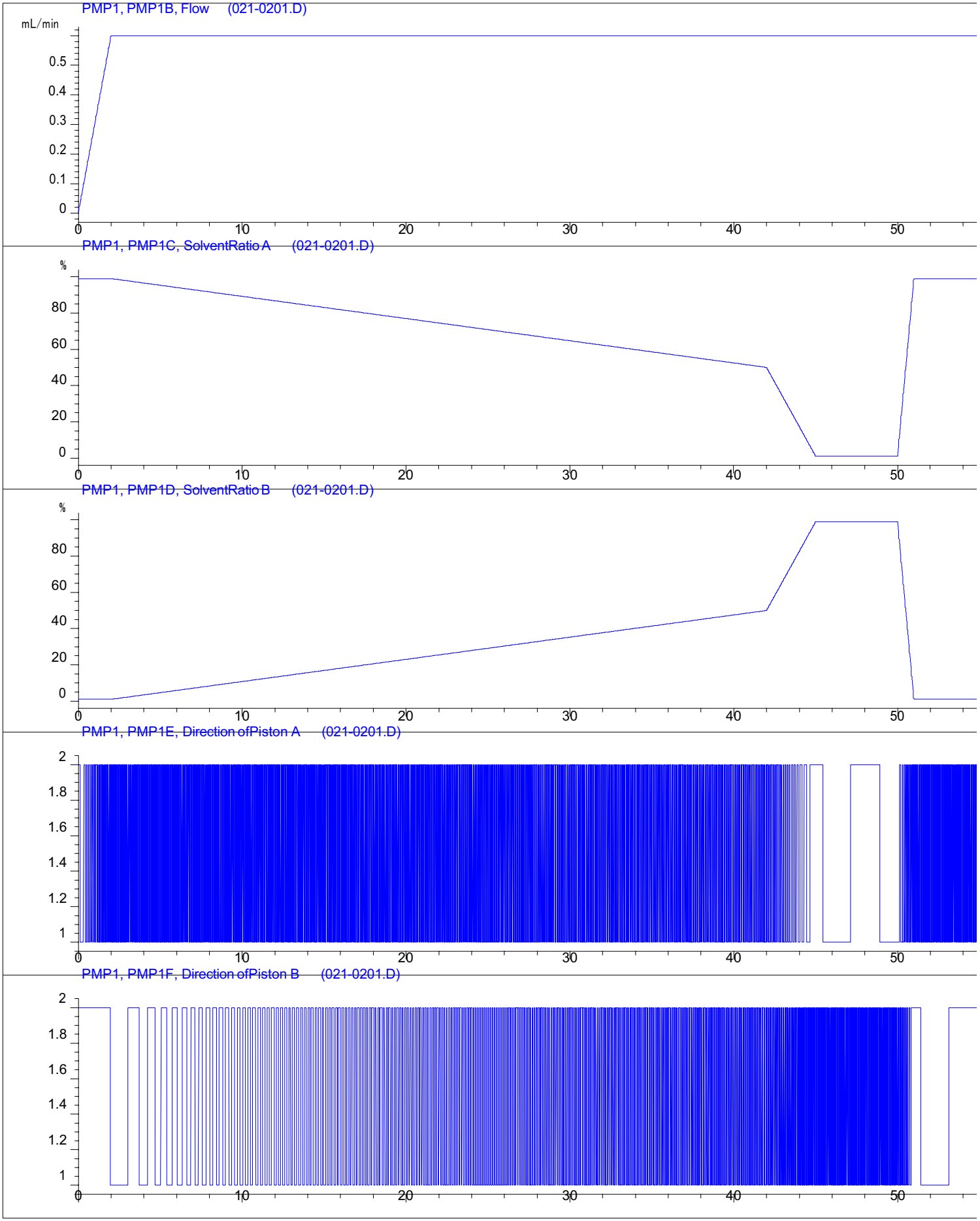
Acq. Operator : SYSTEM Seq. Line : 2
Acq. Instrument : HPLC 4 Location : 21
Injection Date : 5/11/2017 11:05:29 AM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\DEF_LC.S
Method : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\Optimized method = method 12.M
(Sequence Method)
Last changed : 5/11/2017 10:50:25 AM by SYSTEM



mple Name: S4-0,5



mple Name: S4-0,5



mple Name: S4-0,5

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.636	BB	0.1225	9.62458	1.21401	0.0945
2	23.760	BB	0.1739	1.01114e4	881.22125	99.3160
3	31.668	BB	0.2417	60.01377	3.73055	0.5895
Totals :				1.01810e4	886.16581	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.638	BB	0.1245	9.85562	1.19155	0.1711
2	23.760	BB	0.1738	5672.03418	494.58688	98.4759
3	31.668	BB	0.2475	77.93007	4.79837	1.3530
Totals :				5759.81987	500.57680	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	23.760	BB	0.1742	1.80359e4	1568.18176	99.7590
2	29.027	BB	0.1923	14.71484	1.17383	0.0814
3	31.670	BB	0.2463	28.86047	1.76963	0.1596
Totals :				1.80795e4	1571.12523	

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	23.760	BB	0.1742	3433.88965	298.59439	100.0000
Totals :				3433.88965	298.59439	

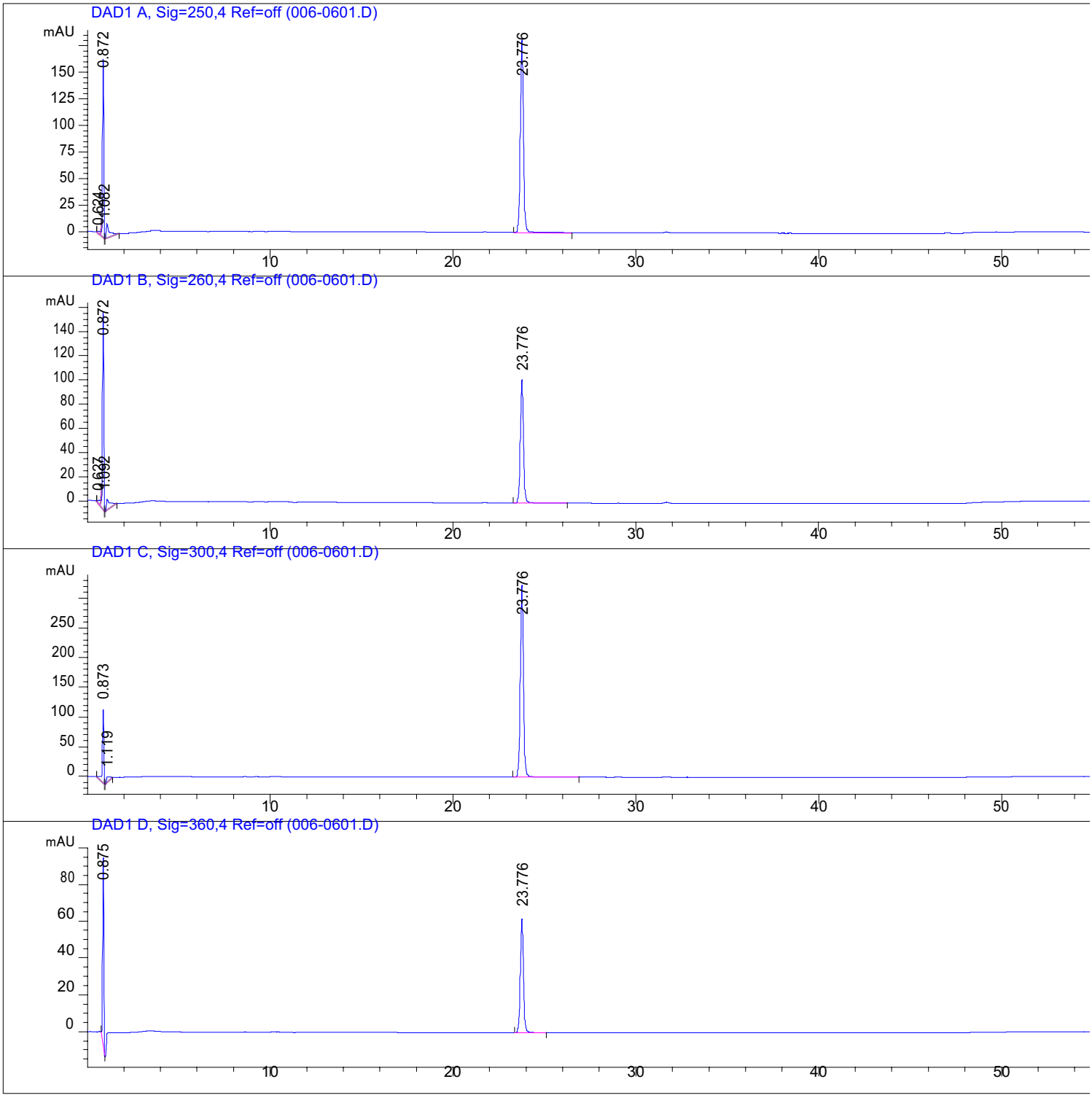
==

*** End of Report ***

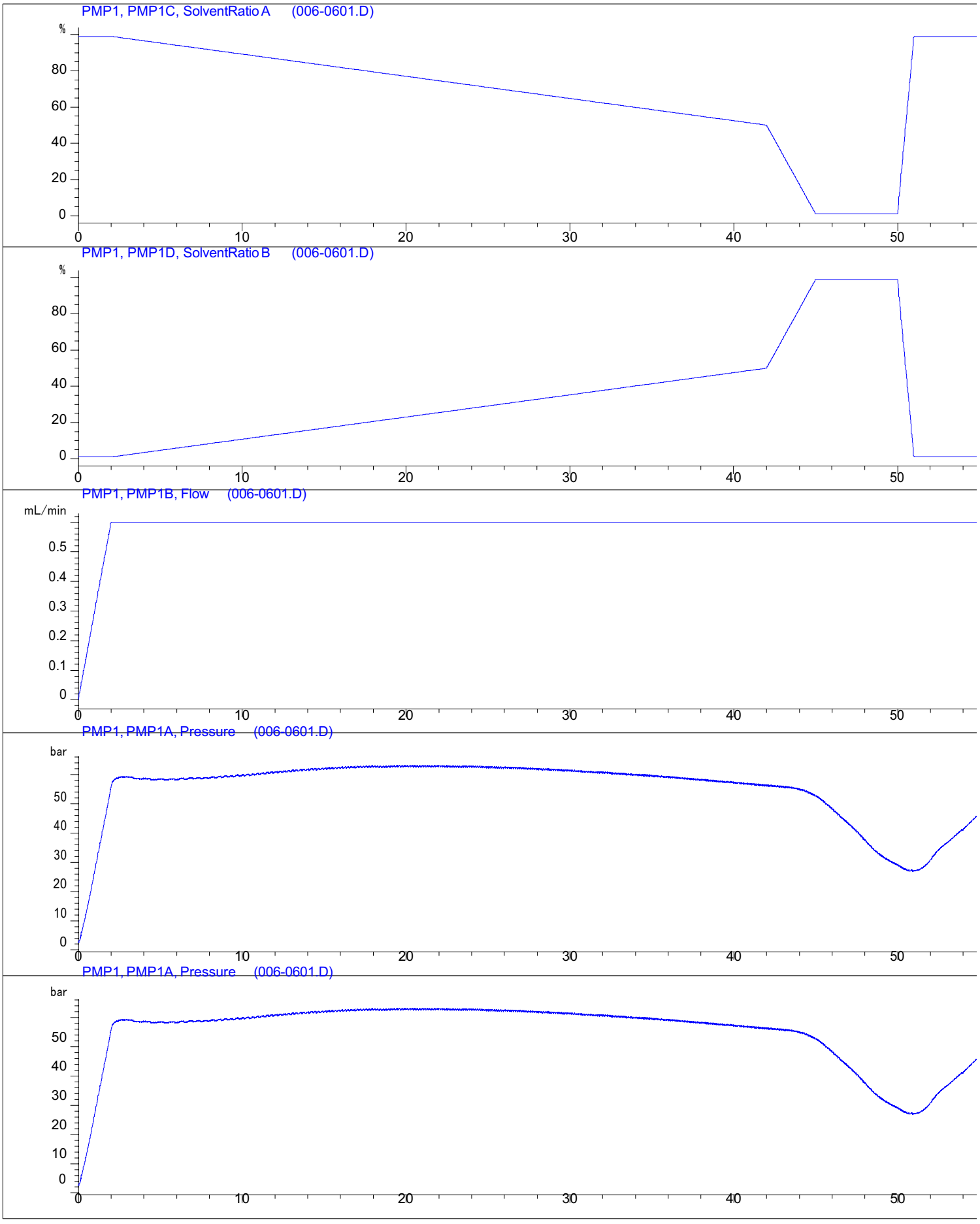
mple Name: S4-1

==========

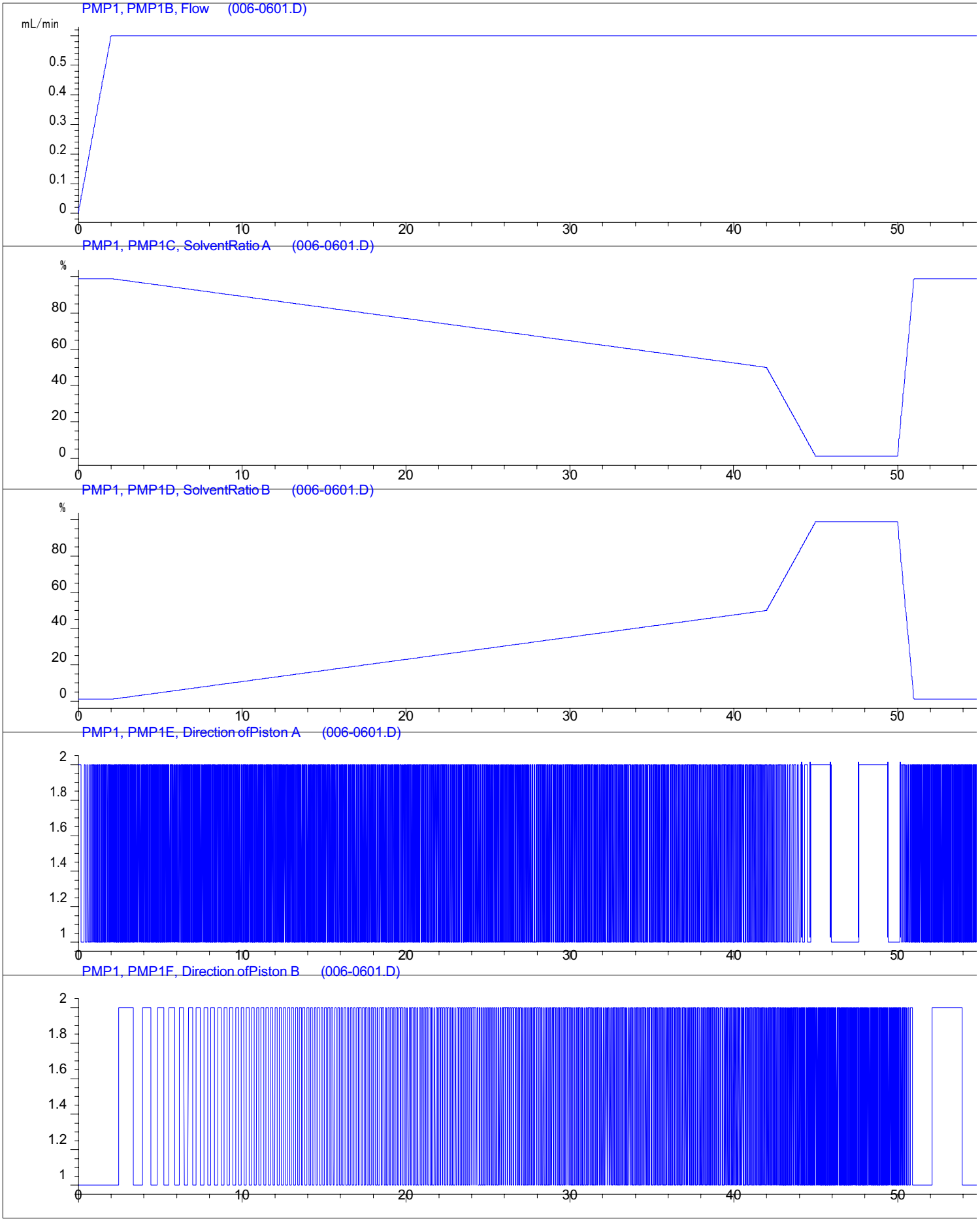
Acq. Operator : SYSTEM Seq. Line : 6
Acq. Instrument : HPLC 4 Location : 6
Injection Date : 5/10/2017 7:21:06 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S4-1



mple Name: S4-1



mple Name: S4-1

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Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.624	BV E	0.2010	33.93164	2.28781	1.1092
2	0.872	VB R	0.0651	706.55072	167.73148	23.0966
3	1.082	BB	0.1764	176.42889	13.86725	5.7673
4	23.776	BB	0.1797	2142.20166	181.49991	70.0269
Totals :				3059.11292	365.38644	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.627	BV E	0.1964	38.03136	2.60319	1.7987
2	0.872	VB R	0.0684	730.64331	162.64673	34.5568
3	1.092	BB	0.2020	141.97522	9.30466	6.7149
4	23.776	BB	0.1800	1203.67493	101.78598	56.9295
Totals :				2114.32482	276.34056	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.873	BB	0.0704	575.92175	123.50809	12.7475
2	1.119	BB	0.1828	120.13378	9.17177	2.6590
3	23.776	BB	0.1797	3821.86572	323.73578	84.5935
Totals :				4517.92126	456.41564	

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S4-1

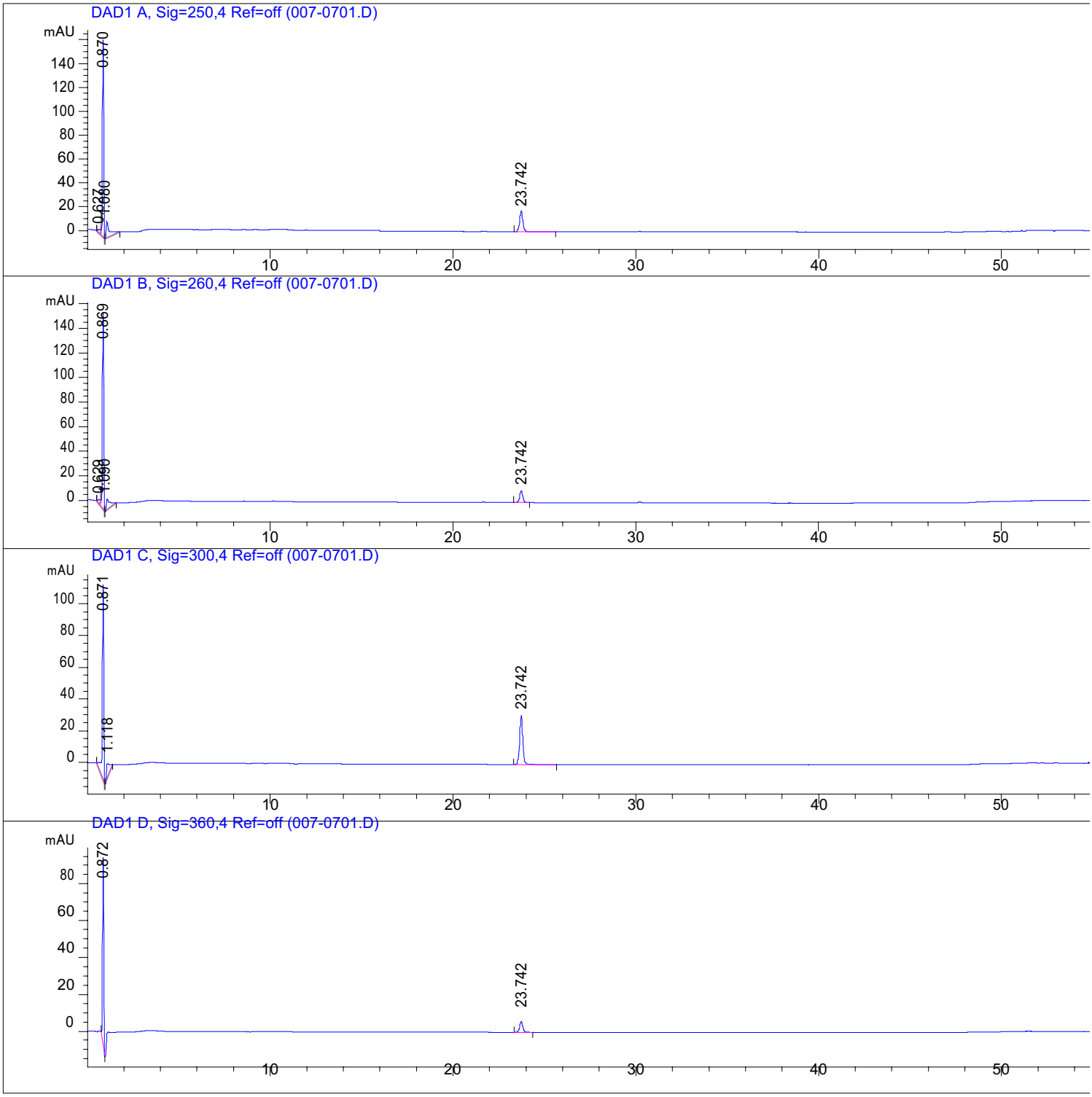
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.875	BB	0.0587	392.64270	102.38562	34.9501
2	23.776	BB	0.1794	730.79633	62.04318	65.0499
Totals :				1123.43903	164.42880	

*** End of Report ***

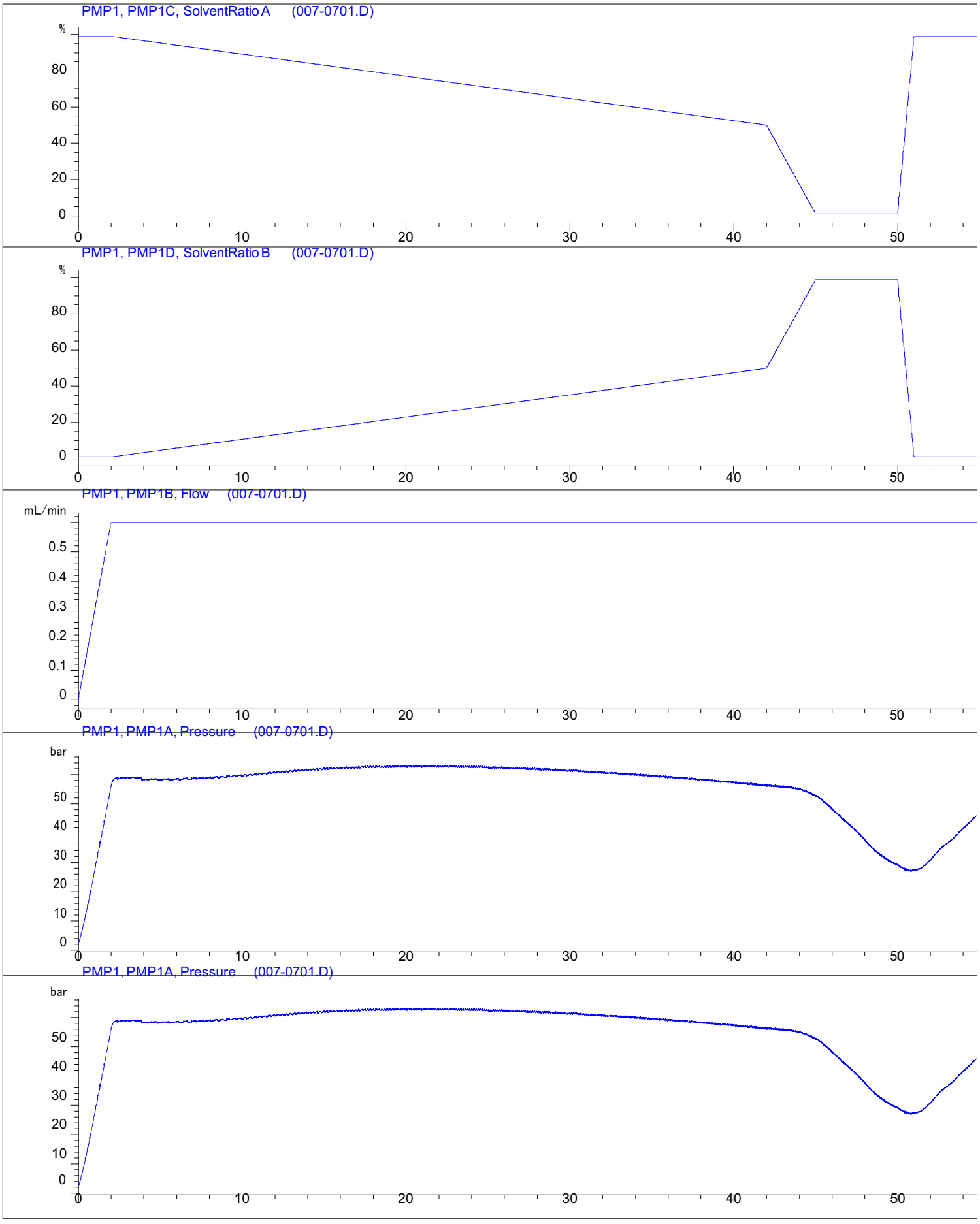
mple Name: S4-2

==========

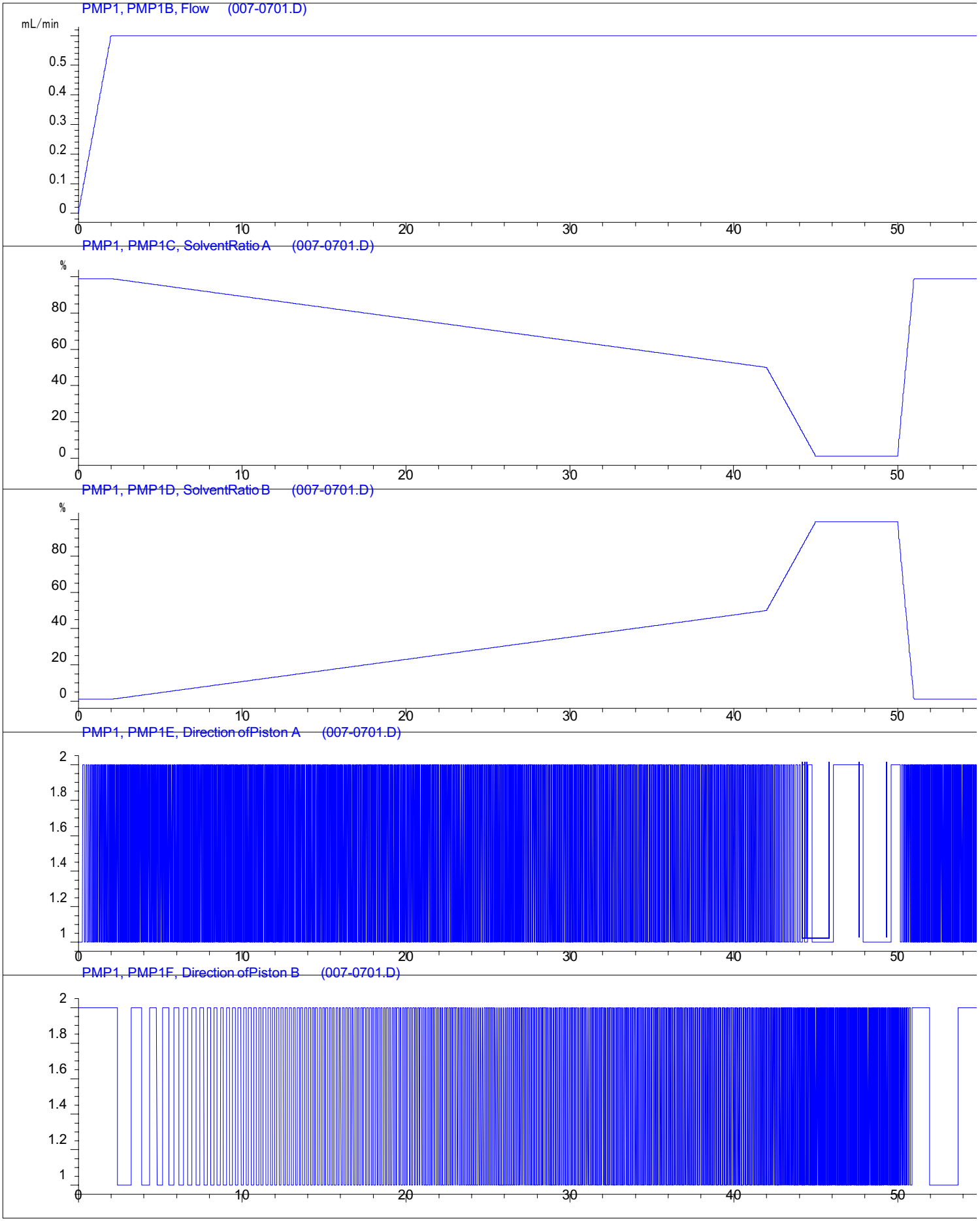
Acq. Operator : SYSTEM Seq. Line : 7
Acq. Instrument : HPLC 4 Location : 7
Injection Date : 5/10/2017 8:18:08 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S4-2



mple Name: S4-2



mple Name: S4-2

==========
Area Percent Report
==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.627	BV E	0.2050	35.19002	2.29328	3.1357
2	0.870	VB R	0.0630	695.79083	165.65689	62.0004
3	1.080	BB	0.1810	184.06531	14.03403	16.4017
4	23.742	BB	0.1795	207.19003	17.32690	18.4622
Totals :				1122.23619	199.31110	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.629	BV E	0.1873	35.53048	2.57052	3.5363
2	0.869	VB R	0.0684	719.40851	160.11333	71.6016
3	1.090	BB	0.1936	136.17955	9.36817	13.5537
4	23.742	BB	0.1767	113.61990	9.69624	11.3084
Totals :				1004.73843	181.74827	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.871	BB	0.0682	567.47705	122.23278	53.9292
2	1.118	BB	0.1799	116.88976	9.09920	11.1084
3	23.742	BB	0.1792	367.89694	30.82935	34.9624
Totals :				1052.26376	162.16134	

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S4-2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.872	BB	0.0587	390.11365	101.80093	84.7646
2	23.742	BB	0.1780	70.11792	5.92615	15.2354
Totals :				460.23157	107.72707	

*** End of Report ***

mple Name: S4-3

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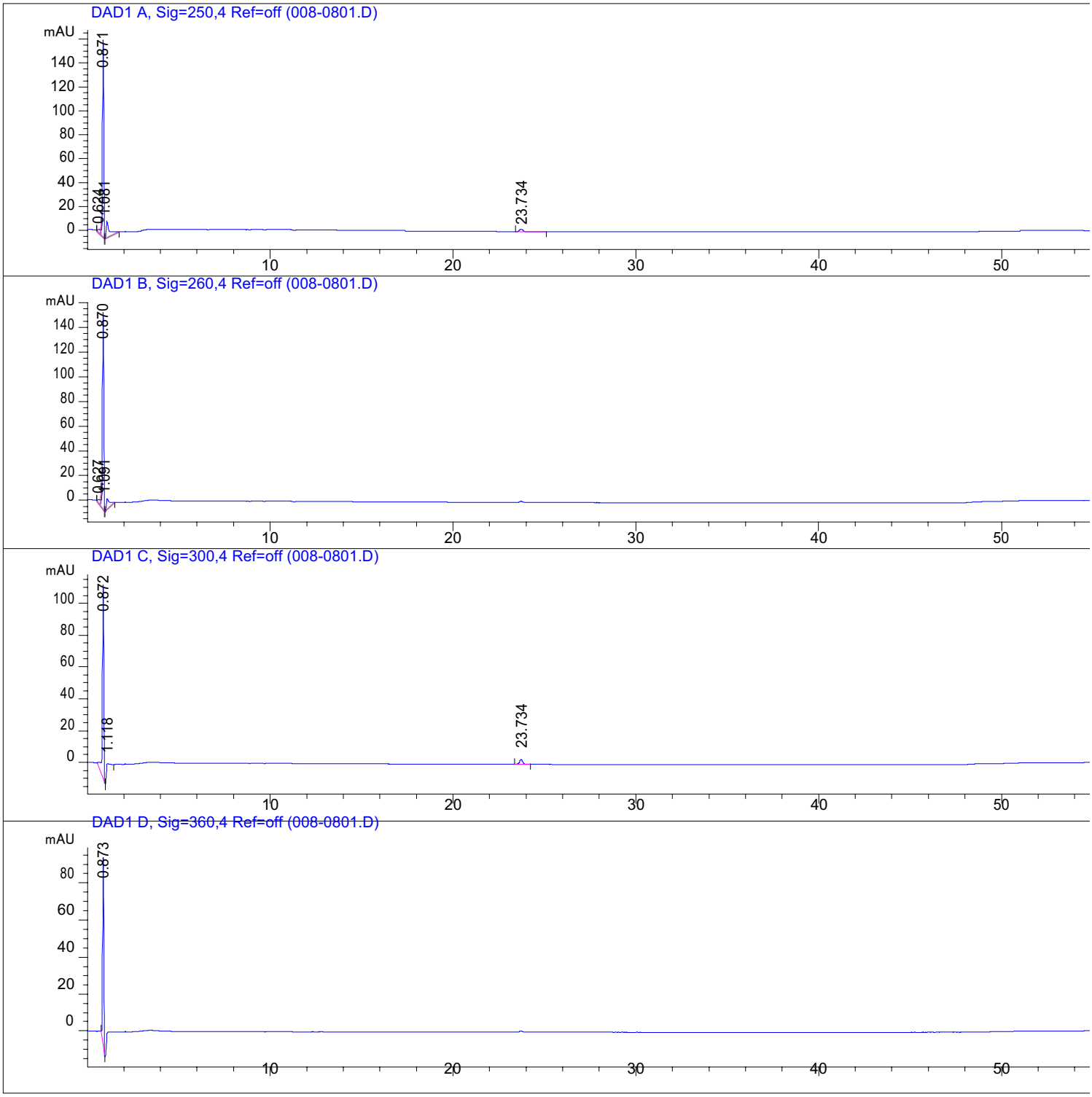
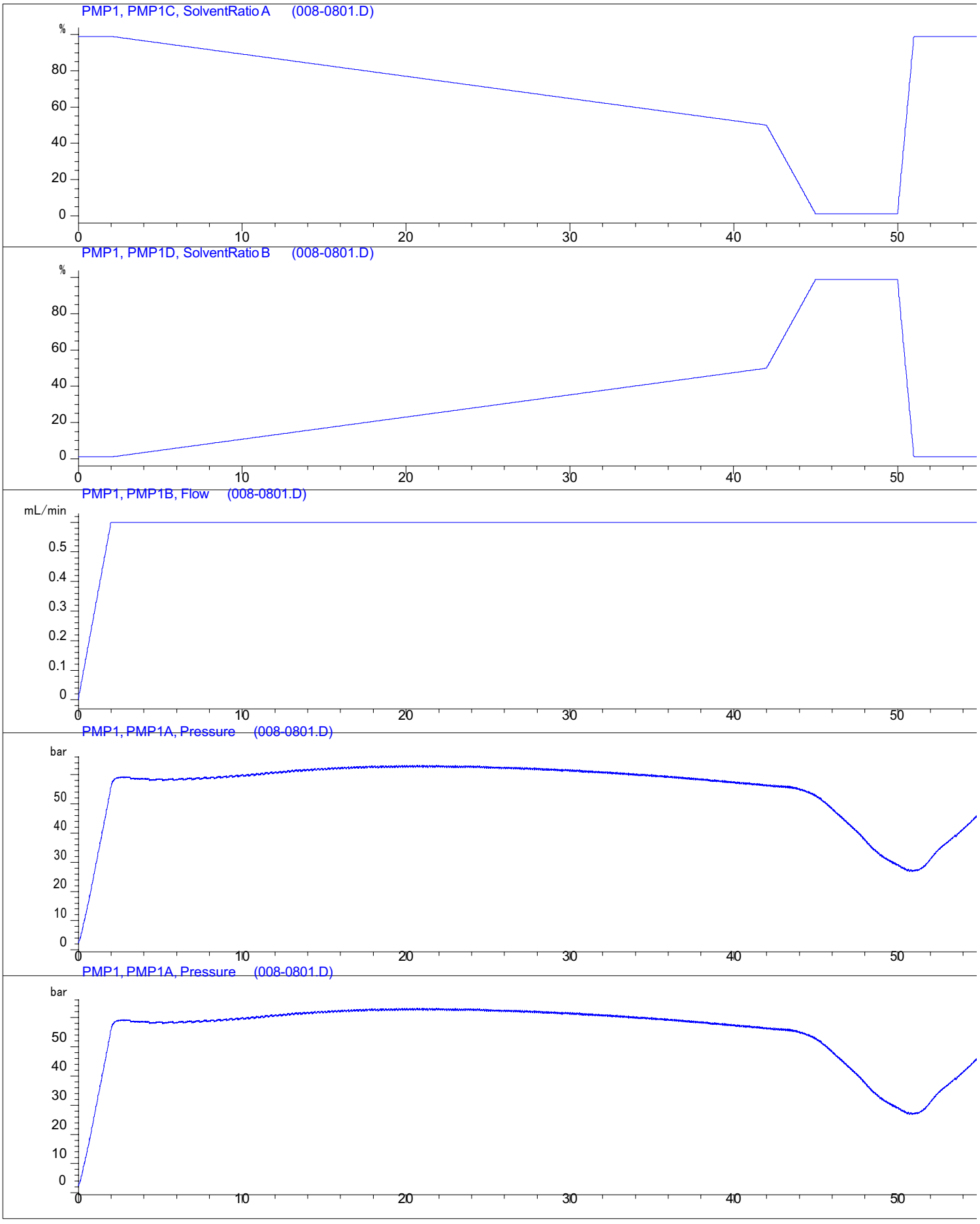
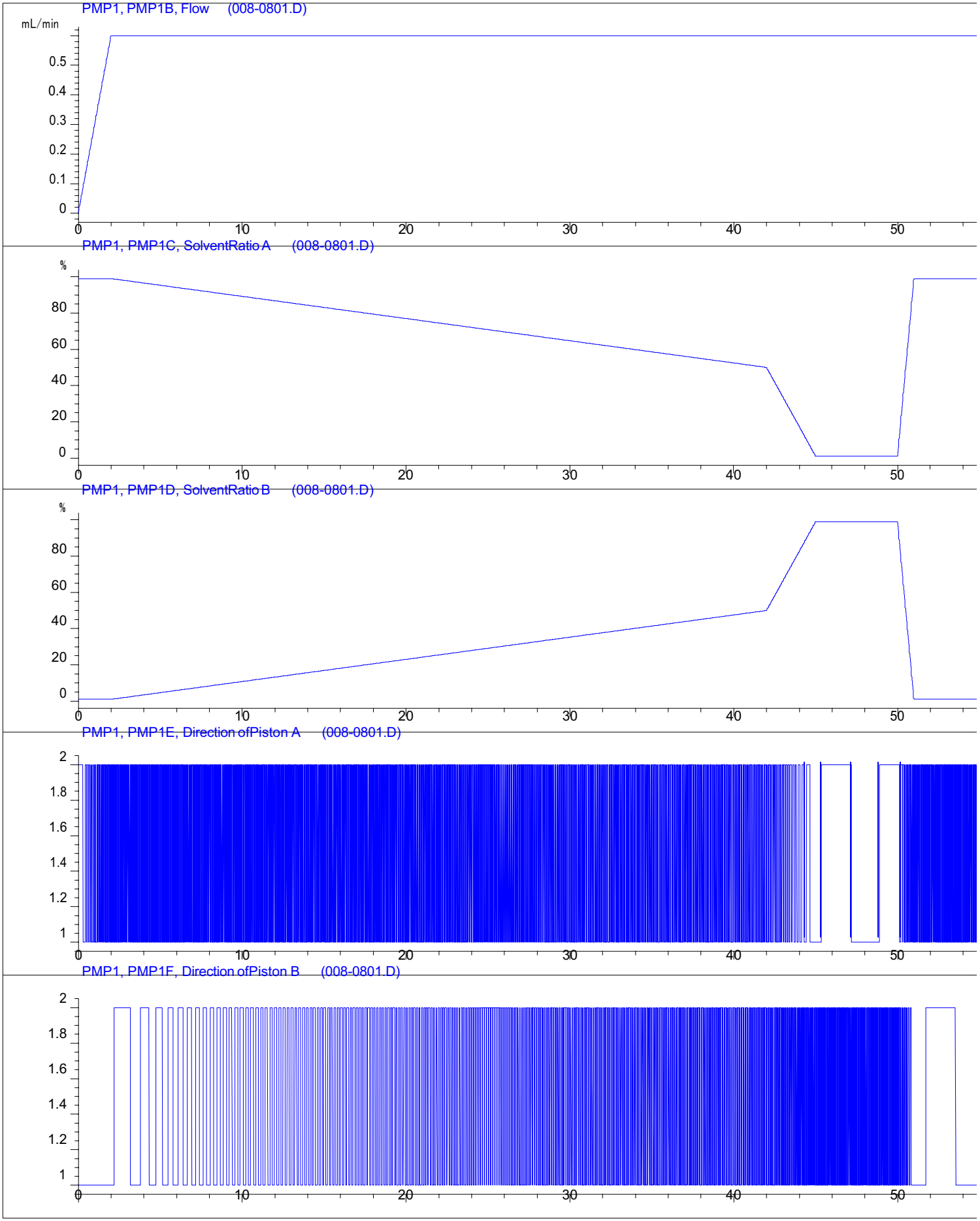
Acq. Operator : SYSTEM Seq. Line : 8
Acq. Instrument : HPLC 4 Location : 8
Injection Date : 5/10/2017 9:15:12 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Sequence - Standards.S
Method : C:\Chem32\1\Data\Sequence - Standards 2017-05-10 15-21-00\Optimized method = method 12.M (Sequence Method)
Last changed : 5/10/2017 3:21:00 PM by SYSTEM



mple Name: S4-3



mple Name: S4-3



mple Name: S4-3

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.624	BV E	0.2033	36.78428	2.39385	3.9471
2	0.871	VB R	0.0628	689.73303	164.91208	74.0110
3	1.081	BB	0.1796	184.07889	14.16309	19.7524
4	23.734	BB	0.1842	21.33745	1.75032	2.2896

Totals : 931.93365 183.21933

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.627	BV E	0.2026	39.91220	2.66682	4.5960
2	0.870	VB R	0.0660	708.00098	158.95673	81.5275
3	1.091	BB	0.1748	120.50613	9.32289	13.8765

Totals : 868.41931 170.94643

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.872	BB	0.0681	565.82416	122.01770	76.6967
2	1.118	BB	0.1972	135.48450	9.56846	18.3647
3	23.734	BB	0.1778	36.43360	3.08534	4.9385

Totals : 737.74226 134.67149

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.873	BB	0.0607	391.36075	102.10653	100.0000

mple Name: S4-3

Totals : 391.36075 102.10653

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*** End of Report ***

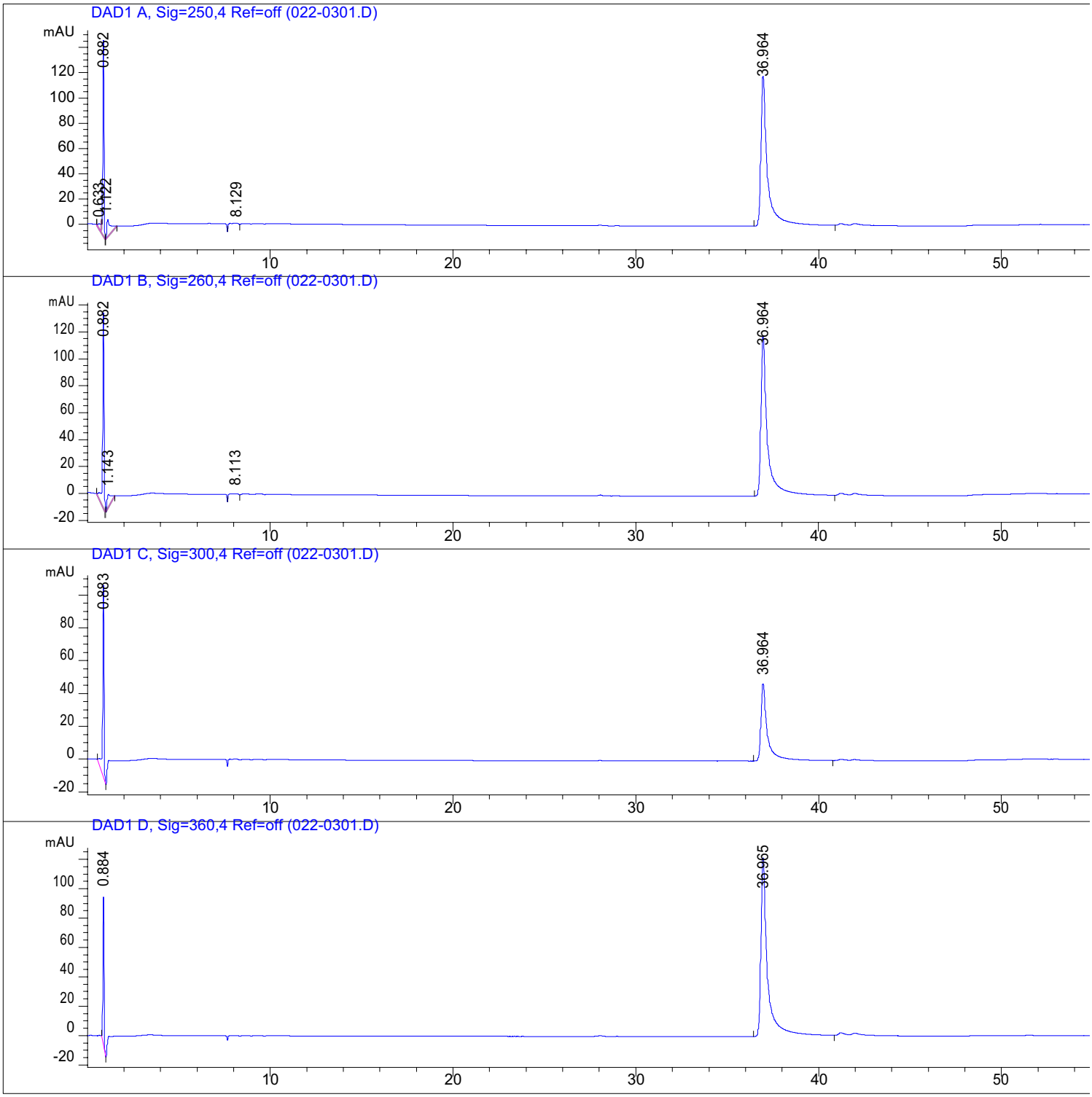
Appendix J

Standard curve quercetin HPLC chromatograms

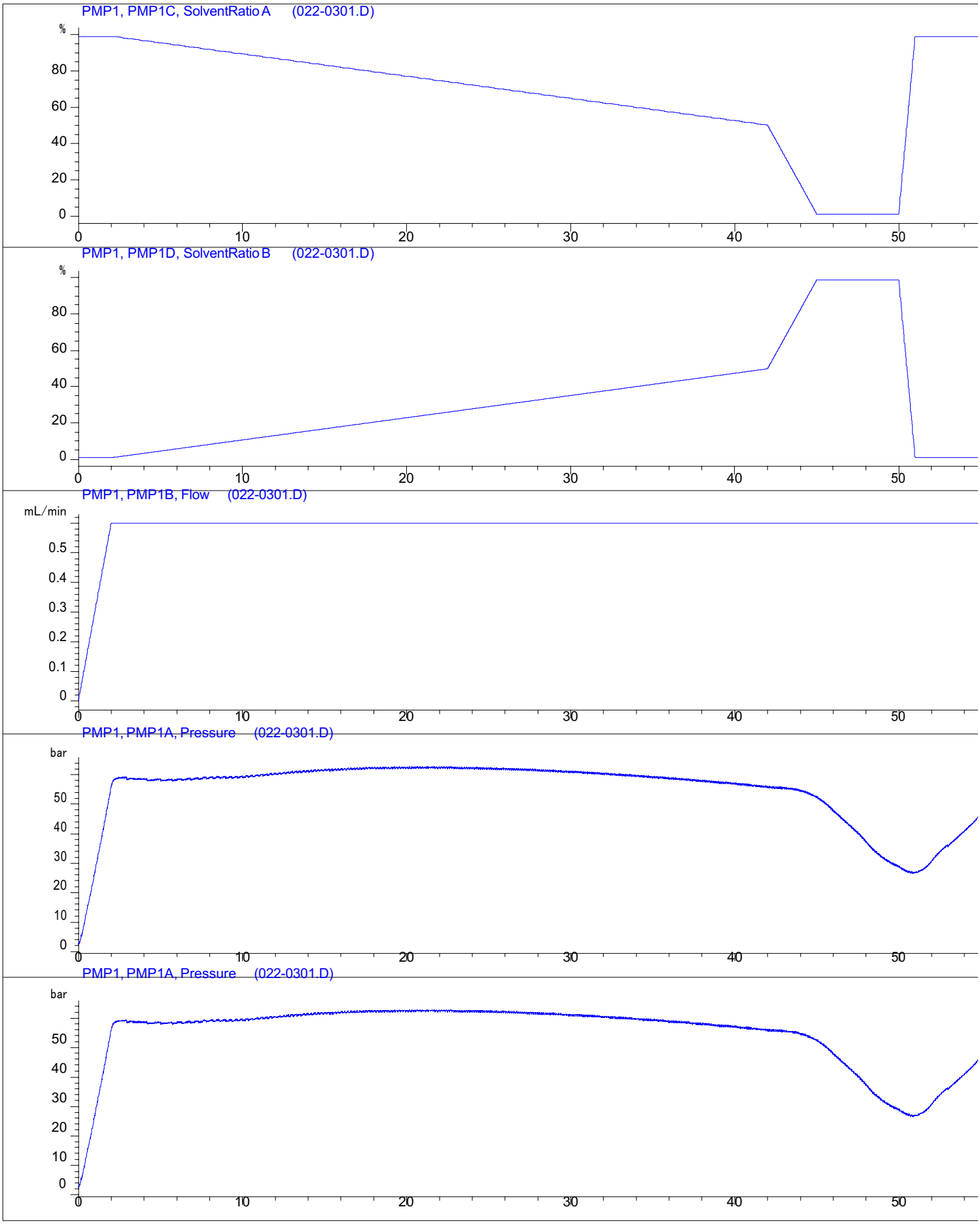
mple Name: S5

==========

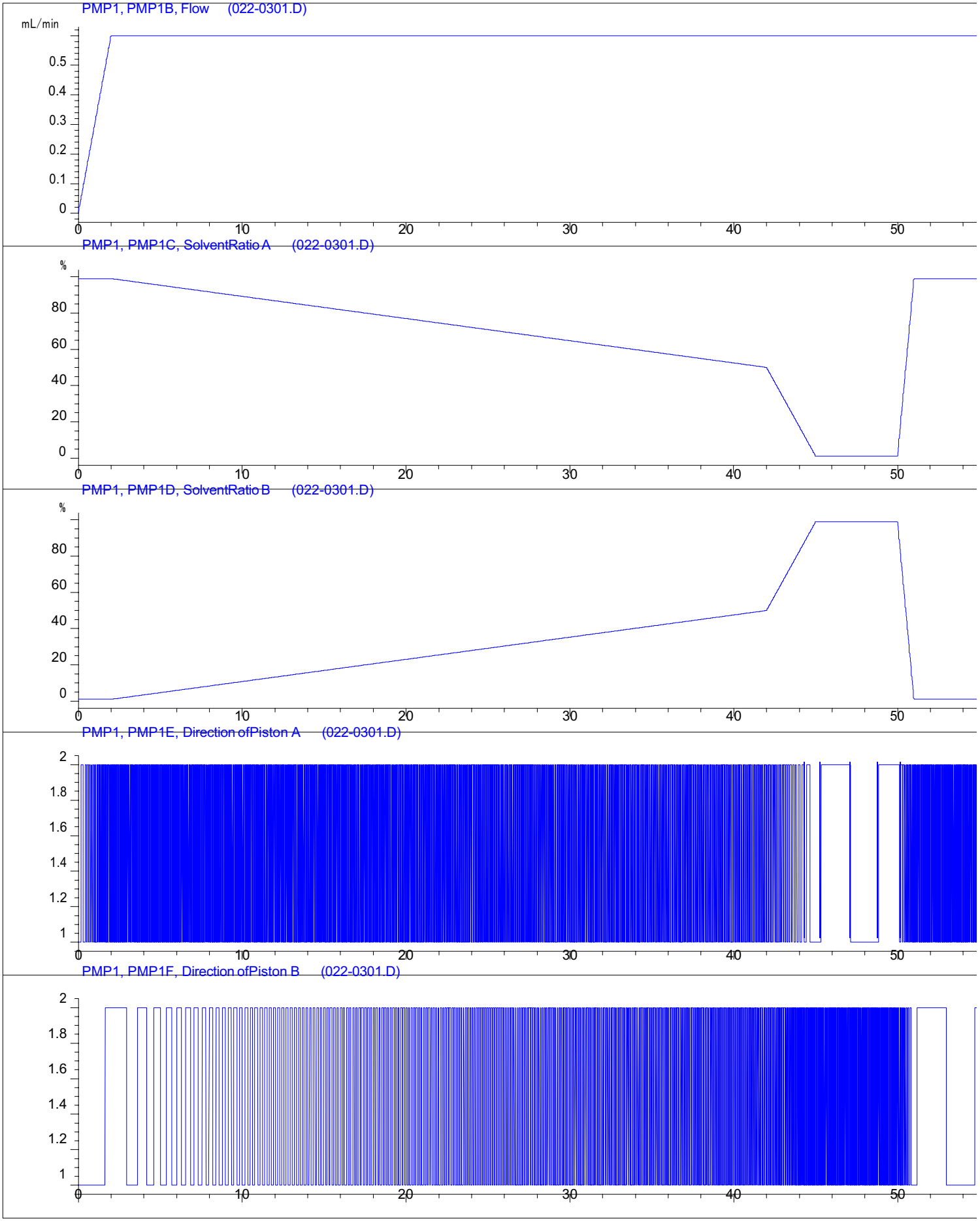
Acq. Operator : SYSTEM Seq. Line : 3
Acq. Instrument : HPLC 4 Location : 22
Injection Date : 5/11/2017 12:02:27 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\DEF_LC.S
Method : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\Optimized method = method 12.M
(Sequence Method)
Last changed : 5/11/2017 10:50:25 AM by SYSTEM



mple Name: S5


PMP1, PMP1C, SolventRatio A (022-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1D, SolventRatio B (022-0301.D)
%
80
60
40
20
0
0
10
20
30
40
50
PMP1, PMP1B, Flow (022-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (022-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50
PMP1, PMP1A, Pressure (022-0301.D)
bar
50
40
30
20
10
0
0
10
20
30
40
50

mple Name: S5


PMP1, PMP1B, Flow (022-0301.D)
mL/min
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
PMP1, PMP1C, SolventRatio A (022-0301.D)
%
80
60
40
20
0
PMP1, PMP1D, SolventRatio B (022-0301.D)
PMP1, PMP1E, Direction ofPiston A (022-0301.D)
2
1.8
1.6
1.4
1.2
1
PMP1, PMP1F, Direction ofPiston B (022-0301.D)

mple Name: S5

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.633	BV E	0.2395	56.90032	3.08105	1.4209
2	0.882	VB R	0.0634	656.13300	154.80637	16.3846
3	1.122	BB	0.1912	198.49310	13.84883	4.9567
4	8.129	BB	0.5743	132.26205	2.74403	3.3028
5	36.964	BB	0.3475	2960.79175	118.51952	73.9351

Totals : 4004.58022 292.99980

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.882	BB	0.0704	696.79718	144.18008	17.8026
2	1.143	BB	0.1842	142.93823	10.41226	3.6519
3	8.113	BB	0.5493	119.78544	2.60113	3.0604
4	36.964	BB	0.3475	2954.50830	118.25484	75.4851

Totals : 3914.02915 275.44831

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.883	BB	0.0701	564.76154	117.40015	32.3839
2	36.964	BB	0.3481	1179.19592	47.10185	67.6161

Totals : 1743.95746 164.50201

Signal 4: DAD1 D, Sig=360,4 Ref=off

mple Name: S5

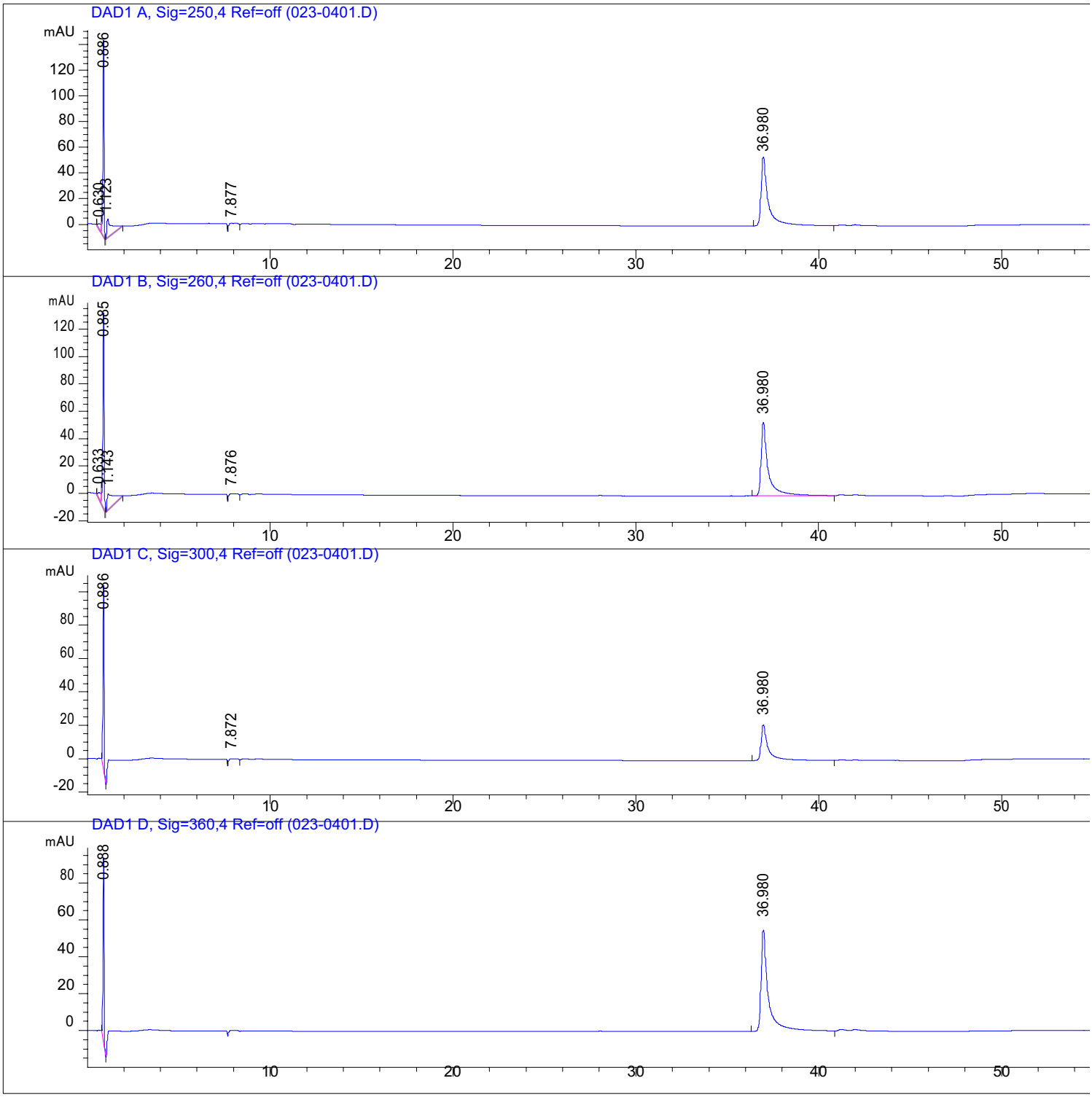
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.884	BB	0.0628	408.84875	102.01109	11.8499
2	36.965	BB	0.3478	3041.38940	121.60992	88.1501
Totals :				3450.23816	223.62101	

*** End of Report ***

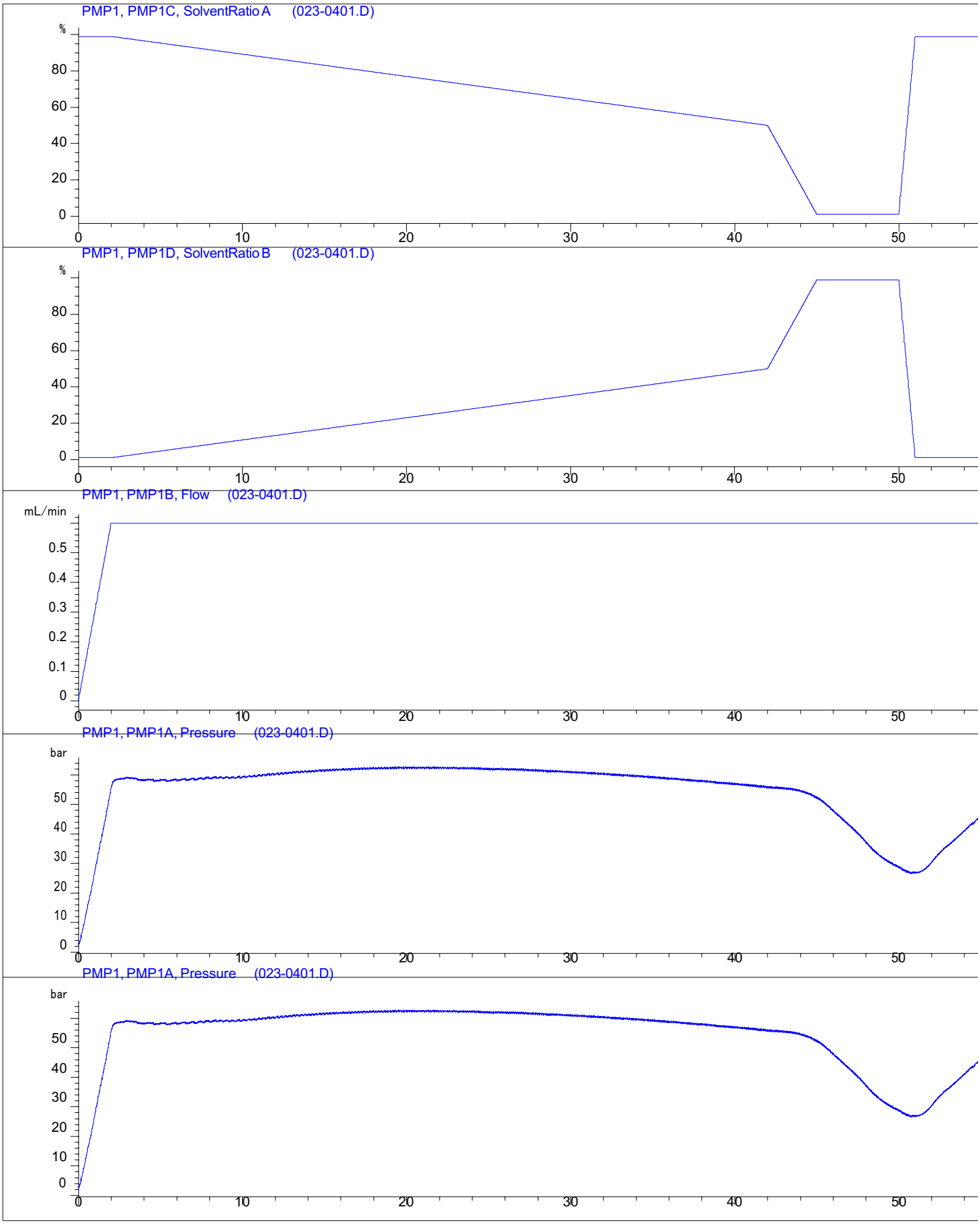
mple Name: S5-0,5

==========

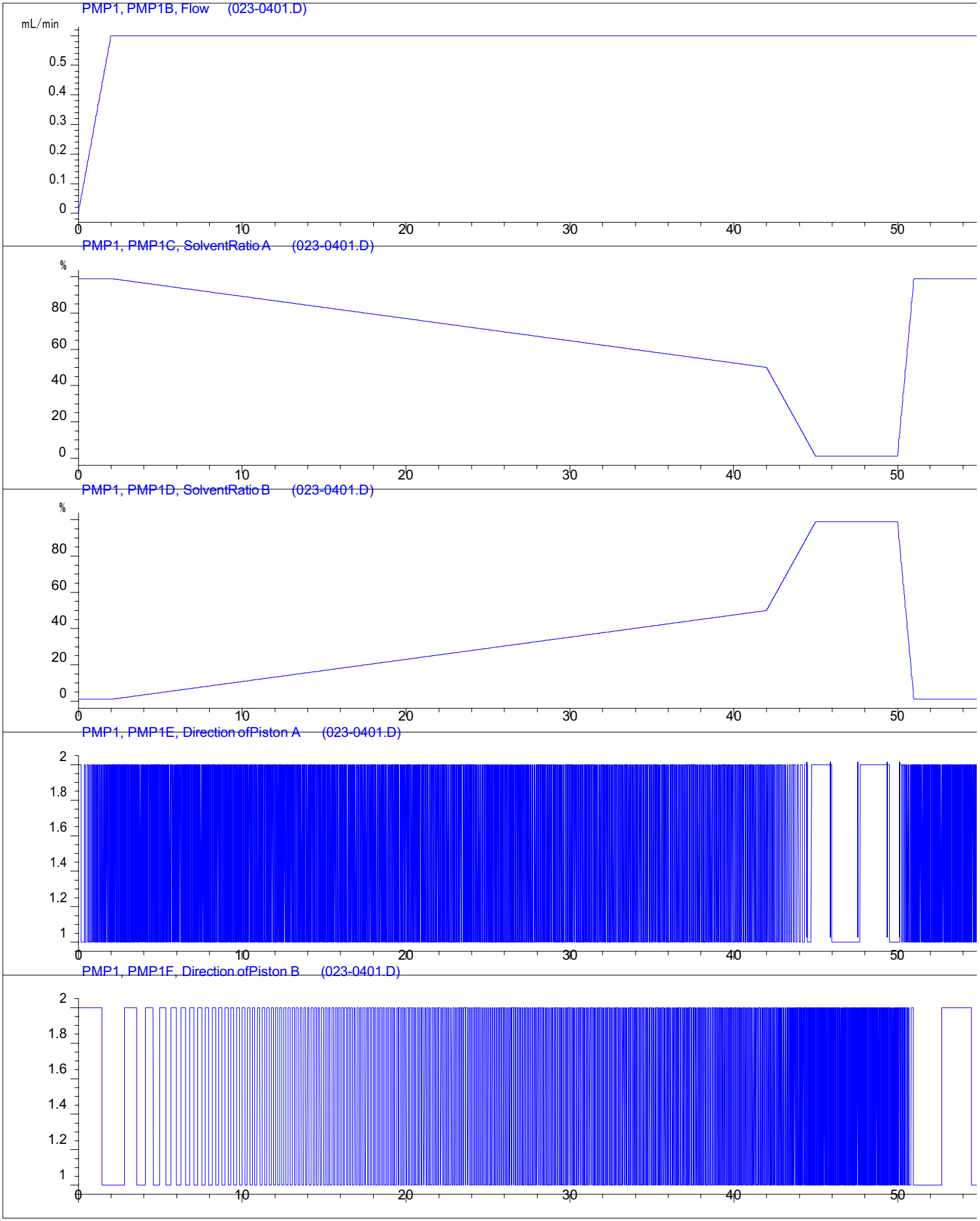
Acq. Operator : SYSTEM Seq. Line : 4
Acq. Instrument : HPLC 4 Location : 23
Injection Date : 5/11/2017 12:59:28 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\DEF_LC.S
Method : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\Optimized method = method 12.M
(Sequence Method)
Last changed : 5/11/2017 10:50:25 AM by SYSTEM



mple Name: S5-0,5



mple Name: S5-0,5



mple Name: S5-0,5

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.630	BV E	0.2694	60.89780	2.91903	2.3623
2	0.886	VB R	0.0647	637.44739	152.71930	24.7272
3	1.123	BB	0.2652	306.62677	14.82842	11.8943
4	7.877	BB	0.3698	122.46102	4.73395	4.7504
5	36.980	BB	0.3758	1450.49109	53.58604	56.2659
Totals :				2577.92407	228.78675	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.633	BV E	0.2650	63.97316	3.14871	2.5088
2	0.885	VB R	0.0669	620.76025	142.17816	24.3439
3	1.143	BB	0.3223	305.12244	11.93283	11.9658
4	7.876	BB	0.3657	111.17622	4.32716	4.3599
5	36.980	BB	0.3761	1448.92664	53.47568	56.8216
Totals :				2549.95871	215.06254	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.886	BB	0.0633	454.42316	112.16961	40.7043
2	7.872	BB	0.3596	81.27874	3.20758	7.2804
3	36.980	BB	0.3777	580.70026	21.32651	52.0153
Totals :				1116.40215	136.70370	

mple Name: S5-0,5

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.888	BB	0.0625	402.20691	100.98257	21.2269
2	36.980	BB	0.3766	1492.59180	55.00674	78.7731
Totals :				1894.79871	155.98931	

*** End of Report ***

mple Name: S5-0,75

==========

Acq. Operator : SYSTEM Seq. Line : 2

Acq. Instrument : HPLC 4 Location : 2

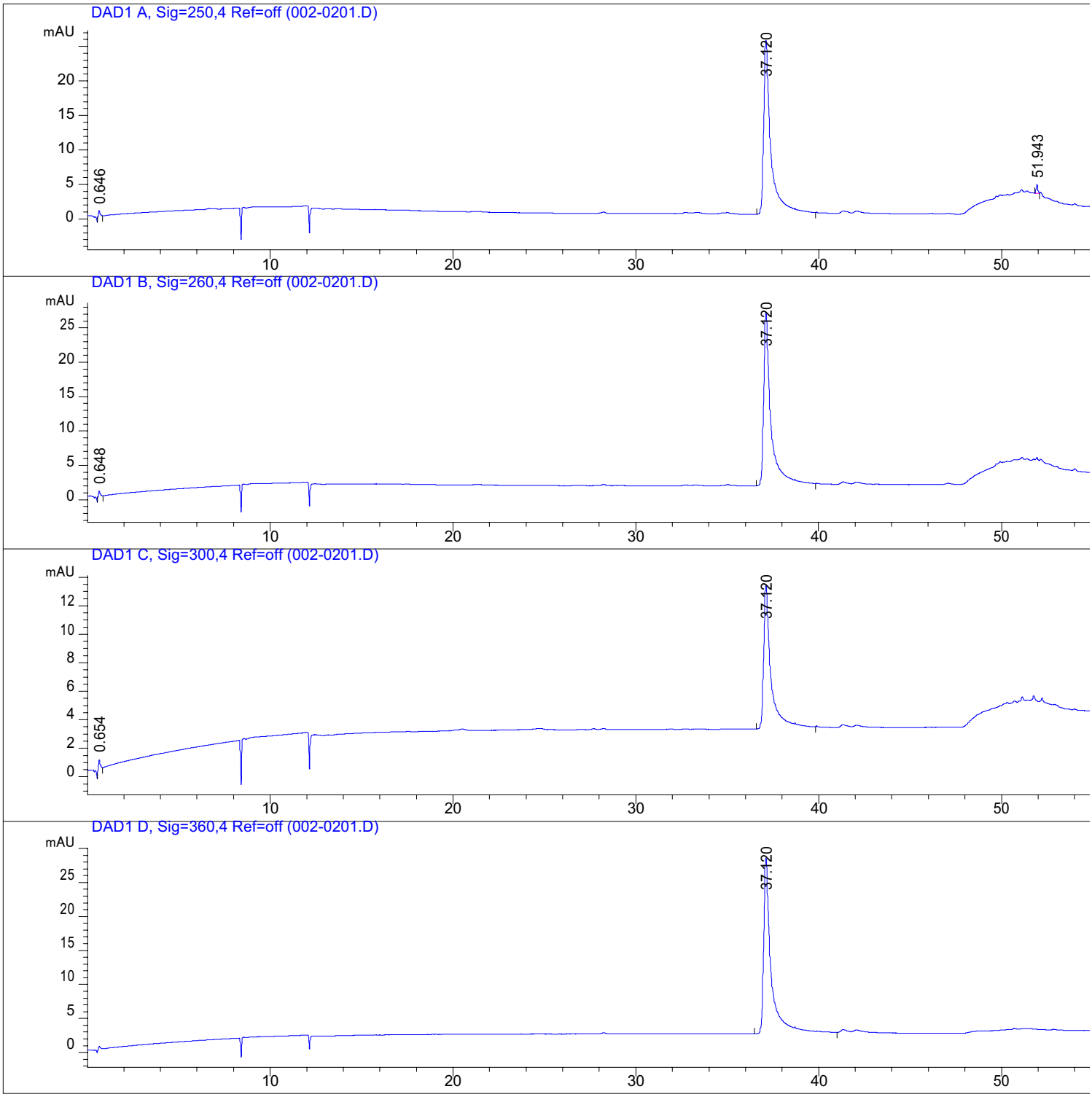
Injection Date : 5/12/2017 1:40:40 PM Inj : 1

Inj Volume : 10.000 µl

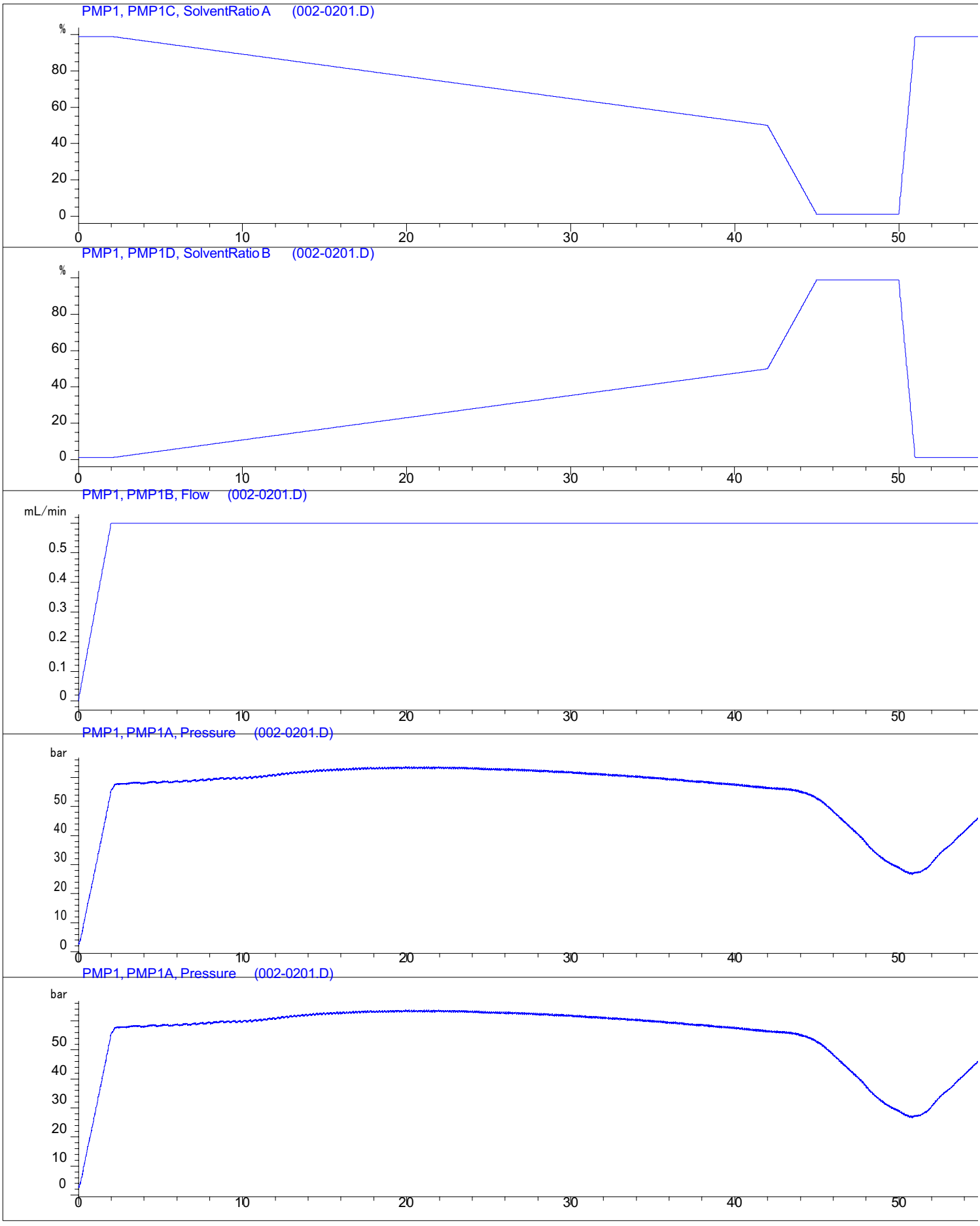
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-12 13-25-22\DEF_LC.S

Method : C:\Chem32\1\Data\DEF_LC 2017-05-12 13-25-22\Optimized method = method 12.M
(Sequence Method)

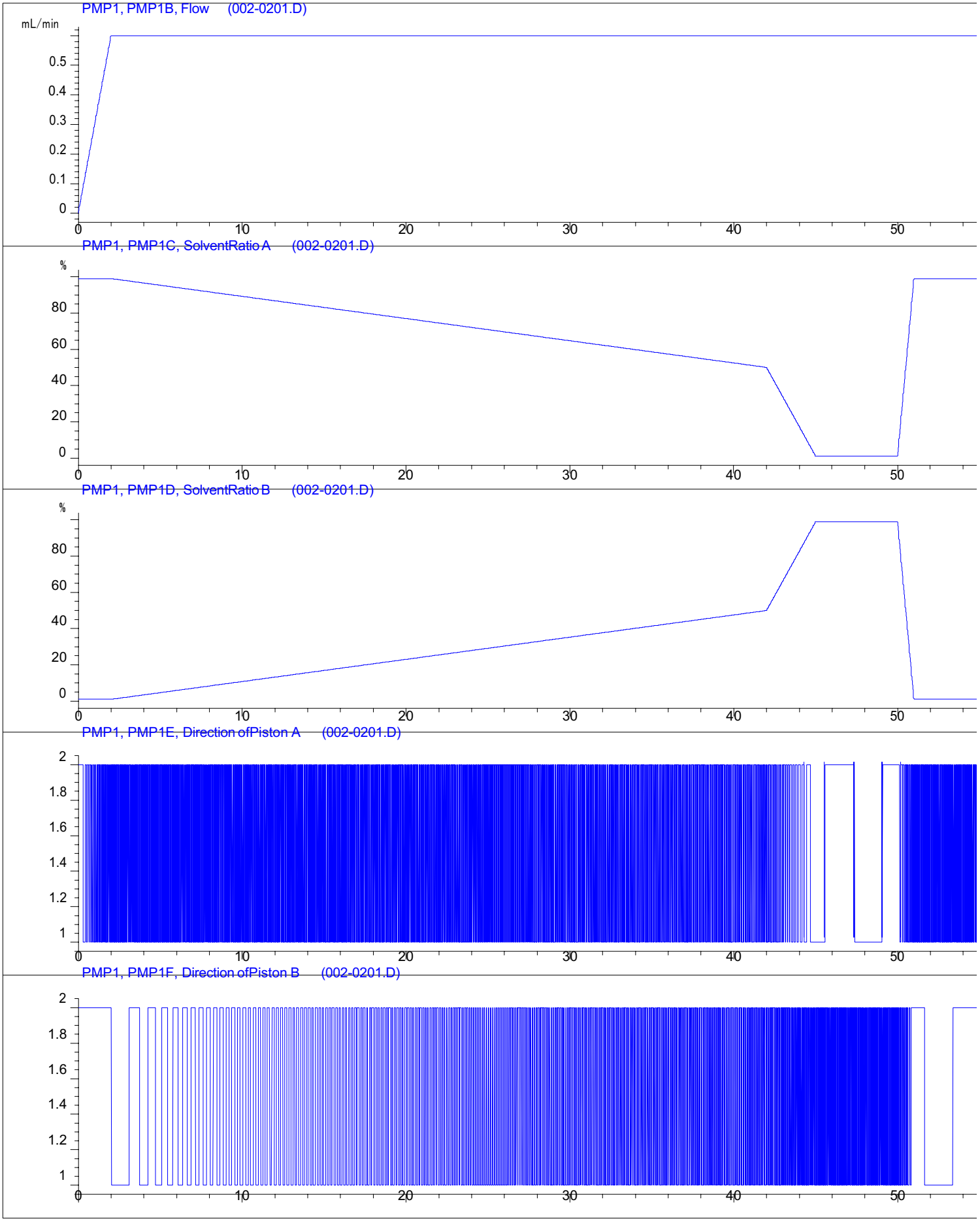
Last changed : 5/12/2017 1:25:23 PM by SYSTEM



mple Name: S5-0,75



mple Name: S5-0,75



mple Name: S5-0,75

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Area Percent Report
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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.646	BB	0.1201	11.09848	1.40627	1.5694
2	37.120	BB	0.3784	687.76337	25.19816	97.2520
3	51.943	BB	0.1005	8.33502	1.30600	1.1786

Totals : 707.19687 27.91043

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.648	BB	0.1242	11.04107	1.36704	1.5832
2	37.120	BB	0.3804	686.34625	25.14561	98.4168

Totals : 697.38732 26.51265

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.654	BB	0.1264	8.72850	1.07829	3.0619
2	37.120	BB	0.3804	276.34354	10.06175	96.9381

Totals : 285.07204 11.14004

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	37.120	BV R	0.3894	730.15515	25.86420	100.0000

Totals : 730.15515 25.86420

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*** End of Report ***

mple Name: S5-0,9

==========

Acq. Operator : SYSTEM Seq. Line : 3

Acq. Instrument : HPLC 4 Location : 3

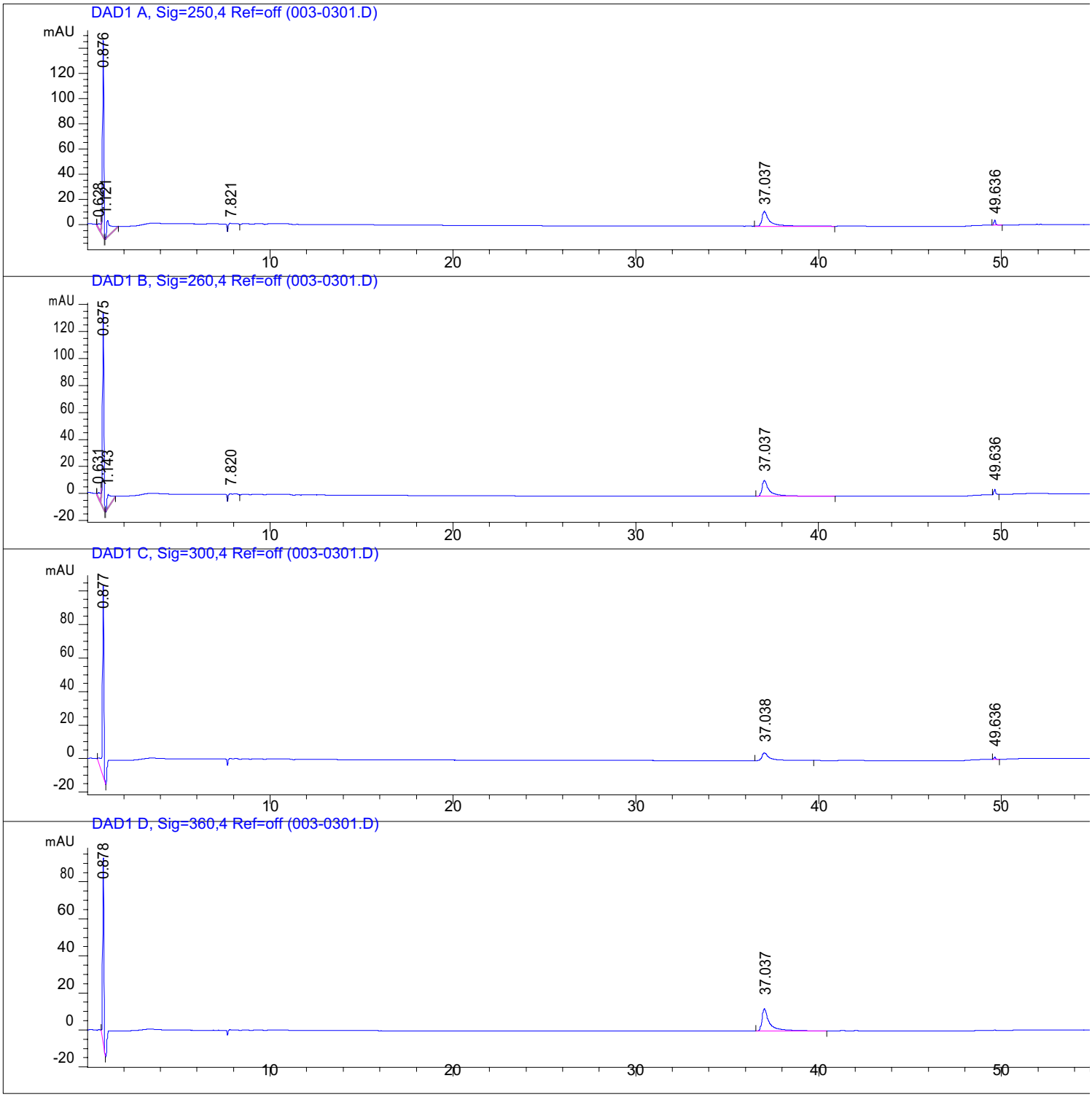
Injection Date : 5/12/2017 2:37:42 PM Inj : 1

Inj Volume : 10.000 µl

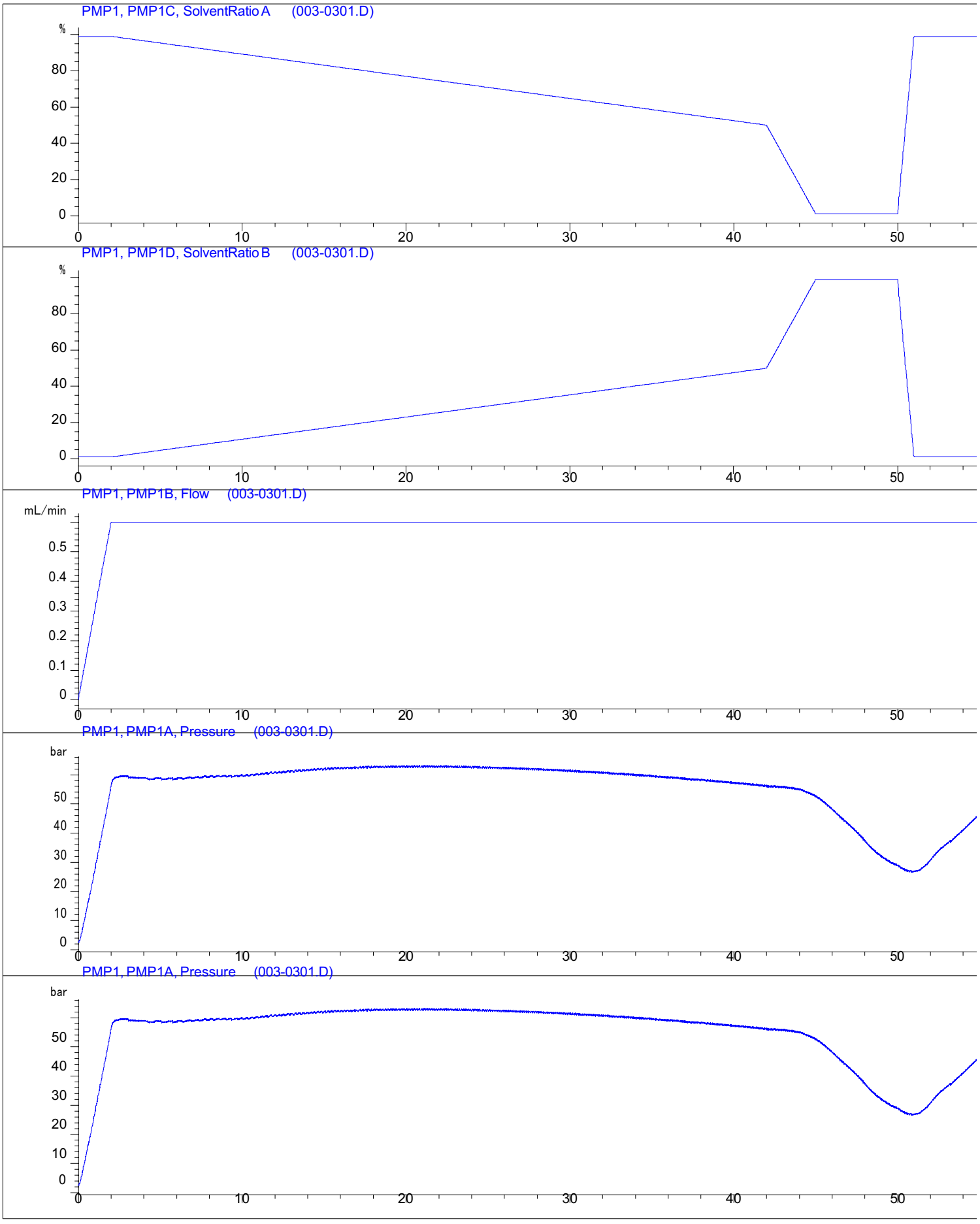
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-12 13-25-22\DEF_LC.S

Method : C:\Chem32\1\Data\DEF_LC 2017-05-12 13-25-22\Optimized method = method 12.M
(Sequence Method)

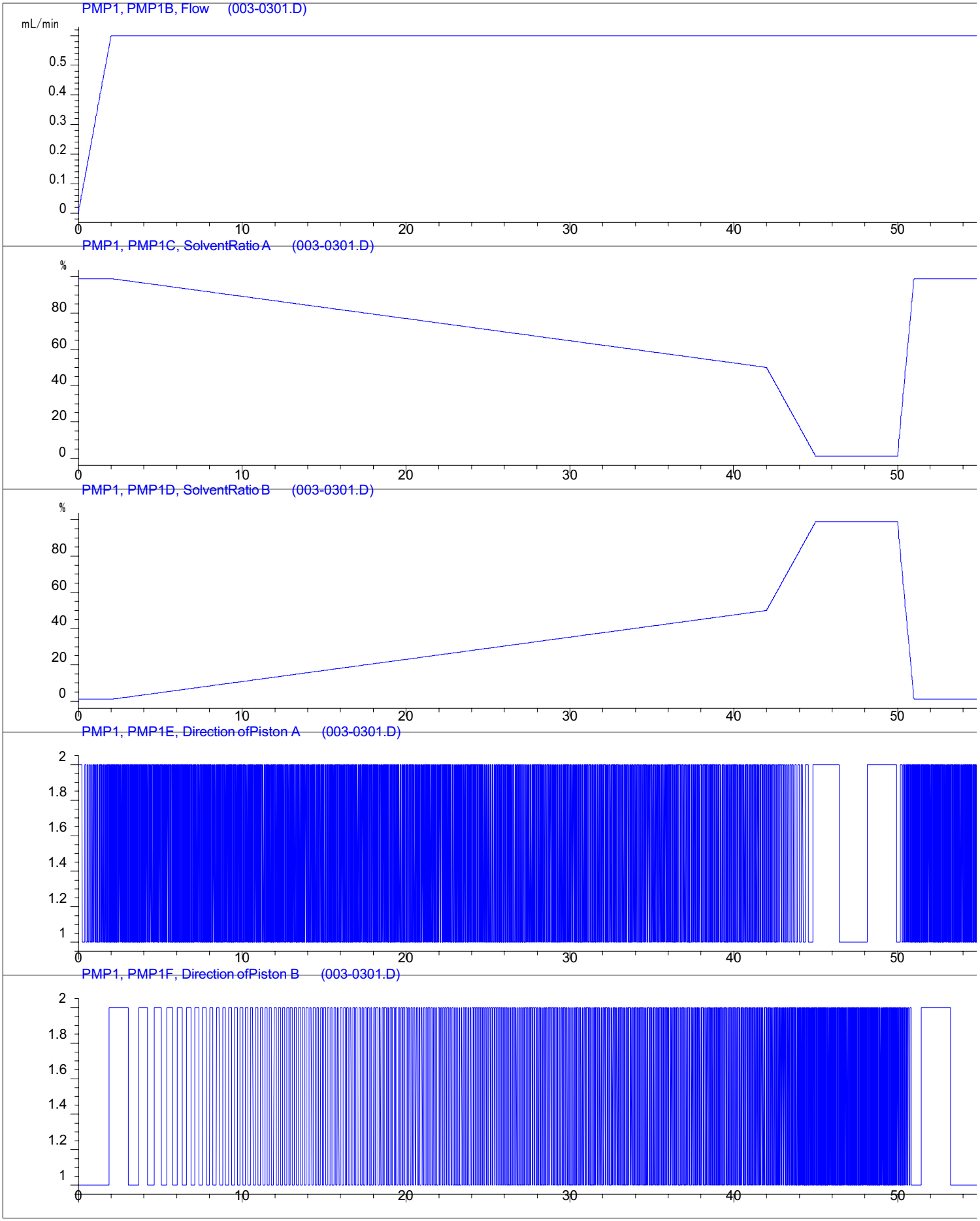
Last changed : 5/12/2017 1:25:23 PM by SYSTEM



mple Name: S5-0,9



mple Name: S5-0,9



mple Name: S5-0,9

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.628	BV E	0.2510	59.02060	3.03394	4.0749
2	0.876	VB R	0.0651	656.31696	156.01930	45.3130
3	1.121	BB	0.2220	228.67795	13.61141	15.7882
4	7.821	BB	0.3388	128.29295	5.25457	8.8575
5	37.037	BB	0.4088	351.19220	11.88294	24.2468
6	49.636	BB	0.0916	24.90778	4.05659	1.7197
Totals :				1448.40843	193.85876	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.631	BV E	0.2471	61.30568	3.23463	4.5556
2	0.875	VB R	0.0674	636.05267	144.32556	47.2645
3	1.143	BB	0.2076	162.54382	10.44513	12.0785
4	7.820	BB	0.3384	113.17124	4.67465	8.4096
5	37.037	BB	0.4084	349.64447	11.84396	25.9817
6	49.636	BB	0.0886	23.01321	3.91384	1.7101
Totals :				1345.73109	178.43778	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.877	BB	0.0724	557.77338	115.34039	79.3852
2	37.038	BB	0.3976	136.74144	4.75353	19.4617
3	49.636	BB	0.0878	8.10170	1.39475	1.1531
Totals :				702.61652	121.48867	

mple Name: S5-0,9

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.878	BB	0.0608	402.03085	100.18069	53.0278
2	37.037	BB	0.4055	356.11990	12.16503	46.9722
Totals :				758.15076	112.34572	

*** End of Report ***

mple Name: S5-1

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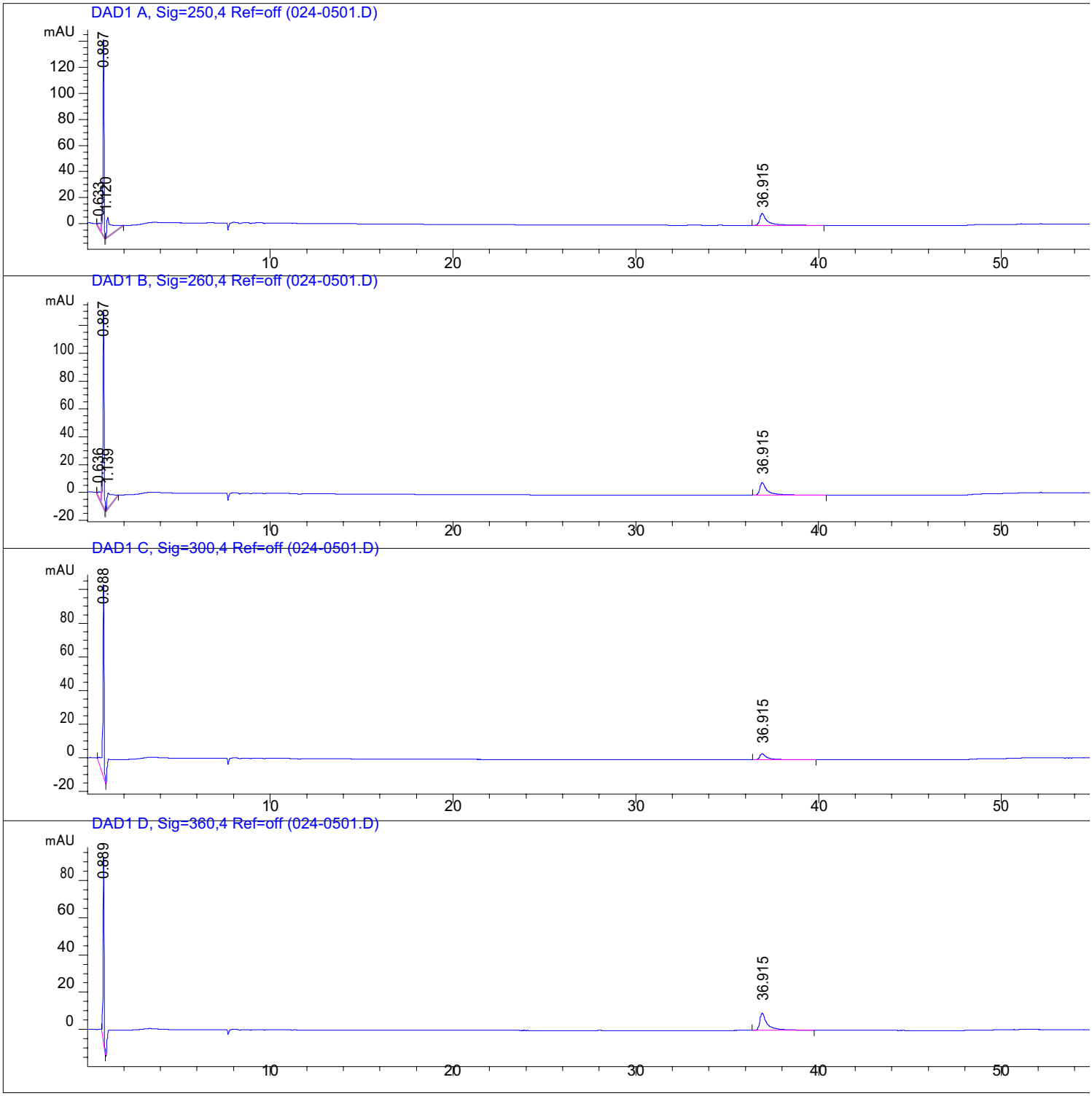
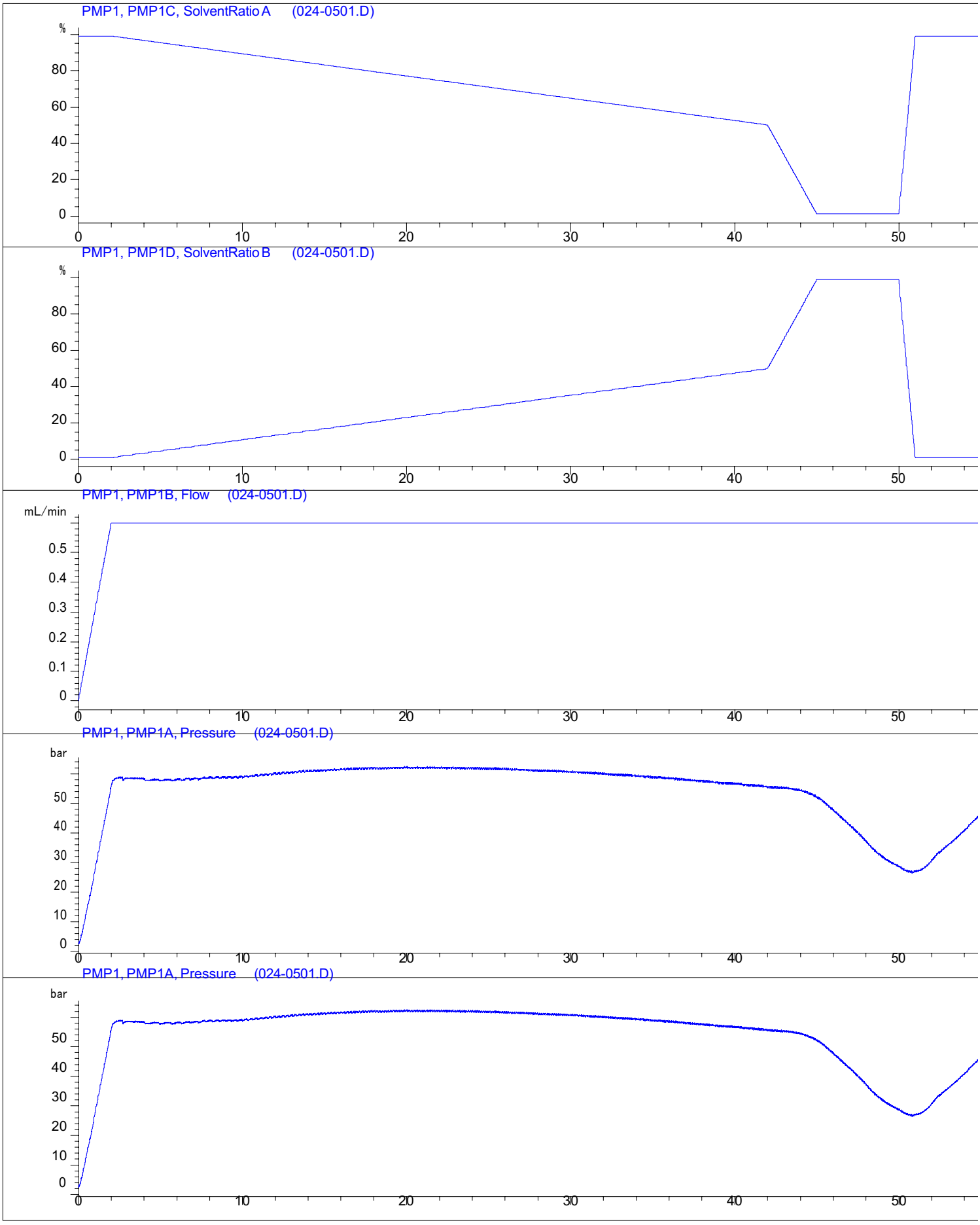
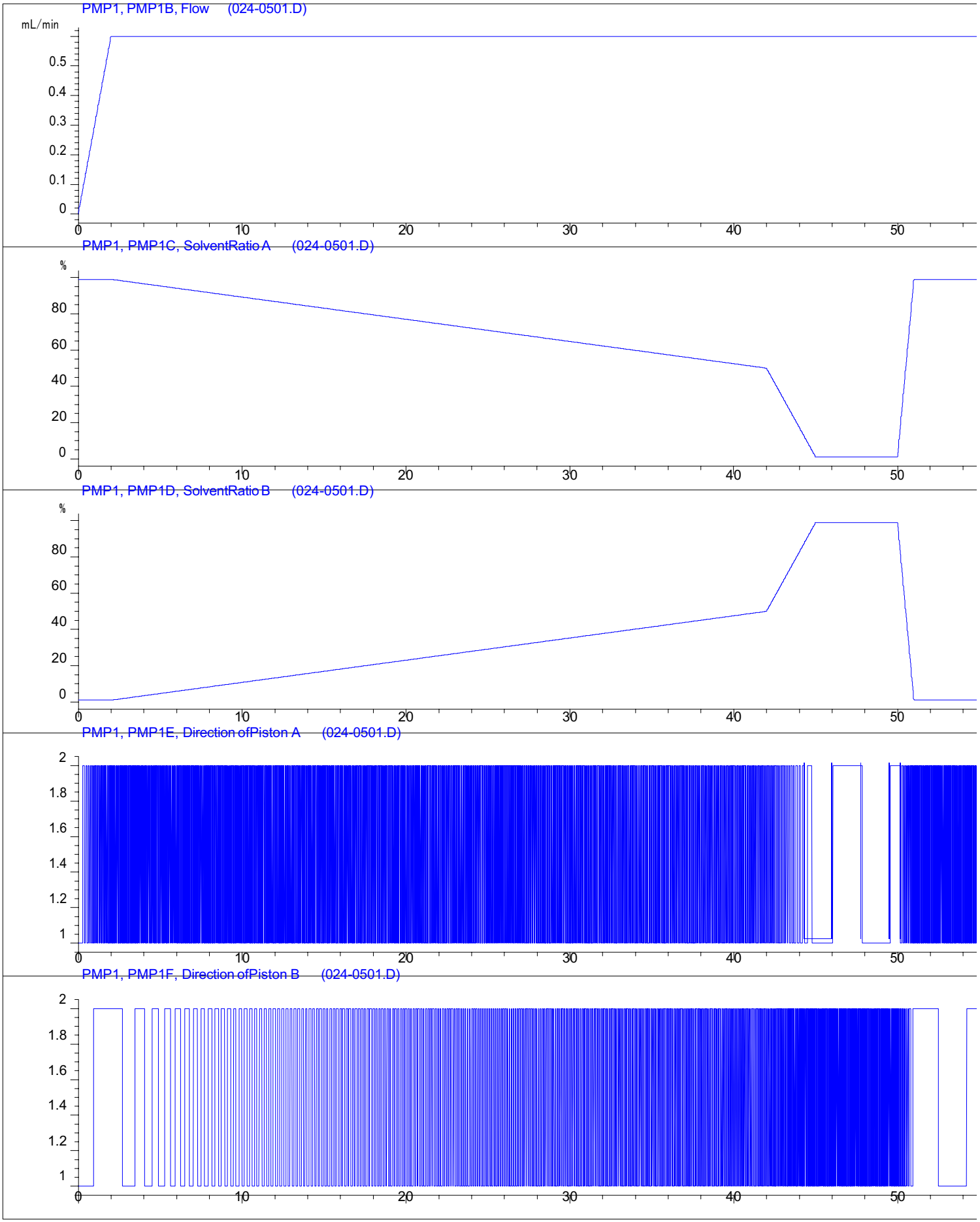
Acq. Operator : SYSTEM Seq. Line : 5
Acq. Instrument : HPLC 4 Location : 24
Injection Date : 5/11/2017 1:56:28 PM Inj : 1
Inj Volume : 10.000 µl
Sequence File : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\DEF_LC.S
Method : C:\Chem32\1\Data\DEF_LC 2017-05-11 10-50-25\Optimized method = method 12.M
(Sequence Method)
Last changed : 5/11/2017 10:50:25 AM by SYSTEM



mple Name: S5-1



mple Name: S5-1



mple Name: S5-1

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=250,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.633	BV E	0.2572	60.17752	3.01028	4.7445
2	0.887	VB R	0.0620	618.34308	150.28706	48.7513
3	1.120	BB	0.2707	321.35205	15.19791	25.3360
4	36.915	BB	0.4106	268.49069	9.09109	21.1683
Totals :				1268.36334	177.58634	

Signal 2: DAD1 B, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.636	BV E	0.2542	63.33312	3.26691	5.4971
2	0.887	VB R	0.0643	602.10858	139.77757	52.2613
3	1.139	BB	0.2473	218.75877	11.42995	18.9876
4	36.915	BB	0.4088	267.91171	9.06637	23.2540
Totals :				1152.11219	163.54080	

Signal 3: DAD1 C, Sig=300,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.888	BB	0.0697	545.71741	114.35739	83.5618
2	36.915	BB	0.4032	107.35336	3.64920	16.4382
Totals :				653.07076	118.00659	

Signal 4: DAD1 D, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.889	BB	0.0596	389.55917	99.61791	58.8336
2	36.915	BB	0.4052	272.57825	9.32164	41.1664

mple Name: S5-1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				662.13742	108.93955	

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*** End of Report ***