Atmospheric pressure Solid Analysis Probe coupled to Quadrupole-time of Flight Mass Spectrometry as a tool for screening and semi-quantitative approach of Polycyclic Aromatic Hydrocarbons, Nitro-Polycyclic Aromatic Hydrocarbons and Oxo-Polycyclic Aromatic Hydrocarbons in complex matrices

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Abstract

A new screening and semi-quantitative approach has been developed for direct analysis of polycyclic aromatic hydrocarbons (PAHs) and their nitro and oxo derivatives in environmental and biological matrices using Atmospheric Pressure Solid Analysis Probe (ASAP) Quadrupole-Time of Flight mass spectrometry (Q-TOF-MS). The instrumental parameters were optimized for the analysis of all these compounds, without previous sample treatment, in soil, motor oil, atmospheric particles (ashes) and biological samples such as urine and saliva of smokers and non-smokers. Ion source parameters in the MS were found to be the key parameters, with little variation within PAHs families. The optimized corona current was 4 µA, sample cone voltage 80 V for PAHs, nitro-PAHs and oxo-PAHs, while the desolvation temperatures varied from 300 ºC to 500 ºC. The analytical method performance was checked using a certified reference material. Two deuterated compounds were used as internal standards for semi-quantitative purposes together with the pure individual standard for each compound and the corresponding calibration plot. The compounds nitro PAH 9-nitroanthracene and oxo-PAH 1,4-naphthalenedione, were found in saliva and urine in a range below 1 µg/g while the range of PAHs in these samples was below 2 µg/g. Environmental samples provided higher concentration of all pollutants than urine and saliva.

Keywords: direct analysis, polycyclic aromatic hydrocarbons, screening, organic pollutants, ambient ionization mass spectrometry, tobacco, cancer.

Introduction

The quality control requirements are more and more demanding, especially in areas of food and health, where consumers have a high concern about the likely contamination or affection to their health. The number of samples requiring analysis has increased dramatically in the last years as well as the required sensitivity of the current analytical procedures [1]. These characteristics imply a high cost of the analysis. For this reason, fast screening procedures are gaining importance, as they permit the evaluation of the presence or absence of specific compounds in the samples that may require a further analysis in depth. These screening methods, most of them based on mass spectrometry (MS), are now promoted in a wide variety of areas.

Ambient mass spectrometry (Ambient MS) analysis has experienced a very rapid development during the last seven years. This technique is defined as mass spectrometric analysis with minimal or no sample preparation using direct sampling and ionization at ambient conditions [2] and it represents the ideal tool for screening analysis. With the introduction of direct analysis in real time (DART) [3] and desorption electrospray ionization (DESI) [4], the analysis of samples without any prior treatment became possible for the first time [2]. In the DART technique, the sample is placed in a stream of helium that contains ionized atmospheric gases (such as water vapor and oxygen), and the analytes in the sample are ionized in open air in the laboratory environment. Desorption Electrospray Ionization (DESI) is carried out by directing charged droplets produced from a pneumatically-assisted electrospray onto a surface to be analyzed at atmospheric conditions. Ions of chemical species present on the surface, usually a paper impregnated in the sample, are produced through the interaction of charged droplets and the sample. DART and DESI are mainly qualitative techniques where the main purpose is to identify the

presence of specific compounds present at the surface of the sample, i.e. targeted analysis. The development of these techniques creates an awareness of the power of Ambient MS and a new sub-field in mass spectrometry. DART and DESI techniques usually coupled to Time of Flight (TOF) detectors have been widely applied as direct screening techniques to a wide variety of samples [5-10].

Atmospheric-pressure solid-analysis probe (ASAP) was developed in 2005 [11]. The sample, usually introduced into the ionization chamber with a glass rod dipped either in the powdered or liquid sample or rubbing the surface, is vaporized and ionized at atmospheric pressure inside a small chamber, thus providing the ions, which arrive at the MS detector [12-13]. The major difference between DART and ASAP is the ionization mode. While DART is open to the environment allowing for water to be part of the chemical ionization process, ASAP takes place in dry atmosphere. DESI, DART and ASAP can be considered as screening techniques to take rapid decision about the further analysis in depth. Their major advantages are the absence of sample treatment, which is usually a tedious, expensive and time consuming step. None of them have a chromatographic separation before detection. This means that all the analytes present in the sample matrix, or formed during the ionization step, reach the source and all of them can be analyzed, thus minimizing any analyte losses. Thus, these techniques are mainly focused on target analysis and constitute powerful tools for screening the absence or presence of target compounds. In a few minutes the sample is injected and processed.

In ASAP, several key parameters, such as corona current, sample cone voltage and gas desolvation temperature need optimization, because they condition the total number of ions arriving at the detector. Important features, like the temperature of the ion source chamber where the sample

is volatilized, remain constant (120ºC) to ensure the maximum volatilization. This technique can be applied to volatile or semi-volatile compounds in solid or liquid complex matrices, without previous sample treatment. The presence or absence of specific compounds at low concentration, and in some cases the order of magnitude of their concentration in the sample, are shown as very interesting characteristics of this technique. However, it is not possible to estimate toxicity from the presence of single compounds by using this method, because too similar compounds in environmental samples superimpose. Using ASAP for screening and semi-quantitative simultaneous analysis of several pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and their nitro and oxo derivatives in complex matrices, can be very useful in health and environmental areas. This is the main purpose of the present paper.

PAHs, nitro-PAHs and oxo-PAHs derivatives are organic compounds listed as priority pollutants by international environmental protection agencies due to their carcinogenic, mutagenic, and toxic effects [14-20]. There are a few applications of Ambient MS methods for direct analysis of PAHs and related compounds [21-26]. However, the possibility of having a semi-quantitative approach using ASAP has not been explored yet. For this reason, the main objective of this work is to evaluate the performance of ASAP-Q-TOF-MS as a screening tool and semi-quantitative approach for PAHs, nitro-PAHs and oxo-PAHs in several liquid and solid complex samples, such as car exhaust, oil, atmospheric dust, urine and saliva. As far as we know, this article represents the first study using direct analysis in one single run through ASAP-Q-TOF-MS for this large series of priority organic pollutants. It is also the first time that a semi-quantitative approach has been proposed using a direct ASAP-MS-Q-TOF in very different complex matrices. A solid certified reference material (CRM) for PAHs

was run in parallel with the samples, in order to check the method performance and the semi-quantitative data. The results obtained are shown and discussed.

Materials & Methods

Chemicals and Reagents

Certified standards of PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, cyclopenta[*cd*]pyrene, benzo [*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo [*a*,*h*]anthracene and benzo[*ghi*]perylene), Nitro-PAHs (1-nitronaphthalene, 2-methyl-1-nitronaphthalene, 1,5-dinitronaphthalene, 2-nitrofluorene, 9-nitroanthracene, 2-nitrofluoranthene, 1-nitropyrene and 6-nitrocrysene) and Oxo-PAHs (1,4-naphthalenedione, benzophenone, 9-fluorenone, anthrone, 9,10-anthracenedione, 7H-benz[*de*]anthracen-7-one, benzo[*a*]anthracene-7,12-dione and naphtacene-5,12-dione) were supplied by Sigma-Aldrich (Madrid, Spain). Acenaphthene-d10 (99 atom % D) and benzo[*a*]pyrene-7,8-d2 (98 atom % D) for being used as internal standards were purchased from Sigma-Aldrich (Madrid, Spain). Detailed information about the molecular weight, chemical formula, CAS number and molecular structure for each of the analyzed compounds of the three families can be found in Table 1 and Figures S1, S2 and S3 in the Supplementary Information material. All the standard solutions were prepared in methanol reagent grade purchased from Scharlab (Barcelona, Spain). Combined solutions of the three families (PAHs, nitro-PAHs and oxo-PAHs) containing different concentrations of each standard were also used to check the performance of the mixtures in ASAP. A sediment as certified reference material (RTC-CRM104-50G, RT-Corp, WY, USA) containing PAHs was supplied by Sigma-Aldrich (Madrid, Spain). The amount of deuterated compounds

added to both the standard mixture solution and the samples was in each case in the required final concentration of 10 ng/g IS in the sample. All standards were under gravimetric control and all concentrations are expressed as w/w basis.

ASAP-Q-TOF-MS instrument

The Atmospheric Solids Analysis Probe (ASAP) was coupled to a quadrupole-time of flight-mass spectrometer (QToF-MS), Xevo G2 QTOF (Waters Corporation, Manchester, UK). The instrument has a mass range up to *m/z* 100 000 and a resolving power of >22,500 full width at half maximum (FWHM). In the ion source atmospheric pressure chemical ionization was used and the chamber temperature is constant at 120ºC. The key ion source parameters to be optimized were: corona current (µA), sample cone voltage (V) and desolvation gas temperature (ºC). Acquisition mode used was MS full-scan data from 70 to 800 amu. Nitrogen was used as desolvation gas at 400 Lh^{-1} flow. Cone flow was not needed for this technique. Currently, ASAP requires manual injection and can neither be processed unattended nor automatically.

ASAP-Q-TOF-MS analysis

As this technique is for target analysis, the first step is to analyze the individual compounds under the optimal experimental conditions. Variables such as cone voltage, desolvation gas temperature and corona current were optimized using standard solutions of pure compounds. Voltage of sampling cone was varied from 20 V to 80 V and voltage of the extraction cone was fixed at 0.1 V. The parameters of acquisition were as follows: a scan time of 1 s and mass range of *m/z* 70 – 800 to cover all PAH, nitro-PAH and oxo-PAH standards [27]. For each MS spectrum, the

characteristic masses were selected and this information was used for searching the presence or absence of each compound in the real samples. Positive identification of the compounds was done using pure standards for all analyzed compounds, using several ions for each compound.

The samples were analyzed in continuous mode (3 min), with a cone voltage of 80 V. The desolvation gas temperature was manually varied from 200 ºC to 500 ºC to ensure the progressive volatilization of all the compounds and thus facilitate the target analysis of the sample. In this way, the three families of compounds were analyzed in only one acquisition. The ions from the lighter compounds appeared first and those from the heavier compounds arrived later at the detector. This procedure facilitated the identification of the compounds, as the corresponding ions were coming progressively according to their size. The ratio between ramp temperature and time scan can be seen in Figure 1. The maximum intensity of each target ion together with the comparison versus the pure individual standards were used for further identification. Atmospheric Pressure Ionization (API) in positive polarity was selected.

For the analyzed samples, the same scan time was used (1s) and the mass range was from *m/z* 70 to 800 to cover all the likely present compounds. A new glass rod was used for each sample. Before the analysis the glass rod was inserted into the source chamber at high temperature for two minutes to remove any contamination from the tip. The rod was then removed, cooled and then dipped into the sample for ten seconds. Solid samples (powdered) sticked to the glass rod, while liquid samples wet the rod. All samples were previously spiked with two deuterated internal standards, as above described, to check the sampling load and the performance of the whole process. In liquid samples the IS were dissolved giving a homogeneous solution. Solid samples (1g) spiked with IS (10 µL of

methanol solution) were homogenized in a vortex and a homogeneous slurry was obtained. Sample load was controlled through these IS, as previous calibration plot under the same experimental conditions was carried out with the IS. Calibration plots were previously obtained for both IS in standard solution. This plot was further used to analyze the sample load and facilitate the semiquantitative approach.

Three replicates of each sample were analyzed. The accuracy and reproducibility (referred to mass accuracy) of all analyses was guaranteed by using a Lockspray. Leucine-enkephalin, generating $[MH]^+$ ions at 556.2771 Da was employed as the lock mass at a concentration of 2 ng mL^{-1} in water/acetonitrile with 0.1% formic acid. Blanks were obtained without loading any sample in the glass rod during the first 30 - 40 sec of the acquisition, while applying the same temperature ramp as in the sample acquisition. Serial diluted solutions (0.1, 0.5, 1, 5, 10, 20 ng/ml) of individual standards at different concentrations were used to establish the limit of detection (LOD) and limit of quantitation (LOQ) under the same experimental conditions as the samples. LOD and LOQ were calculated as three times or ten times respectively the standard deviation of the blank at the same characteristic mass as each compound. The three standard solutions containing the three chemical groups were independently processed. Data were collected and processed using MassLynx software (Waters Corporation). Confirmation with the pure standards processed under the same conditions was carried out. Mass spectra shown in the figures are based on the average of several mass spectra.

Reference material & standards

A solid sediment as CRM for PAHs was used without previous sample treatment to demonstrate the performance of the semi-quantitative approach in direct analysis by ASAP-Q-TOF-MS. The CRM was analyzed under the same experimental conditions as the samples. The glass rod was dipped into the powdered CRM sample and then introduced into ASAP. In order to know the amount of sample loaded into the system as well as to detect any problem related with the matrix effect or ion suppression phenomena, 1 g of all samples was spiked with 10 µL of methanolic solution containing two deuterated PAHs (acenaphthene d10 and benzo[*a*]pyrene 7,8-d2) to get a final concentration of 10 ng/g of each IS. After spiking, the sample was shaken in a vortex and homogenized before the analysis. The IS were analyzed and taken into account for semiquantitative purposes.

Samples

After optimization of the instrumental parameters for the three PAH families (PAHs, nitro-PAHs and oxo-PAHs), different types of samples with complex matrices (motor oil, soil, combustion particles, urine and saliva) were directly analyzed by ASAP-Q-TOF-MS in order to demonstrate the performance of the method. The glass rod was dipped into the solid or liquid sample and directly introduced into the ASAP chamber. As was above mentioned, fine powdered sample or small drops of liquid samples were sticked to the glass rod. The MS spectra acquired by MS-Q-TOF were registered and the abundance (response) of each ion referred to the internal standards was used for semi-quantitative purposes in all cases.

The description of the analyzed samples is as follows:

- mineral motor oil from an automatic generator set

- soil from a parking lot heavily contaminated with vehicle exhaust and carburants
- combustion particles from the exhaust pipe of a diesel central heating system of a building
- biological fluids: a) saliva from a heavy smoker (more than 20 cg/day) taken in the morning before smoking the first cigarette of the day and 1 hour after smoking 1 cigarette; b) urine from a heavy smoker and from a non-smoker

Results and Discussion

ASAP analysis

The sample treatment is usually the bottle neck of any analytical procedure. Working with solid samples, materials or biological samples is always time consuming, as the dissolution, often derivatization and clean-up step are required before the analysis. All these sample treatment steps are usually manually managed, as automatic devices required loading the samples to process them in the right way. The whole analytical procedure (sample treatment + analysis) has to be done for qualitative and quantitative analysis. In this frame, ASAP is an excellent tool for target analysis, as the absence or presence of target compounds can be immediately achieved. This way, the further analysis in depth can be applied to only those samples with positive response from ASAP-MS-Q-TOF. Of course this technique cannot substitute the whole analytical procedure for quantitative analysis, but having the identification/presence of a compound and the idea of order of magnitude for the analyte is very useful to pass/fail criteria.

Due to no sample pre-treatment, direct injection and no chromatographic run, sample and blank are acquired in 3 min. This time can be considered as “fast” analysis.

With the identification of the compounds of interest, the absolute ion intensity can be quantified with respect to the internal standards added to the sample, and the values are later used for semi-quantitative purposes.

Optimization of Ion source parameters

Following, key parameters were explored: Corona current (µA), that affects the ionization; sample cone voltage (V), that influences fragmentation and desolvation gas temperature (ºC), that affects volatilization. Also different values for desolvation gas flow were tested. However, desolvation gas flow was found to have a minor influence for the ASAP generated spectrum, so a fixed value was used for the PAH families. The main purpose was to carry to the detector as much ions as possible, thus diminishing the in-source fragmentation what facilitates the further identification. To achieve this, one key parameter was changed while maintaining the others fixed. As expected, cone voltage was the unique parameter affecting the fragmentation. Three different optimization sets were carried out, one for each family of pollutants (PAHs, nitro-PAHs and oxo-PAHs). Optimal values for each family of analyzed compounds can be seen in Table 1. The target samples were analyzed for about 3min, applying a desolvation temperature ramp and running a blank before and after each of the running samples.

The gas desolvation temperature (300, 400 and 500 ºC) had no effect on the resulting spectra of the PAHs and nitro-PAHs, neither in fragmentation nor in the signal response (i.e., sensitivity). A different trend was found for the oxo-PAH family, where a higher sensitivity/response was found at a lower temperature (300 ºC) for the lighter and medium weight compounds, but not for the heaviest ones (*m/z* 258.0681 benzo[*a*]anthracene-

dione/naphthacene-5,12-dione). For these two congeners the highest sensitivity was obtained at 500 ºC.

With a fixed gas desolvation temperature (500 ºC for PAHs/nitro-PAH and 300 ºC for oxo-PAH) the cone voltage was varied from 20, 40, 60 and 80 V for each compound family. Changing the cone voltage had a minor effect on the intensity of the spectra obtained for the nitro-PAH. Signal and fragmentation increased in parallel as the cone voltage increased. Sampling cone voltages between 20 V and 30 V can be considered typical for small compounds, causing minimal fragmentation. However for oxo-PAHs a similar signal and fragmentation pattern were found between 20 V and 80 V (data shown in Figure 2). As can be seen, the minimum fragmentation and signal were obtained at 20 V, while the maximum was at 80 V. This indicates that oxo-PAHs are more resistant to fragmentation, even at cone voltages as high as 80 V and consequently the relationship between cone voltage and fragmentation of oxo-PAHs cannot be anticipated as a general performance.

Mass spectra for each PAH families

For PAHs, nitro-PAHs and oxo-PAHs, corona current (µA) was investigated over the range from 3 to 6, sample cone (V) at 20, 40, 60 and 80, and desolvation gas temperature (ºC) from 300 to 500. Figure 3 shows the mass spectra obtained in optimum conditions for the analytical standards of PAHs (corona current 4 µA, sample cone at 80 V and desolvation gas temperature 500 ºC).

As there is no previous chromatographic separation, this technique is not capable to differentiate between several PAH isomers (e.g., *m/z* 202.0782 pyrene/fluoranthene, *m/z* 228.0782 benzo[*a*]anthracene/chrysene, etc),

since they have the same mass, which is a drawback of this direct technique. As can be seen in Figure 3, the dominant ion sequence corresponds to the molecular ion (▲) and to the protonated ion (●, MH^+), except for naphthalene. The protonated ion (MH^+) was the most abundant in all the analyzed compounds with the only exception of naphthalene and fluorene. The protonated ion (MH^+) was used for semi-quantitative purposes (except for naphthalene, in which M^+ was used). The ratio between both species depends on sample conditions (e.g., humidity content) and this ratio was stable over the time experiment. According to Ray *et al* [27], who worked with ASAP and steroids, the analysis of steroids by ASAP generally results in the formation of the product ion as either the radical cation $M^{+\bullet}$ or the protonated molecule $(MH)^+$. The formation of the protonated molecule is a result of proton transfer from ionized water clusters in the source. However, if the source is dry, as in ASAP, the formation of the radical cation is the primary ionization mechanism. The same behavior can be observed with PAHs. The mass spectrum from ASAP-MS-QTOF in optimum conditions of a mixture of eight nitro-PAHs is shown in Figure 4. The molecular ion (▲) and the protonated ion (●) were the dominant ion series and again the charged ion series (MH^+) was the most abundant for all the compounds. This performance with Q-TOF-MS has been observed in other works [28].

Figure 5 shows the mass spectrum of a mixture of eight oxo-PAHs from ASAP-MS-QTOF. In a similar fashion as that for the parent PAHs and nitro-PAHs, the molecular ion (▲) and the protonated ion (●, MH^+) were the dominant ion series and again the ion sequence was the most abundant for all the compounds.

Limits of detection, established with the pure compounds in methanol solution, as above described, were found to be around 20 ppb (ng/g) for

PAHs and nitro-PAHs and only 5 ppb (ng/g) for the oxo-PAHs in the methanol solution of the standards. This difference in sensitivity could be explained by the higher ability or affinity of oxygen atoms in oxo-PAHs than in other PAHs to accept protons. The mass resolution and mass accuracy (ppm error), for the three families are shown in Table S3 of the supporting information material.

Matrix influence and ion suppression phenomena can be a serious problem in any conventional analysis. Direct ASAP can be also affected by matrix influence. To investigate this potential problem, two different deuterated internal standards were added to both the samples and the CRM. From the results obtained it can be confirmed that matrix influence was observed but with very low intensity in all samples under study, probably because the analyzed samples were volatilized in the ASAP chamber and the ionization took place without problems (Figures 6 and S4 SI material). Matrix influence and/or ion suppression phenomena can be the reason why some of the analyzed compounds display a higher standard error in the repetitive injections of the oil sample (i.e., *m/z* 154.0782 acenaphthene) (Figure S4).

Qualitative Analysis of Samples

An important advantage of the ASAP-Q-TOF-MS technique is the accurate mass obtained for the compounds of interest, so that the identification of compounds in complex matrices (i.e., oil, soil or saliva) without any prior treatment is much easier. The confirmation with the pure standards of each compound is usually required to confirm the identification.

Direct analysis using ASAP-MS-QTOF provides the exact masses of all the compounds present in the sample. As there is not chromatographic separation those isomers having exactly the same *m/z* values cannot be

confirmed. However, the presence of specific *m/z* value confirms the presence of some of the isomers. Several examples can be highlighted such as *m/z* 178.0782, which is common to phenanthrene and anthracene. For this reason several compounds have been included in Table 2 for each *m/z* value when required.

In the biological samples (i.e. saliva and urine from smokers), three families of contaminants were identified. Urine and saliva samples had constant conditions of humidity, as they are aqueous samples. In these conditions the protonation is improved and better results can be found. PAHs were the most abundant with at least three congeners (*m/z* 154.0782 acenaphthene, *m/z* 178.0782 phenanthrene/anthracene, *m/z* 228.0782 benzo[*a*]anthracene/chrysene) in both types of samples. These results are in accordance with the literature [29, 30]. Meanwhile, only one congener of the nitro-PAH family was found (*m/z* 223.0633 probably, 9-nitroanthracene) in the saliva sample and only one oxo-PAH congener (*m/z* 158.0368 probably, 1,4-naphthalenedione) in the urine sample. In both cases these compounds were found in a heavy smoker just after smoking and not in a non-smoker. These results agree with those anticipated, i.e. that the parental compounds (PAHs) were more abundant than their reaction products (nitro or oxo-PAHs). However, it is important to note that 9-nitroanthracene was present already in the tobacco smoke, probably formed from anthracene during the smoking process, while 1,4-naphthalenedione was the result of oxidation of naphthalene in the human body and therefore excreted in the urine.

Figure S5 shows the spectra for the PAH standards and saliva sample after smoking. The highly carcinogenic aromatic compounds (*m/z* 252.0939 either benzo[*a*]pyrene or benzo[*k*]fluoranthene or both) can be seen in saliva samples, together with many other compounds (e.g. *m/z* 152.0626

acenaphthylene, *m/z* 154.0782 acenaphthene or *m/z* 166.0782 fluorene), from a heavy smoker immediately after smoking [29]. However, benzo[*a*]pyrene is diluted with time in the mouth and disappears, indicating that it is swallowed and assimilated by the body. None of these compounds were found in a non-smoker saliva sample. This finding also confirms that these pollutants are coming from tobacco and not from environmental atmosphere. All these compound groups related to the sample from a heavy smoker are in good agreement with other studies related to the tobacco smoke exposure [31].

The analysis of urine samples also showed many possible PAHs congeners (e.g., *m/z* 154.0782 acenaphthene, *m/z* 178.0782 phenanthrene/anthracene or, *m/z* 228.0782 benzo[*a*]anthracene/chrysene), which were correlated with the congeners found in saliva, as well as an oxo-PAHs congener (*m/z* 158.0368 1,4-naphthalenedione).

A large number of PAH-like pollutants was found in the analyzed environmental samples, as expected and in accordance with the literature [32-35]. The highest number of congeners was found in the soil sample (16) followed by the oil sample (15) and the ash sample with 9 compounds. Within the analyzed chemical families, PAHs account for the vast majority of congeners (14), followed by nitro-PAHs (4) and then oxo-PAHs (2). Figure 7 shows the overlapped spectra of the nitro-PAHs and a polluted soil sample, in which highly toxic nitro-PAH compounds were found (i.e., *m/z* 211.0633 2-nitrofluorene). This compound is a well-known environmental pollutant and has been found as a major component in diesel exhaust [35].

Semi-quantitative approach

A semi-quantitative approach was attempted in order to gain information about the target analyte concentration in the samples. As discussed above, this technique is a powerful tool for the identification of a wide range of target compounds without any prior sample treatment. It can therefore be used as an exploratory approach for unknown samples. If there is evidence of the presence of target compounds, after this screening step, a further sample treatment step may be carried out if necessary. Two internal standards were added to all samples before the analysis, in order to have a better knowledge of sample loaded by the glass rod. The standard solutions used for calibration curves were handled and analyzed in the same manner as the samples, this means by direct analysis by ASAP-Q-TOF-MS, hence the results can be interpolated. This way, semi-quantitative values of these pollutants in the different sample matrices (i.e. solid and liquids) were obtained. It is important to point out that there is a difference between the direct load of a solid sample (the soil or sediment in this case) and the load of target compounds in a methanol solution of standards or in a liquid sample. For this reason only the order of magnitude of each compound can be obtained and not an accurate individual concentration.

The method performance was checked with the analysis of a sediment CRM for PAHs (used as a solid matrix with a "known" target analyte concentration) under the same experimental conditions as the samples. Also a known amount of two deuterated internal standards was added to CRM. The powdered sample was directly analyzed by ASAP-Q-TOF-MS and the semi-quantitative results were in the range given by the certified concentration values for some of the PAHs analyzed (Table S2, Fig. 6). Only in some cases (i.e., *m/z* 228.0782 benzo[*a*]anthracene/chrysene) a lower concentration compared with the minimum reference value was obtained (Figure 6). Probably the volatilization step from the solid

sediment sample affected the heaviest compounds more than the lighter ones. PAHs standard calibration curves are shown in Table S1 (SI material). Table S2 and Figure 6 show the data obtained for the method performance for the sediment CRM. Figure S4 (Supplementary Information material) shows the data obtained for semi-quantitative analysis of five replicates of oil samples with the internal standards. Twelve replicates of the CRM were analyzed and certified values and prediction interval values (min/max), obtained by interpolation in the calibration graph using the IS, were compared. The prediction values are the maximum and minimum value (interval) provided by the certified material. The mean value plus min/max obtained for the CRM by ASAP-Q-TOF-MS were plotted. As can be seen in the graphs (Figure 6) more than 90 % of the values were within the prediction interval values. It can be emphasized that the recommended sample amount for using the CRM is 10 g when applying the sample treatment and further quantitative analysis. However in this case, the sample amount taken for the analysis was much lower, as there is neither sample treatment nor chromatographic separation. Thus, a semi-quantitative approach can be claimed for and the predicted intervals are useful for comparison.

Semi-quantitative analysis (Table 2) of biological samples allowed us to find values below 2 µg/g for PAHs, and about 0.1µg/g for nitro-PAH and oxo-PAHs. Environmental samples (e.g. oil or contaminated soil) showed higher values of PAHs contamination, with values about 10 µg/g for the lighter compound (i.e. naphthalene) and below 1 µg/g for the heaviest ones such as benzo[*k*]fluoranthene and benzo[*a*]pyrene. For the four nitro-PAH congeners the values found were below 10 µg/g, and below 5 µg/g for the oxo-PAH congeners. Unfortunately, as was above mentioned, several common ions are produced from different congeners of each family under

study. Thus the semi-quantitative value in these cases cannot be assigned to only one compound and the total value has been provided, following the same system used in already published studies (e.g., Bamford et al, 2003) [36].

Conclusions

A simultaneous analysis and semi-quantitative approach of PAHs, nitro-PAHs and oxo-PAHs in different matrices, in one single acquisition by direct analysis either in solid or liquid state, without any sample treatment, has been achieved by a new ASAP-Q-TOF-MS technique. The soft ionization and the mass accuracy, together with the high resolution provided by the Q-TOF-MS detector, allowed us to identify a large number of PAHs (15 compound groups), nitro-PAHs (4 congeners) and oxo-PAHs (2 congeners) in complex matrices. Saliva and urine were also directly introduced and analyzed and some nitro-PAHs and oxo-PAHs were found respectively. The method performance was checked for semi-quantitative approach through the analysis of a sediment CRM for PAHs congeners. The addition of two internal standards (deuterated PAHs) with known concentration to the target samples provided information about the sample load and analysis. Semi-quantitative approach of these compounds in soil, oil, car exhaust particles, saliva and urine has been optimized using ASAP-Q-TOF-MS, which represents a new valuable achievement for direct screening and evaluation of these contaminants in many different samples. This method can be used as a first exploratory approach in complex matrices for target compounds. The total analysis only takes some minutes and represents a powerful tool for screening of many samples in environmental and health areas.

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Figure captions

Figure 1. Ramp temperature effect on the obtained mass spectra in an oil sample.

Figure 2. Cone voltage effect on oxo-PAHs standard solution of six ng/ml (20, 40, 60, 80 V) (Abbreviations Table 1). Desolvation temperature: 300ºC

Figure 3. ASAP-Q-TOF-MS spectrum (absolute response) of a standard solution of 18 ng/ml of each PAHs obtained at optimum conditions. (▲ denotes the molecular ion M^+ and ● the MH^+ ion series)

Figure 4. ASAP mass spectrum (absolute response) of a standard solution (eighteen ng/ml) of eight nitro-PAHs (▲ denotes the molecular ion M^+ and ● the MH^+ ion series)

Figure 5. ASAP-Q-TOF-MS mass spectrum (absolute response) of a standard solution (6 ng/ml each) of eight oxo-PAHs (▲ denotes the molecular ion M^+, ● the MH^+ ion series and ★o M ion)

Figure 6. Repetitive "analysis" of CRM (e.g., loads), with minimum and maximum prediction interval values (red line) for different PAHs congeners analyzed.

Figure 7. Overlay mass spectrum (absolute response) of nitro-PAHs standard at 6 ng/ml each and a polluted soil sample, in which a highly toxic nitro-PAH compounds was found (i.e., nitrofluorene).

Atmospheric pressure Solid Analysis Probe coupled to Quadrupole-time of Flight Mass Spectrometry as a tool for screening and semi-quantitative approach of Polycyclic Aromatic Hydrocarbons, Nitro-Polycyclic Aromatic Hydrocarbons and Oxo-Polycyclic Aromatic Hydrocarbons in complex matrices

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Abstract

A new screening and semi-quantitative approach has been developed for direct analysis of polycyclic aromatic hydrocarbons (PAHs) and their nitro and oxo derivatives in environmental and biological matrices using Atmospheric Pressure Solid Analysis Probe (ASAP) Quadrupole-Time of Flight mass spectrometry (Q-TOF-MS). The instrumental parameters were optimized for the analysis of all these compounds, without previous sample treatment, in soil, motor oil, atmospheric particles (ashes) and biological samples such as urine and saliva of smokers and non-smokers. Ion source parameters in the MS were found to be the key parameters, with little variation within PAHs families. The optimized corona current was 4 µA, sample cone voltage 80 V for PAHs, nitro-PAHs and oxo-PAHs, while the desolvation temperatures varied from 300 ºC to 500 ºC. The analytical method performance was checked using a certified reference material. Two deuterated compounds were used as internal standards for semi-quantitative purposes together with the pure individual standard for each compound and the corresponding calibration plot. The compounds nitro PAH 9-nitroanthracene and oxo-PAH 1,4-naphthalenedione, were found in saliva and urine in a range below 1 µg/g while the range of PAHs in these samples was below 2 µg/g. Environmental samples provided higher concentration of all pollutants than urine and saliva.

Keywords: direct analysis, polycyclic aromatic hydrocarbons, screening, organic pollutants, ambient ionization mass spectrometry, tobacco, cancer.

Introduction

The quality control requirements are more and more demanding, especially in areas of food and health, where consumers have a high concern about the likely contamination or affection to their health. The number of samples requiring analysis has increased dramatically in the last years as well as the required sensitivity of the current analytical procedures [1]. These characteristics imply a high cost of the analysis. For this reason, fast screening procedures are gaining importance, as they permit the evaluation of the presence or absence of specific compounds in the samples that may require a further analysis in depth. These screening methods, most of them based on mass spectrometry (MS), are now promoted in a wide variety of areas.

Ambient mass spectrometry (Ambient MS) analysis has experienced a very rapid development during the last seven years. This technique is defined as mass spectrometric analysis with minimal or no sample preparation using direct sampling and ionization at ambient conditions [2] and it represents the ideal tool for screening analysis. With the introduction of direct analysis in real time (DART) [3] and desorption electrospray ionization (DESI) [4], the analysis of samples without any prior treatment became possible for the first time [2]. In the DART technique, the sample is placed in a stream of helium that contains ionized atmospheric gases (such as water vapor and oxygen), and the analytes in the sample are ionized in open air in the laboratory environment. Desorption Electrospray Ionization (DESI) is carried out by directing charged droplets produced from a pneumatically-assisted electrospray onto a surface to be analyzed at atmospheric conditions. Ions of chemical species present on the surface, usually a paper impregnated in the sample, are produced through the interaction of charged droplets and the sample. DART and DESI are mainly qualitative techniques where the main purpose is to identify the

presence of specific compounds present at the surface of the sample, i.e. targeted analysis. The development of these techniques creates an awareness of the power of Ambient MS and a new sub-field in mass spectrometry. DART and DESI techniques usually coupled to Time of Flight (TOF) detectors have been widely applied as direct screening techniques to a wide variety of samples [5-10].

Atmospheric-pressure solid-analysis probe (ASAP) was developed in 2005 [11]. The sample, usually introduced into the ionization chamber with a glass rod dipped either in the powdered or liquid sample or rubbing the surface, is vaporized and ionized at atmospheric pressure inside a small chamber, thus providing the ions, which arrive at the MS detector [12-13]. The major difference between DART and ASAP is the ionization mode. While DART is open to the environment allowing for water to be part of the chemical ionization process, ASAP takes place in dry atmosphere. DESI, DART and ASAP can be considered as screening techniques to take rapid decision about the further analysis in depth. Their major advantages are the absence of sample treatment, which is usually a tedious, expensive and time consuming step. None of them have a chromatographic separation before detection. This means that all the analytes present in the sample matrix, or formed during the ionization step, reach the source and all of them can be analyzed, thus minimizing any analyte losses. Thus, these techniques are mainly focused on target analysis and constitute powerful tools for screening the absence or presence of target compounds. In a few minutes the sample is injected and processed.

In ASAP, several key parameters, such as corona current, sample cone voltage and gas desolvation temperature need optimization, because they condition the total number of ions arriving at the detector. Important features, like the temperature of the ion source chamber where the sample

is volatilized, remain constant (120ºC) to ensure the maximum volatilization. This technique can be applied to volatile or semi-volatile compounds in solid or liquid complex matrices, without previous sample treatment. The presence or absence of specific compounds at low concentration, and in some cases the order of magnitude of their concentration in the sample, are shown as very interesting characteristics of this technique. However, it is not possible to estimate toxicity from the presence of single compounds by using this method, because too similar compounds in environmental samples superimpose. Using ASAP for screening and semi-quantitative simultaneous analysis of several pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and their nitro and oxo derivatives in complex matrices, can be very useful in health and environmental areas. This is the main purpose of the present paper.

PAHs, nitro-PAHs and oxo-PAHs derivatives are organic compounds listed as priority pollutants by international environmental protection agencies due to their carcinogenic, mutagenic, and toxic effects [14-20]. There are a few applications of Ambient MS methods for direct analysis of PAHs and related compounds [21-26]. However, the possibility of having a semi-quantitative approach using ASAP has not been explored yet. For this reason, the main objective of this work is to evaluate the performance of ASAP-Q-TOF-MS as a screening tool and semi-quantitative approach for PAHs, nitro-PAHs and oxo-PAHs in several liquid and solid complex samples, such as car exhaust, oil, atmospheric dust, urine and saliva. As far as we know, this article represents the first study using direct analysis in one single run through ASAP-Q-TOF-MS for this large series of priority organic pollutants. It is also the first time that a semi-quantitative approach has been proposed using a direct ASAP-MS-Q-TOF in very different complex matrices. A solid certified reference material (CRM) for PAHs

was run in parallel with the samples, in order to check the method performance and the semi-quantitative data. The results obtained are shown and discussed.

Materials & Methods

Chemicals and Reagents

Certified standards of PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, cyclopenta[*cd*]pyrene, benzo [*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo [*a,h*]anthracene and benzo[*ghi*]perylene), Nitro-PAHs (1-nitronaphthalene, 2-methyl-1-nitronaphthalene, 1,5-dinitronaphthalene, 2-nitrofluorene, 9-nitroanthracene, 2-nitrofluoranthene, 1-nitropyrene and 6-nitrocrysene) and Oxo-PAHs (1,4-naphthalenedione, benzophenone, 9-fluorenone, anthrone, 9,10-anthracenedione, 7H-benz[*de*]anthracen-7-one, benzo[*a*]anthracene-7,12-dione and naphtacene-5,12-dione) were supplied by Sigma-Aldrich (Madrid, Spain). Acenaphthene-d10 (99 atom % D) and benzo[*a*]pyrene-7,8-d2 (98 atom % D) for being used as internal standards were purchased from Sigma-Aldrich (Madrid, Spain). Detailed information about the molecular weight, chemical formula, CAS number and molecular structure for each of the analyzed compounds of the three families can be found in Table 1 and Figures S1, S2 and S3 in the Supplementary Information material. All the standard solutions were prepared in methanol reagent grade purchased from Scharlab (Barcelona, Spain). Combined solutions of the three families (PAHs, nitro-PAHs and oxo-PAHs) containing different concentrations of each standard were also used to check the performance of the mixtures in ASAP. A sediment as certified reference material (RTC-CRM104-50G, RT-Corp, WY, USA) containing PAHs was supplied by Sigma-Aldrich (Madrid, Spain). The amount of deuterated compounds

added to both the standard mixture solution and the samples was in each case in the required final concentration of 10 ng/g IS in the sample. All standards were under gravimetric control and all concentrations are expressed as w/w basis.

ASAP-Q-TOF-MS instrument

The Atmospheric Solids Analysis Probe (ASAP) was coupled to a quadrupole-time of flight-mass spectrometer (QToF-MS), Xevo G2 QTOF (Waters Corporation, Manchester, UK). The instrument has a mass range up to *m/z* 100 000 and a resolving power of >22,500 full width at half maximum (FWHM). In the ion source atmospheric pressure chemical ionization was used and the chamber temperature is constant at 120ºC. The key ion source parameters to be optimized were: corona current (µA), sample cone voltage (V) and desolvation gas temperature (ºC). Acquisition mode used was MS full-scan data from 70 to 800 amu. Nitrogen was used as desolvation gas at 400 Lh^{-1} flow. Cone flow was not needed for this technique. Currently, ASAP requires manual injection and can neither be processed unattended nor automatically.

ASAP-Q-TOF-MS analysis

As this technique is for target analysis, the first step is to analyze the individual compounds under the optimal experimental conditions. Variables such as cone voltage, desolvation gas temperature and corona current were optimized using standard solutions of pure compounds. Voltage of sampling cone was varied from 20 V to 80 V and voltage of the extraction cone was fixed at 0.1 V. The parameters of acquisition were as follows: a scan time of 1 s and mass range of *m/z* 70 – 800 to cover all PAH, nitro-PAH and oxo-PAH standards [27]. For each MS spectrum, the

characteristic masses were selected and this information was used for searching the presence or absence of each compound in the real samples. Positive identification of the compounds was done using pure standards for all analyzed compounds, using several ions for each compound.

The samples were analyzed in continuous mode (3 min), with a cone voltage of 80 V. The desolvation gas temperature was manually varied from 200 ºC to 500 ºC to ensure the progressive volatilization of all the compounds and thus facilitate the target analysis of the sample. In this way, the three families of compounds were analyzed in only one acquisition. The ions from the lighter compounds appeared first and those from the heavier compounds arrived later at the detector. This procedure facilitated the identification of the compounds, as the corresponding ions were coming progressively according to their size. The ratio between ramp temperature and time scan can be seen in Figure 1. The maximum intensity of each target ion together with the comparison versus the pure individual standards were used for further identification. Atmospheric Pressure Ionization (API) in positive polarity was selected.

For the analyzed samples, the same scan time was used (1s) and the mass range was from *m/z* 70 to 800 to cover all the likely present compounds. A new glass rod was used for each sample. Before the analysis the glass rod was inserted into the source chamber at high temperature for two minutes to remove any contamination from the tip. The rod was then removed, cooled and then dipped into the sample for ten seconds. Solid samples (powdered) sticked to the glass rod, while liquid samples wet the rod. All samples were previously spiked with two deuterated internal standards, as above described, to check the sampling load and the performance of the whole process. In liquid samples the IS were dissolved giving a homogeneous solution. Solid samples (1g) spiked with IS (10 µL of

methanol solution) were homogenized in a vortex and a homogeneous slurry was obtained. Sample load was controlled through these IS, as previous calibration plot under the same experimental conditions was carried out with the IS. Calibration plots were previously obtained for both IS in standard solution. This plot was further used to analyze the sample load and facilitate the semiquantitative approach.

Three replicates of each sample were analyzed. The accuracy and reproducibility (referred to mass accuracy) of all analyses was guaranteed by using a Lockspray. Leucine-enkephalin, generating $[MH]^+$ ions at 556.2771 Da was employed as the lock mass at a concentration of 2 ng mL^{-1} in water/acetonitrile with 0.1% formic acid. Blanks were obtained without loading any sample in the glass rod during the first 30 - 40 sec of the acquisition, while applying the same temperature ramp as in the sample acquisition. Serial diluted solutions (0.1, 0.5, 1, 5, 10, 20 ng/ml) of individual standards at different concentrations were used to establish the limit of detection (LOD) and limit of quantitation (LOQ) under the same experimental conditions as the samples. LOD and LOQ were calculated as three times or ten times respectively the standard deviation of the blank at the same characteristic mass as each compound. The three standard solutions containing the three chemical groups were independently processed. Data were collected and processed using MassLynx software (Waters Corporation). Confirmation with the pure standards processed under the same conditions was carried out. Mass spectra shown in the figures are based on the average of several mass spectra.

Reference material & standards

A solid sediment as CRM for PAHs was used without previous sample treatment to demonstrate the performance of the semi-quantitative approach in direct analysis by ASAP-Q-TOF-MS. The CRM was analyzed under the same experimental conditions as the samples. The glass rod was dipped into the powdered CRM sample and then introduced into ASAP. In order to know the amount of sample loaded into the system as well as to detect any problem related with the matrix effect or ion suppression phenomena, 1 g of all samples was spiked with 10 μL of methanolic solution containing two deuterated PAHs (acenaphthene d10 and benzo[*a*]pyrene 7,8-d2) to get a final concentration of 10 ng/g of each IS. After spiking, the sample was shaken in a vortex and homogenized before the analysis. The IS were analyzed and taken into account for semiquantitative purposes.

Samples

After optimization of the instrumental parameters for the three PAH families (PAHs, nitro-PAHs and oxo-PAHs), different types of samples with complex matrices (motor oil, soil, combustion particles, urine and saliva) were directly analyzed by ASAP-Q-TOF-MS in order to demonstrate the performance of the method. The glass rod was dipped into the solid or liquid sample and directly introduced into the ASAP chamber. As was above mentioned, fine powdered sample or small drops of liquid samples were sticked to the glass rod. The MS spectra acquired by MS-Q-TOF were registered and the abundance (response) of each ion referred to the internal standards was used for semi-quantitative purposes in all cases.

The description of the analyzed samples is as follows:

- mineral motor oil from an automatic generator set

- soil from a parking lot heavily contaminated with vehicle exhaust and carburants
- combustion particles from the exhaust pipe of a diesel central heating system of a building
- biological fluids: a) saliva from a heavy smoker (more than 20 cg/day) taken in the morning before smoking the first cigarette of the day and 1 hour after smoking 1 cigarette; b) urine from a heavy smoker and from a non-smoker

Results and Discussion

ASAP analysis

The sample treatment is usually the bottle neck of any analytical procedure. Working with solid samples, materials or biological samples is always time consuming, as the dissolution, often derivatization and clean-up step are required before the analysis. All these sample treatment steps are usually manually managed, as automatic devices required loading the samples to process them in the right way. The whole analytical procedure (sample treatment + analysis) has to be done for qualitative and quantitative analysis. In this frame, ASAP is an excellent tool for target analysis, as the absence or presence of target compounds can be immediately achieved. This way, the further analysis in depth can be applied to only those samples with positive response from ASAP-MS-Q-TOF. Of course this technique cannot substitute the whole analytical procedure for quantitative analysis, but having the identification/presence of a compound and the idea of order of magnitude for the analyte is very useful to pass/fail criteria.

Due to no sample pre-treatment, direct injection and no chromatographic run, sample and blank are acquired in 3 min. This time can be considered as “fast” analysis.

With the identification of the compounds of interest, the absolute ion intensity can be quantified with respect to the internal standards added to the sample, and the values are later used for semi-quantitative purposes.

Optimization of Ion source parameters

Following, key parameters were explored: Corona current (µA), that affects the ionization; sample cone voltage (V), that influences fragmentation and desolvation gas temperature (ºC), that affects volatilization. Also different values for desolvation gas flow were tested. However, desolvation gas flow was found to have a minor influence for the ASAP generated spectrum, so a fixed value was used for the PAH families. The main purpose was to carry to the detector as much ions as possible, thus diminishing the in-source fragmentation what facilitates the further identification. To achieve this, one key parameter was changed while maintaining the others fixed. As expected, cone voltage was the unique parameter affecting the fragmentation. Three different optimization sets were carried out, one for each family of pollutants (PAHs, nitro-PAHs and oxo-PAHs). Optimal values for each family of analyzed compounds can be seen in Table 1. The target samples were analyzed for about 3min, applying a desolvation temperature ramp and running a blank before and after each of the running samples.

The gas desolvation temperature (300, 400 and 500 ºC) had no effect on the resulting spectra of the PAHs and nitro-PAHs, neither in fragmentation nor in the signal response (i.e., sensitivity). A different trend was found for the oxo-PAH family, where a higher sensitivity/response was found at a lower temperature (300 ºC) for the lighter and medium weight compounds, but not for the heaviest ones (*m/z* 258.0681 benzo[*a*]anthracene-

dione/naphthacene-5,12-dione). For these two congeners the highest sensitivity was obtained at 500 ºC.

With a fixed gas desolvation temperature (500 ºC for PAHs/nitro-PAH and 300 ºC for oxo-PAH) the cone voltage was varied from 20, 40, 60 and 80 V for each compound family. Changing the cone voltage had a minor effect on the intensity of the spectra obtained for the nitro-PAH. Signal and fragmentation increased in parallel as the cone voltage increased. Sampling cone voltages between 20 V and 30 V can be considered typical for small compounds, causing minimal fragmentation. However for oxo-PAHs a similar signal and fragmentation pattern were found between 20 V and 80 V (data shown in Figure 2). As can be seen, the minimum fragmentation and signal were obtained at 20 V, while the maximum was at 80 V. This indicates that oxo-PAHs are more resistant to fragmentation, even at cone voltages as high as 80 V and consequently the relationship between cone voltage and fragmentation of oxo-PAHs cannot be anticipated as a general performance.

Mass spectra for each PAH families

For PAHs, nitro-PAHs and oxo-PAHs, corona current (µA) was investigated over the range from 3 to 6, sample cone (V) at 20, 40, 60 and 80, and desolvation gas temperature (ºC) from 300 to 500. Figure 3 shows the mass spectra obtained in optimum conditions for the analytical standards of PAHs (corona current 4 µA, sample cone at 80 V and desolvation gas temperature 500 ºC).

As there is no previous chromatographic separation, this technique is not capable to differentiate between several PAH isomers (e.g., *m/z* 202.0782 pyrene/fluoranthene, *m/z* 228.0782 benzo[*a*]anthracene/chrysene, etc),

since they have the same mass, which is a drawback of this direct technique. As can be seen in Figure 3, the dominant ion sequence corresponds to the molecular ion (▲) and to the protonated ion (●, MH^+), except for naphthalene. The protonated ion (MH^+) was the most abundant in all the analyzed compounds with the only exception of naphthalene and fluorene. The protonated ion (MH^+) was used for semi-quantitative purposes (except for naphthalene, in which M^+ was used). The ratio between both species depends on sample conditions (e.g., humidity content) and this ratio was stable over the time experiment. According to Ray *et al* [27], who worked with ASAP and steroids, the analysis of steroids by ASAP generally results in the formation of the product ion as either the radical cation $M^{+\bullet}$ or the protonated molecule $(MH)^+$. The formation of the protonated molecule is a result of proton transfer from ionized water clusters in the source. However, if the source is dry, as in ASAP, the formation of the radical cation is the primary ionization mechanism. The same behavior can be observed with PAHs. The mass spectrum from ASAP-MS-QTOF in optimum conditions of a mixture of eight nitro-PAHs is shown in Figure 4. The molecular ion (▲) and the protonated ion (●) were the dominant ion series and again the charged ion series (MH^+) was the most abundant for all the compounds. This performance with Q-TOF-MS has been observed in other works [28].

Figure 5 shows the mass spectrum of a mixture of eight oxo-PAHs from ASAP-MS-QTOF. In a similar fashion as that for the parent PAHs and nitro-PAHs, the molecular ion (▲) and the protonated ion (●, MH^+) were the dominant ion series and again the ion sequence was the most abundant for all the compounds.

Limits of detection, established with the pure compounds in methanol solution, as above described, were found to be around 20 ppb (ng/g) for

PAHs and nitro-PAHs and only 5 ppb (ng/g) for the oxo-PAHs in the methanol solution of the standards. This difference in sensitivity could be explained by the higher ability or affinity of oxygen atoms in oxo-PAHs than in other PAHs to accept protons. The mass resolution and mass accuracy (ppm error), for the three families are shown in Table S3 of the supporting information material.

Matrix influence and ion suppression phenomena can be a serious problem in any conventional analysis. Direct ASAP can be also affected by matrix influence. To investigate this potential problem, two different deuterated internal standards were added to both the samples and the CRM. From the results obtained it can be confirmed that matrix influence was observed but with very low intensity in all samples under study, probably because the analyzed samples were volatilized in the ASAP chamber and the ionization took place without problems (Figures 6 and S4 SI material). Matrix influence and/or ion suppression phenomena can be the reason why some of the analyzed compounds display a higher standard error in the repetitive injections of the oil sample (i.e., *m/z* 154.0782 acenaphthene) (Figure S4).

Qualitative Analysis of Samples

An important advantage of the ASAP-Q-TOF-MS technique is the accurate mass obtained for the compounds of interest, so that the identification of compounds in complex matrices (i.e., oil, soil or saliva) without any prior treatment is much easier. The confirmation with the pure standards of each compound is usually required to confirm the identification.

Direct analysis using ASAP-MS-QTOF provides the exact masses of all the compounds present in the sample. As there is not chromatographic separation those isomers having exactly the same *m/z* values cannot be

confirmed. However, the presence of specific *m/z* value confirms the presence of some of the isomers. Several examples can be highlighted such as *m/z* 178.0782, which is common to phenanthrene and anthracene. For this reason several compounds have been included in Table 2 for each *m/z* value when required.

In the biological samples (i.e. saliva and urine from smokers), three families of contaminants were identified. Urine and saliva samples had constant conditions of humidity, as they are aqueous samples. In these conditions the protonation is improved and better results can be found. PAHs were the most abundant with at least three congeners (*m/z* 154.0782 acenaphthene, *m/z* 178.0782 phenanthrene/anthracene, *m/z* 228.0782 benzo[*a*]anthracene/chrysene) in both types of samples. These results are in accordance with the literature [29, 30]. Meanwhile, only one congener of the nitro-PAH family was found (*m/z* 223.0633 probably, 9-nitroanthracene) in the saliva sample and only one oxo-PAH congener (*m/z* 158.0368 probably, 1,4-naphthalenedione) in the urine sample. In both cases these compounds were found in a heavy smoker just after smoking and not in a non-smoker. These results agree with those anticipated, i.e. that the parental compounds (PAHs) were more abundant than their reaction products (nitro or oxo-PAHs). However, it is important to note that 9-nitroanthracene was present already in the tobacco smoke, probably formed from anthracene during the smoking process, while 1,4-naphthalenedione was the result of oxidation of naphthalene in the human body and therefore excreted in the urine.

Figure S5 shows the spectra for the PAH standards and saliva sample after smoking. The highly carcinogenic aromatic compounds (*m/z* 252.0939 either benzo[*a*]pyrene or benzo[*k*]fluoranthene or both) can be seen in saliva samples, together with many other compounds (e.g. *m/z* 152.0626

acenaphthylene, *m/z* 154.0782 acenaphthene or *m/z* 166.0782 fluorene), from a heavy smoker immediately after smoking [29]. However, benzo[*a*]pyrene is diluted with time in the mouth and disappears, indicating that it is swallowed and assimilated by the body. None of these compounds were found in a non-smoker saliva sample. This finding also confirms that these pollutants are coming from tobacco and not from environmental atmosphere. All these compound groups related to the sample from a heavy smoker are in good agreement with other studies related to the tobacco smoke exposure [31].

The analysis of urine samples also showed many possible PAHs congeners (e.g., *m/z* 154.0782 acenaphthene, *m/z* 178.0782 phenanthrene/anthracene or, *m/z* 228.0782 benzo[*a*]anthracene/chrysene), which were correlated with the congeners found in saliva, as well as an oxo-PAHs congener (*m/z* 158.0368 1,4-naphthalenedione).

A large number of PAH-like pollutants was found in the analyzed environmental samples, as expected and in accordance with the literature [32-35]. The highest number of congeners was found in the soil sample (16) followed by the oil sample (15) and the ash sample with 9 compounds. Within the analyzed chemical families, PAHs account for the vast majority of congeners (14), followed by nitro-PAHs (4) and then oxo-PAHs (2). Figure 7 shows the overlapped spectra of the nitro-PAHs and a polluted soil sample, in which highly toxic nitro-PAH compounds were found (i.e., *m/z* 211.0633 2-nitrofluorene). This compound is a well-known environmental pollutant and has been found as a major component in diesel exhaust [35].

Semi-quantitative approach

A semi-quantitative approach was attempted in order to gain information about the target analyte concentration in the samples. As discussed above, this technique is a powerful tool for the identification of a wide range of target compounds without any prior sample treatment. It can therefore be used as an exploratory approach for unknown samples. If there is evidence of the presence of target compounds, after this screening step, a further sample treatment step may be carried out if necessary. Two internal standards were added to all samples before the analysis, in order to have a better knowledge of sample loaded by the glass rod. The standard solutions used for calibration curves were handled and analyzed in the same manner as the samples, this means by direct analysis by ASAP-Q-TOF-MS, hence the results can be interpolated. This way, semi-quantitative values of these pollutants in the different sample matrices (i.e. solid and liquids) were obtained. It is important to point out that there is a difference between the direct load of a solid sample (the soil or sediment in this case) and the load of target compounds in a methanol solution of standards or in a liquid sample. For this reason only the order of magnitude of each compound can be obtained and not an accurate individual concentration.

The method performance was checked with the analysis of a sediment CRM for PAHs (used as a solid matrix with a "known" target analyte concentration) under the same experimental conditions as the samples. Also a known amount of two deuterated internal standards was added to CRM. The powdered sample was directly analyzed by ASAP-Q-TOF-MS and the semi-quantitative results were in the range given by the certified concentration values for some of the PAHs analyzed (Table S2, Fig. 6). Only in some cases (i.e., *m/z* 228.0782 benzo[*a*]anthracene/chrysene) a lower concentration compared with the minimum reference value was obtained (Figure 6). Probably the volatilization step from the solid

sediment sample affected the heaviest compounds more than the lighter ones. PAHs standard calibration curves are shown in Table S1 (SI material). Table S2 and Figure 6 show the data obtained for the method performance for the sediment CRM. Figure S4 (Supplementary Information material) shows the data obtained for semi-quantitative analysis of five replicates of oil samples with the internal standards. Twelve replicates of the CRM were analyzed and certified values and prediction interval values (min/max), obtained by interpolation in the calibration graph using the IS, were compared. The prediction values are the maximum and minimum value (interval) provided by the certified material. The mean value plus min/max obtained for the CRM by ASAP-Q-TOF-MS were plotted. As can be seen in the graphs (Figure 6) more than 90 % of the values were within the prediction interval values. It can be emphasized that the recommended sample amount for using the CRM is 10 g when applying the sample treatment and further quantitative analysis. However in this case, the sample amount taken for the analysis was much lower, as there is neither sample treatment nor chromatographic separation. Thus, a semi-quantitative approach can be claimed for and the predicted intervals are useful for comparison.

Semi-quantitative analysis (Table 2) of biological samples allowed us to find values below 2 µg/g for PAHs, and about 0.1µg/g for nitro-PAH and oxo-PAHs. Environmental samples (e.g. oil or contaminated soil) showed higher values of PAHs contamination, with values about 10 µg/g for the lighter compound (i.e. naphthalene) and below 1 µg/g for the heaviest ones such as benzo[*k*]fluoranthene and benzo[*a*]pyrene. For the four nitro-PAH congeners the values found were below 10 µg/g, and below 5 µg/g for the oxo-PAH congeners. Unfortunately, as was above mentioned, several common ions are produced from different congeners of each family under

study. Thus the semi-quantitative value in these cases cannot be assigned to only one compound and the total value has been provided, following the same system used in already published studies (e.g., Bamford et al, 2003) [36].

Conclusions

A simultaneous analysis and semi-quantitative approach of PAHs, nitro-PAHs and oxo-PAHs in different matrices, in one single acquisition by direct analysis either in solid or liquid state, without any sample treatment, has been achieved by a new ASAP-Q-TOF-MS technique. The soft ionization and the mass accuracy, together with the high resolution provided by the Q-TOF-MS detector, allowed us to identify a large number of PAHs (15 compound groups), nitro-PAHs (4 congeners) and oxo-PAHs (2 congeners) in complex matrices. Saliva and urine were also directly introduced and analyzed and some nitro-PAHs and oxo-PAHs were found respectively. The method performance was checked for semi-quantitative approach through the analysis of a sediment CRM for PAHs congeners. The addition of two internal standards (deuterated PAHs) with known concentration to the target samples provided information about the sample load and analysis. Semi-quantitative approach of these compounds in soil, oil, car exhaust particles, saliva and urine has been optimized using ASAP-Q-TOF-MS, which represents a new valuable achievement for direct screening and evaluation of these contaminants in many different samples. This method can be used as a first exploratory approach in complex matrices for target compounds. The total analysis only takes some minutes and represents a powerful tool for screening of many samples in environmental and health areas.

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Figure captions

Figure 1. Ramp temperature effect on the obtained mass spectra in an oil sample.

Figure 2. Cone voltage effect on oxo-PAHs standard solution of six ng/ml (20, 40, 60, 80 V) (Abbreviations Table 1). Desolvation temperature: 300ºC

Figure 3. ASAP-Q-TOF-MS spectrum (absolute response) of a standard solution of 18 ng/ml of each PAHs obtained at optimum conditions. (▲ denotes the molecular ion M^+ and ● the MH^+ ion series)

Figure 4. ASAP mass spectrum (absolute response) of a standard solution (eighteen ng/ml) of eight nitro-PAHs (▲ denotes the molecular ion M^+ and ● the MH^+ ion series)

Figure 5. ASAP-Q-TOF-MS mass spectrum (absolute response) of a standard solution (6 ng/ml each) of eight oxo-PAHs (▲ denotes the molecular ion M^+, ● the MH^+ ion series and ★ no M ion)

Figure 6. Repetitive "analysis" of CRM (e.g., loads), with minimum and maximum prediction interval values (red line) for different PAHs congeners analyzed.

Figure 7. Overlay mass spectrum (absolute response) of nitro-PAHs standard at 6 ng/ml each and a polluted soil sample, in which a highly toxic nitro-PAH compounds was found (i.e., nitrofluorene).

Tables

Table 1. Chemical characteristics and optimized parameters of the PAHs, nitro-PAHs and oxo-PAHs analyzed

Compounds	Abbreviation	CAS	MW	Formula	Corona (µA)	Desolvation temperature (ºC)
Naphthalene	NAP	91-20-3	128.0626	$C_{10}H_8$	4	500
Acenaphthylene	ACN	208-96-8	152.0626	$C_{12}H_8$	4	500
Acenaphthene	ANA	83-32-9	154.0782	$C_{12}H_{10}$	4	500
Fluorene	FL	86-73-7	166.0782	$C_{13}H_{10}$	4	500
Phenanthrene	PH	85-01-8	178.0782	$C_{14}H_{10}$	4	500
Anthracene	ANTH	120-12-7	178.0782	$C_{14}H_{10}$	4	500
Fluoranthene	FLAN	206-44-0	202.0782	$C_{16}H_{10}$	4	500
Pyrene	PY	129-00-0	202.0782	$C_{16}H_{10}$	4	500
Cyclopenta [*cd*] pyrene	CPY	27208-37-3	226.0782	$C_{18}H_{10}$	4	500
Benzo[*a*]anthracene	BANTH	56-55-3	228.0782	$C_{18}H_{12}$	4	500
Chrysene	CH	218-01-9	228.0782	$C_{18}H_{12}$	4	500
Benzo[*k*]fluoranthene	BFLAN	207-08-9	252.0939	$C_{20}H_{12}$	4	500
Benzo[*a*]pyrene	BAP	50-32-8	252.0939	$C_{20}H_{12}$	4	500
Dibenzo[*a*,*h*]anthracene	DAN	53-70-3	278.1096	$C_{22}H_{14}$	4	500
Benzo[*ghi*]perylene	BPERY	191-24-2	276.0939	$C_{22}H_{12}$	4	500
1-Nitronaphthalene	1-N-NAP	86-57-7	173.0477	$C_{10}H_7NO_2$	6	500
2-Methyl-1-Nitronaphthalene	2-m-1-N-NAP	881-03-8	187.0633	$C_{11}H_9NO_2$	6	500
1,5-Dinitronaphthalene	1,5-dN-NAP	605-71-0	218.0328	$C_{10}H_6N_2O_4$	6	500
2-Nitrofluorene	2-N-FL	607-57-8	211.0633	$C_{13}H_9NO_2$	6	500
9-Nitroanthracene	9-N-ANTH	602-60-8	223.0633	$C_{14}H_9NO$	6	500
2-Nitrofluoranthene	2-N-FLAN	892-21-7	247.0633	$C_{16}H_9NO_2$	6	500
1-Nitropyrene	1-N-PY	5522-43-0	247.0633	$C_{16}H_9NO_2$	6	500
6-Nitrocrysene	6-N-CH	08-02-7496	273.079	$C_{18}H_{11}NO_2$	6	500
1,4-Naphthalenedione	1,4-O-NAP	130-15-4	158.0368	$C_{10}H_6O_2$	6	300
Benzophenone	BENPHO	119-61-9	182.0732	$C_{13}H_{10}O$	6	300
9-Fluorenone	9-O-FL	486-25-9	180.0575	$C_{13}H_8O$	6	300
Anthrone	ANTHR	90-44-8	194.0732	$C_{14}H_{10}O$	6	300
9,10-Anthracenedione	9,10-O-ANT	84-65-1	208.0524	$C_{14}H_8O_2$	6	300
7H-Benzo[*de*]anthracen-7-one	7H-BANT	82-05-3	230.0732	$C_{17}H_{10}O$	6	300
Benzo[*a*]anthracene-7,12-dione	7,12-BANT	2498-66-0	258.0681	$C_{18}H_{10}O_2$	6	300
Naphthacene-5,12-dione	NAP-5,12-O	1090-13-7	258.0681	$C_{18}H_{10}O_2$	6	300

Table 2. Identified and semi-quantitation range values (µg/g or µg/ml) compounds in complex matrices analyzed in this work (+: 0 – 0.1; ++: 0.1-1; +++: 1-10; ++++: >10; µg/g or µg/ml)

Compounds	Oil	Soil	Ash	Saliva	Urine
Naphthalene	+++	+++	+++		
Acenaphthylene	+++	++	+++		
Acenaphthene		++	++	++	+++
Fluorene	+++	+++	+++		
Phenanthrene/ Anthracene	+++		+++	+++	
Fluoranthene/ Pyrene	+++	++++	+++		
Cyclopenta [*cd*] pyrene					
Benzo[*a*]anthracene/Chrysene	++	++	+	+	++
Benzo[*k*]fluoranthene/ Benzo[*a*]pyrene	++	++			
Dibenzo[*a*,*h*]anthracene					
Benzo[*ghi*]perylene					
1-Nitronaphthalene					
2-Methyl-1-Nitronaphthalene	+++	++			
1,5-Dinitronaphthalene					
2-Nitrofluorene	+++	++			
9-Nitroantracene				+	
2-Nitrofluoranthene/1-Nitropyrene					
6-Nitrocrysene					
1,4-Naphthalenedione					++
Benzophenone					
9-Fluorenone					
Anthrone					
9,10-Anthracenedione	+++		+++		
7H-Benzo[*de*]anthracen-7-one					
Benzo[*a*]anthracene-7,12-dione/ Naphthacene-5,12-dione					

Figure 1

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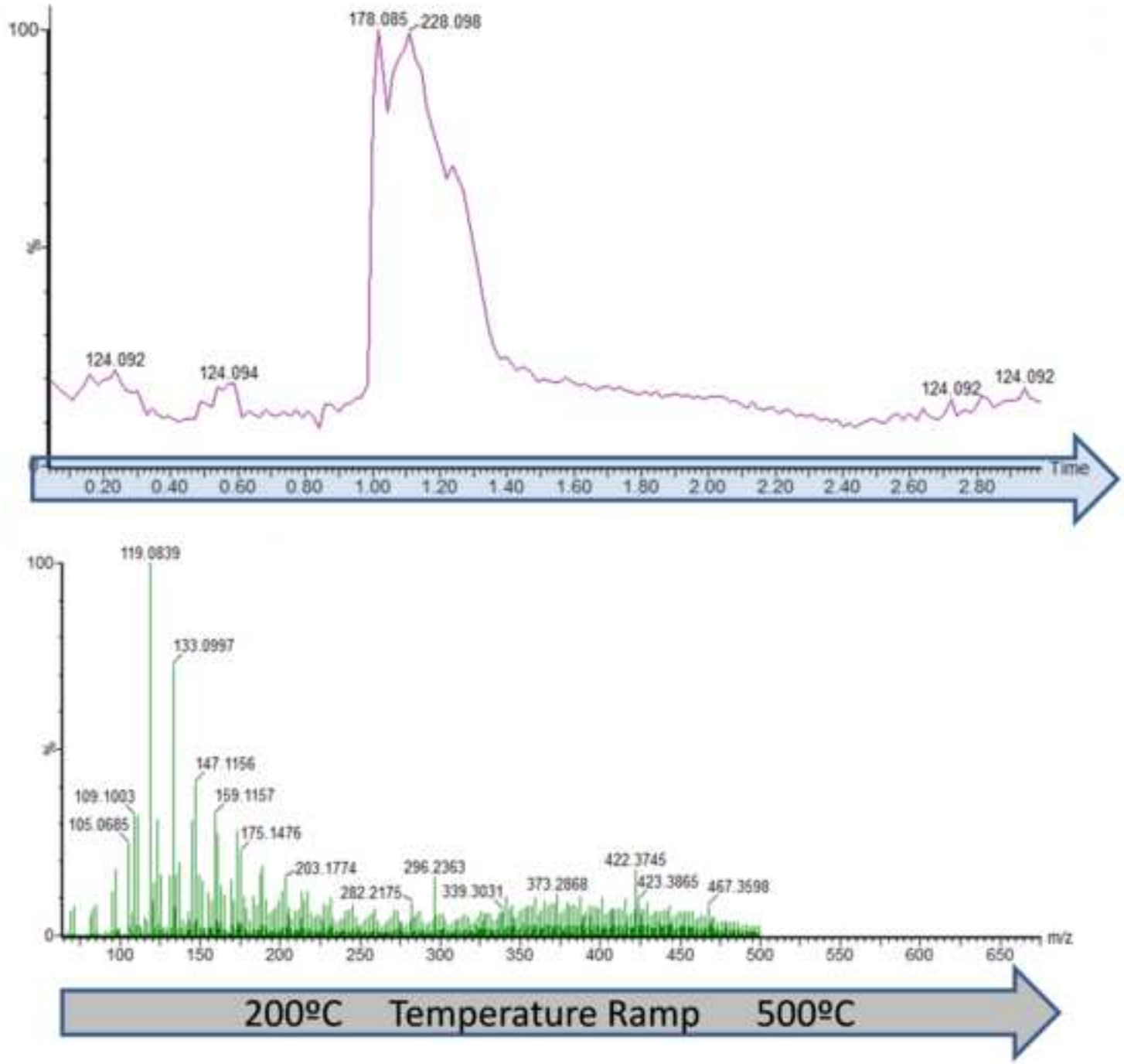

Figure 2

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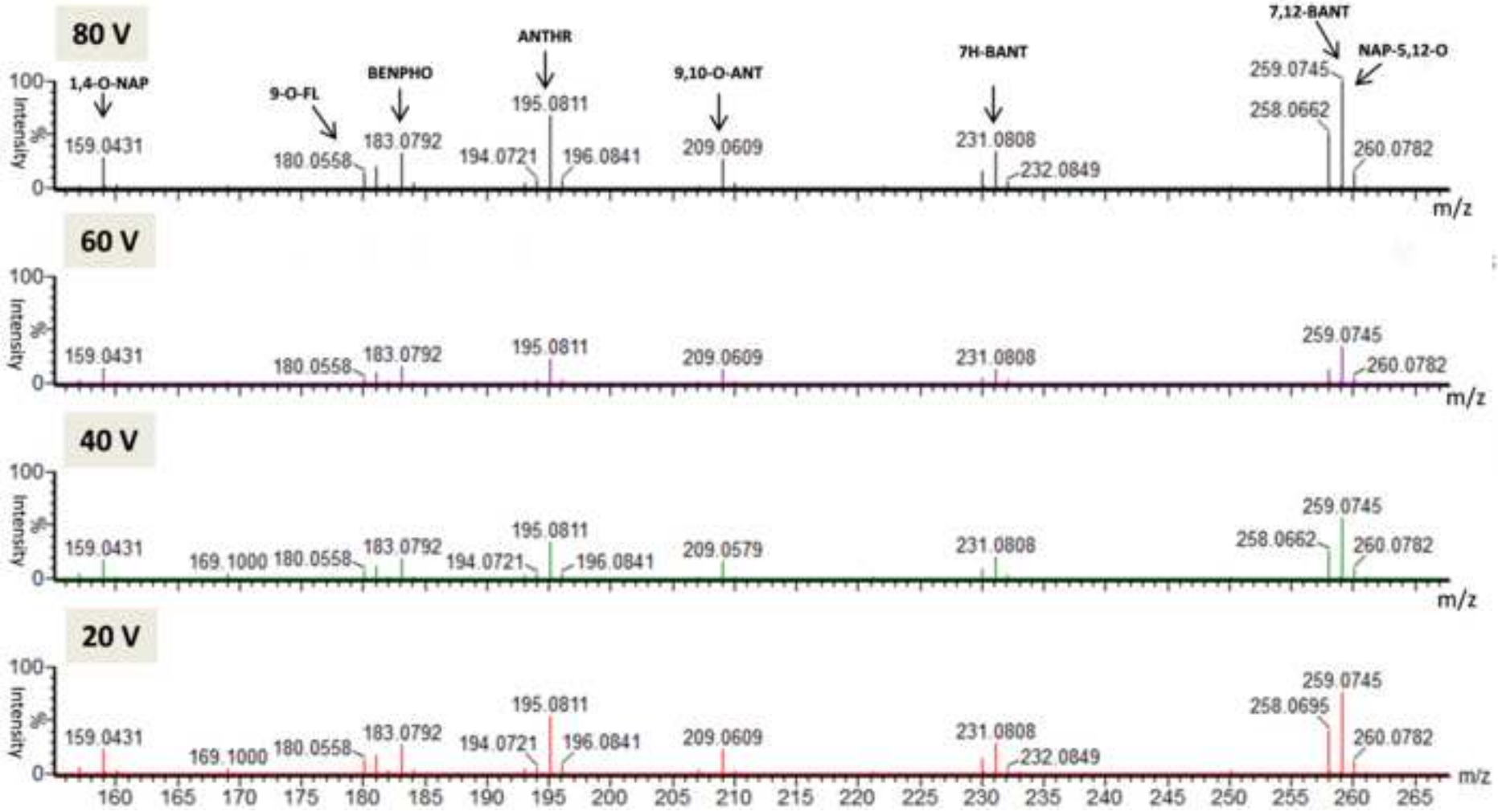

Figure 3

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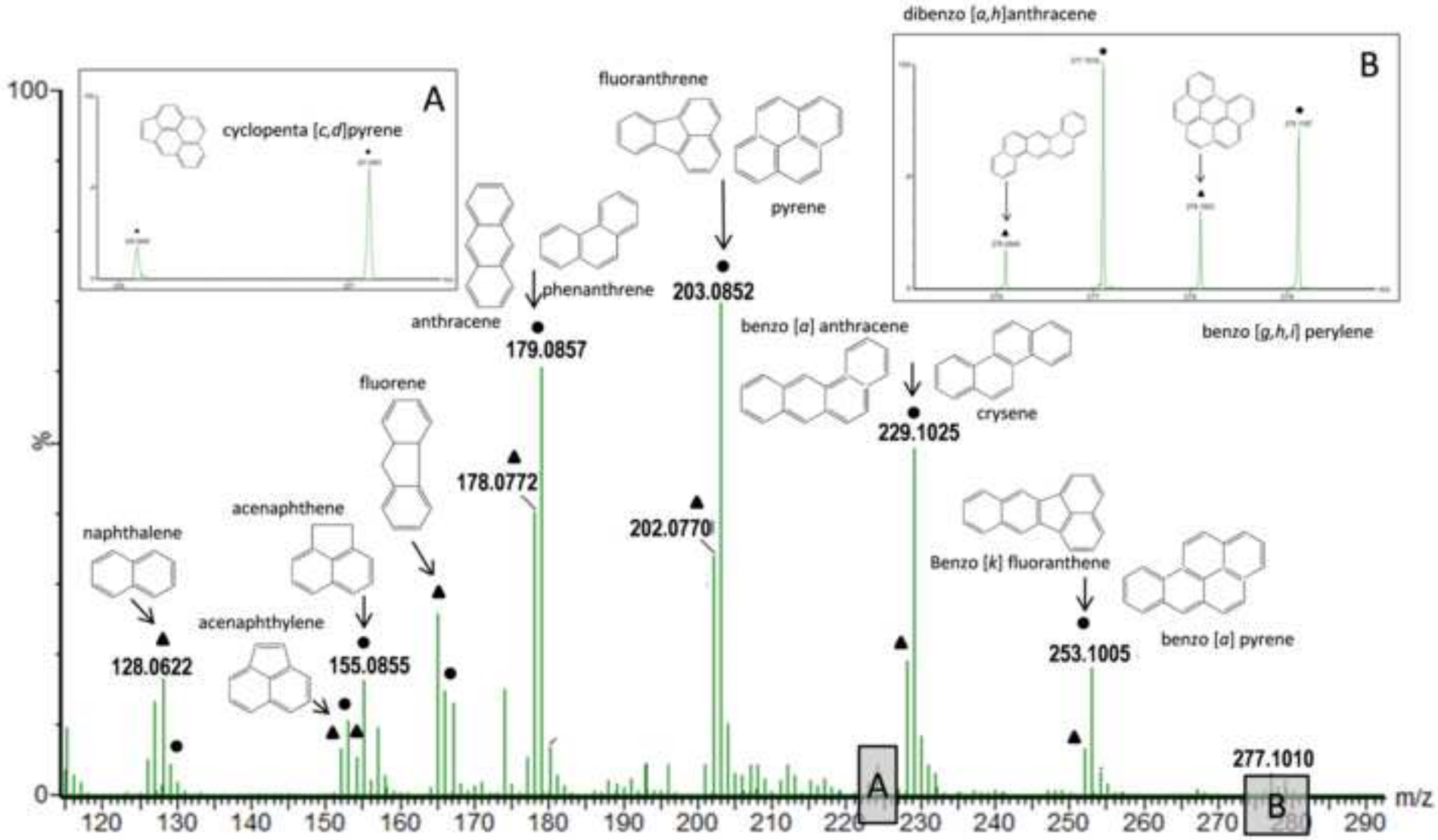

Figure 4

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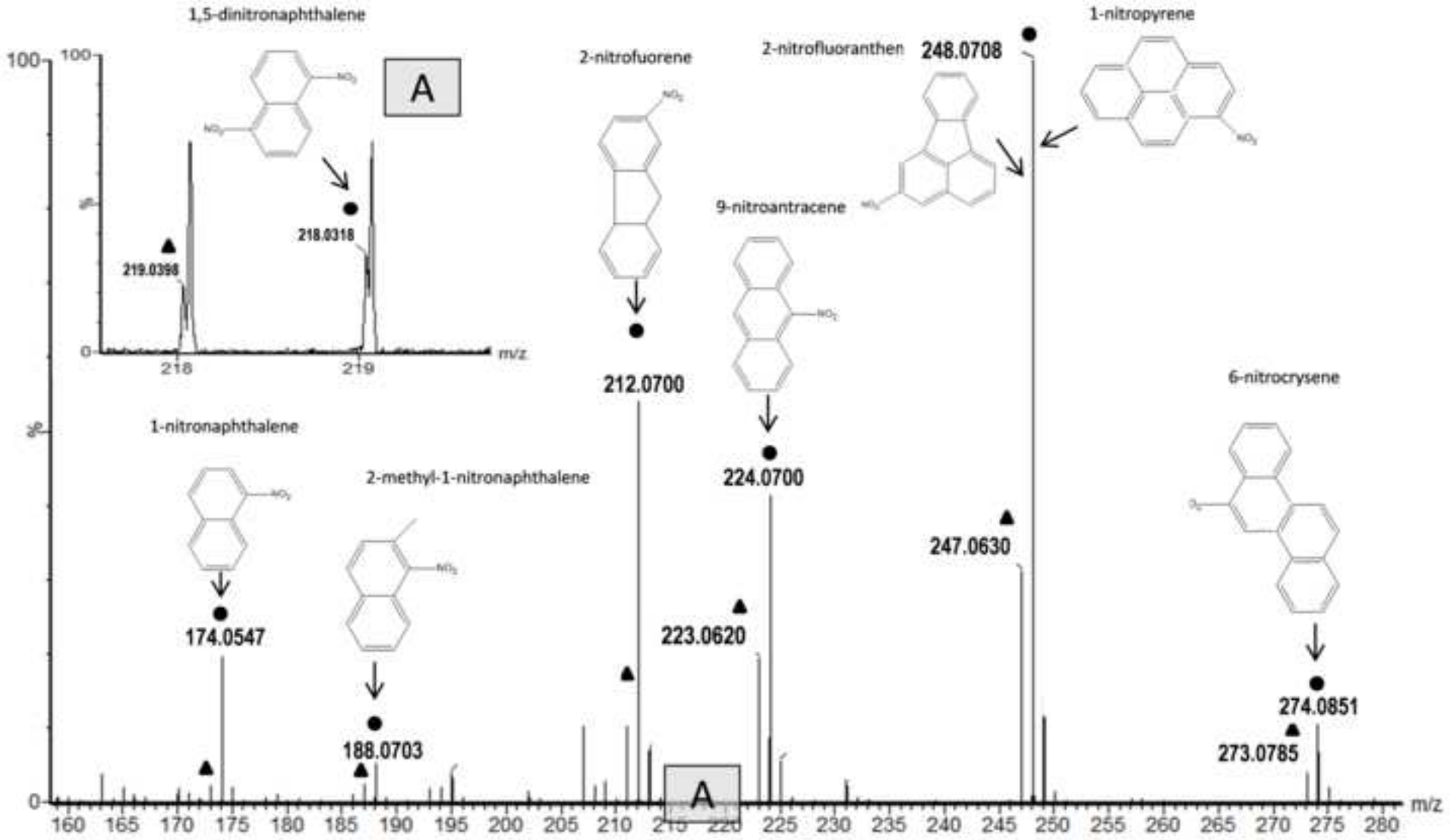

Figure 5

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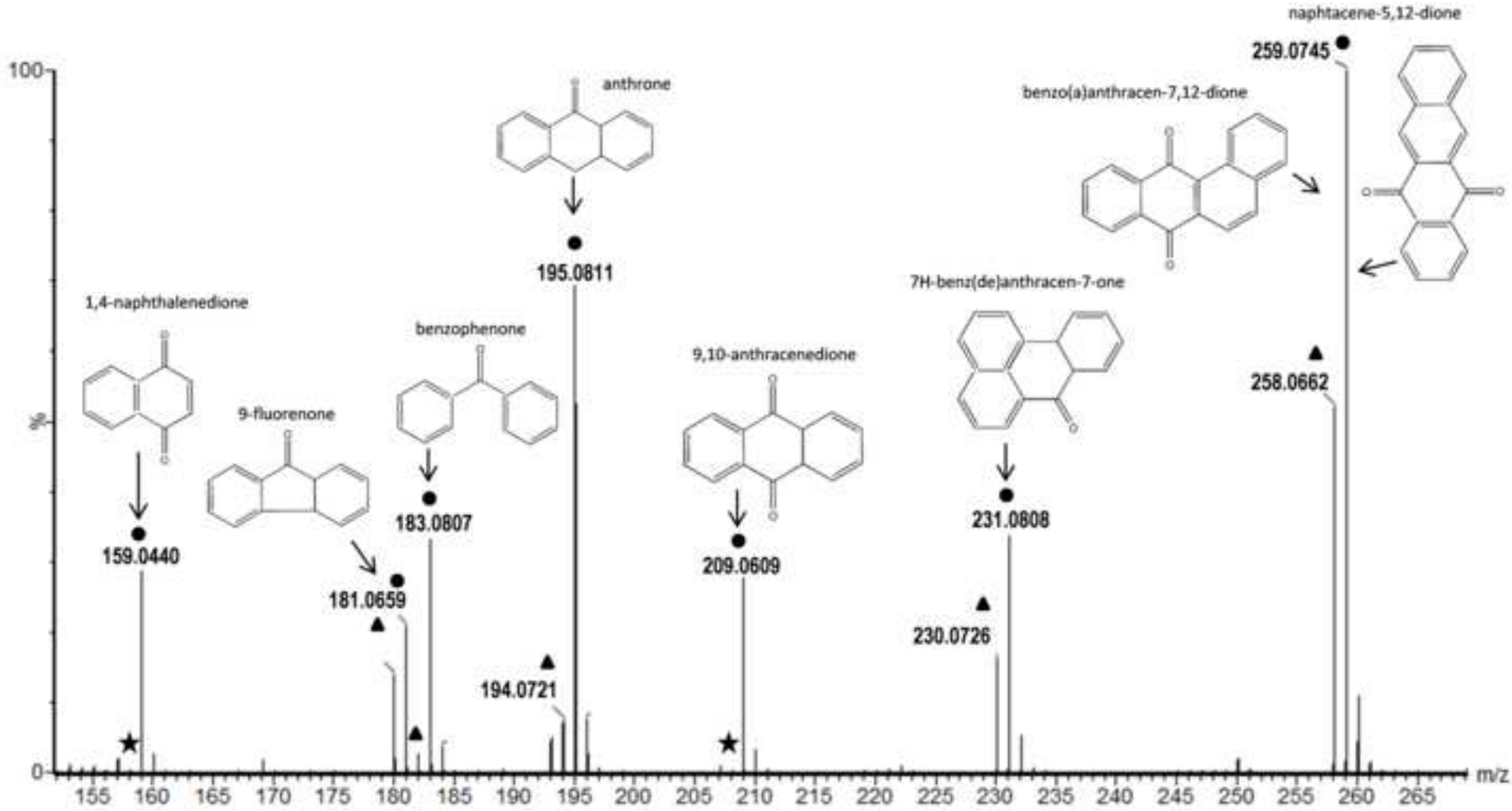

Figure 6

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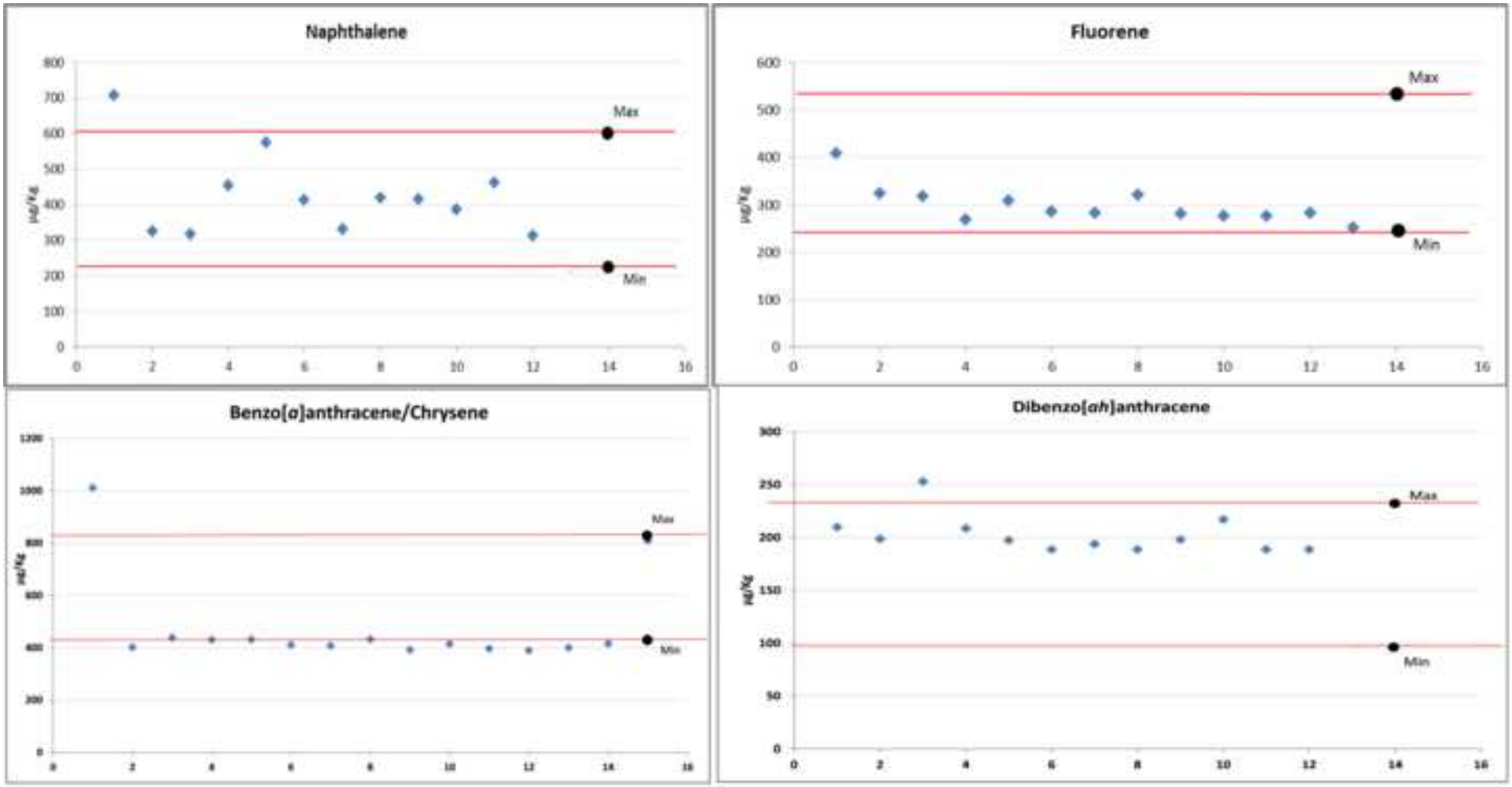

Figure 7

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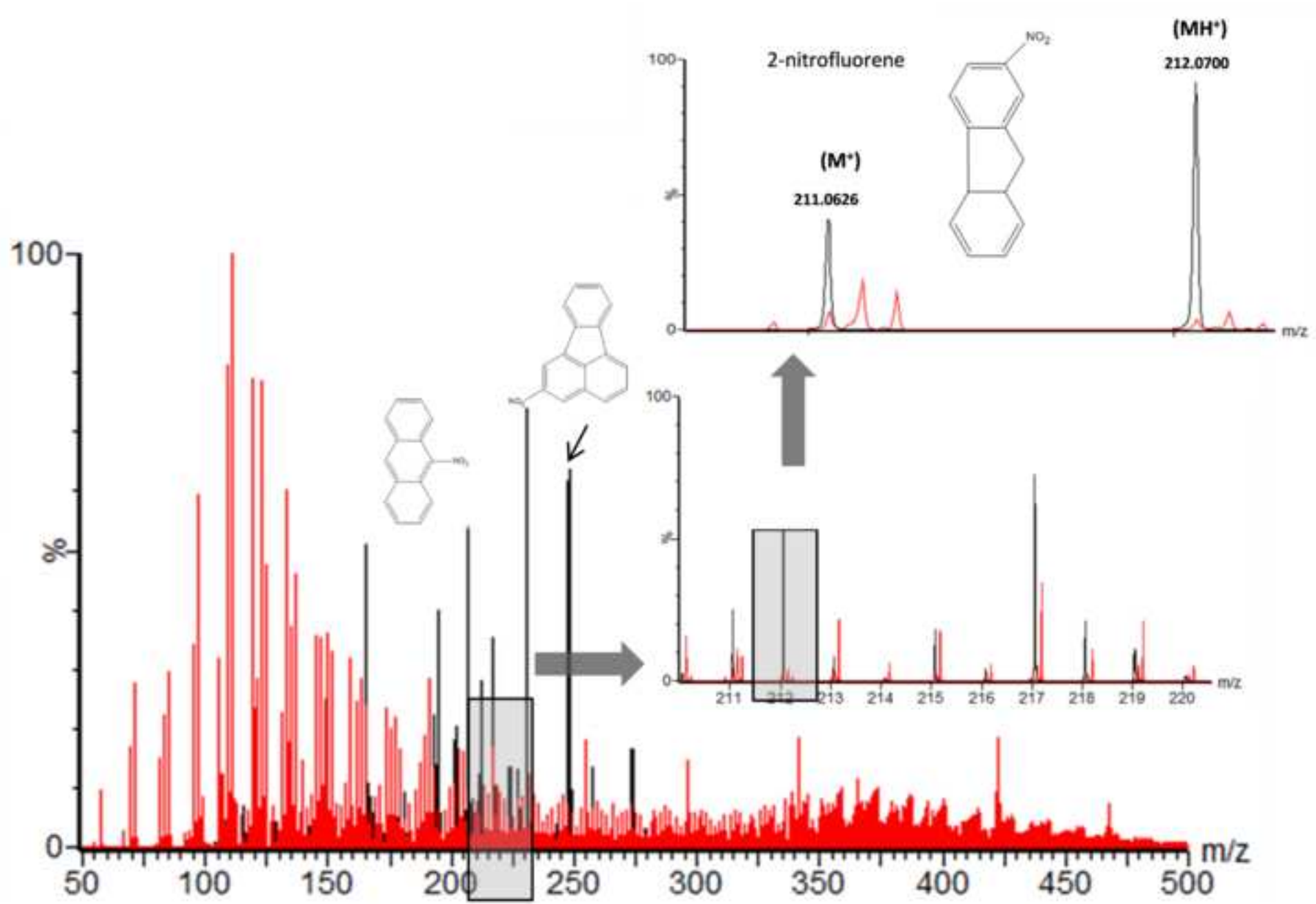

Supplementary Material

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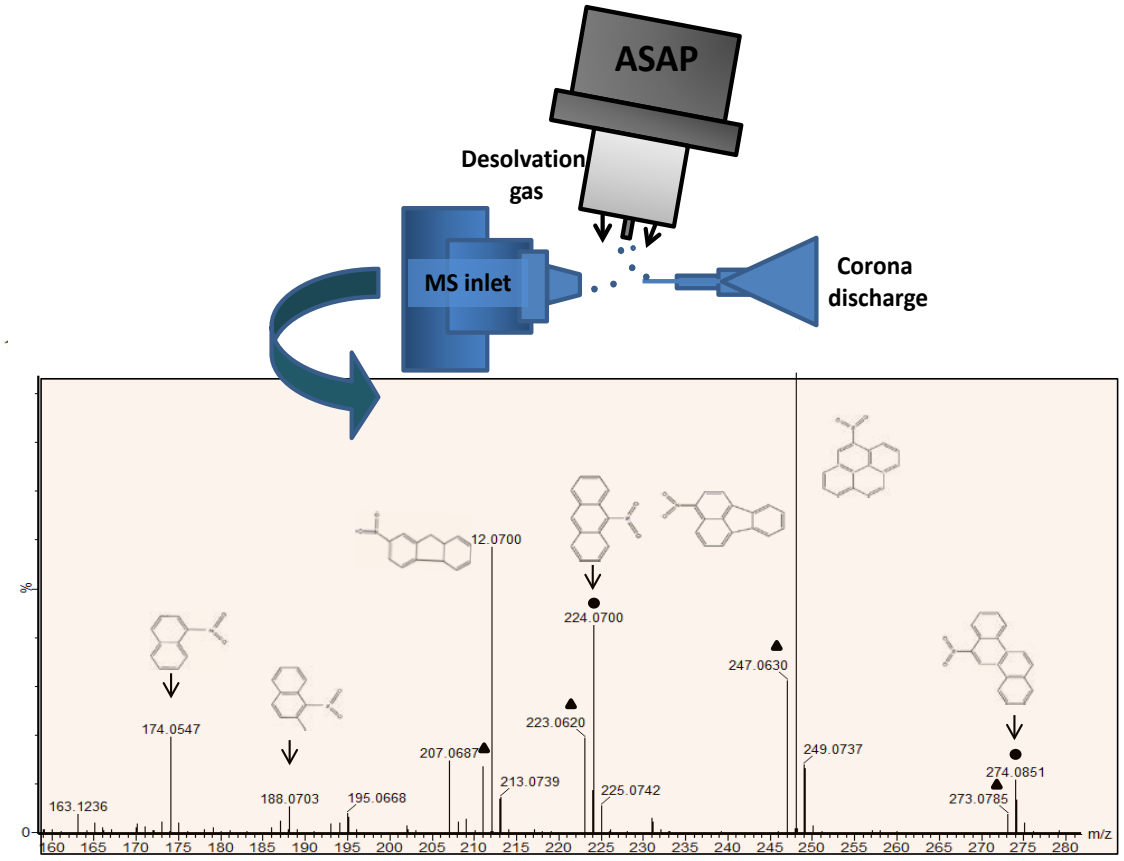
*Graphical Abstract (for review)

Graphical Abstract



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Universidad Zaragoza

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Editor of *Talanta*

Zaragoza, April, 25 th, 2014

Dear Editor,

I'm sending you the manuscript "*Atmospheric pressure Solid Analysis Probe coupled to Quadrupole-time of Flight Mass Spectrometry as a tool for screening and semi-quantitative approach of Polycyclic Aromatic Hydrocarbons, Nitro-Polycyclic Aromatic Hydrocarbons and Oxo-Polycyclic Aromatic Hydrocarbons in complex matrices***" by** Daniel Carrizo, Celia Domeño, Isabel Nerín, Pilar Alfaro, Cristina Nerin✉ for its publication in ***Talanta,*** if it is accepted.

This constitutes an important development in direct analysis for screening and quantitation in difficult samples, either solid or liquid ones. This is the first time that Atmospheric Solid Analysis Probe Mass Spectrometry (ASAP) is explored for this kind of analysis in so different matrices such as soil, car oil particles, urine and saliva. The optimized semiquantitative approach for carcinogenic compounds such as PAHs, nitro-PAHs and oxo-PAHs allows us not only to detect the presence of the contaminants but also to estimate the order of magnitude of concentration in which they are in the samples. This is also the first time that ASAP is proposed for this task and in these aspects reside the main novelty of the work.

I think that this paper is a valuable and innovative contribution to the ambient ionization mass spectrometry and specifically for the Atmospheric Solid Analysis Probe-MS technique. It is important to note that this is the first attempt to use this technique for this wide family of organic pollutants in such complex matrices (i.e. ashes, oil or biological samples such as saliva).

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The paper is unpublished and has not been submitted for publication elsewhere

Looking forward to hearing from you.
Best regards,

Prof. Dr. Cristina Nerin
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Novelty statement

This is the first time that Atmospheric Solid Analysis Probe Mass Spectrometry (ASAP) is explored for this kind of analysis in so different matrices such as soil, car oil particles, urine and saliva.

This is also the first time that an optimized semiquantitative approach for carcinogenic compounds such as PAHs, nitro-PAHs and oxo-PAHs is proposed not only to detect the presence of the contaminants but also to estimate the order of magnitude of concentration in which they are in the samples.

This is also the first time that ASAP is proposed for this task and in these aspects reside the main novelty of the work.

It is important to note that this is the first attempt to use this technique for this wide family of organic pollutants in such complex matrices (i.e. ashes, oil or biological samples such as saliva).

Highlights

- Single and direct analysis of PAHs, nitro-PAHs and oxo-PAHs
- Identification in complex matrices (e.g., soil, ash, saliva, oil, etc.) by ASAP-QTOF-MS
- First time semi-quantitative approach with ASAP-Q-TOF-MS for this wide family of pollutants
- First report on nitro-PAH and oxo-PAH in human urine and saliva samples from smokers

Table 1. Chemical characteristics and optimized parameters of the PAHs, nitro-PAHs and oxo-PAHs analyzed

Compounds	Abbreviation	CAS	MW	Formula	Corona (µA)	Desolvation temperature (ºC)
Naphthalene	NAP	91-20-3	128.0626	$C_{10}H_8$	4	500
Acenaphthylene	ACN	208-96-8	152.0626	$C_{12}H_8$	4	500
Acenaphthene	ANA	83-32-9	154.0782	$C_{12}H_{10}$	4	500
Fluorene	FL	86-73-7	166.0782	$C_{13}H_{10}$	4	500
Phenanthrene	PH	85-01-8	178.0782	$C_{14}H_{10}$	4	500
Anthracene	ANTH	120-12-7	178.0782	$C_{14}H_{10}$	4	500
Fluoranthene	FLAN	206-44-0	202.0782	$C_{16}H_{10}$	4	500
Pyrene	PY	129-00-0	202.0782	$C_{16}H_{10}$	4	500
Cyclopenta [*cd*] pyrene	CPY	27208-37-3	226.0782	$C_{18}H_{10}$	4	500
Benzo[*a*]anthracene	BANTH	56-55-3	228.0782	$C_{18}H_{12}$	4	500
Chrysene	CH	218-01-9	228.0782	$C_{18}H_{12}$	4	500
Benzo[*k*]fluoranthene	BFLAN	207-08-9	252.0939	$C_{20}H_{12}$	4	500
Benzo[*a*]pyrene	BAP	50-32-8	252.0939	$C_{20}H_{12}$	4	500
Dibenzo[*ah*]anthracene	DAN	53-70-3	278.1096	$C_{22}H_{14}$	4	500
Benzo[*ghi*]perylene	BPERY	191-24-2	276.0939	$C_{22}H_{12}$	4	500
1-Nitronaphthalene	1-N-NAP	86-57-7	173.0477	$C_{10}H_7NO_2$	6	500
2-Methyl-1-Nitronaphthalene	2-m-1-N-NAP	881-03-8	187.0633	$C_{11}H_9NO_2$	6	500
1,5-Dinitronaphthalene	1,5-dN-NAP	605-71-0	218.0328	$C_{10}H_6N_2O_4$	6	500
2-Nitrofluorene	2-N-FL	607-57-8	211.0633	$C_{13}H_9NO_2$	6	500
9-Nitroantracene	9-N-ANTH	602-60-8	223.0633	$C_{14}H_9NO$	6	500
2-Nitrofluoranthene	2-N-FLAN	892-21-7	247.0633	$C_{16}H_9NO_2$	6	500
1-Nitropyrene	1-N-PY	5522-43-0	247.0633	$C_{16}H_9NO_2$	6	500
6-Nitrocrysene	6-N-CH	08-02-7496	273.079	$C_{18}H_{11}NO_2$	6	500
1,4-Naphthalenedione	1,4-O-NAP	130-15-4	158.0368	$C_{10}H_6O_2$	6	300
Benzophenone	BENPHO	119-61-9	182.0732	$C_{13}H_{10}O$	6	300
9-Fluorenone	9-O-FL	486-25-9	180.0575	$C_{13}H_8O$	6	300
Anthrone	ANTHR	90-44-8	194.0732	$C_{14}H_{10}O$	6	300
9,10-Anthracenedione	9,10-O-ANT	84-65-1	208.0524	$C_{14}H_8O_2$	6	300
7H-Benzo[*de*]anthracen-7-one	7H-BANT	82-05-3	230.0732	$C_{17}H_{10}O$	6	300
Benzo[*a*]anthracene-7,12-dione	7,12-BANT	2498-66-0	258.0681	$C_{18}H_{10}O_2$	6	300
Naphthacene-5,12-dione	NAP-5,12-O	1090-13-7	258.0681	$C_{18}H_{10}O_2$	6	300

Table 2. Identified and semi-quantitation range values (µg/g or µg/ml) compounds in complex matrices analyzed in this work (+: 0 – 0.1; ++: 0.1-1; +++: 1-10; ++++: >10; µg/g or µg/ml)

Compounds	Oil	Soil	Ash	Saliva	Urine
Naphthalene	+++	+++	+++		
Acenaphthylene	+++	++	+++		
Acenaphthene		++	++	++	+++
Fluorene	+++	+++	+++		
Phenanthrene/ Anthracene	+++		+++	+++	
Fluoranthene/ Pyrene	+++	++++	+++		
Cyclopenta [*cd*] pyrene					
Benzo[*a*]anthracene/Chrysene	++	++	+	+	++
Benzo[*k*]fluoranthene/ Benzo[*a*]pyrene	++	++			
Dibenzo[*ah*]anthracene					
Benzo[*ghi*]perylene					
1-Nitronaphthalene					
2-Methyl-1-Nitronaphthalene	+++	++			
1,5-Dinitronaphthalene					
2-Nitrofluorene	+++	++			
9-Nitroantracene				+	
2-Nitrofluoranthene/1-Nitropyrene					
6-Nitrocrysene					
1,4-Naphthalenedione					++
Benzophenone					
9-Fluorenone					
Anthrone					
9,10-Anthracenedione	+++		+++		
7H-Benzo[*de*]anthracen-7-one					
Benzo[*a*]anthracene-7,12-dione/ Naphthacene-5,12-dione					

Supplementary Material

Click here to download Supplementary Material: 140425-SuppInfo-ASAP-Talanta.docx

Checklist

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8. List of Three Potential Reviewers
9. Supplementary Material

Potential reviewers

1.- Dr. Lourdes Ramos, Instituto de Química Orgánica, CSIC, Spain l.ramos@iqog.csic.es

2.- Dr. Laurence Castle, DEFRA, FERA, UK, laurence.castle@fera.gsi.gov.uk

3.- Dr. Fuwei Xie, Tobacco research Institute of CNTC, Analytical Chemistry, Biochemistry and Environmental Chemistry, China, xiefuwei@sina.com