



Atmospheric pressure gas chromatography with quadrupole time of flight mass spectrometry for simultaneous detection and quantification of polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons in mosses[☆]

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ABSTRACT

Within the family of polycyclic aromatic hydrocarbons (PAHs), nitrated derivatives are of particular interest in environmental science because they have well-known carcinogenic and mutagenic effects. They are in fact more toxic than their parent PAHs. One valuable diagnosis of atmospheric pollution can be obtained using biomonitors such as mosses. These biomonitors can provide information about air pollution over long periods of time in wilderness areas. Thus, they can serve as monitors of the atmospheric transport of pollutants. In this study, atmospheric pressure gas chromatography coupled to a quadrupole hyphenated to a time of flight mass spectrometer (APGC-MS/Q-TOF) has been examined for the identification of target analytes (15 PAHs and 8 NPAHs) for subsequent use in the analysis of mosses. Working ranges in low $\mu\text{g g}^{-1}$ concentration levels were obtained with most correlation coefficients higher than 0.999. All LODs were in the 0.007–0.035 $\mu\text{g g}^{-1}$ range and higher LODs (0.035 $\mu\text{g g}^{-1}$) were obtained for the less volatile PAHs with higher mass and retention times: benzo(g,h,i)perylene, dibenz(a,h)anthracene and indeno(1,2,3-c,d)pyrene. These LODs are of importance for the intended use, biomonitoring, especially taking into account that NPAHs are commonly found at very low concentration levels. Recoveries from mosses ranged from 75 to 98%. Intraday and interday precision ranged from 1.8 to 11.1% RSD and from 2.4 to 16.7% RSD, respectively. Very low concentrations of NPAHs were found in mosses compared to those of PAHs. All these data were used for pattern recognition of the pollutant source. The results are shown and discussed.

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1. Introduction

Within the family of polycyclic aromatic hydrocarbons (PAHs), nitrated derivatives are of particular interest in environmental science because they have well-known carcinogenic and mutagenic effects [1,2]. In fact, they are more toxic than their parent PAHs which require the enzymatic activation of their properties [3].

These compounds can be emitted from combustion sources or formed through the oxidation of PAHs in the atmosphere. NPAH isomers are source specific (combustion or oxidation) [4,5] therefore if they could be used to determine the source of pollution [6]. Nevertheless, other variables must be taken into account as their concentration is influenced by the wind direction, oxidant concentration and source emission (i.e. traffic) [7].

Biomonitoring is a rapid and economical method used for assessing the environmental quality of pollutants in the atmosphere. Various effective biomarkers have been used [8] including mosses and lichens [9], commonly regarded as the best bioindicators of air quality because they can accumulate elements to a far greater extent than is necessary for their physiological needs. A recent study has suggested that mosses are better biomonitors of airborne metals than lichens [10]. They enable the deposition of metals and organic compounds to be evaluated as these substances are accumulated and concentrated in mosses even when present in low concentrations in the local environment. These biomonitors can provide information about air pollution over long periods of time in wilderness areas. Thus, they serve as monitors of the atmospheric transport of pollutants.

Most studies to date have focused on the determination of inorganic elements, especially heavy metals and PAHs, but there are few studies about NPAHs [11–14]. Furthermore, most such studies are focused on a limited number of compounds (generally nitrofluoranthene and nitropyrene isomers).

Additionally, NPAH analysis is conditioned by the low concentrations of the analytes in the samples, which are typically one or

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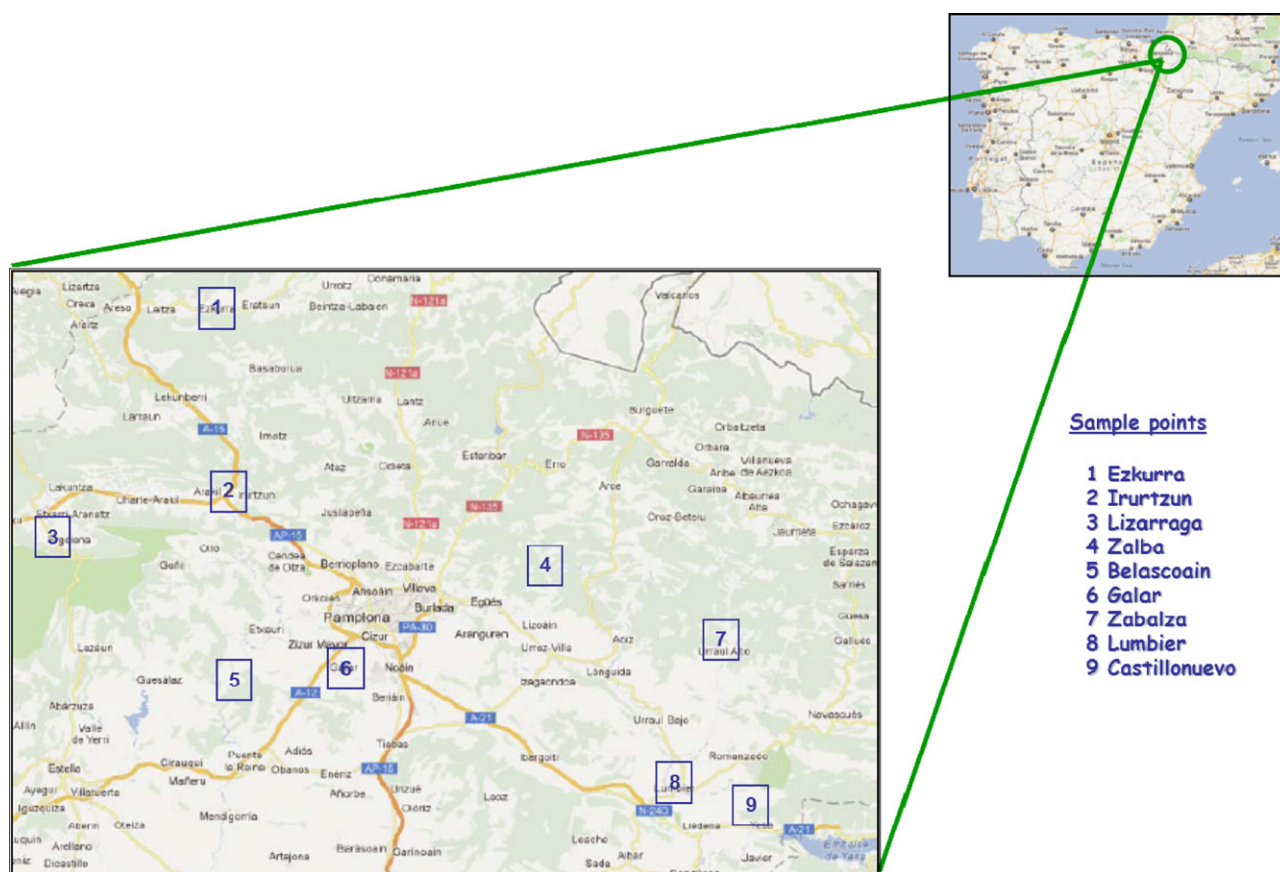


Fig. 1. Sampling area Navarra (Spain).

two orders of magnitude lower than those found for PAHs [4]. Their analysis thus requires several purification and preconcentration steps. Sample preparation is always a critical step in analysis, but in the case of PAH derivatives in vegetable samples the problem is even greater as chlorophylls and other organic matter strongly affect the extraction. Blasco et al. [9,15] optimized a procedure to avoid these problems in the extraction of lichens and simultaneously concentrate the PAHs to enhance the sensitivity of the analysis. Briefly, it consists of the extraction of the lichens by dynamic sonication assisted solvent extraction (DSASE) method and further purification and enrichment of the extract by solid-phase extraction (SPE). However the analysis of PAHs is resolved, the goal is still the analysis of nitroPAH derivatives.

NPAHs may be determined both by gas chromatography (GC) and liquid chromatography (LC). In LC analysis, NPAHs are usually detected by fluorescence [11] after their reduction to amino-derivatives or by chemiluminescence [12,13]. In GC analysis, the only way to determine NPAHs with good sensitivity is by negative chemical ionization. However, PAHs are determined by electron impact ionization, thus two independent analyses are usually required for the GC determination of PAHs and NPAHs. The use of GC–MS with a recently developed atmospheric pressure gas chromatography (APGC) source is an alternative technology for the simultaneous analysis of this type of compound. This technique is based on atmospheric pressure chemical ionization (APCI), and it is therefore a relatively soft ionization process compared to electronic impact (EI). Moreover, APGC operates at atmospheric pressure which avoids the limitation imposed by the use of pumps, allowing a much wider range of flow rates for full GC separations.

This technique can be coupled to time of flight analyzers that provide high sensitivity in full-spectrum-acquisition mode

compared to conventional scanning instruments. As a result, a very reliable identification of the NPAHs analyzed can be achieved and with very low detection limits using one single technique.

The objective of this study is to develop a sensitive and accurate method for the simultaneous analysis of a wide variety of PAHs and NPAHs by using APGC–Q-TOF-MS and to demonstrate its reliability in identifying target compounds at low levels in complex biological samples.

2. Materials and methods

2.1. Survey area and sampling strategy

The survey area was a small region of about 3200 km² in Navarra (Spain). Nine sampling points were selected according to environmental criteria such as climate or proximity to sources of pollution. Fig. 1 shows the sampling sites in the survey area. Collection of the samples was carried out during the summer of 2008 (the driest season).

Moss samples, of the *Hypnum cupressiforme* species, were collected from open areas or small forest clearings. The samples were taken as far as possible from the nearest tree in order to avoid water. Some samples had to be collected from under the canopy tree. 7–10 subsamples were collected from each sampling site, within a 50 m × 50 m area, and were then combined into a single sample. Only specimens located over the ground or dead-tree stumps were sampled.

In the laboratory, the samples were dried in an oven (40 °C) for 2–3 days until reaching constant weight, cleaned from foreign debris such as plants or other moss species and homogenized in a

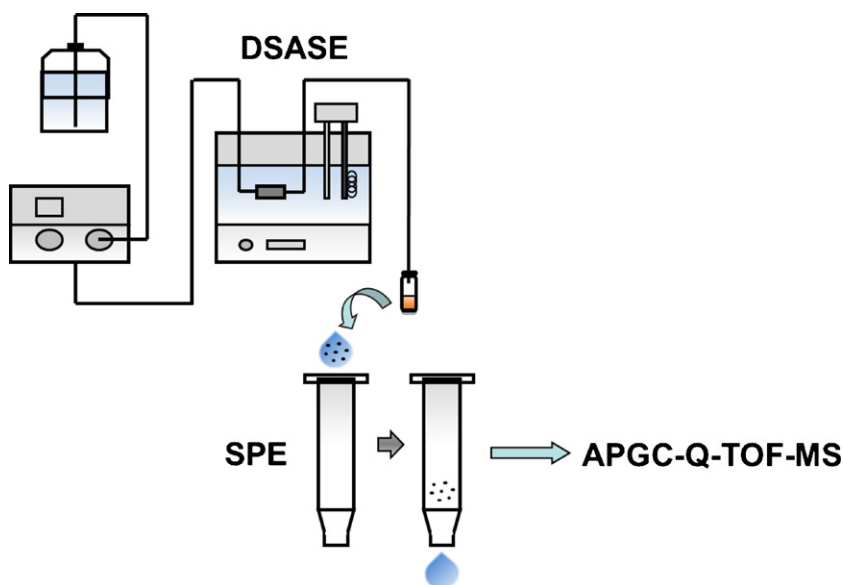


Fig. 2. Sample treatment scheme. Dynamic sonication assisted solvent extraction (DSASE) (first step) in which hexane at 0.2 mL min^{-1} passes through the extraction cell for 10 min followed by a combined Florisil + NH_2 -SPE cartridge (2nd step) is used. Final cleaned extract is analyzed by APGC-Q-TOF-MS.

mill. The samples were stored in PE bags at 4°C until subsequent analysis.

2.2. Chemicals

Certified standards of PAHs and NPAHs were obtained from Sigma–Aldrich (Madrid, Spain): acenaphthene (83-32-9), acenaphthylene (208-96-8), anthracene (120-12-7), benzo (a)anthracene (56-55-3), benzo(a)pyrene (50-32-8), benzo(b)fluoranthene (205-99-2), benzo(g,h,i)perylene (191-24-2), chrysene (218-01-9), dibenzo(a,h)anthracene (53-70-3), fluoranthene (206-44-0), fluorine (86-73-7), indene (1,2,3,cd), pyrene (193-39-5), naphthalene (91-20-3), phenanthrene (85-01-8), pyrene (129-00-0), 1-nitronaphtelene (86-57-7), 2-nitrofluorene (607-57-8), 1,5-dinitronaphtalene (605-71-0), 2-methyl-1-nitronaphtalene (881-03-8), 6-nitrochrysene (7496-02-8), 9-nitroanthracene (602-60-8), 1-nitropyrene (5522-43-0) and 2-nitrofluoranthene (13177-29-2) and acenaphthene d10 (15067-26-2) added to the mosses was used as internal standard for the analytical process. The analytical-grade solvents hexane and dichloromethane were purchased from Scharlab (Barcelona, Spain). Silanized glass wool was purchased from Supelco (Bellefonte, PA, USA) and sodium sulfate anhydrous was supplied by Merck (Darmstadt, Germany).

2.3. Sample treatment

A sample treatment procedure developed and optimized by Blasco et al. for the extraction and isolation of PAHs from lichen samples was used [15]. The procedure consists of the dynamic sonication-assisted solvent extraction (DSASE) of the mosses followed by a SPE clean up step. Three replicates were analyzed for each moss sample.

A 1-mL stainless steel extraction cell (Suprex Corp., Pittsburgh, PA) wool was filled in “sandwich” mode with an aliquot of each moss sample and with silanized glass wool (0.2 g, dry weight). $1 \mu\text{g g}^{-1}$ internal standard was added just before extraction. Both PAHs and NPAHs were dynamically extracted using 0.2 mL min^{-1} flow of pure hexane pumped during 10 min through the samples and under sonication applied by an Ultrasonic LC 130H bath (Elma, Singer, Germany). After extraction, the raw extract (2 mL)

containing the target analytes and interferences, such as vegetal pigments, was collected and further purified by SPE clean up.

SPE cartridges were prepared by adding 0.05 g of sodium sulfate anhydrous and 0.05 of Florisil 100–200 mesh (Sigma–Aldrich) to the top of the commercial 500 mg NH_2 -SPE cartridges (Análisis Vínicos, Tomelloso, Spain). Prior to extraction, the cartridges were placed on a Waters manifold (Milford, USA) and a conditioning step with 6 mL dichloromethane and 3 mL hexane was carried out. For the clean-up step, the raw extract obtained with the DSASE method was loaded on the top of the cartridge and the solid bed was rinsed with 0.5 mL of hexane to remove the retained interferences. Finally, PAHs and NPAHs were eluted using 2 mL of hexane:dichloromethane (65:35). The clean extract, enriched with the analytes of interest, was collected in a glass vial and further concentrated from 2 mL to 0.5 mL (4-fold) under a gentle nitrogen stream before the analysis by APGC-Q-TOF-MS. Fig. 2 shows a scheme of the process.

2.4. APGC-Q-TOF-MS analysis

Analyses of the extracts were performed using an Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA) equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) and coupled to a quadrupole TOF MS, Xevo G2 QTOF (Waters Corporation, Manchester, UK), operating in APGC mode. A HP-5MS (Agilent Technologies) analytical column of $30 \text{ m} \times 0.250 \text{ mm}$ and $0.25 \mu\text{m}$ of film thickness was used. The temperature program for the gas chromatography was as follows: initial temperature, 50°C held for 2 min, linear range of $10^\circ\text{C min}^{-1}$ up to a final temperature of 320°C , held for 12 min. Total time was 41 min. The injector temperature was 250°C . Injection was performed in the splitless mode and the injection volume was $2 \mu\text{L}$. The carrier gas was helium (99.999% purity, 1.0 mL min^{-1}) supplied by Carbueros Metálicos (Barcelona, Spain).

An APGC (atmospheric pressure gas chromatography) source was operated to promote the charge transfer ionization mode mechanism and protonation ionization mechanism. The interface temperature was 150°C using N_2 as the desolvation gas at 200 L h^{-1} flow, and the cone gas flow was set at 16 L h^{-1} . The voltage of the sampling cone was set at 25 V, the voltage of the extraction cone was 3 V, and the APCI corona pin was fixed at a current of 0.4 A. API

Table 1Analytical performance of the APGC–Q-TOF-MS procedure: retention time (min), accurate mass (Da), working range ($\mu\text{g g}^{-1}$), regression coefficient, limit of detection ($\mu\text{g g}^{-1}$) and relative standard deviation (%; $n=3$).

	Retention time (min)	Accurate mass (Da)	Working range ($\mu\text{g g}^{-1}$)	R^2	LOD ($\mu\text{g g}^{-1}$)	Recovery level 1 (%)	Recovery level 2 (%)	Intra-day precision (RSD, %)	Inter-day precision (RSD, %)
<i>PAHs</i>									
Naphthalene	9.59	128.0225	0.115–4.04	0.999	0.007	85	81	5.4	8.9
Acenaphthylene	13.34	152.0176	0.84–4.04	0.999	0.007	89	85	2.2	3.3
Acenaphthene	13.69	154.4000	0.84–4.04	0.999	0.010	81	82	2.1	3.1
<i>d10 acenaphthene (SI)</i>	13.81	164.0913	–	–	0.020	–	–	3.6	4.2
Fluorene	15.01	166.0086	0.84–4.04	0.999	0.010	98	96	1.8	2.4
Phenanthrene	17.27	178.0053	0.84–4.04	0.999	0.007	97	92	1.9	2.6
Anthracene	17.39	178.0053	0.84–4.04	0.999	0.007	97	95	1.8	3.1
Fluoranthene	20.13	202.0027	0.84–4.04	0.999	0.015	91	90	2.8	4.8
Pyrene	20.66	202.0023	0.84–4.04	0.999	0.007	92	91	2.7	5.3
Benzo(a)anthracene	23.58	228.0103	0.84–4.04	0.999	0.015	95	93	1.9	4.2
Chrysene	23.68	228.0104	0.84–4.04	0.999	0.015	92	91	3.1	5.6
Benzo(b)fluoranthene	26.06	252.1734	0.84–4.04	0.999	0.025	87	85	3.5	6.1
Benzo(a)pyrene	26.74	252.0029	0.84–4.04	0.98	0.025	85	84	4.1	10.2
Benzo(g,h,i)perylene	28.91	276.0023	0.84–4.04	0.999	0.035	81	80	8.9	9.9
Dibenz(a,h)anthracene	29.05	278.0345	0.84–4.04	0.999	0.035	80	78	10.7	12.2
Indeno(1,2,3-cd)pyrene	29.25	276.0196	0.84–4.04	0.999	0.035	79	75	11.1	16.7
<i>NPAHs</i>									
1-Nitronaphthalene	15.28	174.0140	0.74–3.57	0.999	0.010	91	89	4.1	6.7
2-Methyl-1-nitronaphthalene	15.60	187.9941	0.74–3.57	0.999	0.015	92	91	3.9	5.1
2-Nitrofluorene	20.59	210.9846	0.74–3.61	0.99	0.007	95	91	4.1	8.9
1,5-Di-nitronaphthalene	19.00	218.9600	0.73–3.53	0.999	0.015	89	87	3.2	7.9
9-Nitroanthracene	20.90	222.9813	0.75–3.65	0.999	0.007	92	91	2.9	5.1
2-Nitrofluoranthene	24.39	246.9794	0.93–4.52	0.999	0.020	89	85	4.2	9.8
1-Nitropyrene	24.85	246.9743	0.80–3.89	0.999	0.020	87	82	5.1	10.8
6-Nitrochrysene	27.00	272.9816	0.70–3.37	0.999	0.020	85	81	9.8	15.7

^a Fortification level: 1.053 $\mu\text{g g}^{-1}$ and 0.930 $\mu\text{g g}^{-1}$ for PAHs and nitro-PAHs, respectively.

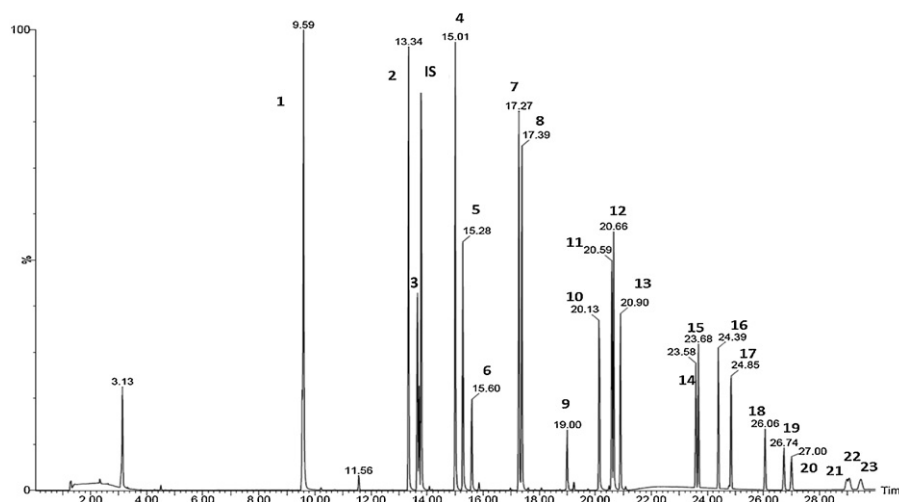


Fig. 3. Chromatogram of standard solution of PAHs and NPAHs ($1.5 \mu\text{g g}^{-1}$). 1, naphthalene; 2, acenaphthylene; 3, acenaphthene, (IS) d10 acenaphthene; 4, fluorene; 5, 1-nitronaphthalene; 6, 2-methyl-1-nitronaphthalene; 7, phenanthrene; 8, anthracene; 9, 1,5-di-nitronaphthalene; 10, fluoranthene; 11, 2-nitrofluorene; 12, pyrene; 13, 9-nitroanthracene; 14, benzo(a)anthracene; 15, chrysene; 16, 2-nitrofluoranthene; 17, 1-nitropyrene; 18, benzo(b)fluoranthene; 19, benzo(a)pyrene; 20, 6-nitrochrysene; 21, benzo(g,h,i)perylene; 22, dibenz(a,h)anthracene; 23, indeno(1,2,3-cd)pyrene.

positive polarity was selected, the probe temperature was 550°C and the temperature of the source was 150°C . The parameters of XEVO G2 QTOF were: scan time 1 s, mass range m/z from 50.0 to 550.0.

2.5. Data treatment

Data were collected and processed using MassLynx software (Waters Corporation). The ChromaLynx XS Application Manager (SCN 714) was used to perform a screening of the compounds in the targeted mode.

3. Results and discussion

3.1. APGC-Q-TOF-MS optimization

The currently used single quadrupole analyzers have limitations in the analysis of complex matrices [16]. The ion trap and, more recently, triple quadrupole analyzers allow operation mode MS2, which reaches an undeniably high degree of sensitivity and selectivity. However, other compounds which may be in the samples are not detected under these conditions and additional tools are necessary for sensitive acquisition methods that facilitate the detection and identification of such compounds in complex samples. This can be achieved with the higher power of resolution and mass measurement accuracy provided by Q-TOF-MS.

The primary screening can be achieved in TOF-MS mode by two techniques: (a) through the retention time of the analyte and that obtained from a standard solution and (b) with accurate mass measurement of molecular ions of the target compounds.

The accurate mass data for the molecular ions were processed in this study by MassLynx, a software program which provided the corresponding elemental composition and the mass errors (i.e. differences between experimental and theoretical masses).

The advantage of GC is the ability to separate peaks in complex samples, but when coupled to QTOF-MS the full accurate mass spectra in MS can be achieved. However, there are very few studies concerning the use of APGC-Q-TOF-MS to identify organic volatile and semivolatile compounds, and none of them contain quantitative data [17,18].

A solution of standards in hexane was used to study the performance of the APGC source in the simultaneous analysis of both

PAHs and NPAHs. Fig. 3 shows the chromatogram of a standard solution of the analytes ($0.5 \mu\text{g g}^{-1}$) and the internal standard. As can be seen, all the target analytes were chromatographically well separated from each other.

As stated in Section 1, the APGC source is a soft ionization interface, thus the obtained spectra should be rich in molecular or quasi-molecular ions. Ionization in APGC takes place through the formation of nitrogen plasma by means of a corona discharge needle. The nitrogen plasma may then either transfer a charge directly to the analyte molecule or by means of an intermediate charged water molecule (H_2O^+). The former is the charge transfer mode and it yields spectra with $\text{M}^{+\bullet}$ as the main peak, while the latter is known as the proton transfer mode. This occurs if there are traces of water in the source or if a modifier is used, and it yields spectra with $\text{MH}^{+\bullet}$ as the main peak [14].

Spectra of the analyte standards showed that both PAHs and NPAHs were softly ionized with almost no fragmentation. The molecular ion $\text{M}^{+\bullet}$ was the base peak in the observed protonated molecular ion ($\text{MH}^{+\bullet}$). Thus, the charge transfer ionization mode was very favored in this case and the use of a modifier to facilitate the proton transfer mechanism was not necessary. Fig. 4 shows the spectra of selected PAHs and NPAHs. As can be seen, no fragmentation occurred and only the molecular ion $\text{M}^{+\bullet}$ as base peak was observed.

From the results presented here it can be concluded that the APCI source allows the simultaneous detection of PAHs and NPAHs by mass spectrometry after GC separation.

Each compound was classified in terms of both retention time (min) and exact mass (Da) of the molecular ion $\text{M}^{+\bullet}$ (Table 1). A data base containing the target analytes (15 PAHs and 8 NPAHs) was generated for subsequent use with the ChromaLynx XS software in the analysis of the real samples. Pure standards of the compounds were also injected in the same conditions to confirm, based on retention times as well as on exact mass fragment, the identity of the compounds. As mentioned above, additional fragmentation could not be achieved as the APCI mode used did not provide more than one stable characteristic mass.

3.2. Analytical performance of the method

The analytical performance of the method was evaluated in terms of linearity, limits of detection, recovery and

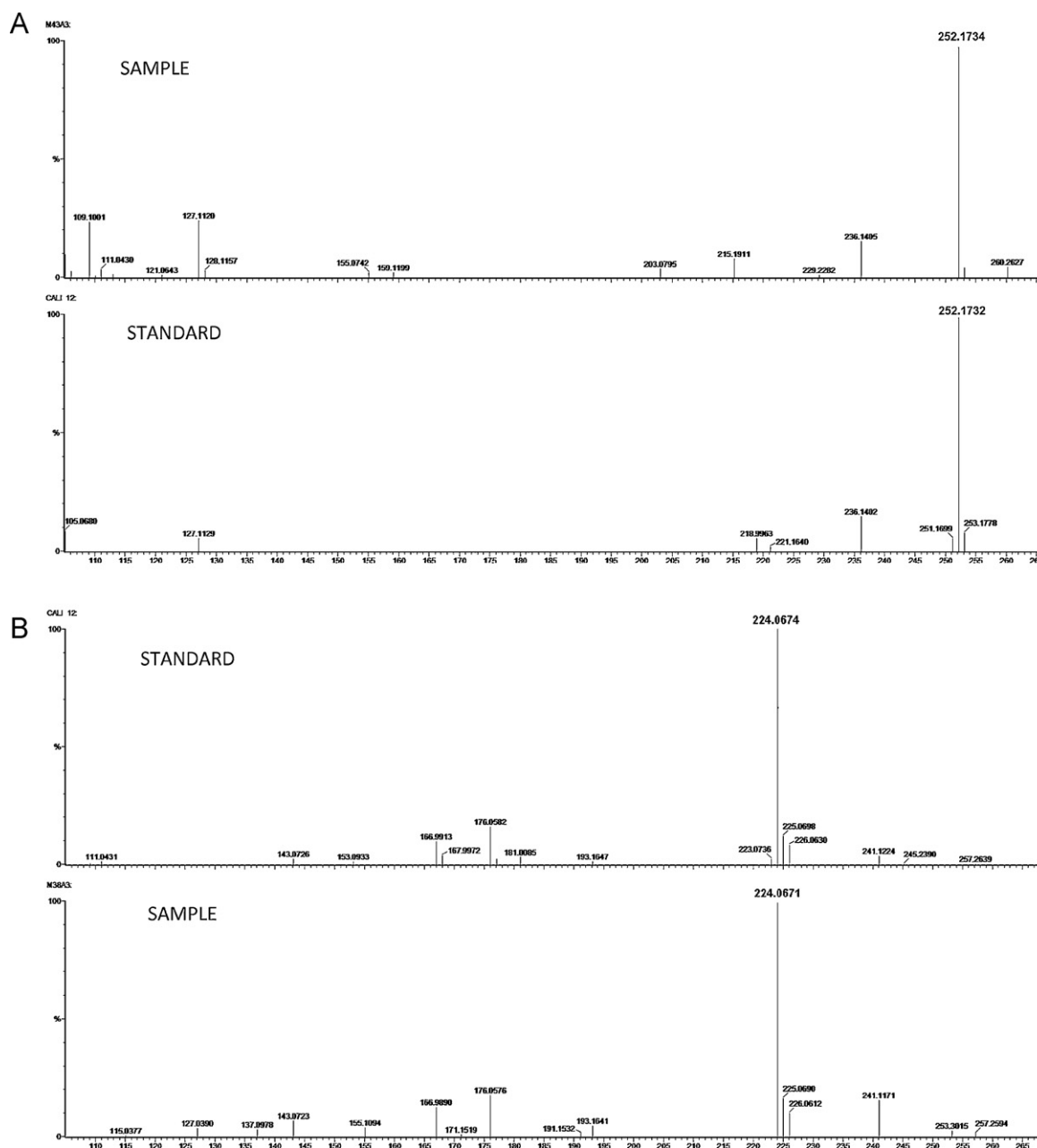


Fig. 4. ChromaLynx spectrum: (a) benzo(b)fluoranthene in Zabalza and (b) 9-nitroanthracene in Irurtzun. In both cases the comparison to the standard MS is provided by ChromaLynx.

reproducibility, and the results are displayed in Table 1. Linearity was obtained by injecting calibration standards of the target analytes in hexane. Working ranges in the lowest ng g^{-1} concentration level were obtained with most correlation coefficients higher than 0.999. Limits of detection (LOD) were obtained by injecting increasingly smaller concentrations of standard solutions. The least concentrated standard yielding a signal/noise ratio greater than 3 was considered as the limit of detection. As can be seen, all LODs were in the $0.007\text{--}0.035 \mu\text{g g}^{-1}$ range and higher LODs ($0.035 \mu\text{g g}^{-1}$) were obtained for the less volatile PAHs, with higher mass and retention times: benzo(g,h,i)perylene, dibenz(a,h)anthracene and indeno(1,2,3-c,d)pyrene. These LODs are of relevance for the intended use, biomonitoring, especially taking into account that NPAHs are commonly found at very low concentration levels.

These samples were analyzed five times on the same day (intra-day precision) and once on five different days (inter-day precision) to evaluate the precision of the chromatographic method.

To determine the method recovery, the moss samples were spiked at two concentration levels: level 1 at $0.96 \mu\text{g g}^{-1}$ for PAHs and $1.4 \mu\text{g g}^{-1}$ for NPAHs, level 2 at 0.66 for PAHs and 0.9 for NPAHs. Deuterated standard (Acenaphthene d10) was added before extraction and used as a standard for the analytical process.

The recoveries ranged from 79 to 98% for the highest level and from 75 to 96% for the lowest. As would be expected, the lowest recoveries were found for the heaviest compounds such as benzo(g,h,i)perylene (80–81%), dibenzo(a,h)anthracene (78–80%) and indene(1,2,3-cd)pyrene (75–79%).

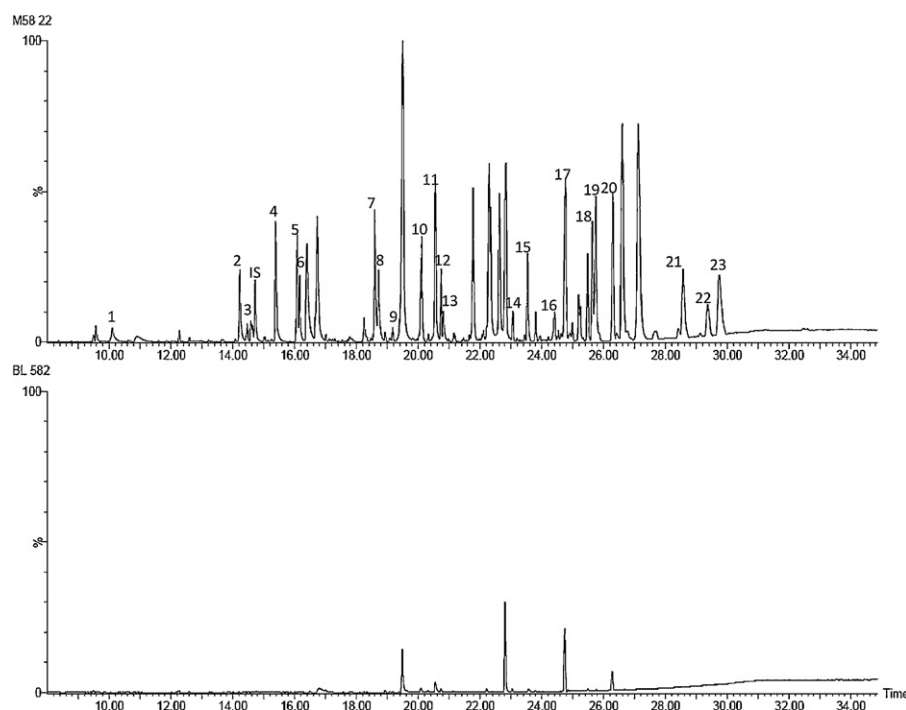


Fig. 5. Chromatogram of sample spiked with PAHs ($0.66 \mu\text{g g}^{-1}$) and NPAHs ($0.9 \mu\text{g g}^{-1}$) and chromatogram of blank. 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, 1-nitronaphthalene; 6, 2-methyl-1-nitronaphthalene; 7, phenanthrene; 8, anthracene; 9, 1,5-di-nitronaphthalene; 10, fluoranthene; 11, 2-nitrofluorene; 12, pyrene; 13, 9-nitroanthracene; 14, benzo(a)anthracene; 15, chrysene; 16, 2-nitrofluoranthene; 17, 1-nitropyrene; 18, benzo(b)fluoranthene; 19, benzo(a)pyrene; 20, 6-nitrochrysene; 21, benzo(g,h,i)perylene; 22, dibenz(a,h)anthracene; 23, indeno(1,2,3-cd)pyrene.

The recoveries found for NPAHs were always higher than those for PAHs. The lowest values were found for 1-nitropyrene (82–87%) and 6-nitrochrysene (81–85%).

Fig. 5 shows the chromatograms of one spiked moss sample and the blank.

3.3. Analysis of mosses

Real samples were analyzed by applying the developed procedure consisting of DSASE extraction followed by SPE cleaning up and further analysis by APGC–Q–TOF–MS. After the analysis, ChromaLynx XS software was used to perform a targeted screening and quantification of the compounds of interest (PAHs and NPAHs).

To confirm the presence of the compounds under study in complex matrices, accurate mass measurements are needed. The errors that can occur depend on the abundance of ions and will be higher when they are of low intensity. In this work, the use of APGC–Q–TOF–MS allowed us to investigate the presence of NPAHs and PAHs in the presence of other contaminants in mosses all analyzed in an objective manner. This required the processing of data to facilitate the proper detection and identification of the analytes. This was supported by the ChromaLynx software, which allowed automatic detection of the sample components and their identification based on the accurate mass spectra acquired with standards.

The ChromaLynx application extracts the ion mass of the analytes with a narrow mass window ($\pm 15 \text{ mDa}$ and a retention time window of $\pm 0.20 \text{ min}$), and then detects the presence or absence of the target analyte from a compound list in a previously generated database. Chromatograms were processed with ChromaLynx XS and a positive hit was generated if the criteria (retention time and exact mass) were met. Deconvolution of the mass spectrum for each compound helped us to confirm the purity of the spectra and thus to search for identification. Comparison of the data with those obtained from the pure standards confirmed the identification of

the compounds. Fig. 4 shows some examples for benzo(a)pyrene in Zabalza and for 9-nitroanthracene in Irurtzun respectively. As can be seen, only using the combination of the analytical and software tools the unequivocal identification was possible.

The developed analytical procedure was applied to simultaneously determine the concentration of PAHs and NPAHs in mosses used as passive biomonitors and collected in the survey area located in Navarra (Spain). The quantification of the identified compounds was carried out to calculate the concentrations of each compound identified by the relationship between the ratio peak area/area internal standard and the peak area standard of the internal standard at known concentrations.

Table 2 shows the results obtained and Fig. 6 shows a chromatogram of a moss sample as an example. The values of PAHs found at various sampling points ranged from 188 to 1733 ng g^{-1} and for NPAHs from 41 to 315 ng g^{-1} .

Previous studies have shown that it is very difficult to establish relationships between the concentrations in these samples due to the number of variables that can influence them [6]. High molecular weight PAHs are related to particles with rapid deposition near the source [19]. In contrast, low molecular weight PAHs can be transported from the source to distant regions (Long Range Atmospheric Transport) because of their persistence and ability to re-volatilize. However, at high latitudes and low temperatures, deposition processes become more pronounced than evaporation, and this affects the final concentrations [20,21]. Comparative studies show that different species have differences in bioaccumulation. The *H. cupressiforme* can accumulate PAHs more efficiently than other moss species [22]. This species contains bioflavonoids, phenyl-substituted aromadendrin derivatives, and kaempferol in its structure, which may have a greater affinity for PAH compounds. There are no references of NPAHs in mosses to compare.

The differences found in PAH concentrations can be attributed to the different locations, since the area under study includes

Table 2Concentration (ng g⁻¹) of PAHs and nitro-PAHs obtained in each sampling point (n = 3). RSD values in Table 1.

	Ezkurra	Irurtzun	Lizarraga	Zabalza	Belascoain	Galar	Zalba	Lumbier	Castillonuevo
Naphthalene	9	14	9	42	39	33	9	88	14
Acenaphthylene	<LOD	<LOD	32	45	86	77	60	172	54
Acenaphthene	<LOD	81	79	<LOD	11	13	82	14	68
Fluorene	13	63	86	181	92	37	62	56	12
Phenanthrene	75	98	52	226	228	61	93	37	40
Anthracene	<LOD	<LOD	41 ± 13	58 ± 4	<LOD	36 ± 2	29 ± 4	123 ± 6	<LOD
Fluoranthene	191	466	25	<LOD	56	<LOD	<LOD	530	<LOD
Pyrene	88	363	174	45	<LOD	52	11	271	<LOD
Benzo(a)anthracene	37	271	48	25	16	16	26	49	<LOD
Chrysene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	40	<LOD
Benzo(b)fluoranthene	78	49	D	37	<LOD	<LOD	D	45	<LOD
Benzo(a)pyrene	<LOD	287	<LOD	110	36	<LOD	<LOD	<LOD	<LOD
Benzo(g,h,i)perylene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dibenz(a,h)anthracene	<LOD	41	<LOD	<LOD	34	<LOD	<LOD	<LOD	<LOD
Indeno(1,2,3-cd)pyrene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Σ PAHs	491	1733	546	769	598	325	372	1425	188
1-Nitronaphthalene	<LOD	18	15	<LOD	49	<LOD	15	<LOD	<LOD
2-Methyl-1-nitronaphthalene	<LOD	38	27	<LOD	20	<LOD	<LOD	41	<LOD
2-Nitrofluorene	D	122	27	<LOD	26	<LOD	44	182	<LOD
1,5-Di-nitronaphthalene	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9-Nitroanthracene	D	33	38	17	36	D	<LOD	25	<LOD
2-Nitrofluoranthene	<LOD	27	53	24	<LOD	47	<LOD	29	<LOD
1-Nitropyrene	<LOD	41	<LOD	<LOD	<LOD	36	<LOD	35	<LOD
6-Nitrochrysene	<LOD	36	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Σ NPAHs		315	160	41	131	83	59	312	

D, detected see LOD in Table 1.

several sampling points either close to urban areas or to natural (wild) ones.

Most of the PAHs found in mosses contain 3 or 4 rings. The most common PAHs were naphthalene, fluorene and phenanthrene which were present in all the samples at different concentrations. The presence of high molecular weight PAHs

was small and the presence of six-ring polyaromatics was not detected.

The highest values of pyrene were found in those areas close to urban environments (Irurtzun, Lizarraga, Lumbier), and the highest value of benzo(a)anthracene was found in Irurtzun. Chrysene was only detected in Lumbier. This finding was expected, as chrysene

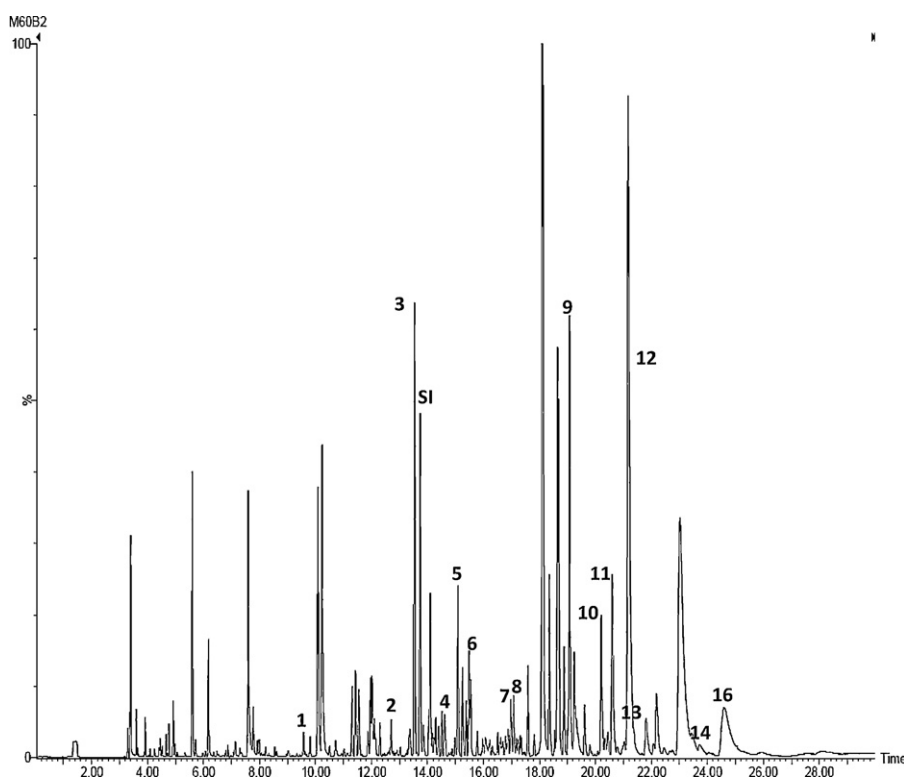


Fig. 6. Chromatogram of sample point in Lizarraga. 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, 1-nitronaphthalene; 6, 2-methyl-1-nitronaphthalene; 7, phenanthrene; 8, anthracene; 9, 1,5-di-nitronaphthalene; 10, fluoranthene; 11, 2-nitrofluorene; 12, pyrene; 13, 9-nitroanthracene; 14, benzo(a)anthracene; 16, 2-nitrofluoranthene.

and pyrene are very reactive compounds and thus their shelf-lives in the atmosphere are shorter than those of the other PAHs. As a result, chrysene and pyrene are usually found near pollution sources.

A similar explanation could apply to pyrene and benzo(a)anthracene which were not found in areas far from urban centers.

Other studies conducted in relatively unpolluted areas have shown similar distribution patterns and similar results have also been found for PAHs in mosses [10,8].

The phenantrene > fluorene > pyrene > anthracene ranking, which is characteristic of unpolluted areas, was clear in the wildest natural sampling points (Castillonuevo and Belascoain).

There is no published data about NPAHs and mosses even though the existence of NPAHs in the atmosphere has been demonstrated. Several mechanisms for the formation of NPAHs in the atmosphere have been proposed but the question remains unclear. The most abundant NPAH found in this work was 2-nitrofluorene, which is the most common in diesel particulates. This reflects the influence of the heavy traffic in the area nearby [23].

Quite high concentrations of 9-nitroanthracene and 2-nitrofluoranthene were found in 5 samples and both compounds were also detected in 2 other samples. Again, these compounds show the influence of nearby urban areas [24]. According to Albinet [6], 9-nitroanthracene could be formed from nitration of anthracene and 2-nitrofluoranthene is only produced in gas phase by the reaction between fluoranthene and NO₂ catalyzed by OH during the day. At night, the reaction takes place with NO₃ instead of NO₂. The area under study in this work is very sunny and probably this is the reason why the concentration of NPAHs found in mosses is higher than that found in the atmospheric particulates [6].

However, 1-nitropyrene is not formed in the atmosphere and can only be found in direct emissions. This compound was only found in the samples taken near the urban areas.

6-Nitrochrysene, the heaviest NPAH, was not detected in any sample.

4. Conclusions

The simultaneous analysis of NPAHs and PAHs in one single run has been achieved thanks to the use of a new technology, namely APGC–Q–TOF–MS. The soft ionization provided by APGC and the accurate mass fragments, together with the help of specific and powerful software, allowed us to identify and later quantify a large number of NPAHs and PAHs in complex matrices. Although APGC–Q–TOF–MS was initially considered as an excellent analytical tool for identification purposes, the wide linear range and the good behavior for quantitative analysis indicate that it is one of the

most promising analytical techniques for resolving difficult tasks. Very small concentrations of NPAHs were quantified in mosses, and these data can be used for pattern recognition of the pollutants, the areas and the likely emissions. Consequently, a better environmental diagnosis can be established, taking into account the pathways of PAHs and NPAHs as well as their distribution in the area under study.

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