Analysis of Polycyclic Aromatic Hydrocarbons in Agricultural Soils by Gas Chromatography-Ion Trap Tandem Mass Spectrometry

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An investigation has been carried out on collision-induced dissociation (CID) in the development of an analytical protocol for the determination of polycyclic aromatic hydrocarbons (PAHs) by ion trap tandem mass spectrometry. Two different considerations were used to choose the optimal CID conditions for complex matrix environmental samples, namely, to determine the highest signal-to-noise (S/N) ratio and the other to eliminate the background interferences originated from complex matrix samples. The PAH content of agricultural soil was measured to estimate overall distribution of PAH in throughout the country, we collected and analyzed 226 soil samples from paddy and upland soil. The average content of total PAH in all samples was 236 μg kg^{-1}, and the range was from 23.3 to 2,834 μg kg^{-1}. The overall distribution of PAH was found to be closely related to the pollution sources, the size of city and the type of industry.

Key words : PAHs, Soil, Monitoring, Mass spectrometry, GC-MS

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among organic compounds which are classified as base/neutrals (extractable) and semi-volatile organics[1]. PAHs are products of combustion processes of carbonaceous fuels. PAHs are among the most hazardous compounds in oil spills[2]. PAHs are found in crude oil, used motor oil, soot, smoke from incomplete combustion, and in various complex mixtures of hazardous chemicals such as creosote. The main sources of human exposure are food intake and inhalation. Food intake can be an important route for fish and wildlife too, along with direct contact in water, sediment and soil. PAHs most often occur in environmental samples as a minute fraction of a complex mixture of hydrocarbons, and it is the exception rather than the rule to find only one or

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two of these compounds occurring together[3].

Analytical methods for PAH analysis have been developed for the past several decades. There is now a wide variety of analytical techniques used for the characterization and quantitation of PAHs, including capillary gas chromatography with flame ionization detection/photo-ionization detection, or mass spectrometric detection, supercritical fluid chromatography, and high performance liquid chromatography (HPLC) using spectrophotometric, mass spectrometric detection (LC-MS), or fluorometric detection[4]. Among these methods, Capillary gas chromatography (GC) was first applied to analyze PAHs in the early 1960s, and its use has progressed to the point that it now comprise one of the standard methods for the determination of these compounds in environmental matrices[5].

Tandem mass spectrometry has been used widely to identify chemical structures, to study reaction mechanism of ion neutral reactions, to obtain thermochemical information of gas phase species, and to improve qualitative and quantitative analysis of complex matrices. With MS/MS, selectivity is obtained by rejecting

matrix interference through the selection of the parent ion, while providing molecular specific information through the daughter ions' spectrum. Combined with the excellent sensitivity of ion trap technology, this method enables low ppb level informative detection under complex mixture conditions[6].

One major aim of this study was to assess the parameters of MS/MS for the quantitative determination of PAHs with the highest sensitivity. The method should, along with other methods, assess the limit of detection (LOD) and standard deviation of replications. Other important aspects were the new approach to eliminate the background noise derived from a sample with complex matrices. This new method was accomplished by combining the characteristics of CID which can control the extent of fragmentation by adjusting excitation energy, q value, and excitation time of a ion trap mass spectrometer and the property of PAHs which are resistant to CID. Surplus excitation energy provided to the ring electrodes eliminates matrix interferences in ion trap tandem mass spectrometry. When the surplus excitation energy is applied to the ion trap mass spectrometer, the matrices are more easily broken down to lower mass compounds than PAHs which are either fragmented or mainly deprotonated, thus the S/N ratio is maximized. In addition to the MS/MS method, the overall monitoring data of PAHs in agricultural soils throughout the Korean peninsula by GC-MS was provided to assess the environmental effect of the rapid industrialization on agricultural environment.

2. Experimental Methods

2.1 Materials

PAHs standards and internal standards were obtained from AccuStandard (New Haven, CT, USA). The PAHs standard includes the following sixteen EPA PAHs: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorine (Fle), phenanthrene (Phe), anthracene (Ant), fluoroanthene (Fla), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoroanthene (BaF), benzo(k)fluoroanthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-

cd)pyrene (IcP), dibenzo(a,h)anthracene (DaA), benzo(g,h,i)perylene (BgP). Deuterated PAHs, namely naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}, were used as internal standards (IS) for the quantitation. The concentration of the IS was 1.0 μ g mL^{-1}. A standard reference material NIST SRM 1944 was obtained from the National Institute of Science and Technology (NIST) and used for method validation.

2.2 Instrumentation

All experiments were performed on a Polaris Q including Trace GC 2000 (ThermoFinnigan, TX, USA), integrated GC-MS/MS system (ion trap with external ionization) fitted with the high temperature ion source.

The GC was equipped with a DB-5ms fused silica capillary column (J&W Scientific, CA, USA), 30 m length x 0.25 mm internal diameter(I.D.) with 0.25 μm bonded film thickness. Helium was used as the carrier gas at a constant velocity of 40 cm/sec. All injections of 1.0 μ L volume were performed in the splitless mode on a split/splitless injector using autosampler. The GC temperature program was 75 ℃ for 5 min, temperature increased to 150 ℃ at 25 ℃/min with no hold time, then increased to 265 ℃ at a 4 ℃/min and finally increased to 285 ℃ at 30 ℃/min, and held at 285℃ for 10 min. The injector temperature was maintained at 285℃ and transfer line temperature was at 295 ℃. The system was fully controlled and all data processing was carried out using a PC with the Xcalibur 1.2 software (ThermoFinnigan, TX, USA).

Electron ionization (EI) spectra were obtained using the full scan mode from m/z 50 to 300 at scan time of 0.36 sec. The analyses of selective ion monitoring (SIM) mode were performed using the detection condition in Table 1.

For MS/MS experiments, collision induced dissociation (CID) was performed in the resonant excitation mode[7]. Precursor ions were isolated using a 1 u isolation window. A CID excitation time of 15 ms or 25 ms was used in all the resonant excitation experiments. Multiple reaction monitoring (MRM) was used for the analysis of sixteen PAHs in a single chromatographic run. Tune setting for full scan

mode is following: Ion source temperature of 200 °C; electron multiplier at 1050-1175 V (Auto tuned to a gain of 3~4 × 10⁵); filament emission current 250 mA at 70 eV; high mass adjustment 50%; and automatic gain control (AGC) 50 (arbitrary unit, range 1~300). Finding the best quantification condition of MS/MS, CID voltage was set from 0 to 5 V and the q_z value was set at 0.225, 0.30, and 0.45.

Table 1. Detection conditions of GC-MS in SIM and CID analysis

Segme nt	Window (min)	Acquired mass /Precursor ion mass	Analyte recorded
1	5.5 ~ 7.0	128, 136	Nap, Nap-d₈
2	7.0 ~ 8.9	152	Acy
3	8.9 ~ 10.0	154, 164	Ace, Ace-d₁₀
4	10.0 ~ 13.0	166	Fle
5	13.0 ~ 19.0	178, 188	Phe, Ant, Phe-d₁₀
6	19.0 ~ 25.0	202	Fla, Pyr
7	25.0 ~ 32.0	228, 240	BaA, Chr, Chr-d₁₂
8	32.0 ~ 38.0	252, 264	BbF, BkF, BaP, Perylene-d₁₂
9	38.0 ~ 45.0	276, 278	IcP, DaA, BgP

2.3 Sample collection

Surface soils were collected in the agricultural areas throughout South Korea, the locations of which are shown in Fig. 2. A total of 226 soil samples (paddy soil, 126; upland soil, 100) were collected for the distribution monitoring of PAHs over a period of two years. A number of samples were collected from each location and bulked together to obtain a representative sample which was used for analysis. To eliminate direct effects of vehicle exhaust emissions the samples were collected at least 30m away from the roads. Some sampling sites were selected purposely to cover specified contamination sources such as power plants and industrial enterprises.

For extraction, samples were mixed with anhydrous sodium sulfate, placed in an extraction thimble, plugged with glass wool and extracted using 250 ml dichloromethane for 16 hours in a Soxhlet extractor according to the EPA 3540C method. Silica gel column clean up was used for purification of the extractant according to EPA

method 3630[1].

2.4 Quantitation

A solution containing 16 PAHs standard and 5 internal standards was prepared by serial dilution of the commercial standard in isooctane. A five-point response factor calibration curve was established demonstrating the linear range of the mass detector. All quantitation was performed by the method of internal standardization by using five internal standards (IS) at 2.00×10^2 ng ml⁻¹ level. The percent relative standard deviation for all calibration analytes did not exceed ± 15 percent with an $R^2 > 0.99$ with a 1st degree fit of the data. The limit of detection (LOD) is a basic performance characteristic for comparing different methods. In this study, a S/N ratio 3 was considered acceptable for estimating the detection limit[8].

3. Results and Discussion

3.1 Optimazation of CID condition

There is a number of approaches to achieve maximum sensitivity and low interference in MS/MS analysis using ion trap mass spectrometry. The observed limit of detection in MS/MS depends on a number of factors, including intensity of the precursor ion, CID, fragmentation, and collection efficiency[9]. Among these, the q value and CID energy are critical parameters, though the ion source temperature and collision time also influence ion trap MS/MS operation. To optimize the CID condition for PAH analysis in environmental samples, three parameters, excitation energy, q value, and excitation time, were investigated. The performance of the ion trap mass spectrometer was tuned along with the S/N ratio, peak area, and spectrum quality for the identification.

The ion abundance curve to the excitation of chrysene was depicted in Fig. 1. The curves were determined after some initial experimentation with the various ion trap CID mass spectrometry conditions. It should be noted that PAHs are unusually stable compounds which are resistant to CID. When the excitation energy was too low, no CID fragmentation occurred. When the CID energy was increased, the molecular ion used as a precursor ion was radically decreased.
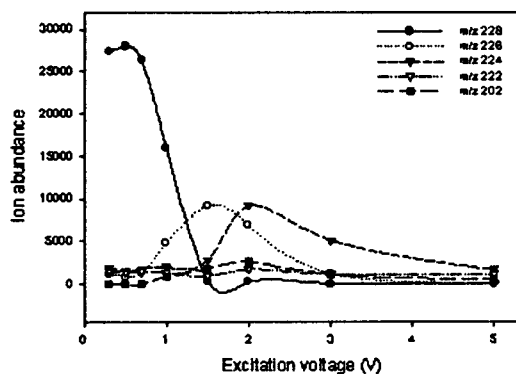
Fig. 1. Decomposition curves of chrysene molecular ion by resonant excitation energy.

Fig. 2 shows the S/N ratio and peak area curves for anthracene by change of excitation energy. The S/N ratio was closely related to the peak area, and the highest around about 0.5V of excitation energy. These results indicate that the precursor ions were effectively trapped and ejected without any decomposition by CID.

The effect of excitation time on the mass fragmentation and the peak intensity was also investigated. The default excitation time is 15 ms in the PolarisQ GC-MS system, but that can be adjusted to any integer value from 1 to 30 ms in the instrument setup. If the isolation width is set to a wide value, increasing the excitation time increases the amount of time each ion in the window can be excited. In this work the excitation time was adjusted to 15 ms and 25 ms, because the compounds with a stable structure requires more time for efficient dissociation in the ion trap[10].

The results are shown in Fig. 3 to depict the peak area versus excitation energy. In the case of benzo(a)pyrene the S/N ratio and peak area only slightly affected by the extended collision time through the whole range of excitation energy.

3.2 Decision of CID condition

As a result of the previous work, two different CID conditions were considered to obtain the maximum sensitivity with lower background interferences. For this purpose, the excitation energy was set at two discrete values, one to allow the highest S/N ratio for the sensitivity (optimum condition) to be obtained, and the other to eliminate complex interferences by supplying surplus energy for the further breakdown while sacrificing signal intensity and S/N ratio (proposed condition).
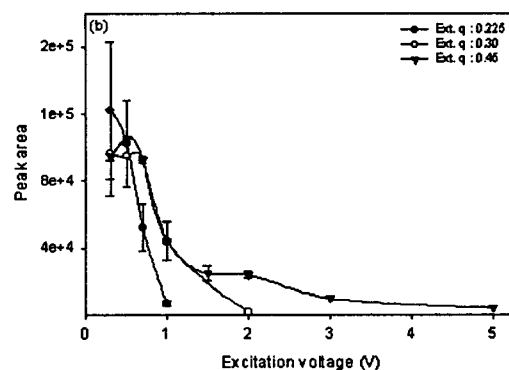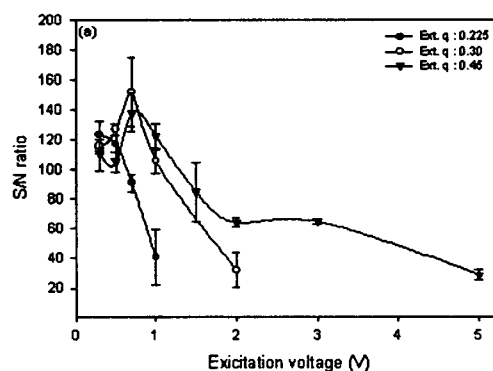

Fig. 2. S/N ratio (a) and peak area (b) of anthracene by change of resonant excitation energy.

Table 2 shows the parameters of the ion trap tandem mass spectrometer for two different conditions. The excitation voltage was in the range between 0.5 and 1.0 in the optimum condition and 1.0 and 2.0 in the proposed condition for matrix elimination. For the matrix elimination condition, the excitation energy was slightly elevated but did not exceed 2V, because the ion intensity was severely affected when the voltage was over 2V. The q value was set at 0.45.
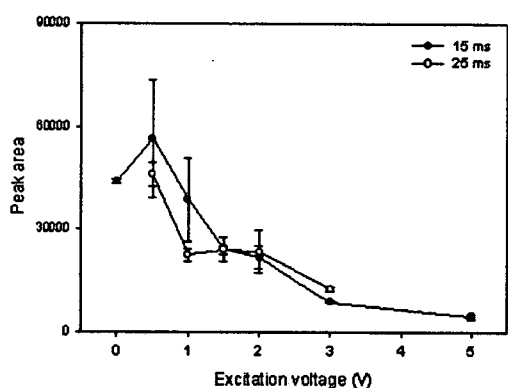
Fig. 3. Peak area of benzo(a)pyrene by change of excitation energy at different excitation time.

Table 2. Parameters of ion trap mass spectrometry in MS/MS analysis for PAHs analysis

Name	Ex. voltage at optimum condition	Ex. voltage at ME condition[1]	Ex. time (ms)
Nap	0.7	1.0	10
Acy, Ace	0.7	1.2	12
Fle	0.7	1.5	12
Phe, Ant	0.5	1.0	12
Fla, Pyr	0.7	1.5	15
BaA, Chr, BbF, BkF	1.0	1.5	15
BaP	1.0	2.0	18
IcP, DaA, BgP	0.7	1.5	20

[1]ME : matrix elimination.

The limit of detection (LOD) for PAH analyses using full scan (EI), SIM, MS/MS modes was investigated, and the result are shown in Table 3. The LOD ranged between 1.22 and 7.26 in EI mode, 2.16 and 4.29 in SIM mode, 0.15 and 2.71 in MS/MS mode for the optimum excitation energy, and 0.62 and 3.20 in MS/MS mode for the proposed excitation energy. This result is similar to the previous work with ion trap mass spectrometry in which the detection limits ranged from 0.92 to 7.99 in EI.[11] However, the present study had up to about 4 times lower LOD than the previous CID work which ranged from 1.32 to 9.37 by Mosi et al[11]. Previous studies have shown that GC-MS in SIM mode

exhibited LOD levels of 0.5~3.5 pg for four PAHs[12], 0.06~9.76 ng mL^{-1} for 16 PAH mixture[13].

Table 3. Detection limits for 16 PAH mixture by EI, SIM, and CID (ng mL^{-1}) in the ion trap mass spectromet

Name	EI[a]	SIM	MS^2 in optimum condition	MS^2 in ME condition[1]
Nap	1.76	1.16	1.16	1.29
Acy	1.46	1.20	0.80	1.42
Ace	1.55	1.34	0.82	0.62
Fle	1.69	1.21	0.75	0.57
Phe	1.43	1.13	0.90	0.89
Ant	1.82	1.30	1.33	1.22
Fla	1.28	1.29	0.72	0.73
Pyr	1.22	1.29	0.70	0.64
BaA	3.08	1.68	0.53	0.94
Chr	2.56	1.74	0.72	0.90
BbF	3.00	2.02	0.15	1.42
BkF	2.96	2.01	0.21	1.83
BaP	4.39	2.41	0.61	2.14
IcP	6.87	3.61	2.31	2.87
DaA	5.46	3.32	2.71	3.20
BgP	7.26	4.29	1.75	1.98

[1]ME : matrix elimination.

3.3 PAH concentration in soils

The total PAH concentration ranged over 2 orders of magnitude from 23.3 to 2834 μg kg^{-1}. The mean concentration of total PAH for all the samples collected was 236 μg kg^{-1}, and the median was 158 μg kg^{-1}. These values are greatly in excess of the reported natural concentration of PAH in soil (1-10 μg kg^{-1}), but slightly lower than those present in rural soil in United Kingdom, the median value of which was 187 μg kg^{-1} [14]. The spatial distribution of total PAH concentrations in relation to geomorphic locations is shown in Fig. 3. The figure shows that PAH were transport from the pollution sources to the mountain area along with the prevailing wind direction.

4. Conclusions

The application of the matrix elimination technique for absolute identification at trace levels in complex matrices, which is readily obtained with ion trap, is a powerful feature for stable

compounds such as PAHs. The method provides good sensitivity for these compounds at the low picogram range and excellent ion ratio confirmation data of the product ion spectra.
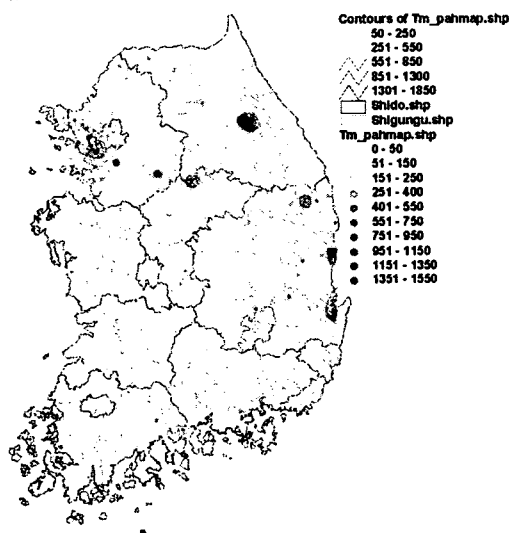

Fig. 4. Distribution map of total PAH in Korea

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