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GC/HRSIR as a complementary technique to GC/ECNIMS

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ABSTRACT

Gas chromatography/electron capture negative ion mass spectrometry (GC/ECNIMS) is a highly selective and sensitive technique for the analysis of appropriate analytes in complex matrices. Its major drawback is often the lack of fragmentation indicative of structure that can be used to confirm the identity of the analyte. Recourse to low resolution EI selected ion recording (SIR) at low resolution can be tried to validate the methodology with additional specificity, but then the selectivity loss relative to ECNIMS and the presence of complex matrix interferences may require additional cleanup that was not needed for GC/ECNIMS. One solution is to use high resolution mass spectrometry as GC/HRSIR to provide the selectivity, sensitivity, and specificity needed to validate the GC/ECNIMS method that will be used for the bulk of the analyses by providing the needed specificity. Several examples are given as to how well this works in practice.

KEYWORDS

Biosolids;
Diesel particulates;
Musk xylene;
Nitro-PNA;
PBDE;
HRSIR;
ECNIMS.

INTRODUCTION

Electron capture negative ion mass spectrometry (ECNIMS) [and the related technique of dissociative EC that we will lump together hereafter as NIMS] has now been in use for over 30 years in various analyses and has been adopted for widespread use[1]. Prized for its sensitivity, selectivity, and relative ease of use, NIMS provides an elegant approach for addressing many types of analytical problems. However, its chief limitation from a specificity standpoint is the lack of fragment ions that add confidence to the confirmation of identity that we expect from a GC/MS method. Indeed, it is not unusual to obtain a single ion such as $M^{-\cdot}$ from a given compound along with the isotope ions[2]. Even when fragment ions occur (by dissociative EC), they often do not arise from significant structural fragmentation as often occurs with EIMS[3].

Thus, one may want to validate the GC/NIMS method by another more specific technique. Herein lies a difficulty because in choosing EIMS, for example, one may lose the selectivity and possibly some of the sensitivity of NIMS. This may result in the need for further cleanup and concentration of sample extracts to achieve the desired result. Recourse to GC/MS/MS might work in some instances using either EIMS or positive ion chemical ionization (CI) MS[4]. Another possibility is to turn to GC/high resolution selected ion recording (GC/HRSIR) for greater specificity such as in the case of EPA Method 1668 that uses HRMS for PCBs[5].

In this work examples of a validation approach for GC/NIMS that uses GC/HRSIR to provide the sensitivity, selectivity, and specificity are shown. Two different complex matrices are used: biosolids and diesel particulates. Substances that provide specific examples

are selected from pharmaceuticals and personal care products, halogenated compounds, and nitro-containing polynuclear aromatics.

Biosolids (sewage sludge) represent the end product of bacterial digestion and applied treatments of raw sewage in a municipal sewage treatment facility[6]. The precipitated material from the aqueous solution consists of a relatively intractable material made up of both inorganic and organic substances that have reached an environmental sink. The characterization of this material remains incomplete but more information is being revealed by various workers throughout the world[7].

A number of papers have now started filling in some of our questions about the types of contaminants in biosolids. The review by Rogers[6] included mention of a variety of compounds including organochlorine pesticides (e.g., aldrin), PCBs, and chlorophenols. A large presence for nonyl phenols and surfactants was mentioned. The large and diverse class of pharmaceuticals and personal care products (PPCPs) was described and most of these compounds (e.g., antibiotics) would be found in the polar fraction of biosolids components[8,9,10]. The synthetic musks are one group of PPCPs that partition with the lipophilic fraction. Methods were given that included extraction and cleanup procedures.

Oberg et al. described the occurrence of PBDEs in over 100 sludge samples from Sweden where the predominant tetrabromo, pentabromo, and hexabromo congeners were found as well as the decabromodiphenyl ether[11]. Ying and Kookana pointed out that high levels of triclosan in biosolids could be a concern in soil applications[12]. Synthetic musks were also determined in biosolids[13]. Nonylphenols, pthalates, and PCBs were determined in biosolids and soil in an effort to follow the fate of such contaminants after soil amendment using biosolids[14].

Diesel particulates, another complex environmental matrix, contain a large number of substances that are of interest from the standpoint of environmental pollution[15,16,17]. In particular, the nitro-containing PNAs have been the subject of analytical study and metabolic study because of their carcinogenic properties that are believed to result from the reducing reaction sequence of the nitro group with further biochemical activation that occurs in humans and other biota.

Diesel particulates also consist of a large number of oxygen-containing aromatic compounds some of which may respond by GC/NIMS, especially if a conjugated carbonyl group is present, although the majority of PNAs (benzo-a-pyrene being a notable exception) and aliphatic hydrocarbons do not respond sensitively. Selective detection by fluorescence spectrometry is not effective for nitro-aromatics (nitro group fluorescence quenching) whereas it is sensitive for amino-aromatic compounds and most non-halogenated PNAs. Particulates in themselves are an area of concentrated investigation because of their role in environmental-related disease[18].

EXPERIMENTAL

Chemicals

Musk xylene and 3-nitrofluoranthene were obtained from Aldrich Chemical Co.; PBDE congeners were obtained from AccuStandard; SRM 1650 was obtained from NIST.

Mass spectrometry

1. GC/NIMS: An Agilent 5975 was operated under negative ion conditions (methane reagent gas at 0.4 flow setting (40% of 2 mL/min), source 150°C, quadrupole 150°C, emission 0.50 mA).

2. GC/HRSIR: A Waters-MicroMass AutoSpec Premier (P) was operated at 10000 resolution in EI mode (500μA trap current, 250°C source, high boiling PFK calibrant, 8 kV accelerating voltage, 350 V photomultipler detector).

Method for Nitro-PNAs

1. Extraction/Cleanup: The method used a SW-846 approved extraction (pressurized liquid extraction) of BS (typical 0.5 g) or diesel particulate (typical 0.05 g) in methylene chloride/acetone by ASE 200 (Dionex)[19]. The ASE conditions were a 10g cell, 80°C, 2500 psi, 15 min extraction. The extraction was repeated and extractants combined. The 3-nitrofluoranthene was spiked at 600μg/kg in diesel particulate (typical 50 mg sample size, 100μL final extract volume) and 100μg/kg in BS (typical 0.5g size, 1 mL final extract volume). An SPE cleanup was used to isolate fractions on silica. A 3 mL Si SPE cartridge (Phenomenex) was washed with hexane; sample was applied in 1 mL of hexane

and then the sample was eluted with 2mL hexane, 2mL hexane/methylene chloride (90/10 v/v), 2 mL hexane/methylene chloride (50/50 v/v), 2 mL methylene chloride, and 2 mL acetone as separate fractions. The nitro-PNA was found in the hexane/methylene chloride (50/50 v/v) fraction.

2. GC/NIMS: A 40 m 0.18μm film 0.18 mm ID column (DB5 Agilent-J&W) was used with temperature programming: 60°C for 1 min followed 60-150°C at 10°C/min, followed by 150-250°C at 4°C/min followed by 250-300°C at 10°C/min. Injector temperature was 250°C. SIM parameters (25 msec dwell) for several classes of nitro-PNAs: m/z 173.05, 174.05, 182.05, 199.05, 211.05, 218.05, 223.05, 224.05, 244.05, 247.05, 248.05, 273.10, 274.10, 292.05, 293.05, 429.85 (IS, PCB#204).

3. GC/HRSIR: A 30 m 0.25μm film 0.25 mm ID column (DB5 Agilent-J&W) was used with temperature programming: 60°C for 1 min followed by 60-300@20°C/min. SIR (30 msec dwell): m/z 201.07043, 217.06534, 242.98563 (PFK lock mass), 247.06333.

Method for PBDEs

1. Extraction/Cleanup: The method used a similar extraction of BS as for nitro-PNAs (typical 0.5g) in methylene chloride/acetone by ASE. The extract was concentrated and then fractionated on silica using SPE to isolate the fraction containing the PBDEs. A 3mL Si SPE cartridge is washed with hexane; sample was applied in 1 mL of hexane and then the sample was eluted with 2mL hexane, 2mL hexane/methylene chloride (90/10 v/v), 2mL hexane/methylene chloride (50/50 v/v), 2 mL methylene chloride, and 2 mL acetone. The PBDEs were found in the hexane and the hexane/methylene chloride (90:10 v/v) fractions (combined) using a typical 0.5 g sample size, 1 mL final extract volume and range from 100μg/kg to several mg/kg depending on congener.

2. GC/NIMS: Same GC parameters as for GC/NIMS for nitro-PNAs. The method used quantitation ions from specific PBDE congeners (congeners 181, 28, 183, 47, 99, 154, 66, 85, 153, 100, 155). SIM parameters (25 msec dwell): m/z 403.8, 405.8, 407.8, 429.8 (IS), 483.7, 485.7, 487.7, 561.6, 563.6, 565.6, 641.5, 643.5, 645.5, 719.4, 721.4, 723.4 .

3. GC/HRSIR: Same GC parameters as for GC/HRSIR for nitro-PNAs. SIR (30 msec dwell) divided into three retention/ion groups: Gp 1: 403.80470, 405.80265, 407.80061, 416.97063 (PFK lock mass), 429.76057 (IS), 483.71385, 485.71112, 487.70907; 5 to 13.6 min; Gp 2: 561.62367, 563.62163, 565.61958, 566.96642 (PFK lock mass), 641.53214, 643.53009, 645.52805; 13.70 to 18.0 min; Gp3: 719.44265, 719.44265 (PFK lock mass), 721.44060, 723.43856; 18.1 to 25.0 min.

Method for musk xylene

1. Extraction/Cleanup: The method used the same extraction of BS as for nitro-PNAs (typical 0.5 g) in methylene chloride/acetone. The extract was concentrated and then fractionated on silica using SPE to isolate the fraction containing the musk xylene. A 3 mL Si SPE cartridge was washed with hexane; sample was applied in 1 mL of hexane and then the sample was eluted with 2 mL hexane, 2 mL hexane/methylene chloride (90/10 v/v), 2 mL hexane/methylene chloride (50/50 v/v), 2 mL methylene chloride, and 2 mL acetone. The musk xylene was found in the methylene chloride fraction. The musk xylene was spiked at 300 μg/kg in BS (typical 0.5 g sample size, 1 mL final extract volume).

2. GC/NIMS: Same GC parameters as for GC/NIMS for nitro-PNAs. SIM parameters (25 msec dwell): 221.10, 265.10, 267.10, 282.10, 297.10,

3. GC/HRSIR: Same GC parameters as for GC/HRSIR for nitro-PNAs. SIR (30 msec dwell): 265.06990, 280.98241 (PFK lock mass), 282.07260, 297.09610.

RESULTS AND DISCUSSION

Overview of results

This work compares analyses for musk xylene, PBDEs, and 3-nitrofluoranthene in either diesel particulates or biosolid matrices using GC/NIMS and GC/HRSIR. Both matrices contain a complex array of substances that complicate determination of target analytes at μg/kg levels. In the case of nitro musks in biosolids, analytes such as galaxolide occur at mg/kg levels. The PBDEs and other analytes occur from low μg/kg levels through mg/kg levels depending on analyte. In diesel particulates, 1-nitropyrene occurs at about 18.2 mg/kg

in the NIST reference material SRM 1650b[20]. The spiking levels used for the example compounds are at or below these naturally occurring levels. The methodologies conform closely to published methods for similar analytes[10,20,21]. Example chromatograms are given to assess selectivity and sensitivity of each technique, GC/NIMS and GC/HRSIR, and enable comparisons between them. The improved specificity in GC/HRMS derives from the ions monitored (given in the Experimental section) and their relative abundances. In general, the NI technique produced predominantly a molecular anion whereas in the EI technique a number of ions were monitored to strengthen confidence in the confirmatory aspect.

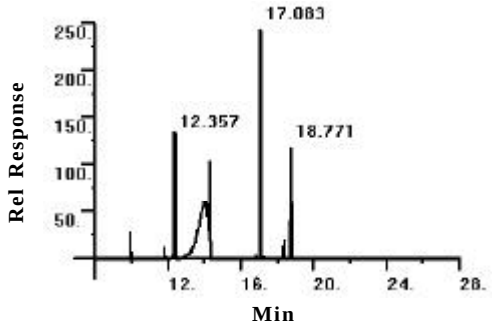

Figure 1: Total ion chromatogram of the negative ion signal for monitoring for PBDEs. The broad peak results from contributions from elemental sulfur isotopes to the monitored ions at m/z 161 and 163; major responses primarily on those ions for early eluters such at RT=12.357, 17.083, and 18.771

PBDEs

A number of congeners of PBDEs occur in biosolids. The lower brominated congeners do not yield significant molecular anions, a behavior they share with polychlorinated compounds such as PCBs. As the bromine number increases, a greater relative abundance of molecular anions are observed with a decrease in the relative abundance of bromine ions monitored at m/z 161 $(HBr_2)^-$ (or at m/z 79 and 81 if those are monitored). Thus, particularly for tri-PBDEs and other congeners giving only low mass bromine-containing ions, the GC/HRSIR technique provides important confirmatory data in the form of ions from the molecular ion cluster. The comparison of selectivity is shown by chromatograms where detection of PBDEs in biosolids by GC/NIMS is illustrated in figure 1 and the same extract analyzed by GC/HRSIR in figure 2.

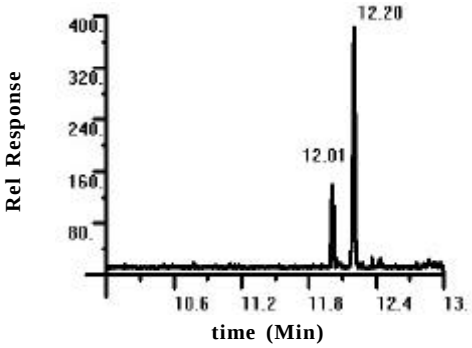

Figure 2: The m/z 403.8047 ion chromatogram of tri BDE by GC/HRSIR at 10000 resolution; RT=12.01 and 12.20 yield molecular ion clusters of the right relative abundance confirming the presence of specific PBDEs

In figure 1, the broad peak results from the response of elemental sulfur that produces a series of ions at m/z 256, 224, 192, 160, etc (beginning at S_8). The ^{33}S isotopic contribution forming M+1 and the corresponding contribution forming M+3 combined with the relatively abundant ^{34}S isotope thus overlap with the bromine containing ions being monitored for the PBDEs. Otherwise, both GC/NIMS and GC/HRSIR show a high level of selectivity for the target analytes. The three ions monitored for each congener molecular ion cluster under GC/HRSIR conditions and their relative abundances provide the confirmatory data for PBDEs together with the retention time. For example, the confirmation of PBDE#100 for m/z 561.62367, 563.62163, and 565.61958 gave relative abundances of 0.51, 100.0, and 99.0 that were within 1% of the theoretical relative abundances (0.86, 100.0, 98.0) and that of PBDE#47 for m/z 483.7138, 485.7112, and 487.7091 were 69.8, 100, and 63.7 in the standard and 68.3, 100, and 64.5 in the biosolids extract.

Nitro-PNAs

Nitro-PNAs usually occur along with other types of PNAs including those that contain oxygen, sulfur, and nitrogen along with a complex hydrocarbon background. Figure 3 and 4 illustrate the selectivity of GC/NIMS and GC/HRSIR for 3-nitrofluoranthene spiked into diesel particulates. Specificity is provided from the GC/HRSIR monitoring of the molecular ion and the losses of 30 and 46 arising from the nitro moiety.

In the spectral confirmation of 3-nitrofluoranthene the observed relative abundances of m/z 201.0704,

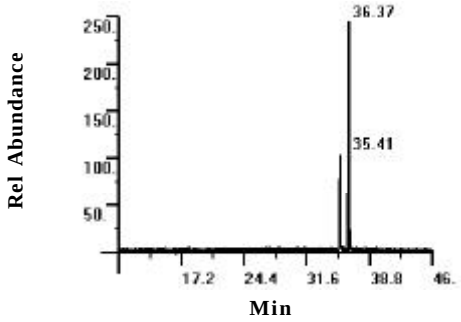

Figure 3: Negative ion chromatogram for m/z 247 of 3-nitrofluoranthene spiked in diesel particulate at 720µg/kg; RT=35.41. A very similar chromatogram was obtained for spiked biosolids. The response for native level of 1-nitropyrene is seen at RT=36.37

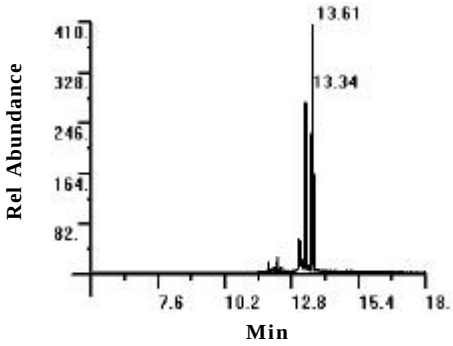

Figure 4: Ion chromatogram for m/z 247.0633 at 10000 resolution from 3-nitrofluoranthene spiked in diesel particulates at 720µg/kg; RT= 13.34 A very similar chromatogram was obtained for spiked biosolids. The response for native level of 1-nitropyrene is seen at RT=13.61

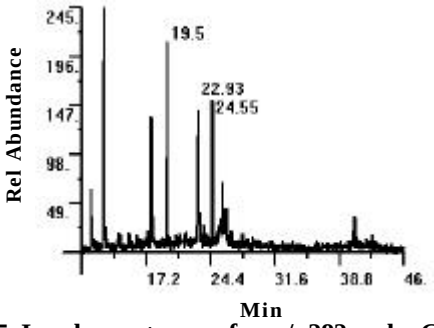

Figure 5: Ion chromatogram for m/z 282 under GC/NIMS conditions for musk xylene spiked in biosolids at 362µg/kg; RT=19.50

217.0653, and 247.0633 were 100, 37.6, and 94.2 in the standard and 97.2, 39.2, 100 in the diesel particulates extract.

Musk xylene

The synthetic musks are subject to a number of potential interferences by virtue of their relatively low

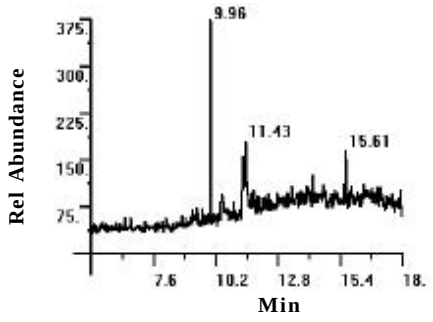

Figure 6: Ion chromatogram for m/z 282.0726 at 10000 resolution from musk xylene spiked in biosolids at 362 µg/kg; RT= 9.96. Note the possible interference from silicone bleed peaks (C13 isotope peak of m/z 281, exact mass 282.0551) that cannot be resolved at 10000 resolution

molecular weight and small positive mass defect. The nitro musks can be determined using GC/NIMS with more selectivity than by EIMS. Figures 5 and 6 compare selectivity of GC/NIMS with GC/HRSIR for musk xylene spiked into biosolids.

One factor to note for GC/HRSIR is that the specificity is not without limitations. The ion monitored for musk xylene at 282.07260 is close to silicone bleed at 282.0551. The example illustrates the fact that GC/HRSIR is not absolutely specific or selective at 10000 resolution, and that multiple ions and their relative abundances need to be monitored to strengthen the confirmation of identity[22]. In this case m/z 265.0699, 282.0726, and 297.0961 gave relative abundances of 3.4, 100, and 3.4 in the standard and 6.9, 100, and 3.9 in the biosolids extract.

CONCLUSION

Applications of GC/NIMS often exhibit high selectivity and sensitivity but reduced specificity as a result of reduced fragmentation. The use of GC/HRSIR as a complementary technique afforded increased specificity while maintaining good selectivity and sensitivity. Appropriate responses within the window of expected exact masses for elemental compositions and relative abundances of at least three ions provided confirmatory data.

Notice

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