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Quantification of complex polycyclic aromatic hydrocarbon mixtures in standard reference materials using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry

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ABSTRACT

This research is the first to quantify complex PAH mixtures in NIST SRMs using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC/ToF-MS), with and without extract cleanup, and reports previously unidentified PAH congeners in the NIST SRMs. We tested a novel, high orthogonality GC column combination (LC-50 × NSP-35), as well as with a commonly used column combination (Rtx-5ms × Rxi-17) for the quantification of a complex mixture of 85 different PAHs, including parent (PAHs), alkyl- (MPAHs), nitro- (NPAHs), oxy- (OPAHs), thio- (SPAHs), bromo- (BrPAHs), and chloro-PAHs (ClPAHs) in extracts from two standard reference materials: NIST SRM1650b (diesel particulate matter), with cleanup and NIST SRM1975 (diesel particulate extract), with and without extract cleanup. The LC-50 × NSP-35 column combination resulted in an average absolute percent difference of 33.8%, 62.2% and 30.8% compared to the NIST certified PAH concentrations for NIST SRM1650b, NIST SRM1975 with cleanup and NIST SRM1975 without cleanup, while the Rtx-5ms × Rxi-17 resulted in an absolute percent difference of 38.6%, 67.2% and 79.6% for NIST SRM1650b, NIST SRM1975 with cleanup and NIST SRM1975 without cleanup, respectively. This GC × GC/ToF-MS method increases the number of PAHs detected and quantified in complex environmental extracts using a single chromatographic run. Without clean-up, 7 additional compounds were detected and quantified in NIST SRM1975 using the LC-50 × NSP-35 column combination. These results suggest that the use of the LC-50 × NSP-35 column combination in GC × GC/ToF-MS not only results in better chromatographic resolution and greater orthogonality for the separation of complex PAH mixtures, but can also be used for the accurate quantification of complex PAH mixtures in environmental extracts, such as diesel particulate matter, without silica gel cleanup.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that constitute a large and diverse class of organic molecules. PAHs are of concern due to their potential persistence, bioaccumulation and toxic effects [1–4]. Some PAH derivatives are more carcinogenic and mutagenic than their parent compounds [5,6].

The extracts from complex environmental samples may contain a variety of PAHs with different molecular sizes and structures including: parent PAHs (PPAHs), alkylated-PAHs (MPAHs), nitro-PAHs (NPAHs), oxy-PAHs (OPAHs), thio-PAHs (SPAHs), chlorinated (ClPAHs) and brominated-PAHs (BrPAHs). The most prominent source of PPAHs and MPAHs is the incomplete combustion of organic material [7,8] in either natural processes, such as forest fires, volcanic eruptions and hydrothermal processes [9–12], or anthropogenic processes, such as the combustion of fossil fuel and biomass [13–15]. Heterocyclic analogs of PAHs, in which one or more carbon atoms are replaced by nitrogen, sulfur, or oxygen, have also been measured as environmental contaminants. NPAHs are formed during the pyrolysis of nitrogen-containing organic materials and significant concentrations are found in industrial and urban atmospheres, tobacco smoke, engine exhaust, coal tar and coal

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gasification residues [8,16]. SPAHs are emitted from most of the same combustion sources as PPAHs and NPAHs [8]. Chemical oxidation and photochemical alteration represent significant sources of OPAH derivatives to the environment [17–19]. Waste incinerators, water chlorination facilities and automobile and diesel exhaust have been shown to form ClPAHs and BrPAHs [4,17–19], in addition to PPAHs, MPAHs, NPAHs, OPAHs, and SPAHs.

The analysis of environmental extracts containing PAHs is often complex and requires cleanup steps and multiple liquid or gas chromatographic methods. Currently, the analysis and quantification of complex PAH mixtures in environmental extracts requires three different one-dimensional GC/MS methods with a total run time of 141.6 min per sample: NPAHs, SPAHs and OPAHs method (45.7 min) [16], PPAH and MPAHs method (46 min) [16], and Cl and Br-PAH method (49.9 min) [20], in addition to the time required for sample cleanup that often includes adsorption, solid phase extraction (SPE) and gel permeation chromatography (GPC).

In order to reduce the analysis time of PAHs contained in a complex environmental mixture, a technique with higher chromatographic peak capacity is needed. Comprehensive two-dimensional gas chromatography (GC × GC) enhances the gas chromatographic separation of complex organic mixtures [21] using two different GC columns, with different retention mechanisms, for the separation of analytes. A GC × GC method with high orthogonality, and low correlation of retention times between dimensions, is preferred.

Quantification in GC × GC/ToF-MS is a more complex process than in one-dimensional GC/MS, where in the latter case a single retention time and peak response are associated with each analyte in the extract. In GC × GC/ToF-MS, a series of modulated peaks (sub-peaks) are generated and detected, and the retention time and response are represented by a distribution of values generated by this process [22,23]. Quantification in GC × GC/ToF-MS is an extension of one-dimensional GC/MS in that these individual sub-peak areas are added together [24]. With GC × GC/ToF-MS, an increase in quantification error occurs because of inaccurate determination of the peak baseline and incorrect identification of peak start and end times, as well as tailing, fronting and overloading of each modulated peak [25], the same sources that also lead to errors in GC/MS compounded by lack of resolution in GC/MS. Peak tailing, fronting and overloading are especially important with GC × GC/ToF-MS because of the shorter and narrower second dimension column. In addition, small variations in integration parameters for the modulated peaks produce variable quantification results with a GC × GC system [26].

Previously, we reported greater separation of complex PAH mixtures in GC × GC/ToF-MS using a liquid crystal column (LC-50) in the first dimension and a nano-stationary phase column (NSP-35) in the second dimension due to its higher orthogonality than the commonly employed combination (Rtx-5ms × Rxi-17) [27]. The objective of this research was to determine if this novel, high orthogonality column combination (LC-50 × NSP-35), as well as the traditional column combination (Rtx-5ms × Rxi-17), resulted in reliable and reproducible quantification of a complex mixture of 85 different PAHs, including PPAHs, MPAHs, NPAHs, SPAHs, OPAHs, BrPAHs and ClPAHs, in two National Institute of Standards and Technology (NIST) standard reference materials (SRM), with and without cleanup. PAHs were quantified in NIST SRM1650b (diesel particulate matter) with silica gel solid phase extraction (SPE) cleanup and in NIST SRM1975 (diesel particulate extract) with and without silica gel SPE cleanup, using both column combinations and a total run times of 54 and 84 min, respectively. The ratio of the summation of the three most intense modulated peaks for each target PAH to the three most intense modulated peak of its corresponding surrogate perdeuterated PAH was used to overcome the quantification problems in atmospheric extracts ($PM_{2.5}$) described above [22,23]. This research is the first to quantify complex PAH mixtures in NIST SRMs using GC × GC/ToF-MS, with and without extract cleanup, and reports previously unidentified PAH congeners in the NIST SRMs.

2. Materials and methods

2.1. Chemicals and reference materials

The standard reference materials, SRM1975 and SRM1650b, were purchased from NIST (NIST, Gaithersburg, MD, USA) [28]. Standard solutions of 18 PPAHs were purchased from ChemService (West Chester, PA, USA), standard solutions of 9 MPAHs, 18 NPAHs and 2 SPAHs were purchased from AccuStandard (New Haven, CT, USA), and neat standards of 17 OPAHs were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard solutions of 15 ClPAHs and 6 BrPAHs were synthesized by Dr. Takeshi Ohura from the University of Shizuoka in Shizuoka, Japan, using published procedures [20,29,30]. The entire list of PAH analytes can be found in Table S-1. Isotopically labeled PAHs, OPAHs, and NPAHs were purchased from CDN Isotopes (Point-Clare, Quebec, Canada) and Cambridge Isotopes Laboratories (Andover, MA) and included d_6-1,4-naphthaquinone, d_4-1,4-benzoquinone, d_{10}-fluorene, d_7-1-nitronaphthalene, d_{10}-phenanthrene, d_8-anthraquinone, d_9-5-nitroacenaphthene, d_{10}-pyrene, d_9-9-nitroanthracene, d_{12}-triphenylene, d_9-3-nitrofluoranthene, d_9-1-nitropyrene, d_{12}-benzo[a]pyrene, d_{11}-6-nitrochrysene, d_{12}-benzo[ghi]perylene as surrogates and d_{10}-acenaphthene, d_8-9-fluorenone, d_{10}-fluoranthene, d_{12}-benzo[k]fluoranthene, d_9-2-nitrobiphenyl, d_9-2-nitrofluorene as internal standards.

2.2. Sample preparation

Three aliquots of NIST SRM1650b and NIST SRM1975 were spiked with known amounts of labeled PAH, OPAH and NPAH surrogates prior to sample preparation. NIST SRM1650b was extracted using a method based on pressurized liquid extraction (PLE) with dichloromethane (DCM) that has been previously described [31–33]. The resulting NIST SRM1650b extracts and the NIST SRM1975 aliquots were cleaned up using 20 g silica gel columns (Mega BE-SI, Agilent Technologies, New Castle, DE) and eluted in three fractions, with 100% hexane (non-polar fraction), 100% DCM (fraction containing PAHs) and 100% ethyl acetate (polar fraction). The DCM fraction was then concentrated to 300 μL under a gentle stream of N_2 using a Turbovap II (Caliper Life Sciences, MA, USA), solvent exchanged to ethyl acetate and spiked with known amounts of internal standards prior to analysis. An aliquot of NIST SRM1975, without cleanup, was also spiked with surrogates and internal standards prior to analysis.

2.3. GC × GC/ToF-MS quantification

A GC × GC/ToF-MS Pegasus 4D (Leco, St. Joseph, MI, USA) was used for this study. The instrument consisted of an Agilent 6890 gas chromatograph (Palo Alto, CA, USA) with a secondary oven, a split/splitless injector, and a non-moving quad-jet dual stage modulator. The two GC columns in the system were connected using an Agilent CPM union (part no. 188-5361) for 0.1–0.25 mm I.D. columns. Two GC column combinations were used. Column combination "A" was a low-polarity Rtx-5ms column (30 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA) integrated with a 5 m guard column, followed by a mid-polarity Rxi-17 column (1.2 m × 0.10 mm × 0.10 μm) (Restek, Bellefonte, PA, USA). Column combination "B" was a liquid crystal LC-50 column (10 m × 0.15 mm × 0.10 μm) (J&K Scientific, Edwardsville, Nova Scotia, Canada), followed by a nano-stationary phase NSP-35