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Modified ion source triple quadrupole mass spectrometer gas chromatograph for polycyclic aromatic hydrocarbon analyses

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ABSTRACT

We describe modified gas chromatography electron-impact/triple-quadrupole mass spectrometry (GC–EI/MS/MS) utilizing a newly developed hydrogen-injected self-cleaning ion source and modified 9 mm extractor lens. This instrument, with optimized parameters, achieves quantitative separation of 62 polycyclic aromatic hydrocarbons (PAHs). Existing methods historically limited rigorous identification and quantification to a small subset, such as the 16 PAHs the US EPA has defined as priority pollutants. Without the critical source and extractor lens modifications, the off-the-shelf GC–EI/MS/MS system was unsuitable for complex PAH analysis. Separations were enhanced by increased gas flow, a complex GC temperature profile incorporating multiple isothermal periods, specific ramp rates, and a PAH-optimized column. Typical determinations with our refined GC–EI/MS/MS have a large linear range of 1–10,000 pg μl^{-1} and detection limits of <2 pg μl^{-1}. Included in the 62 PAHs, multiple-reaction-monitoring (MRM) mode enabled GC-EI/MS/MS identification and quantitation of several constituents of the MW 302 PAH isomers. Using calibration standards, values determined were within 5% of true values over many months. Standard curve r^2 values were typically >0.998, exceptional for compounds which are archetypally difficult. With this method benzo[a]fluorene, benzo[b]fluorene, benzo[c]fluorene were fully separated as was benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene. Chrysene and triphenylene, were sufficiently separated to allow accurate quantitation. Mean limits of detection (LODs) across all PAHs were 1.02 ± 0.84 pg μl^{-1} with indeno[1,2,3-c,d] pyrene having the lowest LOD at 0.26 pg μl^{-1} and only two analytes above 2.0 pg μl^{-1}; acenaphthalene (2.33 pg μl^{-1}) and dibenzo[a,e]pyrene (6.44 pg μl^{-1}).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a widely distributed, highly-monitored class of contaminants, commonly defined as having two or more single or fused aromatic rings with shared carbon atoms and are typically found in complex mixtures. They originate from a number of sources; namely natural biological processes (biogenic), incomplete combustion (pyrogenic) and collection and utilization of fossil fuels (petrogenic). PAHs are semi-volatile with low vapor pressure and are resistant to chemical reaction, thus tending to accumulate rather than degrade [1,2]. Their lipophilic (hydrophobic) nature results in low aqueous solubility and leads PAHs to bioaccumulate across biological membranes. Atmospheric PAHs exist as either free gas-phase molecules, or associated with airborne particulate matter, with higher MW PAHs more likely to be particulate-bound [3–5].

There are three modes of human exposure to PAHs: (1) direct dermal contact, (2) inhalation and (3) ingestion, with inhalation and ingestion typically the primary pathways. Respiratory PAH burden includes both free and particulate-bound fractions. Hassan et al., demonstrated free gas-phase PAHs to comprise 67% of respiratory PAHs at a study site in Giza, Egypt [6]. Upon inhalation, non-particulate bound PAHs are immediately available to partition across biological membranes. Significant effort has been applied to clarify the association of inhaled PAHs with increased incidence of respiratory syndromes, especially asthma and lung cancer [7,8]. Food preparation, particularly smoking or high-temperature grilling of high-fat content meats, provides an immediate pathway to gastro-intestinal exposure to pyrogenic PAHs including

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benz[a]pyrene (BAP), a long-established carcinogen thought to play a role in cancers of the digestive tract [9–12]. To evaluate PAH exposures in humans, methods monitoring metabolites in urine have been established [13,14].

PAHs, including chemically modified oxy- and nitro-polycyclic aromatic hydrocarbons (OPAHs and NPAHs, respectively) as well as alkylated-PAHs (RPAHs), have been shown to undergo wide geographic dispersal far from originating sources [15] and subsets of PAHs have been demonstrated to be mutagenic and/or carcinogenic [16]. RPAH ratios are signature characteristics used to establish PAH sourcing [17]. In typical petrogenic mixtures, RPAHs are more abundant than their unsubstituted parental counterpart. Accurate source identification requires the ability to identify and quantitate both parental and alkylated products. Toxicological fates of PAHs are areas of active research and monitoring programs are widespread [18,19].

In 2010, the US Environmental Protection Agency (EPA) established a relative potency factor (RPF) approach for assessing both individual PAHs and complex PAH mixtures as part of their integrated risk information system (IRIS) [20]. For RPF determinations, potency was assessed relative to BAP using studies which included both BAP and additional PAHs. RPFs for individual PAHs were calculated relative to a BAP value assigned as 1.0. The EPA determined RPFs for 26 PAHs, including many not in the canonical suite of 16 PAHs the EPA has defined as priority pollutants. Several of these PAHs have RPFs significantly greater than BAP, including benz[l]aceanthrylene (RPF 5), dibenz[a,h]anthracene (RPF 10), benzo[c]fluorene (RPF 20), dibenzo[a,l]pyrene (RPF 30), and benz[j]aceanthrylene (RPF 60). Differential RPFs of these magnitudes accentuate the difficulty in accurate risk assessment when evaluating multipart mixtures, especially when limited in the number and type of identifiable PAHs. That PAHs typically occur in mixtures further emphasizes the need for improved methodology to identify and rigorously quantitate individual PAHs within these complex combinations.

The characteristics of PAHs – persistence, bioaccumulation, and pervasive human exposure with associated health risks – make the ability to monitor a wide range of PAHs of interest to a number of regulatory agencies, including the EPA, the National Oceanic and Atmospheric Administration (NOAA), the World Health Organization (WHO), European Committee for Standardization (CEN), European Food Safety Authority (EFSA), the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA), all of whom maintain active monitoring programs. The intellectual effort expended in developing resources for environmental sampling [21,22], extraction [23] and analysis of PAHs [9] from a variety of foodstuffs and environmental matrices are indications of the breadth of interest in PAH detection and quantification.

Historically, PAHs have been analyzed by high-performance liquid chromatography coupled to an ultraviolet (HPLC/UV), diode-array (HPLC/DAD) or fluorescence detector (HPLC/FLU); or gas chromatography with a flame ionization detector (GC/FID), GC/MS or GC/MS/MS [24–26]. Because PAH isomers have the same chemical formula and mass and share significant underlying structural similarities, MS products from isomers often share ion fragments with identical mass to charge ratios (m/z), thus accurate identification requires chromatographic separation of isomers prior to MS detection and final confirmation with appropriate standards.

High-throughput PAH determinations with “off the shelf” instrumentation, particularly single quadrupole systems, are problematic. Many labs encounter difficulties maintaining robust analysis conditions. After relatively few runs, internal standards (ISTD) will give inconsistent response across the calibration range and the range of external standard (ESTD) linearity diminishes. Marginally performing injectors and columns lead to poor resolution and greater peak broadening, limiting rigorous quantitation of detected analytes and preventing identification of additional PAHs of interest.

Additionally, high boiling points, particularly of large PAHs, lead to a marked tendency toward desublimation and deposition within the instrument, limiting sensitivity of detection, reproducibility of quantification and requiring high temperatures and iterative cycles of injector and instrument cleaning to restore performance. The propensity toward deposition also requires high inlet temperatures and the use of liners with glass wool. Efforts to increase separation, especially with high-molecular weight (HMW) PAHs, defined as having molecular weights greater than 300, include coupling liquid chromatographic (LC) separation with a 60 m GC column (LC–GC–MS), which increases separation but requires greater system gas pressures and extends run times [27–29]. Recently Sakuma et al., generated an application note describing an LC-MS/MS system utilizing a fluorescence detection (LC–FLD–MS/MS) method for PAH and RPAH analysis of a limited number of compounds, including 26 PAHs and 11 RPAH derivatives as well as 11 photo-oxidized PAH products [30]. While linearity with FLD detection was reported as four orders of magnitude, there were no r^2 values presented representing that range and the majority of PAHs analyzed were five-ring and smaller. Several additional published methods provide accurate PAH determinations, but are limited to relatively small subsets of analytes [31,32].

Initial efforts to perform rigorous analysis of PAHs using impact-ionization mass spectrometry (EI/MS) were unsuccessful. Using a standard EI source lacking H_2 injection, instrument performance would rapidly degrade. Attempts at rudimentary 3-point calibrations spanning 2 orders of magnitude were unsuccessful. Reinjection of the same standard gave highly variable results, with inter-day and intra-day variation of 25% or more. In its “off the shelf” configuration, the instrument was unsuitable for complex PAH analysis.

We developed a hydrogen-injected, self-cleaning ion source (SCIS) on the GC-EI/MS/MS system to address many of the difficulties encountered in PAH analysis mentioned above. The SCIS introduces hydrogen directly into the ion source through a specially engineered auxiliary pneumatic control module. We changed the extractor lens from the standard 3 mm to 9 mm. The modified instrument was coupled to a novel PAH-select column, and sensitivity and resolution were further refined through iterative adjustment of method parameters. The study presented here utilizes H_2 injection, a 9 mm extractor lens, a PAH-select column and the refined instrument parameters to demonstrate accurate quantitative identification of a suite of 62 PAHs ranging from the two-ring structure of naphthalene with a molecular weight (MW) of 128.17 and a boiling point (BP) of 218 °C, to PAHs in the 302 group with MWs of 302.17 and BPs up to 595 °C (dibenzo[a,l]pyrene). Also included in the suite of 62 are 20 accurately quantified RPAHs, providing the requisite analytical tools for precise PAH sourcing determinations. Additional PAHs within the 302 group were identifiable and application of the method enabled identification of several PAHs not previously identified or quantified from complex mixes, including high relative RPF compounds typically not quantifiable on standard 30 m columns. Only through these modifications were we able to quantify these 62 PAHs with excellent sensitivity and precision.

2. Materials and methods

2.1. GC/MS/MS

The GC/MS/MS instrument was an Agilent 7000B GC/MS/MS. Modifications were made to the instrument to improve the analytical performance in PAH analysis, see Fig. 1. The source was