Journal of Chromatography A, 1459 (2016) 89–100

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of polycyclic aromatic hydrocarbon quinones by gas chromatography-tandem mass spectrometry, following a one-pot reductive trimethylsilyl derivatization

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ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form 9 June 2016
Accepted 11 June 2016
Available online 18 June 2016

Keywords:
Polycyclic aromatic hydrocarbon quinones
GC–MS/MS
Trimethylsilyl derivatization
Atmospheric pollution

ABSTRACT

We developed a sensitive and selective method to simultaneously analyze 37 polycyclic aromatic hydrocarbon quinones (PAHQs) with GC–MS/MS and applied the method to the analysis of standard atmospheric particulate matter samples. PAHQs were reduced with zinc granules and dithiothreitol (DTT) and the reductants were immediately converted to their silylated derivatives in a test tube. Two trimethylsilyl (TMS) groups were introduced into PAHQs through the one-pot reductive TMS derivatization. The PAHQs were derivatized with a mixed silylation reagent (BSA + TMCS + TMSI; (3:2:3)), which is one of the combinations of TMS-derivatization reagents with the highest reactivity. The derivatives produced different fragmentation between *o*-PAHQs and *p*-PAHQs. Therefore, isomers that have the same molecular weight are difficult to separate on a column were separated by the selected reaction monitoring (SRM) mode using the characteristic fragmentations, allowing separation and detection of all PAHQ derivatives in less than 30 min. The instrumental detection limit (IDL) of each PAHQ was 1.2–29 fg/injection and the method quantification limit (MQL) was 0.8–78 μg/kg sample. For quantification, six deuterated PAHQs were used as internal standards to achieve high analytical precision. We applied the developed method to four standard atmospheric particulate matter samples. Results showed that out of 37 PAHQs, 33 compounds were identified and quantified. Moreover, from the 33 PAHQs, 14 were detected for the first time. Similar values were observed for the concentrations of PAHQs that have been quantified in previous reports. This method has the highest practicality in monitoring PAHQs in atmosphere, combustion exhaust gas, and toxicity evaluation. Thus, the method has the potential to become a standard analytical method for such applications.

1. Introduction

Airborne particulate matter (PM), generated from combustion sources such as vehicle emissions, exhaust from factories, and cigarettes, is an important pollutant and is suspected to cause respiratory diseases, allergic diseases, cardiovascular diseases, and cancer due to oxidative stresses [1–6]. Oxidative stress is caused when the balance between oxidation and antioxidation reactions in a body leans toward the former because of overproduction of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide, and hydroxyl radicals, or a deficit in antioxidant capacity [1,7]. Normally, ROS is scavenged by antioxidants such as glutathione or antioxidative enzymes such as superoxide dismutase (SOD). However, excessive ROS oxidizes and degenerates nucleic acid, proteins, and lipids, which inhibits cell functions and causes cancer, respiratory diseases, and cardiovascular diseases [2,3,7,8]. In *in vitro* assays that exposed cells to PM samples from the atmosphere and diesel exhaust, a relationship between the exposure to PM and oxidative stresses on cells has been observed [9–12]. Therefore, it is necessary to determine the causative agents that contribute to oxidative stresses induced by PM.

As a typical organic substance included in PM, polycyclic aromatic hydrocarbons (PAHs) and their derivatives have been reported [13–19]. Polycyclic aromatic hydrocarbon quinones (PAHQs), which are a type of oxygenated PAHs, are generated by incomplete combustion in the same manner as PAHs and sub-

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http://dx.doi.org/10.1016/j.chroma.2016.06.034
0021-9673/

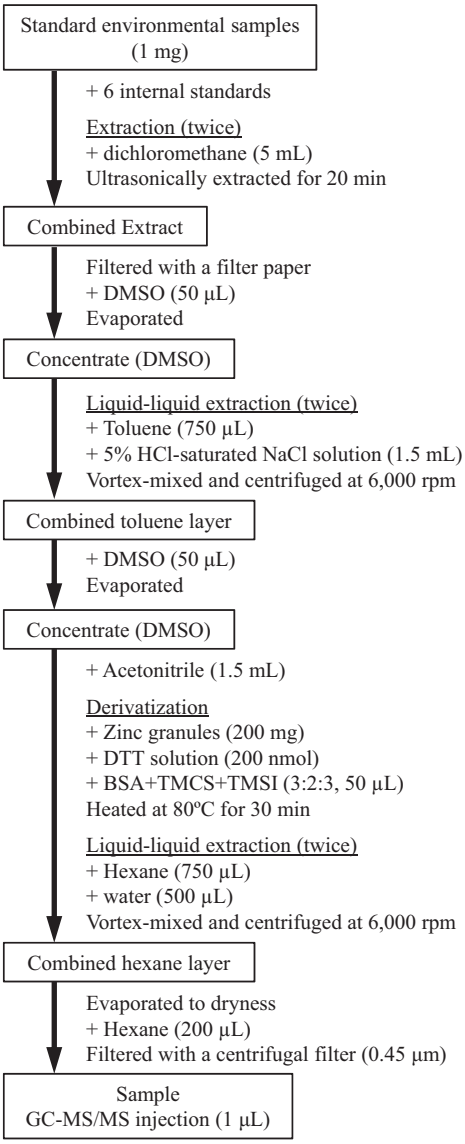

Fig. 1. Flowchart of the pretreatment procedure.

sequently released to the atmosphere [16–21]. There have been reports on PAHQ concentrations in airborne PM and gas phases [20,21]. Although diesel particulate matter is considered as a specific candidate of the primary source of PAHQs [22,23], secondary formation from reactions of PAHs with ozone and radical species in the atmosphere have also been reported [24–26]. However, primary combustion source and secondary formation by atmospheric reactions are not clearly understood.

PAHQs have been reported to overproduce ROS through a redox cycle [27–29]. Unstable semiquinone radicals are produced by enzymatic reduction reactions of PAHQs and subsequently generates $O_2^{\bullet -}$ under the presence of molecular oxygen when being re-oxidized to quinone. In the reaction process, PAHQs function as a catalyst, causing overproduction of ROS. The ROS production has the potential to induce oxidative stresses. Therefore, PAHQs are suspected to be one of the causative agents of diseases associated with PM exposure. To evaluate the health impacts of PAHQ exposure, atmospheric environment monitoring of PAHQ concentrations is necessary. In recent years, a metabolite of PAHQ with the ability to produce ROS, such as 9,10-phenanthrenequinone (9,10-PQ), has been identified in human urine, directly confirming PAHQ exposure through inhalation [30].

PAHQs have many isomers with different parent PAH structures and carbonyl group positions. Although each physiochemical property is similar, ROS productivity and cytotoxicity are different [31]. In the *in vitro* study that evaluated cytotoxicity and ROS production using human A549 cells, *o*-PAHQs overproduced ROS and the cell viability decreased significantly, while *p*-PAHQ did not produce ROS and showed little cytotoxicity. Because PAHQs have completely different toxicity depending on the structure, it is important to achieve complete separation and detection of PAHQ isomers that have similar physiochemical properties in environmental monitoring.

In the PAHQ analysis methods developed so far, liquid chromatography with mass spectrometry (LC–MS) [32–34], liquid chromatography with tandem mass spectrometry (LC–MS/MS) [35–37], gas chromatography with mass spectrometry (GC–MS) [21,22,34,38–41], LC-GC–MS [42] and high performance liquid chromatography (HPLC) [43,44] have been utilized. With the LC–MS (/MS) methods, PAHQs are typically directly ionized with the APCI mode, which is well suited to ionization of small uncharged aromatic molecules [34]. One of the important advantages of LC–MS (/MS) methods is that it does not need derivatization steps. However, the chromatographic resolution of LC is poorer than that of GC. With the GC–MS methods, PAHQs are mainly analyzed directly or through acetyl derivatization. Because of their high polarity, low vapor pressure and the possibility of thermal decomposition [20], derivatization is necessary for higher sensitivity [21,22]. Despite the difficulties, the GC–MS methods preferred over the single LC–MS for precise identification and quantification [34].

The developed analytical methods, especially GC–MS methods, have been applied to atmospheric samples collected in multiple cities and samples derived from combustion sources such as diesel particulate matters. However, analytical targets are limited, and PAHQs that are of interest in verifying environmental dynamics and secondary production and *o*-PAHQs with high ROS productivity are poorly understood. In addition, several of these methods have insufficient sensitivity to detect many of the PAHQs in environmental samples [15]. Based on previous reports [14,16,17,19,21] to analyze PAHQs in atmosphere that is 0.1 pg/m^3 or higher with a routine analysis, high-sensitivity detection with a method quantification limit (MQL) of approximately 500 fg/injection must be achieved. A large number of PAHQ isomers should be targeted for the analysis and a high-sensitive/high-selective analytical method that can analyze all the targets simultaneously is required to evaluate environmental exposure of PAHQs more accurately.

When analyzing many PAHQ isomers with the same molecular weight, the isomers must be sufficiently separated by chromatography and it is more preferable to separate by GC as it has a higher number of theoretical plates compared to LC. On the other hand, as mentioned above, derivatization is necessary for better separation and higher sensitivity in GC analysis. Typically, analysis of PAHQs in selected ion monitoring (SIM) mode does not provide adequate sensitivity and specificity to reliably quantify many of the PAHQs in environmental samples. Therefore, analysis with higher precision using tandem-mass spectrometry is required. However, previously-reported acetyl derivatives easily yield fragmentation of the molecules with EI mode [21,22] and it may be difficult to obtain their molecular ions ([M]$^+$) to develop effective selected reaction monitoring (SRM) transitions, and their high-sensitivity detection seems difficult even with a tandem-mass spectrometry. In general, TMS derivatization tends to produce more product ions based on its multiple fragmentation patterns and is capable of high sensitive and selective detection. In this study, we established the one-pot reductive derivatization method that is capable of trimethylsilyl-derivatizing PAHQs while converting them to their reductants in a single container by zinc granules that can be