



Rapid determination of 9 aromatic amines in mainstream cigarette smoke by modified dispersive liquid liquid microextraction and ultraperformance convergence chromatography tandem mass spectrometry



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ABSTRACT

Aromatic amines in mainstream cigarette smoke have long been monitored due to their carcinogenic toxicity. In this work, a reliable and rapid method was developed for the simultaneous determination of 9 aromatic amines in mainstream cigarette smoke by modified dispersive liquid liquid microextraction (DLLME) and ultraperformance convergence chromatography tandem mass spectrometry (UPC²-MS/MS). Briefly, the particulate phase of the cigarette smoke was captured by a Cambridge filter pad, and diluted hydrogen chloride aqueous solution is employed to extract the aromatic amines under mechanical shaking. After alkalization with sodium hydroxide solution, small amount of toluene was introduced to further extract and enrich aromatic amines by modified DLLME under vortexing. After centrifugation, toluene phase was purified by a universal QuEChERS cleanup kit and was finally analyzed by UPC²-MS/MS. Attributing to the superior performance of UPC²-MS/MS, this novel approach allowed the separation and determination of 9 aromatic amines within 5.0 min with satisfactory resolution and sensitivity. The proposed method was finally validated using Kentucky reference cigarette 3R4F, and emission levels of targeted aromatic amines determined were comparable to previously reported methods. At three different spiked levels, the recoveries of most analytes were ranged from 74.01% to 120.50% with relative standard deviation (RSD) less than 12%, except that the recovery of *p*-toluidine at low spiked level and 3-aminobiphenyl at medium spiked level was 62.77% and 69.37% respectively. Thus, this work provides a novel alternative method for the simultaneous analysis of 9 aromatic amines in mainstream cigarette smoke.

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1. Introduction

Cigarette smoking continues to be a major health hazard worldwide. According to Rodgman and Perfetti [1], over 6000 substances had been identified in cigarette smoke as of 2012, and carcinogenic compounds in cigarette smoke are thought to be responsible for cancers caused by cigarette smoking. Mainstream cigarette smoke is defined as the cigarette smoke that is drawn through the tobacco into an active smoker's mouth [2]. Of all known various classes of constituents, aromatic amines are

found to be present in mainstream cigarette smoke [3–6], such as 2,6-dimethylaniline (2,6-DMA), *o*-toluidine (*o*-ToL), *o*-anisidine (*o*-ASD), *m*-toluidine (*m*-ToL), *p*-toluidine (*p*-ToL), 1-naphthylamine (1-NA), 2-naphthylamine (2-NA), 3-aminobiphenyl (3-ABP), and 4-aminobiphenyl (4-ABP). Studies have revealed that many aromatic amines can form adducts with protein and DNA, and they have been implicated as or possible as human bladder carcinogens [7–10]. *o*-ToL, 2-NA and 4-ABP are classified as group I carcinogens by the International Agency for Research on Cancer (IARC) [11], 1-NA is classified as group 3 carcinogen [12], 2,6-DMA and *o*-ASD are classified as group 2B carcinogens [13,14]. Moreover, as the tobacco controls gathering pace worldwide, reporting and disclosure the emission levels of cigarette smoke constituents has been required by regulatory bodies and legislations. In 2012, U.S. Food and Drug

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Administration established a list of harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke [15], 2,6-DMA, *o*-ToL, *o*-ASD, 1-NA, 2-NA and 4-ABP have been included in it.

Chromatographic technique has played an important role in the analysis of aromatic amines relating to tobacco research studies, including gas chromatography electron capture detection (GC-ECD) [3,16], gas chromatography-mass spectrometry (GC-MS) [4,5], gas chromatography tandem mass spectrometry (GC-MS/MS) [17], and liquid chromatography tandem mass spectrometry (LC-MS/MS) [6,18–20]. Although these methods gave acceptable results, there were several shortcomings for individual technique. GC methods suffered from low sensitivity, GC-MS or GC-MS/MS methods usually required derivatization procedures due to the high polarity and thermal instability of aromatic amines. LC-MS/MS methods were more suitable for the analysis of polar compounds and required less intensive pretreatment, but the resolution of aromatic amine isomers were less than satisfactory. For example, 3-ABP and 4-ABP could not be separated at all in the work reported by Schubert et al. [18], *m*-ToL and *o*-ToL did not reach baseline separation in the work reported by Xie et al. [19]. Much more recently, Zhang et al. [20] reported a two-dimensional online solid-phase extraction combined with LC-MS/MS method (2D SPE-LC-MS/MS), by which the isomers of *o*, *m*, *p*-ToL, 1, 2-NA, 3, 4-ABP were separated with good resolutions. Nevertheless, the total elution program of the 2D SPE-LC-MS/MS method was 30 min. The development of novel methods with faster, simpler sample preparations and excellent results are highly required with regard to these challenges.

Ultrapformance convergence chromatography (UPC²) system is a recent modification of supercritical fluid chromatography introduced by Waters Corporation in 2012. The use of supercritical CO₂ as the primary mobile phase could offer several advantages over liquid mobile phases or carrier gases that are used with traditional LC and GC [21,22]. On the one hand, the low viscosity of CO₂ allows higher flow rates with a lower pressure drop across the column and achieves faster run times. On the other hand, the higher diffusivity of analytes dissolved in supercritical CO₂ could achieve high-efficiency separation due to the improved mass-transfer kinetics. Moreover, unlike LC system which often consumes lot of organic solvents as the mobile phase, UPC² system dramatically reduces the uses of organic solvents, only small amount of co-solvent is consumed, which meet the requirement of green chemistry. As an alternative chromatographic approach, UPC² system coupled with photodiode array detector and MS detector have shown attractive applications in the analysis of structural analogs [23,24], isomers [25], and enantiomeric and diastereomeric mixtures [26,27].

Dispersive liquid-liquid microextraction (DLLME) is a simple, rapid, low cost, and effective preconcentration sample preparation technique first reported by Rezaee et al. in 2006 [28]. Attributing to its superior performance, both classic DLLME and latterly improved DLLME have been widely used in the determination of various pollutants in environmental water samples, such as pesticides [29,30], polycyclic aromatic hydrocarbons [31], metals ions [32], aromatic amines [33,34], etc. Conventional extraction of aromatic amines in mainstream cigarette smoke with acidic water solution motivated us to expand the application of DLLME. To date, no work has been published by applying DLLME in the determination of the above mentioned 9 aromatic amines, not to mention in mainstream cigarette smoke samples.

The aim of present study is to analyze 9 aromatic amines in mainstream smoke by a modified DLLME and UPC²-MS/MS approach. With regard to the modified DLLME sample preparation procedure, in order to avoid the decreased partition efficiencies of analytes into the extraction solvent, vortex-assisted dispersion is employed to replace the use of dispersive solvents and a low density extraction solvent is employed. In addition, considering the

complexity of mainstream smoke, the volume of extraction solvent is scaled up to ease further purification. The use of UPC²-MS/MS detection provides an environmentally friendly approach which allows the fast and efficient separation of 9 aromatic amine analogs within few minutes. To the best of our knowledge, it is the first report on the simultaneous determination of 9 aromatic amines in mainstream smoke by DLLME and UPC²-MS/MS.

2. Experimental

2.1. Reagents and materials

2,6-Dimethylaniline, *o*-toluidine, *o*-anisidine, *m*-toluidine, *p*-toluidine, 1-naphthylamine, 2-naphthylamine, 3-aminobiphenyl, and 4-aminobiphenyl were obtained from Sigma-Aldrich (St. Louis, MO, USA) with a minimum of 98% purity. 2,6-dimethylaniline-D₉, *o*-toluidine-D₉, *o*-anisidine-D₇, 1-naphthylamine-D₇, 2-naphthylamine-D₇, 4-aminobiphenyl-D₉ were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions of aromatic amines were prepared at 1.0 mg/mL in methanol and stored in the dark at -20 °C before use. HPLC grade toluene, *n*-hexane, cyclohexane, acetonitrile, methanol, isopropanol were purchased from Fisher Scientific (Pittsburg, PA, USA). Analytical grade of sodium hydroxide, sodium chloride, sodium sulfate were obtained from Tianjin yongda chemical reagent company (Tianjin, China). Formic acid and 37% hydrochloric acid were obtained from Merck KGaA (Darmstadt, Germany). Ultrapure water was produced from a Milli-Q ultrapure water system from Millipore (Millipore, Bedford, MA, USA). Dispersive SPE clean up tube containing 150 mg anhydrous MgSO₄, 50 mg PSA, 50 mg C18, 7.5 mg GCB was purchased from Agilent (Agilent Technologies, Santa Clara, CA, USA). 3R4F Kentucky reference cigarettes were purchased from the Tobacco and Health Research Institute at the University of Kentucky.

2.2. Sample preparations

Before smoking, cigarettes were conditioned at (22 ± 1) °C and (60 ± 2)% humidity for 48 h. Mainstream cigarette smoke was generated on an Orbit 20 rotary smoking machine (Cerulean, UK) under ISO smoking regimen (35 mL puff volume, 2 s puff duration, 60 s puff interval, no ventilation block) [35] and intense smoking regimen (55 mL puff volume, 2 s puff duration, 30 s puff interval, 100% ventilation block) [36]. The particulate phase of mainstream cigarette smoke was captured on a Cambridge filter pad. 10 cigarettes were smoked. After smoking, the Cambridge filter pad was transferred in a 100 mL screw-capped plastic bottle, then 60 mL 5% hydrochloric acid aqueous solution and 50 μL internal standard solution was added subsequently. Aromatic amines were extracted under mechanical shaking at 300 rpm for 40 min. After that the aqueous extract (approximately 50 mL) was transferred to another 100 mL screw-capped plastic bottle, 4.0 mol/L sodium hydroxide was added to adjust pH to 13.

Aromatic amines in the alkalinized aqueous solution (approximately 75 mL) were further extracted by a modified DLLME. Briefly, 2 mL organic solvent with lower density than water was added into the plastic bottle to extract the free aromatic amines, and 5 g salt was added to facilitate the partition of aromatic amines in the organic solvent. Then the mixture was vortexed for 2 min to assist the dispersion of the organic solvent. The mixture was divided and transferred into two 50 mL PTFE centrifuge tubes. After centrifugation at 10000 rpm for 3 min, the upper organic phase (approximately 1.5 mL) was transferred to a 2 mL dispersive SPE clean up tube (150 mg anhydrous MgSO₄, 50 mg PSA, 50 mg C18,

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