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Determination of trace level genotoxic impurities in small molecule drug substances using conventional headspace gas chromatography with contemporary ionic liquid diluents and electron capture detection

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ABSTRACT

Ionic liquids (ILs) were used as a new class of diluents for the analysis of two classes of genotoxic impurities (GTIs), namely, alkyl/aryl halides and nitro-aromatics, in small molecule drug substances by headspace gas chromatography (HS-GC) coupled with electron capture detection (ECD). This novel approach using ILs as contemporary diluents greatly broadens the applicability of HS-GC for the determination of high boiling (≥130 °C) analytes including GTIs with limits of detection (LOD) ranging from 5 to 500 parts-per-billion (ppb) of analytes in a drug substance. This represents up to tens of thousands-fold improvement compared to traditional HS-GC diluents such as dimethyl sulfoxide (DMSO) and dimethylacetamide (DMAC). Various ILs were screened to determine their suitability as diluents for the HS-GC/ECD analysis. Increasing the HS oven temperatures resulted in varying responses for alkyl/aryl halides and a significant increase in response for all nitroaromatic GTIs. Linear ranges of up to five orders of magnitude were found for a number of analytes. The technique was validated on two active pharmaceutical ingredients with excellent recovery. This simple and robust methodology offers a key advantage in the ease of method transfer from development laboratories to quality control environments since conventional validated chromatographic data systems and GC instruments can be used. For many analytes, it is a cost effective alternative to more complex trace analytical methodologies like LC/MS and GC/MS, and significantly reduces the training needed for operation.

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1. Introduction

Genotoxic impurities (GTIs) are compounds that can induce genetic mutations, chromosomal breaks, and/or chromosomal rearrangements in humans [1]. In addition, these compounds can also exhibit carcinogenic activity [2]. The United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have imposed very strict regulations on the levels of GTIs in pharmaceuticals [3,4]. Depending on the dose and duration of exposure, the allowable daily intake (ADI) can be as low as 1.5 μg/day [5], which in perspective, would be translated to low parts-per-million (ppm) or sub-ppm concentration ranges of GTIs in drug substances, about 1000 times lower than typical thresholds on non-genotoxic impurities [6–8]. This highly conservative threshold also applies to pharmaceutical impurities containing structurally alerting functional groups that may possess genotoxic activity, namely potential genotoxic impurities (PGIs) [2]. Although GTIs that enter human body may come from drug substances, excipients, degradants, or metabolites, the major source of GTIs is usually active pharmaceutical ingredient (API) manufacturing, which may require the use of genotoxic reagents, solvents, and catalysts [9–11]. Thus, monitoring the presence of various GTIs in drug substances is of great importance.

The trace-level analysis of GTIs in drug substances is often a great analytical challenge for the pharmaceutical industry [8,12]. Many traditional approaches, such as HPLC–UV and GC-FID, are usually not effective for impurity analysis at sub-ppm or lower level. In recent years, a number of orthogonal analytical methods

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that employ various sample preparation, separation, and detection technologies for the determination of GTIs in different matrixes have been developed [12–14]. Chemical derivatization has been employed for enhancing sensitivity [12,15–20]. Matrix deactivation and exploitation of coordinating metal ions for electrospray ionization mass spectrometry (ESI-MS) have allowed for superior analyte response by minimizing the chemical reactivity of the matrix and increasing the overall ionization efficiency of the detector [21,22]. Moreover, the application of sorbent-type extraction techniques, such as solid-phase microextraction, has resulted in ultra-trace level quantification of GTIs in the parts-per-billion (ppb) and even parts-per-trillion (ppt) ranges [23].

Static headspace sampling (SHS) is a common technique for various gas chromatographic analyses in pharmaceutical industry. This approach minimizes the amount of non-volatile matrix components that can be introduced to the gas chromatographic system by sampling only the gaseous components in a sample vial. As a result, low chromatographic background interference and high method sensitivity can be achieved due to a greatly reduced matrix effect [24]. In SHS-GC, the partitioning of volatile analytes between the sample vial headspace and the sample matrix reaches equilibrium. Thus, optimization of the HS oven temperature can result in a dramatic increase in the amount of analytes partitioning in the headspace, thereby leading to high sensitivity.

Recently, a number of studies on the analysis of volatile GTIs/PGIs by SHS-GC have been reported, such as the determination of alkylating agent derivatives by HS-GC/MS [25], the analysis of volatile alkyl/aryl halides using either HS-GC/ECD or HS-GC/MS [26,27], the quantification of hydrazine derivatives by HS-GC/MS [28], and the analysis of a free-radical reagent 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) by HS-GC/MS [29]. Although these studies have successfully applied HS-GC for the analysis of various GTIs and/or PGIs, the chosen analytes or their chemical derivatives were sufficiently volatile at or below 100 °C. To the best of our knowledge, the quantification of GTIs possessing high boiling points above 100 °C by HS-GC has not been reported, probably because the organic diluents used in HS-GC analyses are usually not applicable at the high headspace oven temperatures which are needed to sufficiently volatilize these GTIs. The commonly used high-boiling organic diluents such as dimethyl sulfoxide (DMSO), N,N-dimethylacetamide (DMAC), or N,N-dimethyl formamide (DMF) can significantly contribute to chromatographic background at elevated headspace oven temperatures, leading to instrumentation contamination and carryover issues, while also affecting the overall method sensitivity. Moreover, these solvents can build up substantial internal pressure when reaching their boiling points and breach the sample vial septa or even rupture the vial itself and cause safety issues [30].

Within the last two decades, studies on ionic liquids (ILs) in analytical chemistry have increased exponentially [31]. By definition, ILs are salts which exhibit melting points at or below 100 °C. These compounds are typically composed of organic cations and a variety of anions. Compared to traditional organic solvents, ILs exhibit excellent unique physicochemical characteristics such as high thermal stability, negligible vapor pressure, varying viscosity, non-flammability, and tunable solvation properties. All these combined features make ILs highly attractive for many applications in analytical chemistry [32]. Due to their high thermal stability and low volatility, ILs has been previously applied as diluents in SHS-GC for the analysis of high-boiling residual organic solvents [33–35]. The application of ILs as diluents enables high headspace oven operating temperatures while contributing to low chromatographic background and excellent method sensitivities. Therefore, it is necessary to investigate the application of ILs as contemporary diluents for the determination of high-boiling GTIs by SHS-GC.

We report, for the first time, the application of ILs as modern diluents in the SHS-GC/ECD analysis of two classes of high-boiling GTIs, namely, alkyl/aryl halides and nitroaromatics. A number of ILs with different combinations of cations and anions were screened to determine their suitability as diluents for the analysis of these selected GTIs by HS-GC/ECD. The effects of the HS oven temperature on the analyte response, analytical performance comparison for different IL diluents, and method validation are discussed in detail.

2. Experimental

2.1. Chemicals

The sample information for all GTIs and IL diluents used in this study is listed in Table 1. The following chemicals were purchased from Sigma–Aldrich (MO, USA): 1-bromo-3-chloropropane (99%), benzyl chloride (99%), 2,4-dimethylnitrobenzene (98%), 2-nitrobenzaldehyde (98%), 4-nitrobenzaldehyde (98%), 1-nitronaphthalene (99%), 2-nitrofluorene (98%), 1-butyl-3-methylimidazolium tetrafluoroborate (≥98%), 1-butyl-2,3-dimethylimidazolium tetrafluoroborate (≥97%), trihexyltetradecylphosphonium bis[(trifluoromethyl)sulfonyl]imide (≥95%), 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide (≥98%), Warfarin (≥98%), and dimethyl sulfoxide (DMSO, Grade puriss. p.a., ≥99.9% by GC). The following chemicals were purchased from Acros Organics (NJ, USA): benzotrichloride (98%), 1-chloro-2-nitrobenzene (>99%), 1,6-dibromohexane (98%), 2-chloro-5-nitroaniline (99%). Benzyl bromide (>98%) and 1-butyl-2,3-dimethylimidazolium bis[(trifluoromethyl)sulfonyl]imide (>98%) were purchased from TCI (Tokyo, Japan). Iodobutane (99%) and *n*-propyl bromoacetate (97%) were purchased from Alfa Aesar (Heysham, England). The chemicals 1-(2-chloro-5-nitro-phenyl)ethanone (97%) and 1-butyl-1-methylpyrrolidinium tetracyanoborate (>99%) were purchased from Oakwood Chemical (SC, USA) and EMD Chemicals (NJ, USA), respectively. Deionized water was obtained by using a Milli-Q water purification system (Millipore, MA, USA) and API Compound 1 was a Genentech internal developmental compound.

2.2. Sample preparation

The GTIs were grouped into two sets, namely, alkyl/aryl halides and nitroaromatics, as shown in Table 1. Standard solutions were prepared by dissolving 10 mg g^{-1} of either analyte set in a selected IL. From this standard solution, a series of additional standards were prepared by diluting with the same IL. Working standards were prepared by spiking a certain volume of a standard solution to a 10 mL headspace vial and using the selected IL to further dilute the sample until a total volume of 500 μL is achieved. The same sample preparation method was performed for relative recovery studies; however, 50 mg (10%, w/v) of a drug substance was also added to the solution.

2.3. Instrumentation and chromatographic conditions

Chromatographic separations were achieved on VF-624ms capillary column (30 m × 0.32 mm I.D. × 1.8 μm film thickness, Agilent/Varian Inc., Santa Clara, CA, USA) in an Agilent 7890 GC/ECD with an Agilent G1888 headspace sampler oven (Santa Clara, CA, USA). The HS oven was operated at an optimal sampling temperature, which was determined through optimization. The sample loop and transfer line was operated at 230 °C and 240 °C, respectively. The equilibration time was 10.0 min. The GC injector was maintained at 250 °C with 1:1 split ratio. The high temperatures of

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