Journal of Chromatography A, 1217 (2010) 2726–2735

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review

Novel techniques for enhancing sensitivity in static headspace extraction-gas chromatography

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ARTICLE INFO

Article history:
Available online 11 January 2010

Keywords:
Headspace
Gas chromatography
Derivatization
Ionic liquids

ABSTRACT

Static headspace extraction-gas chromatography (SHE-GC) is one of the most commonly used techniques for the analysis of volatile compounds. It is considered by most to be a mature technique and to an extent this is true: there are many users from outside the traditional chromatography research community developing and publishing SHE-GC methods and there are numerous instruments and devices for SHE-GC commercially available. However, research on new SHE-GC methods continues. In this review, several interesting new developments in SHE-GC are described using examples from the past three years' literature. First, the fundamental theory of SHE-GC is reviewed to provide a basis and common theme for the discussion of new methods. Next, several areas of SHE-GC research are explored: new sampling configurations, analyte derivatization and ionic liquids as solvents. These are all means for enhancing partitioning of the analyte into the vapor phase, thus improving analytical sensitivity of the overall SHE-GC method. Ideally, partitioning of analytes into the vapor phase is increased while partitioning of matrix components is not, or is decreased. There are many aspects of the seemingly straightforward process in SHE-GC that require further fundamental research to extend the application range of SHE-GC and to make method development more systematic.

Contents

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doi:10.1016/j.chroma.2010.01.005

1. Introduction

Static headspace extraction (SHE) is one of the most important sample preparation techniques for gas chromatography. Ease of sample preparation, automation and instrument sensitivity make static headspace extraction the technique of choice for the analysis of volatile compounds in a wide range of concentrations and sample types, from part-per million and lower to percentages and from many liquids and solids. Since development of the first automated systems nearly 30 years ago and the development of theory and fundamentals by analytical chemists, static headspace extraction is now routinely used by scientists in a wide range of disciplines. While static headspace extraction generally offers straightforward initial sample preparation, users who analyze complex samples often notice differences in instrument response, depending on the sample matrix. In this article, fundamentals of static headspace extraction are reviewed with an eye toward explaining the reasons behind this variable sensitivity, followed by discussion of new techniques and examples from the recent literature, including instrumental improvements, derivatization and ionic liquids, that apply these fundamentals to enhancing sensitivity in headspace extractions.

2. Theory of static headspace extraction

The theory of static headspace extraction is thoroughly described in three texts, by Kolb and Ettre [1], Ioffe and Vitenberg [2] and Hachenberg and Schmidt [3]. It is summarized below, with conclusions that provide a basis for discussion of new techniques. In SHE-GC extraction, the vapor phase directly above and in contact with a liquid or solid sample in a sealed container is sampled and an aliquot is transferred to a gas chromatograph for separation on a column, detection and quantitation. A schematic of the equilibrium that must be generated is shown in Fig. 1 and is described using the simple equilibrium constant expression shown in Eqs. (1) and (2), describing partitioning between vapor and solution phases.

$$\mathrm{A(g)} \leftrightarrow \mathrm{A(sol'n)} \tag{1}$$

$$K = \frac{[\mathrm{A(sol'n)}]}{[\mathrm{A(g)}]} \tag{2}$$

The variable A refers to an analyte and K refers to the concentration equilibrium constant for that analyte partitioning between the solution and vapor phases. Note that in keeping with the convention described in all three texts, this equilibrium expression is written with the gas phase as reactant and the sample phase as product; in the strictest sense, since the analyte is in the sample phase when initially placed in the vial, this equation should be reversed. Throughout this article, the traditional notation with the vapor phase as reactant is maintained. In any event, it is assumed in nearly all static headspace extraction experiments that equilibrium is reached within the container prior to and during removal of the aliquot of the vapor phase for analysis. *The partition coefficient and the related drive to equilibrium, therefore are critical in controlling sensitivity, reproducibility and instrument response in static headspace extraction-gas chromatography.*

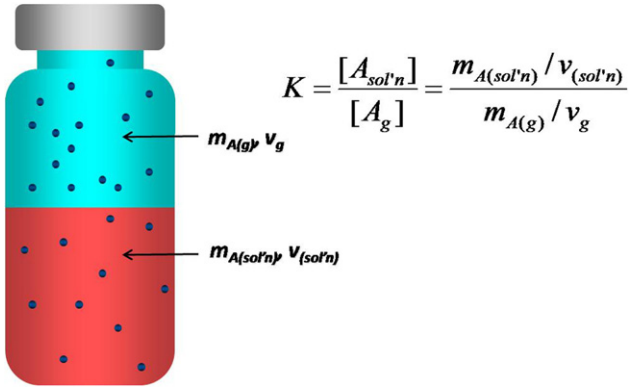

Fig. 1. Diagram of a vial for SHE. This illustrates the key variables of the volumes of the sample phase and headspace phase and the equilibrium constant of the phase transfer between the two phases.

2.1. Fundamental relationships

The ability to determine the amount of a substance within a liquid or solid sample by analyzing the headspace vapor above it in a closed vessel derives from three critical fundamental principles:

1. Dalton's Law of Partial Pressure states that the total pressure of a mixture of gases (the headspace found in every vial), is the sum of the partial pressures of each component.

$$p_{\mathrm{total}} = \sum_{i=1}^{n} p_i \tag{3}$$

The total pressure inside the vial is given as p_{total} and p_i refers to the partial pressure of the ith component in the mixture. In combination with the ideal gas law, this also states that the number of moles of each gas in the mixture is similarly additive, permitting accurate quantitative analysis of gas mixtures.

2. The partial pressure of a gas is related to the amount of volatile solute in a solution beneath the headspace using Raoult's Law, which states that the vapor pressure of a dissolved solute (p_i) is proportional to the mole fraction of the solute in the solution (x_i). For an ideal solution, the proportionality constant (k) is the vapor pressure of the pure solute at the given temperature (p_i^{o}).

$$p_i = kx_i \quad \text{where } k = p_i^{\mathrm{o}} \tag{4}$$

In most SHE-GC methods, analytes are in solution, and most SHE-GC authors carefully justify choices of solvents. The need to dissolve analytes and matrix components to form an ideal or nearly ideal solution is the driver for much SHE-GC research, including the development of ionic liquids as solvents, presented in this article. Further, Raoult's Law demonstrates the critical importance of temperature in the vial, which determines the vapor pressure of both analytes and matrix components. Generating differences in vapor pressure is the main goal of vial temperature adjustment in SHE-GC.

3. For ideal dilute solutions, Raoult's Law is often expressed in the form of Henry's Law which relates the partial vapor pressure of a solute in dilute solution (p_i) to its mole fraction in the solution (x_i).

$$p_i = Hx_i \tag{5}$$

H, the Henry's Law constant is often determined and quoted in method development and as evidence that the solution is ideal. Deviations from Henry's law are generally seen as evidence that the solution is not ideal. Non-ideal solution behavior is one of the main causes of deviations from linear calibration behavior in SHE-GC and of disagreement between responses for standards and samples.

2.2. Non-ideal analyte solutions

Raoult's Law, and by extension Henry's Law, apply only to ideal solutions. For non-ideal solutions, which are present in many "real" samples, it must be modified by considering activity, so an activity

coefficient is added to Eq. (4).

$$p_i = p_i^o \gamma_i x_i \tag{6}$$

The activity coefficient, γ, modifies the mole fraction, expressing the actual availability of the analyte to vaporize, which may be quite different than its mole fraction in the sample. In many cases of complex sample matrices, γ is less than one in the initial sample, thus hindering vaporization. One driver behind solvent choices is therefore, to increase γ. In the best case, the solvent would strongly attract matrix components, decreasing γ for them, while repelling analytes, increasing γ for them. This is one reason that SHE of many organic compounds from aqueous solutions is often extremely effective.

When Eqs. (3)–(5) are combined with the equilibrium expression described in Fig. 1 and Eqs. (1) and (2), the most commonly used expression for relating the concentration of analyte in a liquid sample within the vial to the instrument response in static headspace gas chromatography is obtained. This is shown in Eq. (7), modified from the form presented by Kolb and Ettre with the addition of a response factor term that accounts for the instrumental contribution to the response.

$$\text{Area} = \frac{(RF)C_o}{K + \beta} \tag{7}$$

The peak area is a function of instrument response factor (RF), initial concentration of the analyte within the sample (C_o), the solution–vapor partition coefficient (K) and the vapor:liquid phase ratio (β). Eq. (7) holds true for ideal solutions, however it does not account for varying activity coefficients found in non-ideal solutions and solids. All three texts discuss the influence of activity coefficient in SHE. This can be summarized using the expression below from Kolb and Ettre (Eq. (2.29)) which relates the actual observed partition coefficient from Eq. (7) above to the vapor pressure of the analyte and the activity coefficient.

$$K = \frac{p_{\text{total}}}{p_i^o \gamma_i} = \frac{[\text{A(sol'n}]}{[\text{A(g)}]} \tag{8}$$

The partition coefficient (K) is therefore a function of the total pressure in the vial (p_{total}), the vapor pressure of the pure analyte and the activity coefficient. If Eqs. (6)–(8) are combined, the following expression is obtained, which includes both instrument response factor and activity coefficient as variables that need to be controlled in SHE-GC method development.

$$\text{Area} = \frac{(RF)C_o}{p_{\text{total}}/p_i^o \gamma_i + \beta} \tag{9}$$

2.3. Implications of the fundamental relationships on SHE

Eq. (9) shows that detector response in static headspace gas chromatography can be increased by increasing vapor pressure or the activity coefficient of the analyte(s) relative to those of matrix components. It also demonstrates the ideal characteristics of solvents used to dissolve solid samples or to modify liquid samples in preparation for SHE-GC analysis.

1. The ideal solvent generates a low partition coefficient for the analyte and a high partition coefficient for the matrix components and interferences. Eq. (9) shows that this will generate higher instrument response for analytes and lower response for interferences.
2. The ideal solvent generates an ideal solution ($\gamma = 1$) with the analyte and interfering components, thus obeying Henry's Law for both samples and standards. This simplifies Eq. (9) and ensures that equal response is generated for both standards and samples.
3. The ideal solvent has low vapor pressure itself, so it does not interfere with the analysis. This will also lower the total pressure in the vial, p_{total}, reducing K and increasing response.

Eq. (9) also reminds that instrumental response factor and phase ratio (if similar in magnitude to K) may be important factors in determining instrument response in SHE-GC. In all cases, the injection process from the SHE sampler to the GC and the performance of the gas chromatographic column and detector should be optimized and controlled. In cases where K is of similar magnitude, the phase ratio must also be carefully controlled.

In the remainder of this article, new techniques for increasing the instrumental response factor, decreasing the partition coefficient and increasing analyte activity coefficient in static headspace gas chromatography are reviewed. The literature is generally application oriented, with much practical advice but relatively little in terms of fundamental and systematic guidance for method development. Classically, an increase in temperature may be used to decrease the partition coefficient of most analytes by increasing vapor pressure. However, this is problematical if the solvent is also volatile. For liquid samples, "salting out" is a common technique for increasing the activity coefficient of an analyte and therefore sensitivity and instrument response. In many classical applications, most notably pharmaceutical residual solvents analysis, solvent mixtures are used both to enhance solubility of the analytical matrix and to reduce interference in the instrumental analysis.

New SHE techniques that increase sensitivity include improvements in instrumentation, mostly through better interfacing of the headspace sampling device to the gas chromatograph. Derivatization of the analytes can also be used to either decrease the partition coefficient of semi-volatile analytes, or for the analysis of extremely volatile substances, to increase it. Recently, ionic liquids have been demonstrated as potentially useful solvents for pharmaceutical solvent analysis. This review focuses on SHE-GC using traditional instruments and glassware. Headspace-solid-phase micro-extraction (HS-SPME) [4], stir-bar sorptive extraction (SBSE) [5], dynamic headspace extraction (purge and trap) [6], and multiple headspace extraction (MHE) [7], are not covered. These are all important techniques in their own right, but are mature, require specialized devices or instrumentation and have been discussed recently in other venues.

3. Instrumental techniques

The classical means for adjusting sensitivity in SHE-GC involve using temperature (usually increased), adding salts to the analyte solution (salting out) and using solvent mixtures to fully dissolve the sample and/or enhance vaporization of the analytes. The fundamentals of these techniques are treated thoroughly in the textbooks, so they are not repeated here. While the bulk of articles employing SHE-GC use traditional method development and instruments, several authors have added new or better optimized instruments. Some recent examples include the use of a programmed temperature vaporizing inlet [8] in the gas chromatograph as interface between the headspace sampler and GC, addition of a sorbent trap as a concentrator between the headspace extraction and the GC and straightforward new extraction techniques, such as single drop micro-extraction for collecting and concentrating the headspace vapors.

3.1. SHE-PTV-GC–MS

Perez Pavon et al. improved the instrument response factor by using a programmed temperature vaporization (PTV) inlet as the

interface between SHE and GC–MS for the analysis of ICH/USP Class 1 residual solvents in pharmaceuticals [9–11]. They further demonstrated improved response factor by using selected ion monitoring detection. They obtained detection limits under 10 part-per-trillion with RSD less than 12% relative for these solvents. They present a thorough evaluation of the PTV inlet as interface and MS as detector for optimizing response-related factors including comparisons of split, splitless and solvent vent injections and full scan versus selected ion monitoring for detection. The significant improvements in response that they observe are attributed to the longer sampling times allowed by trapping vapor from the SHE-GC transfer line in the cold PTV inlet and improved chromatographic performance inherent in the PTV-large volume injection process itself. In the solvent vent-PTV injections, the headspace was sampled for 1.7 min, clearly allowing a much larger volume of headspace vapor to be transferred to the GC than in traditional methods. Fig. 2 shows contour plots demonstrating this multidimensional analysis. Each analyte is represented by a region in this plot, facilitating unambiguous qualitative analysis in the same manner as in GC × GC. Although common in GC × GC, this type of data presentation is less common in GC–MS, yet as in GC × GC, it is quite powerful.

3.2. SHE-MS

For the rapid screening of off flavors in food by SHE-GC–MS, Carrillo-Carrion et al. used the gas chromatograph as a nearly direct interface between SHE and MS [12]. By holding the column at 200 °C isothermal throughout the analysis, minimizing gas chromatographic retention and separation, they performed what they termed as a vanguard analysis on yogurt for the presence of aldehydes. At this temperature, the capillary column showed very little analyte retention, so in practice the GC acted as a rapid transfer device between the headspace sampler and the mass spectrometer. For samples that demonstrated ions characteristic of aldehydes in the rapid vanguard MS analysis, the same system could then be used with temperature programmed GC–MS, without changing the column, to perform a "rearguard" analysis to determine the specific aldehydes that may be present. The vanguard analysis requires a 15 min extraction followed by a 5 min analytical run, while the rearguard analysis required the same 15 min extraction followed by a 30 min GC run. If most samples are negative for aldehydes, this results in about 50% reduction in analysis time with no additional instrumentation required. This is a straightforward but elegant combination of rapid screening and detailed quantitative analysis into a single system and method.

3.3. Distillation–SDME-GC–MS

In an interesting combination of classical and new techniques, Jalali Heravi and Sereshti combined steam distillation and single drop micro-extraction (SDME) in a classical glassware system with GC–MS to identify 56 essential oils in *Artemisia haussknechtii* Boiss, which grows wild throughout Iran [13–15]. A diagram of their glassware setup is shown in Fig. 3, showing the multiple equilibrium processes involved in this system: evaporation in the distillation pot, enhanced by the addition of water, condensation in the distillation column and solvent-vapor equilibrium in the single drop. Note that complete distillation and SDME can be performed with straightforward glassware found in most organic chemistry laboratories. Plant matter was refluxed in water to generate vapor containing essential oils. The SDME was performed using a 1 μL drop of hexadecane suspended from the syringe needle for 4 min after equilibrium was reached.

In this case, as is the case in all headspace-SDME, SPME and sorptive extraction analyses, there are multiple equilibria involved, with the classical headspace equilibrium (small K) desired in the reaction pot to drive analytes into headspace combined with collection using the reverse (large K) to trap analytes from the vapor phase into the solvent drop. These authors present a solvent optimization study, showing the responses of several analytes in several solvents for the SDME that roughly tracks instrument response with analyte solubility in those solvents. In SDME, the use of a very small volume

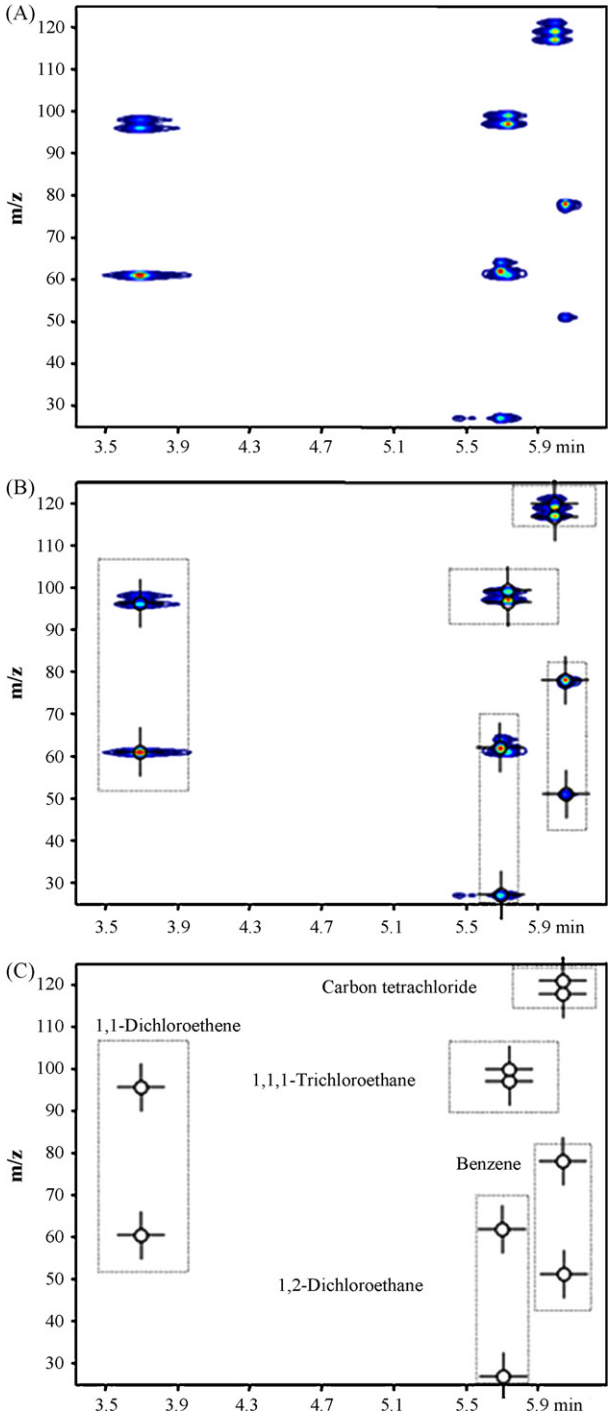

Fig. 2. GC–MS contour plots for ICH Class 1 pharmaceutical solvents. (A) Contour plots for a solution containing the five solvents in water. (B) Zones selected for each solvent. (C) Template generated for the identification of solvents in pharmaceutical product. Note closely eluting pairs: benzene and carbon tetrachloride; 1,2-dichloroethane and 1,1,1-trichloroethane. Reprinted with permission from Ref. [9, Fig. 5]. Copyright 2007 Elsevier Science.

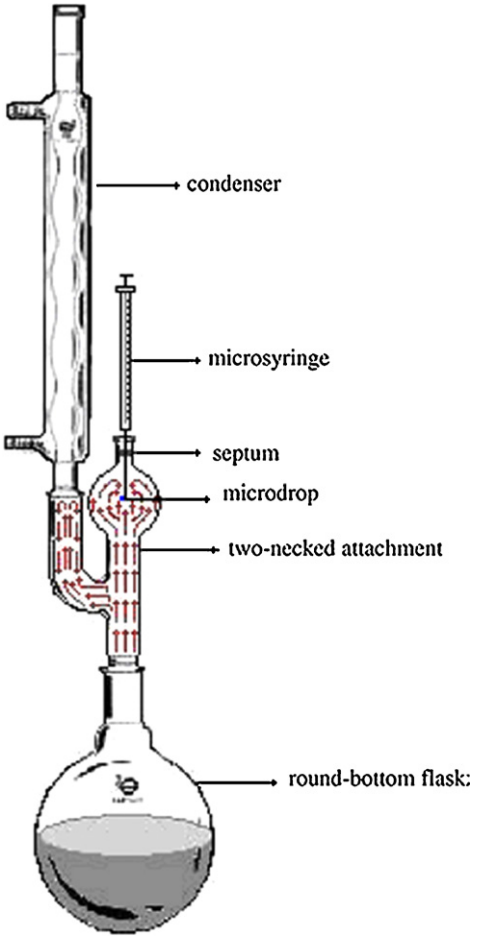

Fig. 3. Diagram of hydrodistillation apparatus showing location of syringe and micro-drop. Note vapor flow around the drop. Reprinted with permission from Ref. [13, Fig. 1]. Copyright 2007 Elsevier Science.

drop as a trapping medium also has significant concentrating effect and therefore a strong positive effect on the instrumental response and sensitivity.

3.4. SHE-Trap-GC–MS

Another way in SHE to improve response by trapping analytes from the vapor phase is to place a solid-phase trap in line between a classical headspace sampler and the gas chromatographic inlet. This technique, called, headspace trap, was initially developed in 2004 [16,17]. In a beverage application, Schulz et al. used a sorbent trap between the vial and GC to concentrate and determine volatile components of the headspace of spirits [18]. They reported 35–55-fold increase in response versus traditional SHE-GC without the trap. A schematic of the headspace trap process is shown in Fig. 4. Initially, SHE-GC is carried out as usual, followed by a decay period in which the pressure inside the vial is slowly released through the trap, which is at lower temperature than the vial. The trap is then heated to desorb analytes into the GC. A comparison of headspace trap with SHE for the analysis of volatile components in a German beverage is shown in Fig. 5. Clearly the addition of the sorbent trap improves sensitivity for these analytes. This type of trapping has been used for additional analyses including volatile organic compounds, fuel oxygenates and aromatic solvents, mostly at very low concentrations in water [19–22]. The choice of trapping sorbent also potentially provides an additional degree of selectivity.

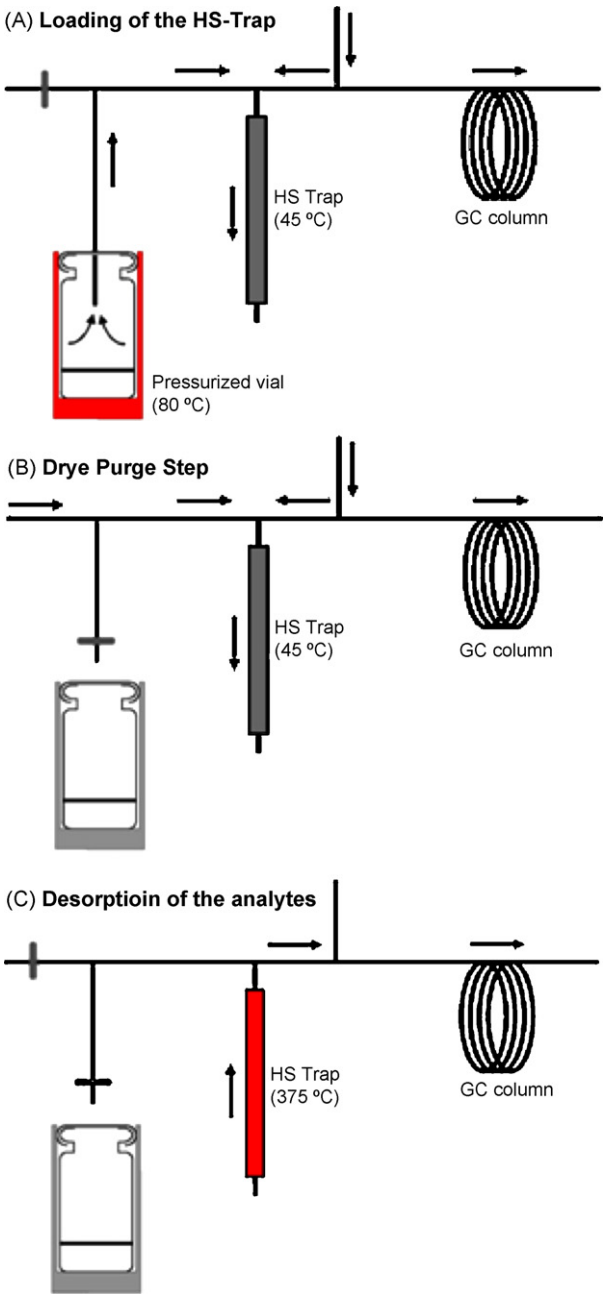

Fig. 4. Steps in static headspace extraction with headspace trap. (A) Trap loading. (B) Drying. (C) Analytes are transported by the carrier gas into the gas chromatographic inlet. Reprinted with permission from Ref. [18, Fig. 1]. Copyright 2007 Elsevier Science.

4. Derivatization

The vapor pressure of a compound may be changed by reacting it with a suitable reagent to form a more volatile derivative that may also have additional physical properties more conducive to the analytical method being used. There are numerous classical derivatization reactions, summarized by Knapp [23] and Wells [24]. In gas chromatography, most often, polar compounds are derivatized to form non-polar analogues to enhance their solubility in the non-polar solvents and stationary phases most associated with gas chromatographic methods. In SHE, derivatization may be used to reduce analyte partition coefficient, therefore increasing the portion in the vapor phase and the instrument response. Small polar molecules can be both volatile and water soluble, making extraction

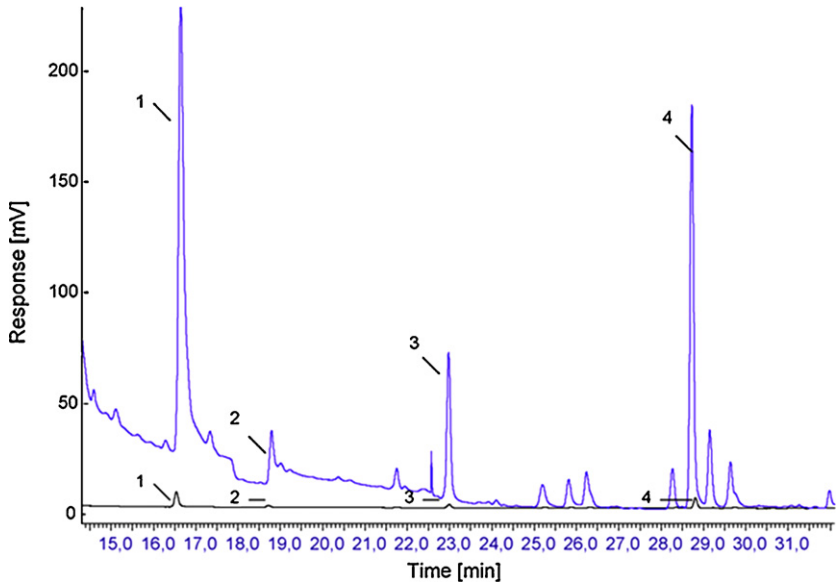

Fig. 5. SHE-GC–MS analysis of a German fruit spirit beverage acquired using static headspace (lower chromatogram) and headspace trap (upper chromatogram) (1 = ethyl lactate, 2 = 1-hexanol, 3 = benzaldehyde, 4 = ethyl octanoate). Reprinted with permission from Ref. [18, Fig. 3]. Copyright 2007 Elsevier Science.

from aqueous matrices difficult and making them reactive on the glass and metal surfaces commonly found in headspace samplers and gas chromatographs. These may be derivatized to form somewhat larger, less-polar analogues, making them less reactive inside the vial and on surfaces in the instrument and more amenable to SHE from matrices in which the parent compounds may have been highly soluble.

Alzaga et al. provided a thorough description of the many issues, both positive and negative involved in the use of derivatizing reagents with SHE-GC methods [25]. They used SHE-GC to determine traces of alkylating agents (derivatizing reagents) themselves in pharmaceutical formulations also containing active pharmaceutical ingredients, residual solvents and matrix components. By their nature, derivatizing reagents are reactive, the alkylating agents examined by these authors are also semi-volatile and the matrix can generate numerous background interferences, making analysis difficult. Following dissolving of samples in dimethylsulfoxide (DMSO)/water mixtures, they derivatized the alkylating agents *in situ* to form pentafluorothiophenol derivatives, which were both more volatile and provided much better response in GC–MS analysis than the parent compounds.

They also examined several compositions of the DMSO/water solvent combination, which is similar to that used in traditional pharmaceutical residual solvents analysis. DMSO/water mixtures are often used in pharmaceutical analysis as they provide one of the best possibilities for dissolving the many compounds present in pharmaceutical samples. Their results, presented in Fig. 6, demonstrate a major challenge in optimizing solvent systems in SHE-GC method development. The authors concluded that their method provided the best results with a 12.5% ratio of DMSO to water, however some analytes showed the best response at other concentrations and others showed little variation in response at all. While the authors' conclusion in using the 12.5% DMSO percentage is supported by the phenomenological data, it is difficult to systematically explain. This article illustrates the challenges and compromises in choosing a solvent during SHE-GC method development. In this case that compromise is further exacerbated by the addition of the derivatization reaction.

In a similar derivatization reaction application, Jacq et al. used an automated SHE-GC–MS system to monitor the progress of the formation of ethyl methane sulfonate from ethanol and methane sulfonic acid [26]. They used a dual-rail robotic system to conduct the reaction, carry out derivatization with pentafluorothiophenol and finally to perform SHE-GC–MS on the derivatives. They present a useful examination of the method development steps involved with using a derivatization reaction, especially the reaction time and the presence of water, in SHE-GC.

Cardador et al. combined liquid micro-extraction with methylation to determine haloacetic acids in water by SHE-GC [27]. These acids are by-products from the use of hypochlorite-based disinfectants and are considered Stage 1 contaminants by the United

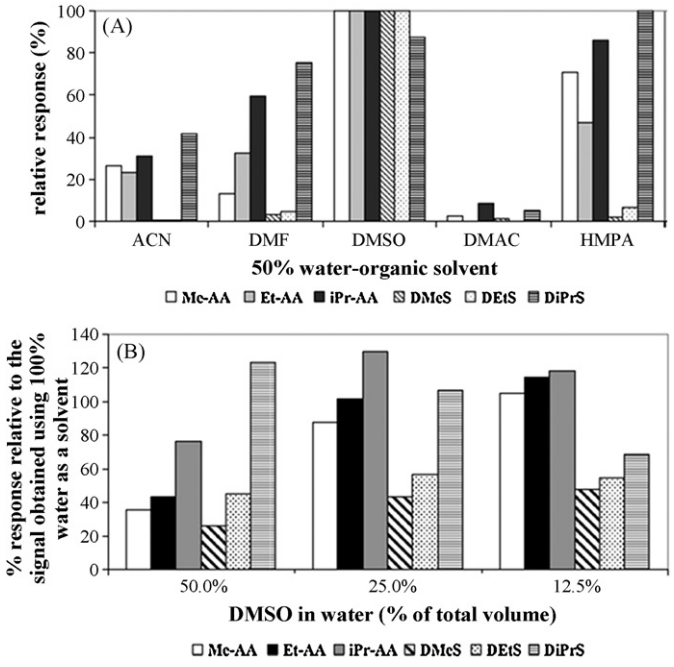

Fig. 6. Effect of solvent composition on response for *in situ* derivatization–HS–GC–MS using pentafluorothiophenol as derivatization agent with different alkylating agents at (A) 50% water–organic solvent (acetonitrile, ACN; dimethylformamide, DMF; dimethyl sulfoxide, DMSO; *N*,*N*-dimethyl acetamide, DMAC; hexamethyl phosporamide, HMPA) and (B) different DMSO–water ratios. Reprinted with permission from Ref. [25, Fig. 1]. Copyright 2007 Elsevier Science.

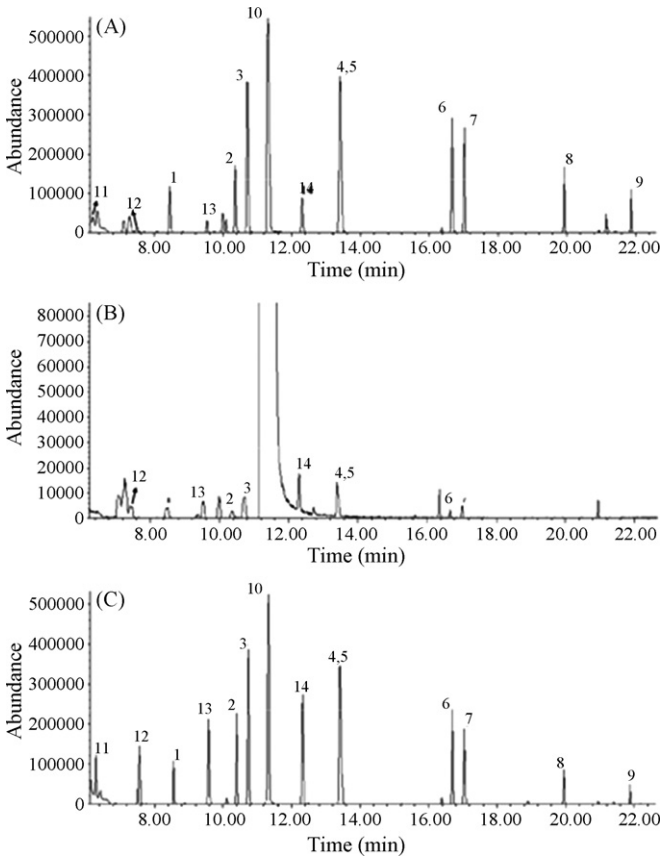

Fig. 7. SHE-GC–MS chromatograms for haloacetic acid derivatives. (A) Two-phase system; (B) single organic phase; (C) aqueous phase. Peak identification: nonochloroacetic acid (MCAA) (1); monobromo-AA (2); dichloro-AA (3); trichloro-AA (4); bromochloro-AA (5); dibromo-AA (6); bromodichloro-AA (7); dibromochloro-AA (8); tribromo-AA (9); IS (10); chloroform (11); bromodichloromethane (12); dibromochloromethane (13); bromoform (14). Reprinted with permission from Ref. [27, Fig. 3]. Copyright 2008 Elsevier Science.

States Environmental Protection Agency. Haloacetic acids have low volatility and high polarity, making them excellent candidates for derivatization-based methods. Haloacetic acids in aqueous solution were simultaneously converted to the methyl esters using dimethylsulfate and extracted into a small amount of pentane, providing about 70-fold concentration of the derivatives. Chromatograms showing haloacetic acid analysis in several systems are seen in Fig. 7. For many of the analytes, especially the less volatile, later eluting compounds, the two-phase system ultimately chosen by the authors provides the best response, but this is not true for all analytes. In this case, the solvent system allows the derivatization reaction to occur in the aqueous phase, followed by the derivatives extracting into the organic phase. In systems with multiple analytes, even those as closely structurally related as haloacetic acids, partition and/or activity coefficients may vary widely among analytes.

In separate reports, simple *in situ* derivatization reactions were used by Perez Pavon et al. to determine ibuprofen and chlorophenols in aqueous systems, followed by SHE and programmed temperature vaporization injection-GC [28,29]. They used a traditional automated headspace sampler interfaced through a heated transfer line to the gas chromatograph. A diagram of this setup, which is emblematical of other SHE-GC methods involving derivatization is shown in Fig. 8. In the diagrammed system, salting out was first used to increase analyte activity coefficient, enhancing vaporization of the polar analyte, ibuprofen. Next acid and methanol were added to form a methyl derivative under acidic conditions, generating an analog with higher vapor pressure. The system was then heated to complete the reaction and reach equilibrium. This is an elegant example of using derivatization to make a volatile derivative of ibuprofen, a compound not generally considered volatile, thus extending the utility of SHE-GC.

In the second example, using a similar procedure, chlorophenols were derivatized under basic conditions using acetic anhydride to form acyl derivatives. Use of the PTV inlet as interface allowed much longer transfer times and larger total volume transfer from the headspace sample vial to the inlet. During sample transfer, the PTV inlet was operated in cold solvent vent mode, trapping the analytes of interest in the liner. The PTV vent was then closed and the PTV was heated while transferring the analytes in splitless mode to the column. This resulted in a significant increase in sensitivity when compared to more traditional methods such as liquid–liquid extraction and solid-phase extraction. Detection limits were similar to those reported for headspace-SPME [30]. In these studies, a combination of enhanced analyte partitioning to the vapor phase using derivatization and increased instrument response using the PTV inlet are combined to provide large improvements in sensitivity. Both examples show possibilities for using SHE-GC for semi-volatile analytes.

5. Ionic liquids as solvents

Ionic liquids (ILs) are simply defined as ionic substances that exist as liquids at convenient temperatures, most commonly room temperature. Although they have been known for about a century, they have received have received significant attention in the chemistry literature over the past decade as “green” alternatives to traditional organic solvents for myriad applications including synthesis, materials research, catalysis and extraction. For separation scientists interested in ionic liquids, Yao and Anderson presented a particularly thorough discussion and analysis of the relevant physical properties of room temperature ionic liquids used as chromatographic stationary phases, including discussion of basic physical properties, solubility and interaction energy related parameters: Kovats retention index, Rohrschneider–McReynolds constants and gas chromatographic retention behavior [31–33].

About 10 years ago, Armstrong et al. first demonstrated the use of 1-butyl-3-methylimidazolium hexafluorophosphate and its chloride salt as stationary phases for gas chromatography [34]. They determined classical chromatographic figures of merit including

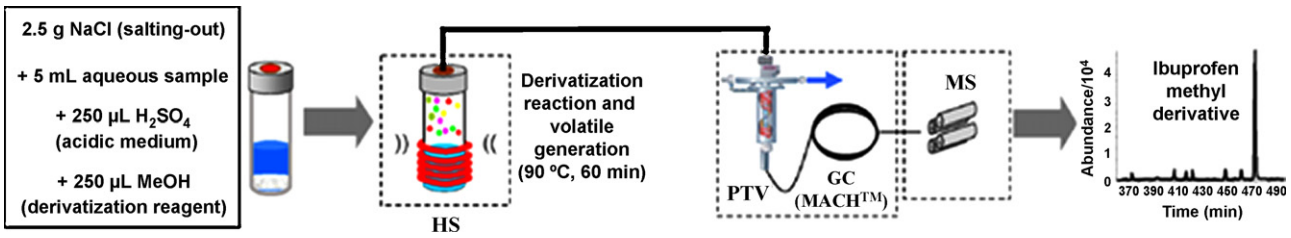

Fig. 8. Derivatization scheme for *in situ* SHE-GC–MS analysis. Reprinted with permission from Ref. [29, Fig. 1]. Copyright 2009 Elsevier Science.

Kovats retention indices, Rohrschneider–McReynolds constants and retention factors for several analyte compound classes. In additional studies, Anderson and Armstrong developed thermally stable ionic liquids and evaluated them as stationary phases for traditional capillary gas chromatography [35,36]. While these materials are themselves not especially appropriate as solvents for SHE, as they are highly viscous, the work demonstrates the potentially useful dual-nature properties of ionic liquids as solvents for SHE. They can be simultaneously retentive to both polar and non-polar solutes and can be highly retentive to specific functional groups. For one ionic liquid, they report a gas chromatographic retention factor of 1.1 for toluene and 35.8 for nitrobenzene. The data presented in these references are replete with similar examples.

In 2004, Poole thoroughly reviewed and discussed chromatographic methods for determining the solvent properties of ionic liquids [37]. This review included study of the physical properties of over 200 ionic liquids reported in the literature, including melting point, density, viscosity and upper temperature limit. Interestingly there seems to be more data available on more advanced properties such as polarity, measured by various techniques. Disappointingly, relatively little physical property data were available for many ionic liquids. Physical properties that were determined seem to be tailored to the specific needs of the experiment for which the ionic liquid was developed, rather than for understanding of the ionic liquid's basic physical properties. Until more physical property data are readily available, it is likely that development of ionic liquids as solvents for chromatographic sample preparation will continue based primarily on empirical experimentation.

5.1. Ionic liquids for SHE of residual solvents

The first demonstration of the use of ionic liquids as solvents for static headspace extraction was by Liu and Jiang for the determination of pharmaceutical residual solvents [38]. They used 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF4) to prepare samples containing acetonitrile, dichloromethane, *N*-methyl-2-pyrrolidone (NMP), toluene, dimethylformamide (DMF) and *n*-butyl ether, solvents involved in the synthesis of Adefovir Dipivoxil, a water insoluble drug substance. They noted that two of their solvents of interest, NMP and DMF, have relatively low vapor pressure, limiting the available solvents for preparing the samples. They compared analytical figures of merit for samples dissolved in [bmim]BF4 to DMSO and found increased peak intensity for all of the solvents studied in [bmim]BF4 at constant temperature. Examining this observation in light of Eqs. (8) and (9) and assuming that the vapor pressure of each analyte is constant, is it seen that the ionic liquid increases the activity coefficient, thereby decreasing the partition coefficient and enhancing sensitivity. There is an interesting research opportunity in the further study of this phenomenon.

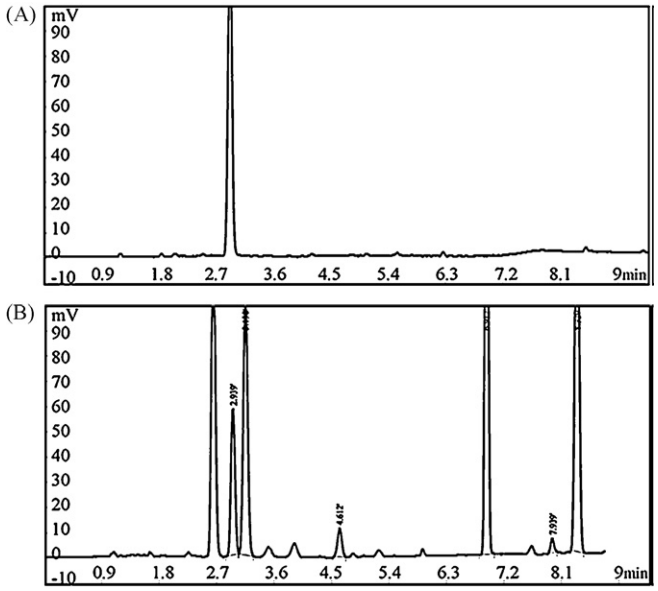

Fig. 9. (A) Chromatogram of blank matrix medium, the peak of acetone residual in [bmim]BF4. (B) Chromatogram of analytes. Analytes listed in order of elution: acetonitrile, dichloromethane, NMP, toluene, DMF, *n*-butyl ether. Reprinted with permission from Ref. [38, Fig. 3]. Copyright 2007 Elsevier Science.

Chromatograms from this first work are shown in Fig. 9. It was observed that background signals from the ionic liquid matrix do not interfere with analysis of the analytes of interest. However, as has been often noted with the use of many classical solvents such as DMSO for residual solvents analysis, these interfering peaks do exist. This was examined in more detail by Von Wald et al. in 2008, using SHE-GC to examine the residual solvents in several ionic liquids themselves [39]. They studied six of the most common ionic liquids under conditions involving no treatment, sparging the liquid with heat, vacuum and purging the vial headspace with nitrogen. The headspace above each ionic liquid with no treatment was found to contain several common solvents at concentrations ranging from 3 to 8700 ng/mL of vapor. The sparged ionic liquids were the cleanest, with residual solvent concentrations mostly less than 10 μg/mL of vapor, roughly translating to less than 10 ppm. For ionic liquids to be useful as solvents for static headspace extraction, it is likely that they will routinely need to be sparged with pure nitrogen prior to use.

Von Wald et al. also determined solution–vapor partition coefficients for several solvents in six ionic liquids. A portion of their data is shown in Table 1. They illustrate that extreme selectivity is available (for better and for worse) for SHE with ionic liquids as it is for GC. For triethylamine, *K* ranged from 32 to 15,000, while for

Table 1
Solution–vapor partition coefficients of several solvents in various ionic liquids. Specific identities of the solvents and ionic liquids are given in the reference. Uncertainties given by the authors not provided here for brevity. Adapted from Ref. [39, Table 9].

Ionic liquids							
Cpd	BP (°C)	TTPdC	TTPBSI	BMIBSI	BMIMS	BDIHF	THMAS
MTBE	55	18	22	13	4	7	1
IPA	82	115	30	44	99	29	26
TEA	90	32	1027	106	1425	2521	15,000
Heptane	98	31	38	8	2	3	0
Toluene	111	126	127	103	51	64	6
Pyridine	115	225	223	263	227	254	123
Hexylamine	131	300	200	585	2000	3000	2,000
DMF	153	651	932	3000	940	1354	834
PG	187	800	400	700	900	611	800
NMP	202	944	1800	2000	1700	2000	1,800

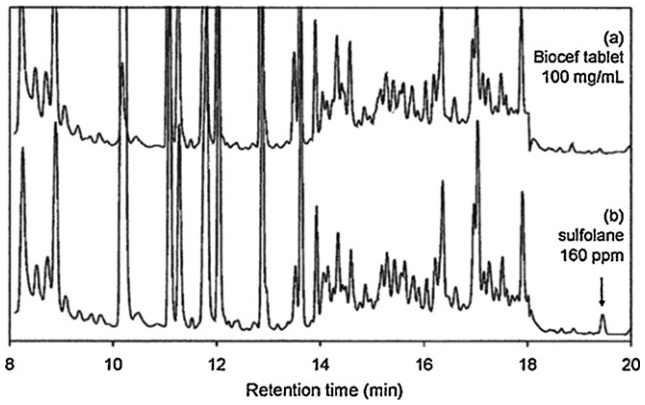

Fig. 10. Headspace gas chromatograms using ionic liquids: (A) whole pharmaceutical tablet with an estimated level of 20 μg/g sulfolane, and (B) spiked with sulfolane at 160 μg/g. Reprinted with permission from Ref. [41, Fig. 4]. Copyright 2009 Elsevier Science.

isopropanol, the range is much narrower, from 26 to 115. Practically, this has potential benefit in that an ionic liquid can be chosen to enhance sensitivity for particular analytes of interest, however a single ionic liquid may not be available for general analysis of a large number of compounds at once, as is common in SHE-GC. It is especially interesting that the analyte partition coefficients do not directly track the analyte boiling points (vapor pressure) for any of the ILs. This indicates the widely varying activity coefficients that are possible when using ILs as solvents for SHE-GC. The solvent properties and partitioning of compounds in ionic liquids have been studied by several authors, but it is clear that, given the large number of ionic liquids available, this deserves further study [40].

Recently, Laus et al. used an ionic liquid, 1-*n*-butyl-3-methylimidazolium dimethyl phosphate (BMIM DMP), as the solvent for determining several of the least volatile pharmaceutical residual solvents in the presence of excipients and an active pharmaceutical component [41]. They observed low-part-per million detection limits for DMSO, *N*-methylpyrrolidone, sulfolane, tetralin, and ethylene glycol, which are among the most difficult pharmaceutical solvents to quantify. Besides favorable liquid–vapor partition coefficients for the analytes, to be suitable for SHE-GC, an ionic liquid must also be able to fully dissolve expected matrix components. BMIM DMP gave a transparent, but colored solution when a mixture of starch, guar derivatives of cellulose, or fatty acid salts such as magnesium stearate were dissolved. Fig. 10 shows an SHE-GC analysis of sulfolane in a pharmaceutical tablet dissolved in BMIM DMP. In this analysis, the source of the many additional peaks is not stated; it is expected that some came from the ionic liquid which was not purified prior to use.

5.2. Next steps in the study of IL in SHE

Ionic liquids are clearly promising as solvents for SHE-GC. There is additional research needed in several areas.

1. Purification by sparging with high purity nitrogen as discussed by von Wald et al. is effective but inconvenient. Ionic liquids will need to be available in better purity or simpler methods of purifying them in the lab need to be developed.
2. There are numerous ionic liquids available, a strength in that it is possible to find an ionic liquid that will effectively dissolve both analyte and matrix components. However, systematic IL selection principles, based in study of physical and solvation properties need to be developed.
3. Solution–vapor partition coefficients vary considerably for analytes dissolved in ionic liquids. Much further study of liquid–vapor partitioning is therefore needed to aid in the selection and method development process.
4. Compared to other solvents, ILs have relatively high cost to purchase and purify. This will need to be addressed for IL to significantly replace traditional solvents.

6. Conclusions

Static headspace gas chromatography is one of the most important tools for chemical analysis among users in many fields developing new applications and among researchers interested in fundamental aspects of the technique. Several very interesting new supporting techniques have been developed in the past few years, all with the goal of improving sensitivity through decreasing solution–vapor partition coefficient, increasing the activity coefficient or increasing the instrument response factor. Instrumental methods include use of a PTV inlet as interface between headspace sampling and the column, use of sorbent traps between the sampling vial and the GC and use of the GC as a direct interface between headspace and MS. Fundamentally, these do not alter the extraction process but they enhance sensitivity by increasing the instrument response factor. Derivatization of analytes in the vial can lower the partition coefficient (raise the analyte's vapor pressure) by converting less volatile analogs into more volatile derivatives, also enhancing sensitivity. Finally, room temperature ionic liquids are emerging as new solvents that raise the activity coefficient of analytes versus interfering components toward evaporation, exhibiting interesting selectivity and also potentially improving sensitivity. All of these areas require more research to see their full potential and to extend the range of compounds amenable to static headspace gas chromatographic analysis.

Acknowledgments

The authors gratefully acknowledge financial support from the Sanofi Aventis Foundation which provided financial support for the Center for Academic Industry Partnership. NHS acknowledges support in the form of a sabbatical leave during the writing of this manuscript from Seton Hall University. We also gratefully acknowledge instrumental support for our work with static headspace gas chromatography from PerkinElmer and many technical conversations about static headspace gas chromatography with Drs. Andrew Tipler of PerkinElmer and Leslie Ettre of Yale University.

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