Utilization of a Matrix Effect to Enhance the Sensitivity of Residual Solvents in Static Headspace Gas Chromatography

Zhi Chen[1,2*], Wei X Huang[1], Shenjiang Yu[1,2], Jing Yang[1], Hui Liu[1], Abu M Rustum[1], Daisy Richardson[2], Mohammed Shameem[2]

[1]Global Quality Services, Analytical Sciences, Legacy Schering-Plough Corp., Merck & Co., Inc., 1011 Morris Ave, Union NJ, USA
[2]Sterile Product and Analytical Development, BioProcess Development, Merck & Co., Inc., 2000 Galloping Hill Rd, Kenilworth NJ, USA

Abstract

A novel approach to enhance the sensitivity of residual solvent analysis in static headspace gas chromatography (HS-GC) was developed. During the investigation of matrix effects on the recovery of residual solvents using HS-GC analysis, we found that the spiking of a particular additive in DMSO decreased the partition coefficients (K) of various common residual solvents, and thus substantially increased their concentrations in the gas phase (headspace). Further study also found that other compounds structurally similar to the additive could produce the unique matrix effect. By utilizing this matrix effect, the sensitivities of 1,1-dichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane were increased by 67%, 67%, 80%, and 64%, respectively, compared to the analyte solution without the additive. Other residual solvents, which also showed enhancement of sensitivities, are ethanol, isopropanol, tert-butanol, methylene chloride and chloroform (enhanced up to 40%, 50%, 57%, 42%, and 66%, respectively). The results of the study indicates that intermolecular interactions, particularly hydrogen-bonding interaction among the additive, residual solvent and diluent, may play a key role in this matrix effect, as the strongest effect is observed for analytes that possess strong hydrogen bond acidity and/or low basicity, i.e., alcohols and chlorinated compounds.

Key words: Residual Solvents; Matrix Effect; Static Headspace Gas Chromatography (HS-GC); Hydrogen-Bonding

Introduction

Organic volatile solvents are widely used in the synthesis, purification, and manufacturing of active pharmaceutical ingredients (APIs) and final drug products. These process-related solvents may not be completely removed during the manufacturing process, and trace levels of these solvents may be retained in APIs and final drug products [1]. Because of their potential toxicity [2], it is important to ensure that the residual solvents remaining in APIs and final drug products are below the safety thresholds, which are mandated by regulatory authorities [3-5].

Static headspace gas chromatography (HS-GC) is a commonly used technique in pharmaceutical laboratories to determine process-related residual solvents in APIs [6-8]. In HS-GC analysis, API is dissolved in a diluent in a sealed vial and thermostated until the partitioning of residual solvents is equilibrated between the liquid phase (analyte solution) and gas phase (headspace) in the sealed vial. A known aliquot of the headspace is then injected into the GC system for analysis. When the phase ratio of liquid and gas phase is fixed in the vial, the detector response of a residual solvent is related to the nature of its composition, initial concentration in the analyte solution and partition coefficient K (K is defined as the analyte liquid phase concentration versus its gas phase concentration) [6]. In general, the detector sensitivity of HS-GC is sufficient for such an analysis, but may become a problem in some situations. For instance, when the solubility of an API is limited in the diluent, or when extremely low levels of residual solvents (e.g., ICH class 1 solvents) are of interest [9]. In addition, the high partition coefficient (K) or low response of a residual solvent can lead to extremely low HS-GC sensitivity and cause more challenges related to the detection limit of a method [10].

There are a number of ways to enhance the HS-GC sensitivity. One of the most straightforward methods is to optimize the GC instrumental parameters, or using more sensitive detectors, such as MS [10-12], ECD [13,14], NPD [14], etc. Besides, various other options

have been attempted to decrease the analyte partition coefficient (K) and hence increase its concentration in the headspace. For instance, elevating temperature at the equilibrium of the sample thermostated stage advances the evaporation of residual solvents and decreases their K values [6,15,16]. However, the use of high temperature is restricted by several factors, such as the potential of vial leakage due to high pressure, injection repeatability, and thermal stability of APIs, etc. Another approach to decrease the K value is the use of "salting-out" effect, which has been applied to increase the sensitivity of residual solvents in aqueous solutions. In this approach, a large quantity of electrolyte is added in the analyte solution in order to decreases the K values of polar compounds [6, 15,17]. Similarly, pH adjustment of the analyte solution can also change the partition coefficients of polar compounds [15]. In the case of using an organic diluent, adding a small amount of water into the analyte solution can decrease the K values of non-polar compounds [6,16,18]. However, using aqueous or aqueous/organic mixture diluent may not be suitable for APIs, especially for lipophilic drug substances [19,20]. In such cases, high boiling-point organic solvents, such as dimethyl sulfoxide (DMSO) and N-methyl-2-pyrrolidone (NMP) owning superior solubility for most APIs, are widely employed as diluents in generic HS-GC methods, which are commonly used in pharmaceutical laboratories for in-process control and batch release tests [2,21]. Currently, only a handful of publications

have been dedicated to the improvement of residual solvent sensitivity using high boiling-point organic diluents. Among them, the effect of different diluents on the partition behavior of residual solvents was studied [22]. In addition, ionic liquids were reported capable of decreasing the K value in some cases [10,21].

To analyze trace level residual solvents in APIs, dissolving a large amount of API in the analye solution (typically about 50 to 500 mg/mL) is inevitable. The high API concentration may substantially change the activity coefficient (α) of an analyte from its α value in a standard solution without the API. Such a change is usually referred to as the matrix effect which can cause a bias signal of the analyte (i.e., low or high recovery) in HS-GC analysis [23,24], if an external standard is used for calibration. According to historical data in our laboratory, the matrix effect usually decreases the signals of residual solvents in HS-GC and needs to be compensated by pre-determined correction factors. However, a rare case was recently discovered in which the matrix effect caused by trichlormethiazide (compound 1 in Chart 1) could significantly increase the analyte signals. Further study revealed that a couple of other compounds, which are structurally similar to trichlormethiazide, could also generate this type of matrix effect in DMSO and in other high boiling-point organic diluents. By utilizing this matrix effect in HS-GC analysis, the sensitivity of a large number of common residual solvents, including ICH class one solvents, could be greatly enhanced. A possible mechanism of this matrix effect was also proposed and discussed.

Experimental

Chemicals and reagents

Trichlormethiazide (1), diazoxide (2), loratadine (3), and tolnaftate (4) were obtained from Schering-Plough Corporation. Benzenesulfonamide ($\geq$ 98%; 5) was purchased from Alfa Aesar. Hydroflumethiazide (about 97%; 6), benzthiazide (>99%; 7), and meticrane (>99%; 8) were purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO; HPLC grade, 99.9+%; 9) was purchased from Burdick and Jackson. N-methyl-2-pyrrolidone (NMP; 99.0%; 10) was purchased from Mallinckrodt. N,N-dimethylacetamide (DMA; 99%; 11) and benzyl alcohol (BA; 99%; 12) were purchased from Acros. The chemical structures of compounds 1 to 12 are shown in Chart 1. All the residual solvents were purchased from commercial sources with purities greater than 99%. Headspace vials (10-mL) with 20-mm PTFE/silicone aluminum seals were purchased from Agilent Technologies.

Sample preparations

To prepare stock solutions, appropriate amount of residual solvents were accurately weighed in volumetric flasks and mixed with a diluent (DMSO, NMP, DMA or BA). Standard solutions with various concentration levels of residual solvents were prepared by appropriate dilutions from the stock solutions. For analysis of residual solvents in a standard solution, 1 mL of the solution was pipetted into a 10-mL headspace sample vial and then sealed. For analysis of residual solvents in an API, or in a solution spiked with an additive, desired amount of the API, or the additive was weighed directly in the sample vial before the standard solution was pipetted.

Equipment and HS-GC Conditions

A gas chromatographic system (6890N, Agilent Technologies) equipped with a headspace autosampler (G1888, Agilent Technologies) was used to analyze the samples for residual solvents. Data acquisition and analysis were conducted by Agilent ChemStation (version 3.2).

A DB-624 GC column [30 m × 0.32 mm (ID), 1.80 μm film thickness; J&W Scientific] was employed for the separation. Sample vials were loaded into the headspace oven and heated to 110 °C with shaking for 11 minutes. The injections were made by pressure via a 1-mL sample loop. The split ratio of helium carrier gas was 1: 40 and the inlet temperature was set at 160 °C. The oven program at the initial column temperature was set at 35 °C for 15 minutes, then ramped at the rate of 10 °C/minute to 90 °C, and continuously ramped at the rate of 45 °C/minute to 200 °C, and held for 5 minutes. A Flame Ionization Detector (FID) was used for the detection and its temperature was set at 250 °C. The gas flow rate for the detector was set at 40 mL/minute for hydrogen, 400 mL/minute for air, and 30 mL/minute for the make-up gas. Figure 1 shows a representative GC chromatogram of residual solvents.

Determination of Correction Factors

A series of standard solutions with various concentration levels of residual solvents were analyzed, and the peak area (A) of each residual solvent in the chromatograms was recorded. The peak areas of individual residual solvents were plotted versus their concentrations, and the slope of each linear curve was determined by the least-square method. When no additive (and/or API) was spiked in standard solutions, the peak area was designated as $Slope_{std}$ and the slope was designated as $Slope_{add}$. When an additive (and/or API) was spiked in the standard solutions, the peak area was designated as $Slope_{add}$ and slope was designated as $Slope_{add}$. The correction factor (CF) excluding the matrix effect for each residual solvent was calculated using the following equation unless otherwise mentioned:

$$CF= Slope_{std} /Slope_{add}$$

In case a residual solvent was already presented in an additive and/or API, its amount was subtracted during the data processing. Blank samples, i.e., clean DMSO spiked with the additive and/or API, were tested for this purpose.

Chart 1: Chemical structures of compounds 1 to 12.
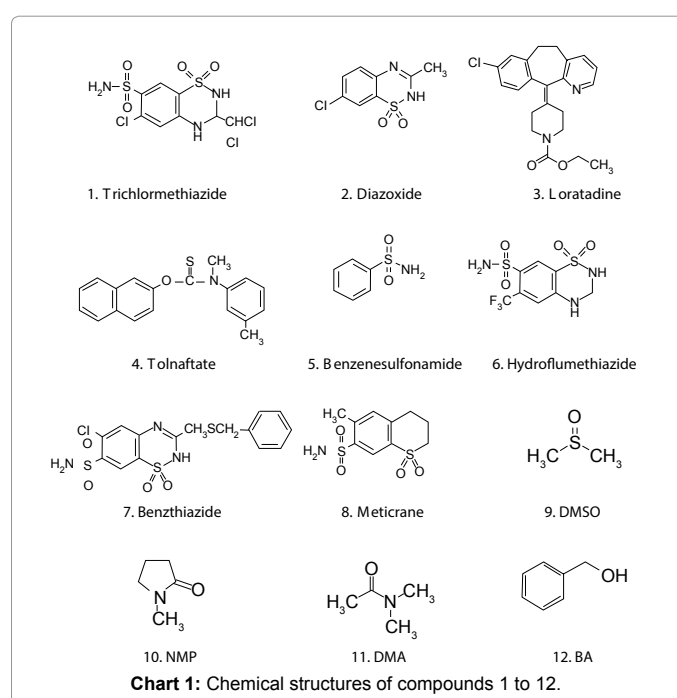
Utilization of the matrix effect for residual solvent analysis

For residual solvent analysis in a specific API, desired amount of the API (e.g. 500 mg) and additive (e.g. 500 mg) was weighed directly in the sample vial and then dissolved with 1 mL of DMSO. Residual solvent peak areas in the chromatograms were used for the quantitation purpose against external calibration curves. Correction factors of these residual solvents were pre- determined to compensate the matrix effect caused by the combination of API and additive. Additionally, clean DMSO spiked with the same amount of additive only was tested in case blank subtraction is needed.

Results and discussion

Matrix effect on residual solvents analysis

Correction factors for individual residual solvent are usually used to rectify bias signals caused by the matrix effect in HS-GC analysis. The correction factor value of a residual solvent reflects the degree of molecular interactions between the API and residual solvent in a specific diluent. A correction factor larger than one (>1) indicates that the matrix effect increases the K value of the corresponding residual solvent and consequently decreases its sensitivity in HS-GC analysis. On the contrary, a correction factor less than one (<1) indicates that the matrix effect reduces the K value of the corresponding residual solvent and consequently enhances its sensitivity. The historical data in our laboratory shows that the correction factors for most common residual solvents in various APIs in DMSO are greater than one. These results strongly indicate that most API matrixes in DMSO diluent tend to retain the residual solvents in the liquid phase and hence reduce their sensitivity in HS-GC analysis.

To investigate the cause of matrix effect, the correction factors of 18 common residual solvents were determined in the presence of trichlormethiazide, diazoxide, loratadine, and tolnaftate. Table 1 summarizes the results of this investigation. The correction factor for each individual residual solvent was the smallest in the trichlormethiazide spiked solution, compared to the solutions spiked with other three APIs. In the trichlormethiazide spiked solution, the correction factors for most of the residual solvents were less than one except for 1,4-dioxane and THF, whose values were equal to or slightly larger than one. In the diazoxide spiked solution, the correction factors were less than one for alcohols and chlorinated solvents, and were equal to or above one for other solvents. Table 1 also shows that alcohols and chlorinated solvents in both trichlormethiazide and diazoxide spiked solutions had the smallest correction factors among the 18 residual solvents. However, in loratadine and tolnaftate spiked solutions, almost all residual solvents had correction factors larger than one except for methanol, isopropanol, and acetone whose correction factors were close to one. Obviously, the correction factor values in Table 1 reflect different kinds of matrix effects caused by different APIs in DMSO diluent.

To explore the matrix effects further, the correction factors of 18 residual solvents in tolnaftate, trichlormethiazide, and diazoxide spiked solutions were plotted as a function of their respective API concentrations. The correlation profiles observed in Figures 1-3 indicate three types of matrix effects. Figure 1 shows the plot of the correction factors versus different tolnaftate concentrations (mg/mL) in the spiked solution. As expected, the correction factors for all residual solvents are larger than or close to one. The correction factors in the spiked solution increased almost linearly when the tolnaftate concentration increased from zero to 1000 mg/mL. Slopes for most of the residual solvents were positive in Figure 1, indicating that this type of

matrix effect has a negative impact on the sensitivity in residual solvent analysis by HS-GC. For example, sensitivities of 1-butanol, chloroform, methylene chloride and toluene lost approximately 16%, 27%, 22% and 45%, respectively, when the tolnaftate concentration was 1000 mg/mL in the spiked solution. Figure 1 shows the most common matrix effect due to the presence of API in the sample solution, except for a few solvents such as methanol, ethanol, and acetone. The correction factors for all other solvents are significantly larger than one, indicating that the equilibrium of the residual solvents is favored in the liquid phase rather than the gas phase (i.e., headspace of the sample vial).

When trichlormethiazide was spiked in the analyte solution containing the 18 residual solvents, the impact of matrix effect was very much opposite to the typical matrix effect observed in HS-GC analysis. Figure 2 shows the matrix effect that is quite different from the matrix effect shown in Figure 1. The residual solvents in the trichlormethiazide spiked solution could be classified into three groups. Alcohols and chlorinated solvents fell in group A, in which their correction factors were less than one and the smallest compared to residual solvents in other groups. When the concentration of trichlormethiazide in the spiked solution increased from zero to 1000 mg/mL, the correction factors of alcohols and chlorinated solvents decreased almost linearly with respect to the concentration of trichlormethiazide in sample solution. The residual solvents in group B had correction factors less than one, but changed moderately when the concentration of trichlormethiazide in sample solution increased. THF and 1,4-dioxane in group C had the largest correction factors, which were close to, or above one. Their correction factors decreased slightly at the beginning, and then increased linearly with the additional amount of trichlormethiazide. The unusual matrix effect shown in Figure 2 can increase the signals of a large number of common residual solvents in HS-GC analysis. For instance, the signals of methanol, ethanol, isopropanol, 1-butanol, *tert*-butanol, methylene chloride and chloroform (group A solvents) increased by approximately 54%, 52%, 65%, 65%, 73%, 54% and 84%, respectively, when the amount of trichlormethiazide in the sample solution was about 1000 mg/mL. For group B residual solvents, the signals increased in a range of approximately 10% to 35%.

As shown in Figure 3, the matrix effect caused by diazoxide is between the matrix effects caused by tolnaftate and trichlormethiazide. In the presence of diazoxide, the group B and group C residual solvents (as shown in Figure 2) merged into one group (group B' in Figure 3). The correction factors for these solvents were larger than one and increased slightly with an increasing amount of diazoxide in the spiked solution, and leveled off after 700 mg/mL concentration. THF and 1,4-dioxane had the largest correction factors among all residual solvents in the presence of diazoxide, which is similar to the previous observation, i.e., in the presence of trichlormethiazide. Alcohols and chlorinated solvents continued to show a similar pattern to that in Figure 2 and stayed in the same group (group A'). Since diazoxide has a similar structure to trichlormethiazide and its effect on group A' residual solvents is comparable to that of trichlormethiazide, it can be assumed that the chemical structure of trichlormethiazide is the root cause of the unusual matrix effect.

Molecular interactions in matrix effects

To support the previous assumption that the chemical structure of trichlormethiazide led to the unique matrix effect, a number of additional compounds, which have similar chemical structures to trichlormethiazide were selected for further investigation. Benzenesulfonamide, benzthiazide, meticrane and hydroflumethiazide were purchased from commercial sources to determine their role in the
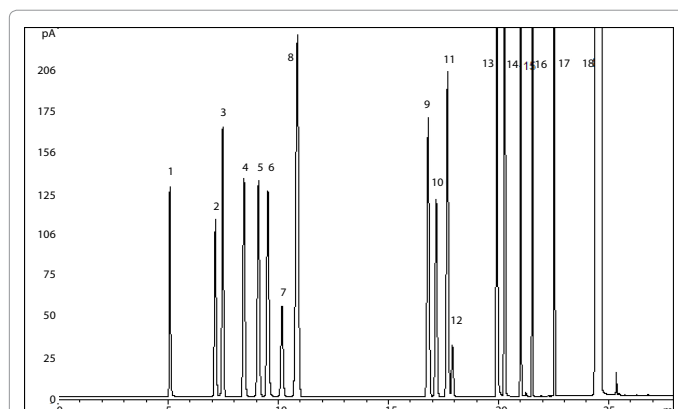
Figure 1: Representative GC chromatogram of residual solvents. 1: methanol; 2: ethanol; 3: ether; 4: acetone; 5: isopropanol; 6: acetonitrile; 7: methylene chloride; 8: t-butanol; 9: methyl ethyl ketone; 10: ethylacetate; 11: tetrahydrofuran (THF); 12: chloroform; 13: trimethylamine; 14: heptane; 15: butanol: 16: 1,4-dioxane; 17: toluene; 18: dimethyl sulfoxide (DMSO).
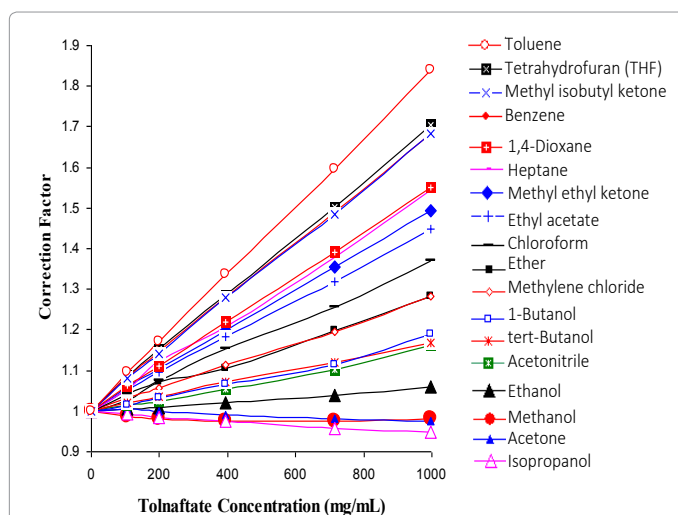

Figure 2: A representative correlation profile of the correction factors versus various tolnaftate concentrations in DMSO diluent. The reported correction factor result is the average from three replicate measurements.
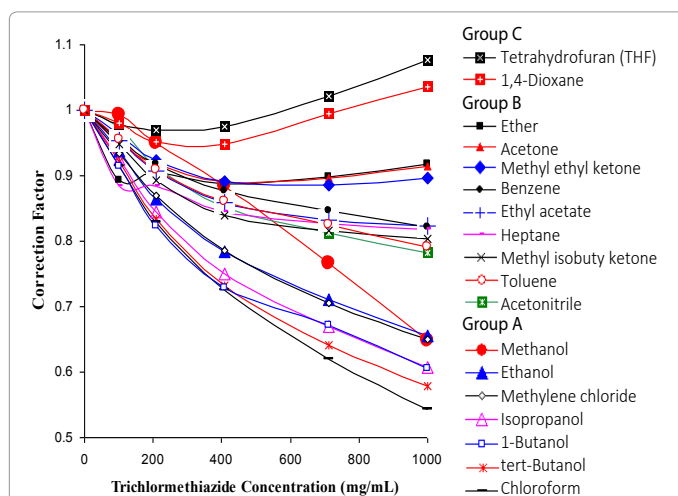

Figure 3: A representative correlation profile of the correction factors versus various trichlormethiazide concentrations in DMSO diluent. The reported correction factor result is the average from three replicate measurements.

matrix. As shown in Table 2, when one of the trichlormethiazide-like compounds (excluding diazoxide) was added into the diluent (DMSO), almost all residual solvents used in the study showed correction factors smaller than one except for THF and 1,4-dioxane. Alcohols and chlorinated solvents showed the smallest correction factors in all spiked solutions, including the diazoxide spiked solution. The data obtained from this investigation indicated that these compounds can also generate the matrix effect that is similar to the trichlormethiazide matrix effect. By carefully comparing the chemical structures of all additives used in this study, it is clear that the trichlormethiazide-like compounds contain at least one hydrogen bond (H-bond) donor in their molecular structures while tolnaftate and loratadine do not have any. Table 3 lists the number of H-bond acceptors and donors for each additive. Trichlormethiazide and hydroflumethiazide each has four H-bond donors and their matrix gave the smallest average correction factors as indicated in Table 2. Benzthiazide, meticrane and benzenesulfonamide each has two to three H-bond donors and their matrix gave moderately small correction factors. Diazoxide has one H-bond donor, and only the correction factors of group A solvents (alcohols and chlorinated solvents) are smaller than one. Loratadine and tolnaftate do not possess any H-bond donor, and almost all residual solvents in the presence of these two compounds had correction factors larger than one. This correlation shows that the correction factors of residual solvents increase with the decrease of H-bond donor number of the spiked additive (API) in the sample solution, indicating that the H-bonding ability of the additives have a great impact on the matrix effect.

The following study investigated the role of diluent in the matrix effect. Three diluents in addition to DMSO, i.e., NMP, DMA, and BA, were selected. These four diluents own various hydrogen-bond capabilities, as shown in Table 3. In this study, a set of four representative residual solvents (THF, acetone, 1-butanol, and chloroform) along with a trichlormethiazide-like additive (benzenesulfonamide) was diluted by these diluents, respectively. Table 4 summarizes the correction factors of THF, acetone, 1-butanol, and chloroform in the presence of benzenesulfonamide measured in NMP, DMA, DMSO and BA diluents. The results of this study show that all four residual solvents had the smallest correction factor values in NMP diluent and had the largest values in BA diluent. In NMP, DMA, and DMSO diluents, THF and acetone had correction factors larger than one (THF had the largest values). On the other hand, 1-butanol and chloroform had correction factors much smaller than one and chloroform had the smallest values. However, all four residual solvents in BA diluent had correction factors larger than one and acetone had the largest value. As shown in Table 3, BA is the only one among the four diluents that has both H-bond donor and acceptor. Therefore, it can be claimed that the unique matrix effect can only take place in a diluent that has no H-bond donor.

The correction factor of an individual residual solvent also relates to its own H-bonding ability. The H-bond acidity (α_2^H) and basicity (α_2^H) data published in the literature [25-27] are used to map the 18 residual solvents in Figure 4, including their correction factors in trichlormethiazide-spiked solution. These residual solvents span a wide range of H-bond acidity (α_2^H) and basicity (β_2^H) and can be basically divided into two regions. Alcohols and chlorinated compounds that have larger β_2^H values and/or smaller β_2^H values are classified in region I, in which they have smaller correction factors compared with those residual solvents in region II which have smaller α_2^H values and/ or larger β_2^H values, This classification shows a good agreement with the grouping of various residual solvents shown in Figure 2 and Figure 3. Based on the above discussion, it is obvious that the H-bonding

Residual Solvent˙	Trichlormethiazide	Diazoxide	Loratadine	Tolnaftate
Methanol	0.72	0.74	1.07	0.98
Ethanol	0.68	0.86	1.15	1.02
Isopropanol	0.64	0.86	1.22	0.98
1-Butanol	0.64	0.89	1.29	1.10
tert-Butanol	0.61	0.87	1.30	1.10
Methylene chloride	0.67	0.98	1.34	1.15
Chloroform	0.58	0.94	1.47	1.20
Heptane	0.78	1.13	1.75	1.25
Acetonitrile	0.78	1.02	1.13	1.07
Benzene	0.82	1.17	1.64	1.36
Toluene	0.80	1.21	1.79	1.43
Ethyl acetate	0.81	1.08	1.43	1.24
Ether	0.86	1.08	1.41	1.13
Acetone	0.89	1.06	1.29	0.99
Methyl ethyl ketone	0.87	1.13	1.42	1.26
Methyl isobutyl ketone	0.80	1.16	1.62	1.36
1,4-Dioxane	1.00	1.23	1.47	1.29
Tetrahydrofuran (THF)	1.02	1.24	1.59	1.37
Additive Concentration (mg/ mL)	800	700	800	500

Table 1: Correction factors of the residual solvents in trichlormethiazide, diazoxide, loratadine, and tolnaftate spiked DMSO solutions. ˙Concentration levels of residual solvents are 100, 300 and 1000 ppm, and correction factors were determined by the ratios of slopes. The reported result is the average from two replicate measurements.

Residual Solvent*	Additive					
	A	**B**	**C**	**D**	**E**	**F**
Methanol	0.76	0.75	0.79	0.81	0.85	0.74
Ethanol	0.73	0.74	0.77	0.79	0.83	0.86
Isopropanol	0.71	0.71	0.76	0.77	0.80	0.86
1-Butanol	0.64	0.67	0.72	0.71	0.78	0.89
tert-Butanol	0.70	0.69	0.74	0.75	0.77	0.87
Methylene chloride	0.72	0.75	0.84	0.82	0.80	0.98
Chloroform	0.64	0.68	0.77	0.75	0.72	0.94
Heptane	0.84	0.86	0.91	0.85	0.85	1.13
Acetonitrile	0.82	0.81	0.86	0.90	0.95	1.02
Benzene	0.83	0.87	0.95	0.94	0.94	1.17
Toluene	0.80	0.84	0.94	0.91	0.92	1.21
Ethyl acetate	0.84	0.85	0.89	0.88	0.94	1.08
Acetone	0.92	0.91	0.92	0.96	1.02	1.06
Methyl ethyl ketone	0.89	0.89	0.93	0.93	1.00	1.13
Methyl isobutyl ketone	0.81	0.82	0.88	0.85	0.92	1.16
1,4-Dioxane	0.92	0.96	0.99	0.98	1.07	1.23
Tetrahydrofuran (THF)	0.96	1.00	1.03	1.00	1.08	1.24
Average	0.80	0.81	0.86	0.86	0.90	1.03

Table 2: Correction factors of the residual solvents in hydroflumethiazide (A) trichlormethiazide (B) benzthiazide **(**C) meticrane (D) benzenesulfonamide **(**E) diazoxide and (F) spiked solutions. *Concentration levels of residual solvents are 100, 300 and 1000 ppm, and correction factors were determined by the ratios of slopes. The reported result is the average from two replicate measurements.

	Compounds	Acceptors	Donors
Additives	Trichlormethiazide	7	4
	Hydroflumethiazide	7	4
	Benzthiazide	7	3
	Meticrane	5	2
	Benzene sulfonamide	3	2
	Diazoxide	4	1
	Loratadine	3	0
	Tolnaftate	3	0
Diluents	NMP	2	0
	DMA	2	0
	DMSO	1	0
	BA	1	1

Table 3: The number of hydrogen bond acceptors and donors in individual additives and diluents.

Residual Solvent*	NMP	DMA	DMSO	BA
1-Butanol	0.63	0.86	0.71	1.31
Chloroform	0.50	0.57	0.63	1.09
Acetone	1.05	1.10	1.08	1.83
Tetrahydrofuran (THF)	1.07	1.12	1.15	1.41

Table 4: Benzenesulfonamide matrix effect on correction factors in different diluents. *Concentration levels of residual solvents are 100, 300 and 1000 ppm, and correction factors were determined by the ratios of slopes. The reported result is the average from two replicate measurements.

interaction among the additives, diluents, and residual solvents in the sample solution plays an important role in the observed matrix effect. However, other intermolecular forces, such as dipole-dipole, π-π, dipole-induced dipole, induced dipole-induced dipole, etc., may also contribute to the matrix effect.

Utilization of the matrix effect for residual solvent analysis

Because of the unique molecular structure of trichlormethiazide and its similar compounds, matrix effect caused by these compounds can be utilized to increase the sensitivity of residual solvent analysis in HS-GC method. One of the useful applications is to analyze 1,1-dichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane, which are defined as class 1 residual solvents by regulatory authorities. Because of their high toxicity, the class 1 residual solvents are strictly limited at a few ppm level in API, or drug product except for 1,1,1-trichloroethane. In addition to their trace level limits, these compounds have low responses on FID detector due to their chlorination, particularly for carbon tetrachloride. It is very challenging to enhance the sensitivity of class 1 residual solvents in HS-GC analysis using a FID system. The detector signals in the original analyte solution that contained approximately 2 to 5 ppm (μg/mL) each of 1,1-dichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane were 1.2 pA, 0.54 pA. 0.05 pA and 0.42 pA in peak height, respectively. After adding approximately 800 mg of trichlormethiazide into the analyte solution, the signals were significantly increased to 2.0 pA for 1,1-dichloroethene, 0.90 pA for 1,1,1-trichloroethane, 0.09 pA for carbon tetrachloride, and 0.69 pA for 1,2-dichloroethane. In other words, the matrix effect caused by trichlormethiazide increased the sensitivity of 1,1-dichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane by approximately 67%, 67%, 80%, and 64%, respectively. Figures 5 and 6 shows the comparison chromatograms of the residual solvent solutions with and without spiking trichlormethiazide in the sample solution.

In another example, we applied the matrix effect to analyze ethanol, isopropanol, *tert*-butanol, methylene chloride and chloroform in tolnaftate API solution. Because of the matrix effect caused by tolnaftate itself, the signal intensities of these residual solvents were reduced compared with the standard solution without tolnaftate. To improve the sensitivity, different amounts of trichlormethiazide were spiked into the tolnaftate sample solution. The sensitivities of ethanol, isopropanol, *tert*-butanol, methylene chloride and chloroform increased by approximately 24%, 29%, 33%, 24% and 35%, respectively, when trichlormethiazide was spiked at 500 mg/ml in the sample solution. When increased the trichlormethiazide concentration to 1000 mg/ml, the sensitivities of ethanol, isopropanol, *tert*-butanol, methylene chloride and chloroform increased by approximately 40%, 50%, 57%, 42% and 66%, respectively. Table 5 shows the summarized data.

Compared with the other similar techniques including "salting-out", the utilization of this matrix effect has the following advantages. First, the matrix effect can increase the sensitivity of most common
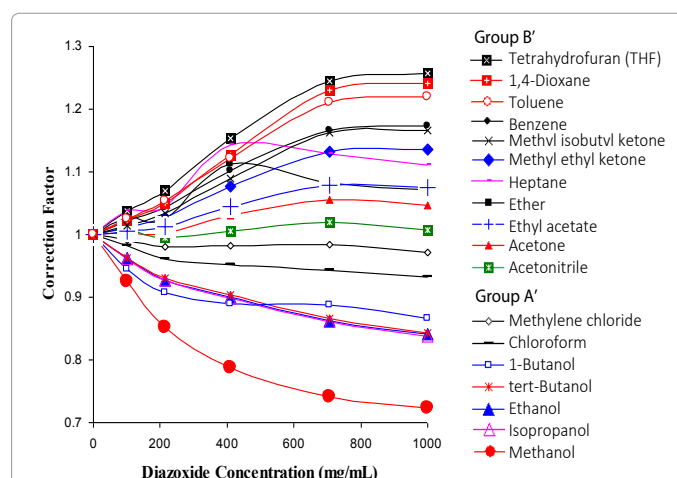

Figure 4: A representative correlation profile of the correction factors versus various diazoxide concentrations in DMSO diluent. The reported correction factor result is the average from three replicate measurements.
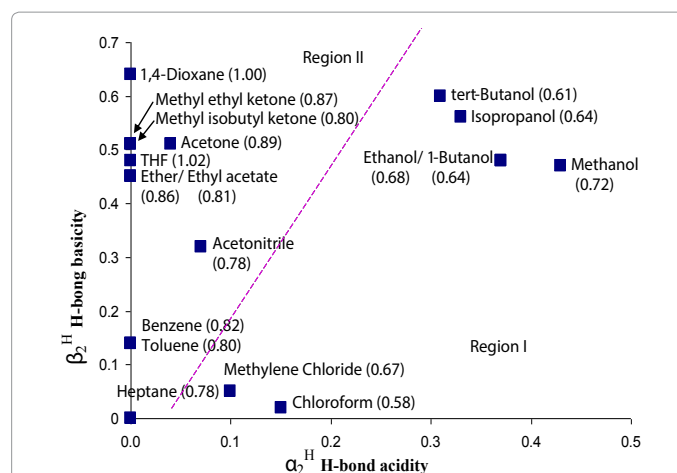

Figure 5: Hydrogen-bond acidity (α_2^H) and basicity (β_2^H) map of the residual solvents.
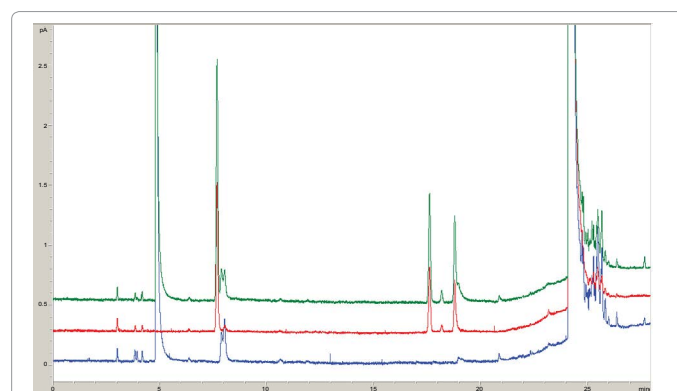

Figure 6: A comparison GC chromatogram of class 1 residual solvents by spiking trichlormethiazide; a) 800 mg/mL trichlormethiazide in DMSO as background; b) class 1 residual solvents standard solution containing 1,1-dichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, and 1,2-dichloroethane only; c) class 1 residual solvents standard solution spiked with 800 mg/mL trichlormethiazide.

Residual Solvents	% Signal increased by trichlormethiazide amount		
	0 (mg/mL)*	500 (mg/mL)	1000 (mg/mL)
Ethanol	100%	124%	140%
Isopropanol	100%	129%	150%
1-Butanol	100%	117%	134%
tert-Butanol	100%	133%	157%
Methylene chloride	100%	124%	142%
Chloroform	100%	135%	166%

Table 5: Enhancement of HS-GC signal of residual solvents in tolnaftate sample solution by spiking of trichlormethiazide. *The concentration of residual solvents is 300 ppm each, and tolnaftate concentration is 500 mg/mL. The reported result is the average from three replicate measurements.

residual solvents used in pharmaceutical productions except for few with strong H-bond basicity and low acidity. Second, unlike the "salting out" technique, it is possible to use this new approach to predict the effectiveness of the matrix effect based on the H-bonding abilities of the diluent, additive and residual solvents. In addition, the additives used in this study are compatible with high boiling-point organic diluents and there are no solubility issues in the residual solvent analysis. Finally, this approach might bring a new concept providing an alternative to enhance the sensitivity of residual solvents in HS-GC analysis. Since many compounds that are structurally similar to trichlormethiazide can also generate this type of matrix effect, it is possible to identify other similar compounds that may have much stronger matrix effect than trichlormethiazide and dramatically enhance the sensitivity of residual solvents analysis using HS-GC. As this study focuses on the root cause and potential applications of matrix effect, we do not include method evaluation or validation results here. However, critical method attributes such as accuracy, precision, specificity, sensitivity, and robustness have been demonstrated in our previous research [28,29].

Conclusion

A unique matrix effect caused by specific molecules (additives) has been identified. This matrix effect has the capability to enhance significantly the sensitivity of many common residual solvents in HS-GC analysis. The matrix effect generated by trichlormethiazide or by other compounds that are structurally similar to trichlormethiazide has the ability to decrease the partition coefficients (K) of most common residual solvents in high boiling-point diluents, such as DMSO, DMA and NMP, resulting in substantial increase of sensitivity in HS-GC analysis. The data obtained during our study is consistent with the proposed postulation on the molecular mechanism of the matrix effect, in which hydrogen-bonding interaction and competition among the additive, residual solvent and diluent play a key role. Application of this new approach would be most effective for analytes possessing strong H-bond acidity and/or weak H-bond basicity. Compared with the "salting-out" (which is limited for polar compounds) and other similar methods, the unique matrix effect reported in this paper is more advantageous and practical in terms of applicability and theoretical predictability (for a given set of additive, diluent, and residual solvents). Its utilization would readily enhance the sensitivity of residual solvents (specifically for alcohol and chlorinated solvents) in HS-GC analysis. To the best of our knowledge, this is the first paper reporting the enhancement of HS-GC sensitivity for residual solvents analysis using non-polar additives in the sample preparations.

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