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# Analysis of organic volatile impurities in pharmaceutical excipients by static headspace capillary gas chromatography

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**Abstract**

A systematic approach for the identification and quantification of organic volatile impurities (OVIs) in pharmaceutical excipients is described. Analytical procedures utilizing static headspace capillary gas chromatography coupled with flame-ionization and MS detection techniques were developed for the analysis of toxic ICH class 1 solvents and US Pharmacopeia OVIs at sub-ppm levels, and commonly used organic solvents in a wide range of concentrations. Chromatographic conditions and headspace parameters for the methods were optimized for separation, sensitivity, and speed. The proposed methodologies were demonstrated to be selective, accurate, and reproducible, and were successfully applied to the rapid screening of OVIs in typical excipients.


*Keywords:* Headspace analysis; Pharmaceutical analysis; Volatile organic compounds; Excipients

## 1. Introduction

There is a growing appreciation of the role that pharmaceutical excipients play in enhancing stability, bioavailability, and delivery of active drugs. Purity characterization of excipients is becoming increasingly important in the development and manufacture of pharmaceutical products as the quality of excipients can have a significant impact on the safety and efficacy of drug products [1,2]. Organic solvents, which are widely used in the production of excipients for synthesis and purification, may not be completely removed by practical manufacturing techniques. The residue of these solvents offers no therapeutic benefit, but can present a serious potential hazard to human health. Therefore, it is essential to ensure that the residual solvents (or organic volatile impurities (OVIs)) in excipients are below the acceptable levels stipulated in worldwide regulatory standards, such as ICH [3] and USP [4]. The use of some highly toxic/carcinogenic solvents (ICH class 1) has to be controlled to extremely low levels and demands extremely sensitive detection methods. Analysis of OVIs is known to be one of the most challenging analytical tasks in pharmaceutical analysis and control. Excipients present an even greater challenge due to the fact that information regarding the presence of OVIs in excipients may be difficult to obtain from a supplier who is unwilling to provide comprehensive data on the material because of fear that the information could be exploited by a competitor. Unknown OVIs are often detected during the routine quality control testing of OVIs in excipients using existing official methods, resulting in lengthy laboratory investigations which can cause costly delays in manufacturing. Hence, the situation necessitates the development of a rapid, sensitive, and reliable analytical method to screen, identify, and quantitate any OVIs in excipients.

The most appropriate method for analyzing organic volatile compounds is capillary gas chromatography (cGC) due to its unique features, which include extremely high separation efficiency, the availability of a sensitive universal flame ionization detection (FID) system for quantification, and the ability to be coupled with mass spectrometry (MS) for unknown identification. Static headspace-cGC (sHS-cGC) has become the preferred technique as it offers distinct advantages over the direct injection technique [5]. In headspace analysis, a liquid or solid sample is placed in a sealed vial, which is then thermostated until a thermodynamic equilibrium between the sample and gas phase is reached. A known aliquot of the gas phase is then introduced

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into the GC for analysis. Consequently, only volatile components are introduced into the sHS-cGC system, resulting in an extended lifetime of the column and reduced instrument maintenance. Automated sHS sampling also provides superior sensitivity and reproducibility.

Extensive research has been conducted on the headspace analysis of OVIs in pharmaceuticals and is well documented in the scientific literature [6–13]. However, most of the published studies have focused primarily only on USP OVIs (chloroform, 1,4-dioxane, dichloromethane, 1,1,2-trichloroethene) and/or commonly used ICH class 2 and 3 solvents, and no efforts to date have been made to address ICH class 1 solvents (benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, 1,1,1-trichloroethane), or excipients specifically. This work presents an evaluation of existing methodologies and proposes a systematic approach for the identification and quantification of OVIs in pharmaceutical excipients. It was the primary objective of the study to develop a simple and sensitive technique for the rapid analysis of ICH class 1 solvents, USP OVIs, and other organic solvents commonly used in chemical processes. For this purpose, two sHS-cGC procedures were developed. One method is a general screening procedure employing a 60 m × 0.53 mm i.d. Rtx-1301 capillary column and an FID system (sHS-cGC–FID), which can be used for the quantification of OVIs at or well below the ICH limits. In addition, an sHS-cGC–MS procedure employing electron impact (EI) ionization could be utilized for the identification of unknown OVIs. Development of chromatographic conditions and optimization of headspace parameters are discussed in detail. The procedures presented have been shown to be simple, sensitive, and reproducible. Typical pharmaceutical excipients have been analyzed using these methods to demonstrate the suitability of the proposed methodologies for their intended purpose for the control of OVIs in excipients.

## 2. Experimental

### 2.1. Chemicals

Solvents used were of ≥99% purity and were purchased from the following sources: methanol, acetone, acetonitrile, 2-propanol, ethyl acetate, tetrahydrofuran from Fisher Scientific (Fair Lawn, NJ, USA); ethanol from Aaper (Shelybgville, KY, USA); benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1,1-trichloroethane, chloroform, 1-propanol, *tert*-butanol, dichloromethane, 1,1-dichloroethene, 1,4-dioxane, hexane, toluene, 1,1,2-trichloroethene, and dimethyl sulfoxide (DMSO) from Aldrich (Milwaukee, WI, USA). All excipients used were pharmaceutical grade and were purchased from the following sources: butylated hydroxyanisole (Eastman), butylparaben sodium (Nipa), carbomer (B.F. Goodrich), citric acid (Spectrum), hydroxypropylcellulose (Hercules), lactose (Foremost), Mannitol (SPI Polyols), microcrystalline cellulose (FMC), magnesium stearate (Mallinckrodt), polyethylene glycol (Dow), poloxamer (BASF), polysorbates (Croda), propyl gallate (Clariant), sucrose (J.T. Baker), tartaric acid (Mallinckrodt), tromethamine (Angus Chemicals).

### 2.2. Instrumentation

The sHS-cGC–FID study was performed with a Hewlett-Packard (Agilent, Wilmington, DE, USA) Model 6890 series gas chromatography (total electronic pneumatic control of gas flow) equipped with a volatiles interface, an HP 7694 headspace autosampler, and an FID system. The headspace transfer line was directly connected to the volatiles interface. An auxiliary EPC (electronic pressure control) module was used for vial pressurization. Chromatographic data were collected and handled via the Multichrom Chromatographic Data management system (Thermo LabSystems, Cheshire, WA, UK) available in the laboratory.

The sHS-cGC–MS study was performed with a Thermo Finnigan Trace GC-PolarisQ system (Thermo Finnigan, San Jose, CA, USA) equipped with a capillary split–splitless inlet and COMBI-PAL liquid and headspace GC injection system (Leap Technologies, Carrboro, NC, USA). Chromatographic data were collected and handled via Thermo Finnigan Xcalibur Data System Software (Thermo Finnigan, San Jose, CA, USA).

### 2.3. sHS-cGC–FID instrumental conditions

The sHS sampling was performed with the HP 7694 headspace sampler. A 1 ml sample loop was employed. The headspace autosampler conditions were set as follows: oven temperature, 80 °C; transfer line temperature, 85 °C; loop temperature, 85 °C; vial equilibration time, 60 min; shaking (mixing) speed, high; loop fill time, 0.15 min; injection (or vent) time, 0.3 min; vial pressure, 10 psi (1 psi = 6894.76 Pa); pressurization time, 0.1 min; loop equilibration time, 0.05 min.

A 60 m × 0.53 mm i.d. Rtx-1301 column with 3.0 μm film thickness (Restek, Bellefonte, PA, USA) was utilized for chromatographic separation of the OVIs. The carrier gas was helium at a constant flow rate of 43 cm/s. The volatiles interface was maintained at 140 °C with a split ratio of 1:3. The FID system was set at 250 °C and nitrogen was used as the make-up gas. The column oven temperature program involved an initial temperature of 40 °C for 1 min, increased at 1 °C/min to 50 °C, then to 150 °C at 20 °C/min, then to 240 °C at 45 °C/min and held for 2 min.

### 2.4. sHS-cGC–MS instrumental conditions

The sHS sampling was performed with the COMBI-PAL headspace sampler. A 2.5 ml gas-tight syringe with a 26 gauge pt 5 needle was used (Microliter Analytical Supplies, Suwanee, GA, USA). Carryover in the gas-tight syringe was

eliminated by an automatic syringe flush performed after each injection. The headspace autosampler conditions were set as follows: incubation temperature, 80 °C; incubation time, 60 min; syringe temperature, 85 °C; agitation speed, 500 rpm; syringe injection volume, 1.0 ml; syringe fill speed, 0.5 ml/s; syringe injection speed, 0.5 ml/s.

A 60 m × 0.32 mm i.d. Rtx-1301 column with 1.0 μm film thickness (Restek) was utilized for chromatographic separation of the OVIs. The carrier gas was helium at a constant flow rate of 2.0 ml/min. The capillary split–splitless inlet was maintained at 140 °C with a split ratio of 1:10. The column oven temperature program involved an initial temperature of 35 °C for 4 min; increased at 10 °C/min to 50 °C and held for 2 min; then increased at 40 °C/min to 90 °C; then increased at 5 °C/min to 120 °C; and then increased at 40 °C/min to 200 °C.

MS scanning experiments were conducted using a solvent delay of 4.5 min and a mass range of 20–150 *m/z* with a scan time of 0.50 s per scan. The EI ion source was operated at electron energy of 70 eV. All other mass spectrometer conditions were set as follows: filament emission, 200 mA; ionization waveform, On; ion source temperature, 200 °C; transfer line temperature, 250 °C. The ion trap was calibrated using the autotune routine of the PolarisQ software.

### 2.5. Standard preparation

A stock standard solution of OVIs was prepared in DMSO at the concentrations presented in Table 1. A working standard was prepared by dilution of 5.0 ml stock solution to 100 ml with water. Headspace standard solutions (containing 0.8% DMSO) were prepared by transferring 1.0 ml of working standard and 5.0 ml of water into a 20 ml headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap (Agilent). The concentrations of working standards are listed in Table 1 along with the equivalent OVI contents in a sample that would be obtained by dissolving 50 mg of sample in 6.0 ml diluent (see Section 2.6). A series of standard solutions for method evaluation was prepared by diluting the stock standard solution with water. Quantification was performed by the method of external standardization.

Table 1
The concentrations of standard solutions

| OVI | Stock standard solution (mg/l) | Working standard (mg/l) | Equivalent concentration in sample (μg/g)[a] |
|---|---|---|---|
| Methanol | 1600 | 80 | 1600 |
| Ethanol | 1600 | 80 | 1600 |
| 1,1-Dichloroethene | 8 | 0.4 | 8 |
| Acetone | 400 | 20 | 400 |
| 2-Propanol | 400 | 20 | 400 |
| Acetonitrile | 400 | 20 | 400 |
| Dichloromethane | 50 | 2.5 | 50 |
| *tert*-Butanol | 400 | 20 | 400 |
| Hexane | 50 | 2.5 | 50 |
| 1-Propanol | 400 | 20 | 400 |
| Ethyl acetate | 250 | 12.5 | 250 |
| Tetrahydrofuran | 50 | 12.5 | 50 |
| Chloroform | 60 | 3 | 60 |
| 1,1,1-Trichloroethane | 10 | 0.5 | 10 |
| Carbon tetrachloride | 4 | 0.2 | 4 |
| Benzene | 2 | 0.1 | 2 |
| 1,2-Dichloroethane | 5 | 0.25 | 5 |
| 1,1,2-Trichloroethene | 80 | 4 | 80 |
| 1,4-Dioxane | 180 | 9 | 180 |
| Toluene | 25 | 1.25 | 25 |

[a] Concentration (μg/g) expressed on a weight basis relative to a 50 mg sample weight = {[working standard] (mg/l)/50 mg} × 1000.

### 2.6. Sample preparation

For water-soluble excipients, 500 mg of sample was transferred into a 50 ml volumetric flask and brought to volume with water. Headspace sample solutions (containing 0.8% DMSO) were prepared by transferring a 5.0 ml aliquot of the sample solution along with a 1.0 ml aliquot of 5% DMSO in water into a 20 ml headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap. For water insoluble excipients, 500 mg of sample was transferred into a 50 ml volumetric flask. A small amount of DMSO (0.5 ml) was added to dissolve the sample, and the resultant solution was brought to volume with water. Headspace sample solutions (containing 0.8% DMSO) were prepared by transferring a 5.0 ml aliquot of the sample solution along with a 1.0 ml aliquot of water were transferred into 20 ml headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap.

## 3. Results and discussion

### 3.1. sHS-cGC–FID method development

It is essential in the development of an sHS-cGC method for OVI analysis that the experimental conditions and instrument parameters for sample preparation, chromatography, and headspace sampling be carefully selected and optimized for selectivity, sensitivity, and speed [9].

#### 3.1.1. Sample preparation

Sample dissolution medium plays an important role in the sensitivity and accuracy of headspace analysis and requires careful consideration. Water has been found to be the best hydrophilic sample dissolution medium for OVI analysis because it is generally free of organic contaminants and gives no FID response [14]. In addition, water offers much higher sensitivities for most OVIs because of their relatively smaller partition coefficients in water than in organic media. Therefore, water has been used as a sample dissolution medium in most published sHS-cGC OVI procedures. However, water is not a good dissolution medium for hydrophobic samples which account for a significant portion

of the vast variety of excipients in use. Another problem encountered during the preliminary experiments in this study was that stock standards prepared with pure water exhibited poor solution stability for class 1 solvents. To avoid these problems associated with water, a small amount of DMSO (0.8%, see Sections 2.5 and 2.6) was then introduced in all standard and sample preparations. DMSO is commonly used for water-insoluble samples in OVI analyses because of its solubilizing properties and high boiling point [12]. Experiments conducted using standards containing different levels of DMSO indicated (data not shown) that no significant loss of sensitivity was observed when less than 8% DMSO was introduced in headspace solutions. The addition of DMSO not only resulted in acceptable solution stability, but also enhanced the extraction of OVIs from hydrophobic excipients. In addition, the use of co-solvents improved precision by limiting the loss of volatile analytes during standard preparation and handling.

Another important aspect to be taken into account for headspace analysis is the determination of sample size. It is well known that sample matrix may have a significant effect on the accuracy of sHS analysis and result in the need for a tedious standard addition quantitation technique [4]. Hence, the sample concentration in headspace analysis is usually kept very low to minimize the matrix effects so that quantitation may be performed by an external standard method, which significantly simplifies the analytical procedure. It has been reported that diluted aqueous samples (20 mg/ml) did not affect responses of solvent standards for sHS analysis [15]. Since many excipients exhibit low solubility in water, an even more diluted sample solution, 8 mg/ml, was employed in this study to ensure complete sample dissolution and full OVI recovery. However, the very small sample size selected inevitably resulted in low method sensitivity, and presented a great challenge to the subsequent cGC method development as discussed below.

### 3.1.2. Chromatographic conditions

Selecting an appropriate column is a critical factor in the success of GC method development, and can be a difficult and sometimes frustrating task considering the fact that hundreds of GC columns are now commercially available. Four major column parameters: stationary phase, internal diameter (i.d.), length ($L$), and film thickness ($d_f$), need to be carefully examined in terms of resolution, quantitation sensitivity, and analysis speed. The most popular stationary phases used for aqueous samples are methyl, phenyl, or cyanopropyl substituted polysiloxanes due to the fact that they exhibit a longer lifetime and are less susceptible to damage upon exposure to water vapor than other types of stationary phases. Differences in the polarity of the substituted groups and their relative amounts determine the selectivity of these stationary phases. Polysiloxanes with low polarity, such as (6%-cyanopropyl-phenyl)methylpolysiloxanes (RTX-1301, DB-624, etc.), have been found to be the best suited for the analysis of OVIs with a great variety of column dimensions and film thickness employed [6–15]. Columns with smaller internal diameters ($\leq$0.32 mm) offer higher separation efficiency and shorter run time, but have limited sample loading capacities. As a result, it would be difficult to achieve the high sensitivity required for the quantitation of the low levels of class 1 solvents in diluted sample solutions of excipients as discussed in the preceding section. On the other hand, capillary GC columns with a 0.53 mm i.d., commonly referred to as megabores, have the highest sample capacities while still maintaining the superior separation efficiency characteristics of capillary columns. Another advantage of using megabore columns pertains to the fact that they are preferred for headspace analysis, as they can be operated at higher carrier gas flow rates to reduce peak broadening due to dead volumes in headspace sampler components [9]. Film thickness can also influence separation efficiency and capacity of a capillary column. Thicker film columns are typically used for volatile compounds as increased retention results in better resolution for very volatile solutes. Thicker film columns also have higher solute capacities. Therefore, megabore (6%-cyanopropyl-phenyl)methylpolysiloxanes columns with the thickest film available were investigated in this study.

A preliminary study was conducted using a 30 m $\times$ 0.53 mm i.d. $\times$ 3.0 μm RTX-1301 column. In order to achieve the separation of all analytes of interest, an isothermal oven temperature program was used. The resultant chromatogram is shown in Fig. 1A. Although good resolution has been observed for most analytes, satisfactory sensitivity was not achieved for carbon tetrachloride ($CCl_4$) due to its low FID response and low concentration limit (4 ppm) as evidenced by a small, broad peak (No. 15) in the chromatogram. It was found that the sensitivity could be improved significantly by sharpening the $CCl_4$ peak with an oven temperature thermogradient (see Fig. 1B). However, co-elution of several analytes (Peaks 6–8, 12 and 13) were observed as a result of fast elution. Longer columns were then investigated to improve sensitivity for $CCl_4$ without losing the resolution. Fig. 1C shows the optimal separation achieved on a 60 m RTX-1301 column. Compared to the 30 m column, the 60 m column offered much better resolution for all solutes and still retained satisfactory sensitivity for $CCl_4$. A longer, 90 m column was also tried. A slight improvement in resolution was obtained, but with a longer run time. The 60 m $\times$ 0.53 mm i.d. 3.0 μm RTX-1301 column was chosen for further method development as it provided a good compromise between acceptable resolution and short analysis time. The final chromatographic conditions are given in Section 2.3.

### 3.1.3. Optimization of headspace parameters

There are many instrumental parameters of a headspace sampler that can affect the sensitivity and reproducibility of static headspace analysis. Common instrument parameters, such as oven temperature and vial equilibration time, exist for different types of headspace autosamplers. However,

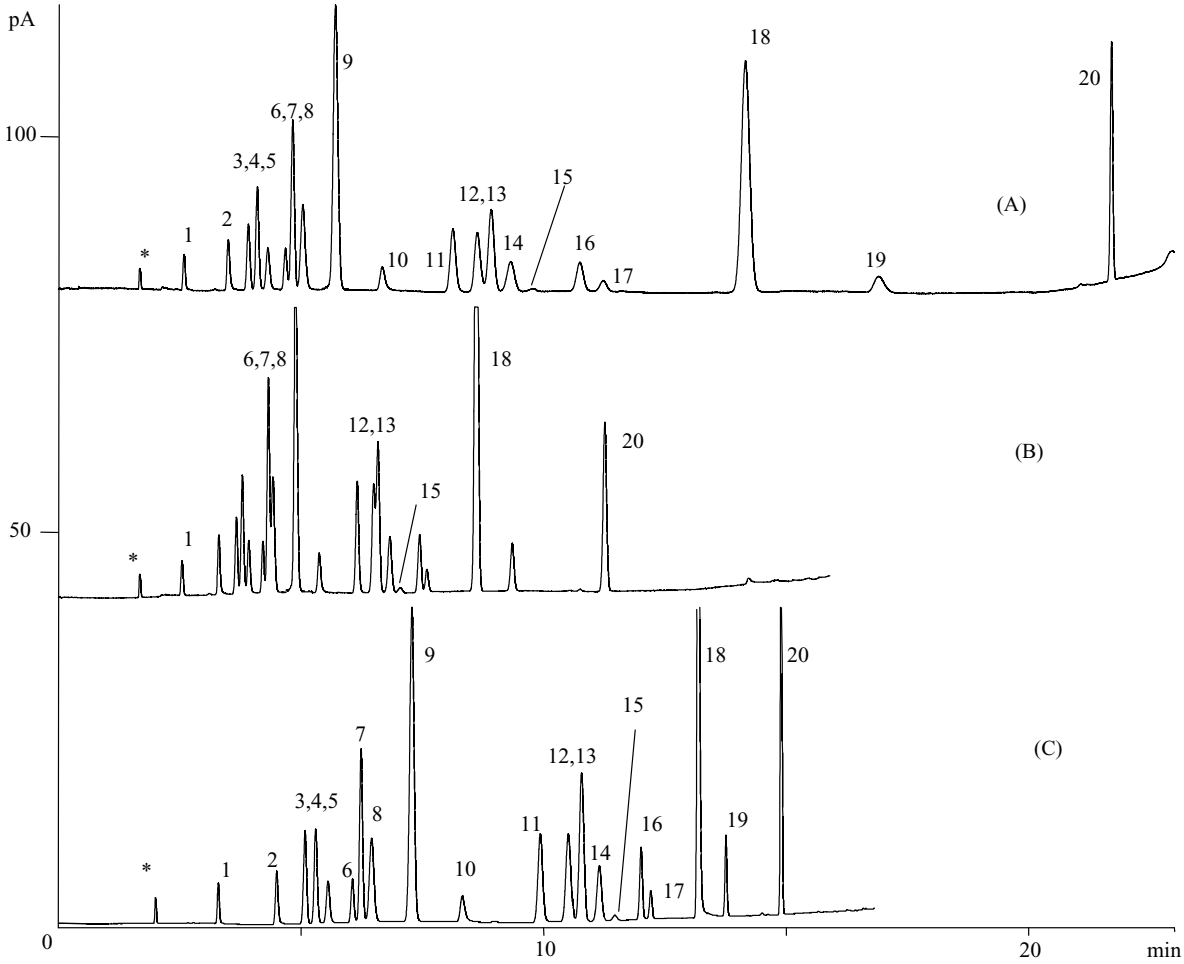


Fig. 1. sHS-cGC–FID chromatograms of working standard (see Section 2.5). Method conditions (injector, detector, and headspace sampler conditions given in Section 2.3). (A) Restek Rtx-1301, 30 m × 0.53 mm i.d., 3.0 μm, carrier gas as He at 35 cm/s measured at 40 °C, column oven temperature 40 °C for 20 min, then 40–240 °C at 40 °C/min. (B) Restek Rtx-1301, 30 m × 0.53 mm i.d., 3.0 μm, carrier gas as He at 35 cm/s measured at 40 °C, column oven temperature 40 °C for 1 min, then 40–120 °C at 5 °C/min, and then 120–240 °C at 40 °C/min. (C) Restek Rtx-1301, 60 m × 0.53 mm i.d., 3.0 μm, carrier gas as He at 43 cm/s measured at 40 °C, column oven temperature 40 °C for 1 min, then 40–50 °C at 1 °C/min, then 50–150 °C at 20 °C/min, and then 150–240 °C at 45 °C/min and held for 2 min. Peaks: (1) methanol, (2) ethanol, (3) 1,1-dichloroethene, (4) acetone, (5) 2-propanol, (6) acetonitrile, (7) dichloromethane, (8) *tert*-butanol, (9) hexane, (10) 1-propanol, (11) ethyl acetate, (12) tetrahydrofuran, (13) chloroform, (14) 1,1,1-trichloroethane, (15) carbon tetrachloride, (16) benzene, (17) 1,2-dichloroethane, (18) 1,1,2-trichloroethene, (19) 1,4-dioxane, (20) toluene. (∗ injection artifact).

each model also has its unique sets of parameters, as a result of different instrument configurations. The HP7694 HS autosampler used in this study employs a loop-based HS sampling device and the critical parameters needed to be considered are loop fill time, vial pressure, and pressurization time. For each parameter, the two most commonly employed setting values were selected after a survey among the published studies using HP7694 HS autosampler [9]. A series of experiments with eight sets of possible combinations of these settings was then designed and conducted to find the best set of conditions for optimal sensitivities and precision for ICH class 1 solvents. The results obtained are shown in Table 2. It is evident from the results that Sets 1 and 5 generated the highest OVI area responses. It can also be seen that higher vial pressure produced lower responses, probably due to the dilution of analytes in the gas phase at a high pressure. Set 1 exhibited slightly higher sensitivity for benzene while Set 5 produced better overall precision. The small difference in sensitivity might be due to experimental error. Therefore, the parameter settings of Set 5 were chosen for further studies.

For aqueous HS samples, oven temperature is normally set at either 80 or 85 °C (the maximum temperature allowed for samples containing a large amount of water). No significant difference in peak responses was observed between the two temperature settings during the experiments (data not shown). The lower oven temperature, 80 °C, was selected for this study to minimize any potential vial septum bleed and/or sample degradation [10], which may interfere with the chromatographic analysis of the low levels of OVIs. It has been demonstrated that the distribution equilibrium of OVIs between HS and aqueous sample solutions can be reached in less than 20 min [5,9]. A 60 min vial equilibration time was chosen in this study as the extended vial equilibration

Table 2
Optimization of headspace autosampler parameters

| | Experiment no. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Loop fill time (min) | 0.10 | 0.10 | 0.10 | 0.10 | 0.15 | 0.15 | 0.15 | 0.15 |
| Vial pressure (psi) | 10 | 10 | 18 | 18 | 10 | 10 | 18 | 18 |
| Pressurization time (min) | 0.1 | 0.5 | 0.1 | 0.5 | 0.1 | 0.5 | 0.1 | 0.5 |
| Peak response | | | | | | | | |
| Benzene | 11513 | 10954 | 10830 | 8918 | 11380 | 11215 | 10056 | 8303 |
| Carbon tetrachloride | 552 | 250 | 432 | 381 | 551 | 526 | 509 | 464 |
| 1,2-Dichloroethane | 10413 | 10026 | 9860 | 8203 | 10142 | 10004 | 9179 | 7759 |
| 1,1-Dichloroethene | 7196 | 7105 | 7020 | 5667 | 7147 | 7167 | 6446 | 5373 |
| 1,1,1-Trichloroethane | 7996 | 7671 | 7588 | 6261 | 7923 | 7827 | 7005 | 5850 |
| Mean R.S.D. (%)[a] | 4.4 | 3.9 | 3.2 | 2.8 | 2.3 | 2.4 | 3.3 | 5.6 |

[a] Mean R.S.D. is calculated by averaging the R.S.D.s obtained for five OVIs.

time improves sHS analysis precision and accuracy. The additional time for vial equilibration does not significantly increase the analysis time, which is only limited by the GC run time since the HP 7694 headspace autosampler allows the staggering of the start of the vial incubation time. The final sHS conditions are given in Section 2.4.

### 3.2. sHS-cGC–FID method evaluation

Representative sHS-cGC–FID chromatograms of the diluent blank, working standard, and samples using optimized experimental conditions are illustrated in Fig. 2. All analytes of interest are well resolved from each other and no

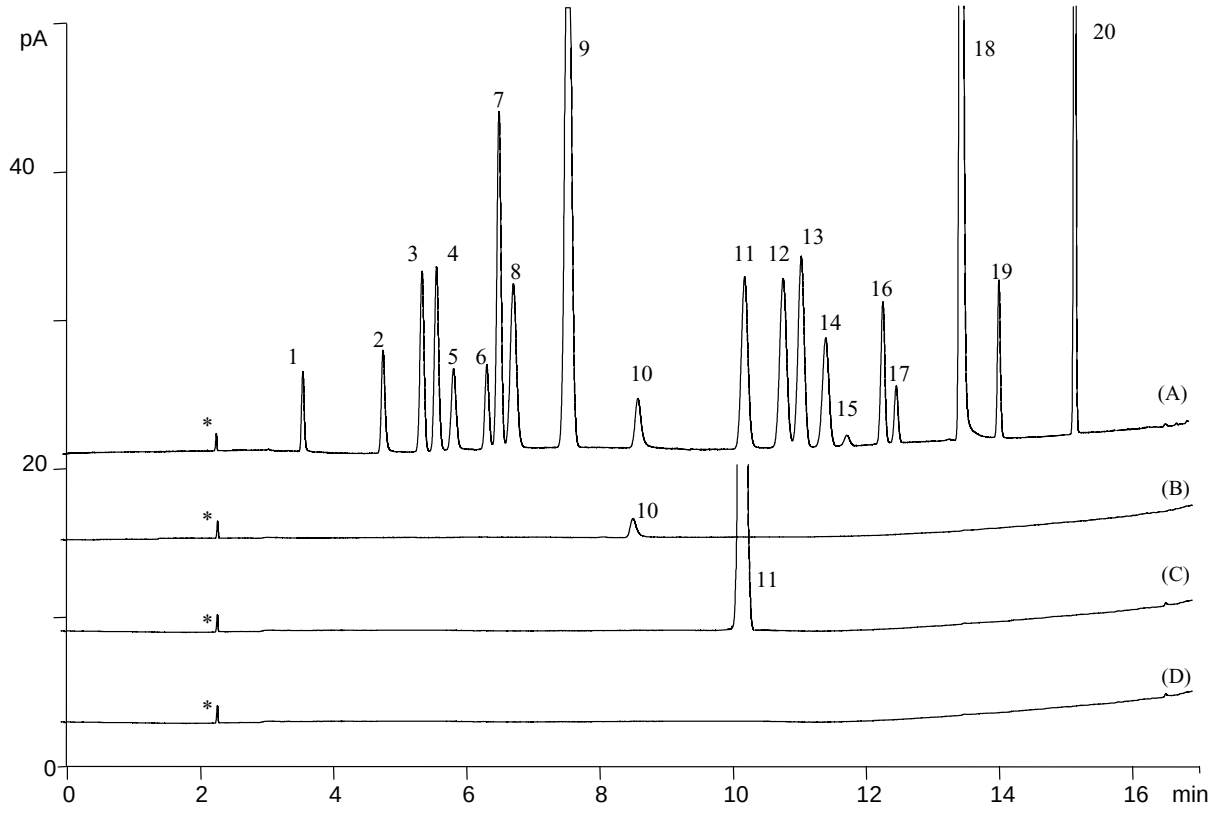


Fig. 2. sHS-cGC–FID chromatograms for analysis of OVIs in excipients. Method conditions are shown in Section 2.3. (A) The working standard (see Section 2.5). (B) A sample of propyl gallate. (C) A sample of carbomer. (D) The diluent blank. Peaks: (1) methanol, (2) ethanol, (3) 1,1-dichloroethene, (4) acetone, (5) 2-propanol, (6) acetonitrile, (7) dichloromethane, (8) *tert*-butanol, (9) hexane, (10) 1-propanol, (11) ethyl acetate, (12) tetrahydrofuran, (13) chloroform, (14) 1,1,1-trichloroethane, (15) carbon tetrachloride, (16) benzene, (17) 1,2-dichloroethane, (18) 1,1,2-trichloroethene, (19) 1,4-dioxane, (20) toluene (∗ injection artifact).

significant matrix peaks other than an injection artifact were observed in the blank chromatogram. No significant carry-over was observed for each analyte (data not shown). Complete separation of all OVIs was achieved within 16 min with a total GC cycle time of about 20 min compared to the typical 30–45 min run time needed for official methods [4].

The performance characteristics of the sHS-cGC–FID procedure were evaluated with respect to linearity, range, detection limit, precision, and accuracy. The linearity of the method was evaluated from triplicate injections of a series of standard solutions prepared for each analyte over the concentration range listed in Table 3. The concentration range for each analyte was from 10 to 1000% of the working standard. The upper end of concentration range for most ICH class 2 and 3 solvents was set below the ICH limits because historical data in excipient release indicated that the levels of these OVIs in the samples were for the most part well below ICH limits. In the rare case that individual OVIs fall outside the linear range, re-analysis can be easily performed by diluting the sample solution. Therefore, the linearity ranges in Table 3 were considered sufficiently wide for the intended purpose of the method. Results for linearity are summarized in Table 3 along with the determined detection limits (DLs) and method precision data. Each analyte showed excellent linear behavior over the examined concentration range with coefficient of determination ($R^2$) values of 0.9987–0.9999. The DLs were determined based on the standard deviation ($\sigma$) of the blank and the slope ($S$) of the calibration curve ($DL = 3.3\sigma/S$) as defined in the ICH guideline [16]. DLs as low as 0.1–0.6 μg/g (ppm) for class 1 solvents have been obtained. DLs for other analytes were also sufficiently low enough to enable estimation of OVI levels well below ICH limits. The lower end of the linear range is defined as the quantitation limit (QL) for each analyte, e.g. 1 ppm for $CCl_4$ and 0.2 ppm for benzene, demonstrating that the methodology provides outstanding overall sensitivity for the sHS-cGC–FID procedure. Method precision was assessed by evaluating the obtained relative standard deviations (R.S.D.s) of replicates for each analyte over the linearity ranges. All R.S.D.s observed (Table 3) are equal to or less than 3.6%, which is well below the USP mandated limit of R.S.D. 15% for OVI analysis [4], indicating that the method provides excellent precision for OVI HS analysis. The accuracy of the method was verified on several excipient samples spiked with known concentrations of OVIs, and was reported as percent recovery of the known amount of analyte added to the sample. Mean recoveries found for each OVI are given in Table 3. Recoveries obtained for each of the OVIs are all within 85–115%, indicating sufficient accuracy for trace analysis.

### 3.3. Pharmaceutical excipient sample analysis

#### 3.3.1. sHS-cGC–FID analysis

This newly developed sHS-cGC–FID method was applied to screen some of the commonly used excipients in pharmaceutical products for possible presence of toxic ICH class 1 solvents and USP OVIs. Sixteen excipients covering a wide

Table 3
Figures of merit for the sHS-cGC–FID method

| OVI | ICH limit (μg/g) | Linearity | | Precision (R.S.D. (%))[b] | Accuracy (Recovery (%))[c] | Detection limit[d] (μg/g[a]) |
|---|---|---|---|---|---|---|
| | | Range (μg/g[a]) | $R^2$ | | | |
| Methanol | 3000 | 160–16000 | 0.9997 | 2.2 | 103.9 | 48 |
| Ethanol | 5000 | 160–16000 | 0.9996 | 3.2 | 101.5 | 48 |
| 1,1-Dichloroethene | 8 | 0.8–80 | 0.9994 | 0.8 | 102.9 | 0.4 |
| Acetone | 5000 | 40–4000 | 0.9999 | 1.1 | 111.3 | 12 |
| 2-Propanol | 5000 | 40–4000 | 0.9998 | 1.7 | 101.8 | 12 |
| Acetonitrile | 410 | 40–4000 | 0.9995 | 3.6 | 95.4 | 12 |
| Dichloromethane | 600 | 5–500 | 0.9998 | 0.6 | 102.0 | 2 |
| *tert*-Butanol | 5000 | 40–4000 | 0.9999 | 1.1 | 100.9 | 12 |
| Hexane | 290 | 5–500 | 0.9987 | 1.0 | 85.7 | 2 |
| 1-Propanol | 5000 | 40–4000 | 0.9998 | 3.0 | 104.1 | 12 |
| Ethyl acetate | 5000 | 25–2500 | 0.9991 | 2.5 | 114.4 | 7.5 |
| Tetrahydrofuran | 5000 | 5–500 | 0.9999 | 0.9 | 100.7 | 1.5 |
| Chloroform | 60 | 6–600 | 0.9998 | 0.9 | 101.6 | 1 |
| 1,1,1-Trichloroethane | 1500 | 1–100 | 0.9996 | 1.1 | 99.2 | 0.6 |
| Carbon tetrachloride | 4 | 1–40 | 0.9996 | 3.1 | 96.4 | 0.4 |
| Benzene | 2 | 0.2–20 | 0.9998 | 2.7 | 101.0 | 0.1 |
| 1,2-Dichloroethane | 5 | 0.5–50 | 0.9999 | 2.2 | 92.6 | 0.2 |
| 1,1,2-Trichloroethene | 80 | 8–800 | 0.9998 | 0.7 | 101.9 | 3 |
| 1,4-Dioxane | 380 | 18–1800 | 0.9995 | 2.1 | 101.2 | 5 |
| Toluene | 890 | 2.5–250 | 0.9998 | 0.8 | 99.6 | 0.8 |

[a] Concentration expressed on a weight basis relative to a 50 mg sample weight (see Table 1 for calculation).
[b] Pooled R.S.D. ($n = 18$) of R.S.D.s at six concentration levels over the linearity range for each OVI [16].
[c] Mean of recovery (%) ($n = 9$) obtained for each OVI [16].
[d] DL = 3.3 × (standard deviation of blank)/(slope of calibration curve) [16].

Table 4
Pharmaceutical excipient sample analysis

| Excipients | | Residual solvents detected | | |
|---|---|---|---|---|
| Name | Formulation functionality | Class 1 solvents | USP OVIs | Others |
| Butylated hydroxyanisole | Antioxidant | None | None | Methanol (~140 ppm) |
| Butylparaben sodium | Antimicrobial preservative | None | None | none |
| Carbomer | Emulsifying/suspending agent | None | None | Ethyl acetate (1500 ppm) |
| Citric acid | Acidifying/buffering agent | None | None | None |
| Hydroxypropylcellulose | Coating agent/tablet binder | None | None | None |
| Lactose | Tablet/capsule diluent | None | None | None |
| Mannitol | Tablet/capsule diluent; sweetener | None | None | None |
| Microcrystalline cellulose | Tablet/capsule diluent; tablet disintegrant | None | None | None |
| Magnesium stearate | Tablet/capsule lubricant | None | None | None |
| Polyethylene glycol | Solvent | None | None | None |
| Poloxamer | Emulsifying/solubilizing/wetting agent | None | None | None |
| Polysorbates | Emulsifying/solubilizing/wetting agent | None | None | None |
| Propyl gallate | Antioxidant | None | None | 1-Propanol (80 ppm) |
| Sucrose | Tablet/capsule diluent; sweetener | None | None | none |
| Tartaric acid | Acidifying agent | None | None | none |
| Tromethamine | Alkalizing agent | None | None | Methanol (200 ppm); acetone (trace) |

range of formulation functionality were analyzed using the procedure. The screening results are summarized in Table 4. No detectable amounts of any ICH class 1 solvents or USP OVIs were found in any of the excipient samples examined. An ICH class 3 solvent, 1-propanol, was found at 80 μg/g (ppm) in a sample of propyl gallate. Another ICH class 3 solvent, ethyl acetate, was found to be present at a relative high level of 0.15% in carbomer. Both solvents were presumably used and not completely removed in the manufacturing process [17]. The ICH limit for class 3 solvents is 5000 ppm or 0.5%. Therefore, both materials met ICH requirements and were deemed to be suitable for pharmaceutical use. Methanol, an ICH class 2 solvent with a limit of 3000 ppm, was found in both butylated hydroxyanisole (~140 ppm) and tromethamine (200 ppm). A trace amount of acetone was also detected in the tromethamine sample. The origin of methanol and acetone in these two excipients was not known. Manufacturers confirmed that these solvents

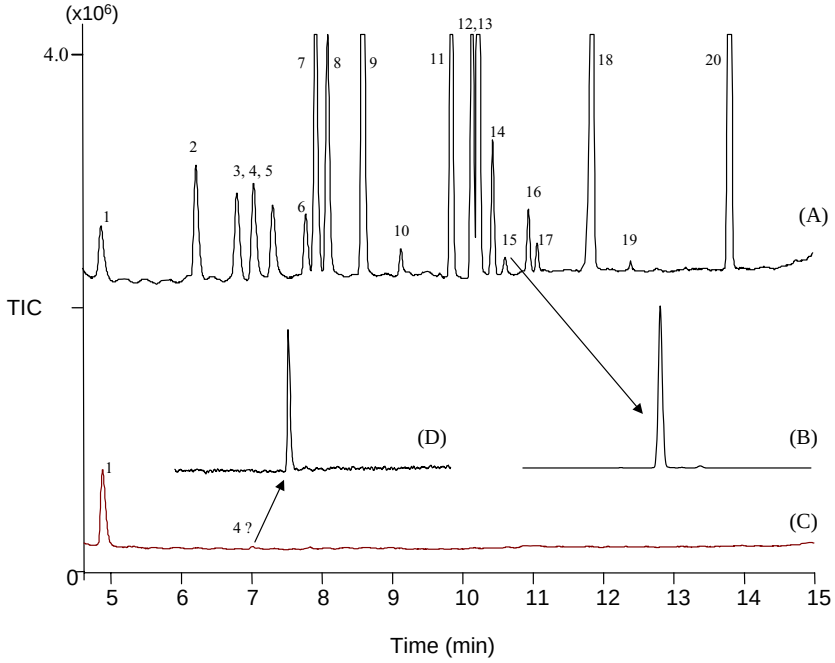


Fig. 3. sHS-cGC–MS chromatograms for analysis of OVIs in excipients. Method conditions are shown in Section 2.4. (A) Total ion chromatogram of the working standard (see Section 2.5). (B) Selected ion chromatogram of $CCl_4$ in standard. (C) Total ion chromatogram of a sample of tromethamine. (D) Selected ion chromatogram of acetone in tromethamine. Peaks: (1) methanol, (2) ethanol, (3) 1,1-dichloroethene, (4) acetone, (5) 2-propanol, (6) acetonitrile, (7) dichloromethane, (8) *tert*-butanol, (9) hexane, (10) 1-propanol, (11) ethyl acetate, (12) tetrahydrofuran, (13) chloroform, (14) 1,1,1-trichloroethane, (15) carbon tetrachloride, (16) benzene, (17) 1,2-dichloroethane, (18) 1,1,2-trichloroethene, (19) 1,4-dioxane, (20) toluene.

were not used in their production processes. It is possible that they might have originated in raw materials used in the manufacture of the excipients. The screening analyses were easily performed and completed in a short period of time, and all detected OVIs were clearly identified based on unique retention times for each OVI.

*3.3.2. sHS-cGC–MS analysis*

To further confirm the identities of the OVI peaks found in the sHS-cGC–FID screening analyses, an sHS-cGC–MS procedure was developed. Due to the limited content of this text, method development and optimization for GC–MS are not discussed. A 60 m × 0.32 mm i.d., 1.0 μm RTX-1301 column was employed so that an elution profile comparable to the sHS-GC–FID procedure could be obtained (see Fig. 3A) while the flow rate in the mass spectrometer was reduced to an acceptable level for MS vacuum pumps. An injection split ratio of greater than 10 was required to prevent band broadening, resulting in lower sensitivity for most OVIs in the scanning mode compared to the sHS-cGC–FID analysis. However, it was found that GC–MS could provide higher sensitivity for OVIs with low FID responses such as $CCl_4$. When combined with the selected ion monitoring (SIM) mode, the detectability for $CCl_4$ increased by a factor of ∼100. A typical SIM chromatogram of $CCl_4$ is shown in Fig. 3B along with a total ion comment (TIC) chromatogram obtained for the tromethamine sample (Fig. 3C). Since peak identification was the primary goal of the GC–MS analysis, a much larger sample size, 50 mg/ml, was used in an attempt to confirm the presence of a trace amount of acetone in the tromethamine sample. The acetone peak is almost undetectable in the TIC chromatogram, but clearly detected in the single ion chromatogram of 43 Da with a high signal-to-noise ratio. All OVIs identified by the sHS-cGC–FID procedure were confirmed by the GC–MS analyses. Peak identifications were conducted by a search of the mass spectrum of peaks obtained from the sample solutions against the US National Institute of Standards and Technology (NIST) mass spectral library. Although GC–MS is a powerful tool for unknown identification and offers excellent sensitivities in SIM mode, it has its disadvantages as a routine quality control (QC) procedure in that sophisticated and expensive MS instrumentation is not readily available in QC laboratories. Furthermore, the proper use of the instrument also demands a well trained and experienced operator. The results presented in this work demonstrate that, in the most cases, the sHS-gC–FID procedure can be an excellent means for fast and reliable identification and quantitation of OVIs in excipients without the necessity for more complicated hyphenated detection techniques.

## 4. Conclusions

A systematic analytical approach for the identification and quantification of organic volatile impurities in pharmaceutical excipients is described in this article. A simple general method utilizing static headspace capillary gas chromatography coupled with FID was developed and provided an effective means for rapid screening of organic volatile impurities in excipients. A 60 m Rtx-1301 megabore capillary column was found to provide the optimal chromatography for headspace analysis. Sample preparation and headspace parameters were optimized to enable the quantification by external standardization of ICH class 1 solvents, USP OVIs, and most common organic solvents. The sHS–cGC–FID procedure has been shown to be specific, sensitive, precise, and accurate. In addition, an sHS–cGC–MS procedure was developed for the identification of OVIs detected by the general procedure, and proven to be an excellent tool for peak identity confirmation. Typical pharmaceutical excipients were analyzed to demonstrate the suitability of the proposed methodologies for the qualitative and quantitative determination of OVIs in a wide range of concentrations from 100 ppb to 1.6%. All excipients examined were found to be free of any ICH class 1 solvents and USP OVIs. The other OVIs detected were quantitated to be well below the ICH limits. A systematic study of OVIs in excipients from various sources is beyond the scope of this work; however, it is expected that the presence and amount of OVIs in commercial materials will vary by manufacturer and should warrant continued comprehensive study using the presented methodologies.

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