

Organic Volatile Impurities by Headspace-Gas Chromatography

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Introduction

Many pharmaceutical products must be analyzed for organic volatile impurities (OVIs) at different stages of their development (raw materials, intermediate products and final product). One method of choice for OVI analysis is U.S. Pharmacopoeia method, USP<467>Method IV. The minimum detectable analyte levels currently specified by this method, which requires a 1000-fold sample dilution, are not difficult to achieve. However, some compounds are not soluble at this high a concentration and require further dilution. Others may be expensive or scarce, requiring lower detection limits to enable the use of less sample. Therefore, having a method that is both flexible and that can meet aggressive detection limits is desirable.

The purpose of these experiments was to develop analytical method parameters which could achieve a 1000-fold improvement in detection limits from those specified by the USP method. An improvement of this magnitude would allow the current detection limits to be met, while diluting the sample by an additional factor of 1000, consuming far less sample in the analysis.

Instrumentation and hardware setup

The OVI method described here was implemented using a PerkinElmer® TurboMatrix™ HS 40 Automated Headspace Sampler and a Clarus® 500 Gas Chromatograph (GC) configured with a Flame Ionization Detector (FID), as shown in Figure 1 (Page 2).

The sample effluent from the headspace vial is delivered to the analytical column via a deactivated fused-silica transfer line, which is threaded through a heated, hard, Teflon®-lined tube to prevent condensation of analytes and to protect the fused silica. The deactivated fused-silica transfer line can be connected to the analytical column in two ways, as follows:

- Introduced into the injector port where the effluent is allowed to enter the column via the injector port liner similar to a direct liquid injection. The column carrier gas can then be controlled via the GC Programmable Pneumatic Control (PPC) of the injector port; or
- Directly connected to the analytical column using a glass-lined or deactivated column connector. With this connection, a GC injector port is not required.

By attaching the analytical column directly to the transfer line, the analytes are completely transferred to the column. When using the injector port, the transfer line is placed into the center of the injection liner. Since the dead volume of the liner may cause band broadening, a PerkinElmer Zero Dilution Liner (Part Numbers N1011445 and N1011446) is recommended to prevent this.

The USP recommended column is a 6% cyanopropyl-phenyl/94% dimethyl polysiloxane (Elite-1301 – Part Number N9316687) 30 M x 0.53 mm x 3.0 µm column. In addition, the USP recommends a 5 M x 0.53 mm guard column be connected at the front of the analytical column, which also functions as a retention gap. When installing the transfer line directly to the analytical column, the guard column is unnecessary because the transfer line provides this function. In addition, with

this installation, one may attain chromatographic benefits (peak sharpening) by connecting a 2 M x 0.18 mm section of deactivated fused silica to the end of the analytical column by providing increased back pressure. Installation via the injector port would require the guard column to comply with the method. However, the 2 M x 0.18 mm section at the end of the column is unnecessary.

Experimental

Standards were prepared by weighing 0.0500 g of dioxane, trichloroethylene (TCE), benzene, chloroform and methylene chloride in a 50-mL volumetric flask, using methanol as the solvent (note: if methanol is an analyte, use dimethylformamide – DMF, as the solvent). A 0.500-mL aliquot of this 1000-ppm stock solution was diluted with deionized water in a 50-mL flask creating a 10-ppm

Table 1. Headspace Conditions.

Sample Temperature:	85 °C
Needle Temperature:	120 °C
Transfer line Temperature:	130 °C
Equilibration Time:	20 min
Pressurization Time:	1.0 min
Injection Time:	0.1 min
Withdrawal Time:	0.0 min

Table 2. Gas Chromatograph Conditions.

Initial Temperature:	40 °C
Time 1:	6 min
Rate 1:	2 °C/min
Final Temperature:	50 °C
Column Flow Rate*:	10 mL/min
Detector:	250 °C
Range and Attenuation:	1

*Note: When installing via the injector port, a pressure-pulsed injection is beneficial using a 12-mL/min column flow rate at injection for 0.5 min and reducing this flow to 6-mL/min analytical flow rate. The initial headspace pressure required is 15 psi.



Figure 1. TurboMatrix Automated Headspace Sampler (right) with the Clarus 500 Gas Chromatograph (left).

stock solution in water which was used for the remaining dilutions. A nine-point calibration was performed over the range of 0.1 ppb to 1000 ppb ($\mu\text{g/L}$). The concentration range used in this experiment is to demonstrate enhanced detection limits and dynamic range.

The headspace and gas-chromatograph conditions used for the analysis are outlined in Tables 1 and 2 (Page 2), respectively. Figure 2 represents a 45-ppb standard chromatogram analyzed under these conditions.

Experiments using a 10-mL sample volume and a 5-mL sample volume were conducted. In order to facilitate the release of dioxane from the water, a 40% sodium sulfate saturated solution is required. 4 grams and 2 grams of sodium sulfate were added to the vials, respectively. The results obtained from the 10-mL volume will be discussed first.

Results

The results for the 10-mL sample volume are documented in Table 3. The linear range and correlation coefficients were determined via a nine-point calibration curve between 0.1 ppb and 1000 ppb. Because of the different partition coefficients and detector responses of the

analytes, benzene saturated the detector above 400 ppb and dioxane's minimum detection limit (MDL) was 5.0 ppb using the 10-mL sample volume.

Table 3 also reports the precision, recovery and detection levels for all components using a 10-mL sample volume. Precision was investigated at a 45.0-ppb level, resulting in 1 to 2% RSDs. Recovery data was generated on three separate spikes, achieving 102 to 99% recoveries.

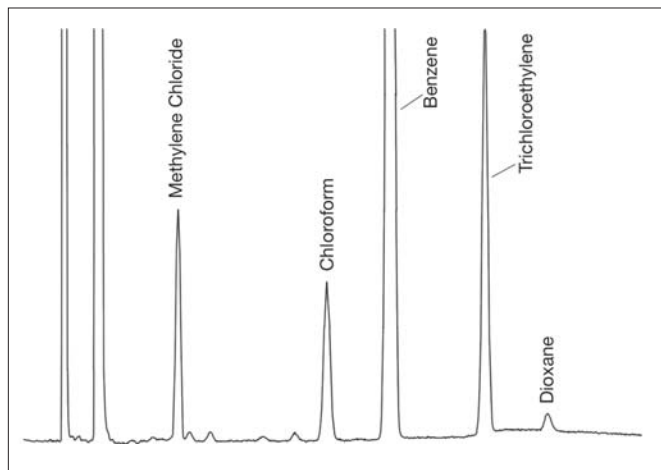


Figure 2. 45-ppb standard.

Table 3. Linearity and Repeatability for Various Recovery and Minimum Detection Limits (MDLs) for Various Components Using 10-mL Liquid Sample Volume.

Analyte	Linearity 0.1 to 1000 ppb Correlation Coefficient R ²	Repeatability at 45 ppb (n=6) % RSD	Average Recovery from 3 samples %	MDL ppb
Methylene Chloride	0.99989	1.5	102	0.5
Chloroform	0.99997	1.8	101	0.5
Benzene	0.99991	1.5	99.5	0.05
Trichloroethylene	0.99935	2.0	99.7	0.1
Dioxane	0.99995	1.5	99.7	5

Table 4. Linearity and Repeatability for Various Components Using 5-mL Liquid Sample Volume.

Analyte	Linearity 0.5 to 1000 ppb Correlation Coefficient R ²	Repeatability at 45 ppb (n=6) %RSD
Methylene Chloride	0.99993	0.5
Chloroform	0.99994	1.1
Benzene	0.99959	1.3
Trichloroethylene	0.99975	1.8
Dioxane	0.99891	1.8

Linearity and precision data for the 5-mL sample volume can be found in Table 4 (Page 3). The range was 0.5 ppb to 1000 ppb and 8 points were investigated. Benzene did not saturate the detector at this level. Data was collected on both volumes because the USP recommends a 5-mL sample size. However, one can enhance the detection limits more than 2-fold by using a 10-mL sample volume. Another way to attain lower detection limits is to use a sample temperature of 90 °C instead of 85 °C and a faster column flow.

Conclusions

We investigated USP<467>Method IV used for the determination of organic volatile impurities present at various stages of the pharmaceutical production process. We accomplished our goal of improving the detection limits of this method by a factor of 1000, which enables

lower detection of target analytes and/or allows for the reduction of the sample amount used.

In addition, the data generated in this study demonstrates that headspace-gas chromatography is a quantitative technique providing a dynamic range of 3-4 orders of magnitude, exceeding the requirements of the USP method. The data also demonstrates very low relative standard deviations, high precision and very good recoveries. In addition, this application enabled the analysis of analytes in matrices which are not otherwise amenable to gas chromatography.

Using the PerkinElmer headspace-gas chromatographic system, the analyst will enjoy an inert sample path with zero carryover. The technique of headspace-gas chromatography is an accurate, precise, fast and easy-to-maintain tool when investigating residual solvents in pharmaceutical matrices, including intermediates and end products.

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