

ANALYTICAL METHODS FOR RESIDUAL SOLVENTS DETERMINATION IN PHARMACEUTICAL PRODUCTS

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Abstract: Residual solvents (RS) are not desirable substances in the final pharmaceutical product and their acceptable limits have been published in pharmacopoeias and ICH guidelines. The intension of this paper was to review and discuss some of the current analytical procedures including gas chromatographic (GC) and other alternative techniques which are used for residual solvents determination. GC methods have been developed to monitor this kind of impurities routinely. The most popular techniques of sample introduction into the gas chromatograph include direct injection, static or dynamic headspace, solid-phase microextraction and single drop microextraction. Different separation techniques like two dimensional chromatography or multicapillary chromatography were compared with classical separation mode with use of single capillary column. Also alternative methodologies for residual solvents testing were discussed in this review. In conclusions, gas chromatography-based procedures were described as the most appropriate because of the lowest detection limits, ease of sample preparation and specificity.

Keywords: residual solvents, analytical methods, gas chromatography, headspace, solid-phase microextraction, single drop microextraction

Organic solvents are routinely applied during synthesis of drug substances, excipients, or during drug product formulation. They are not desirable in the final product, mainly because of their toxicity, their influence on the quality of crystals of the drug substance and their odor or taste, which can be unpleasant for patients. To remove them, various manufacturing processes or techniques (usually under increased temperature or/and decreased pressure) are in use. Even after such processes, some solvents still remain, yet in small quantities. These small quantities of organic solvents are commonly known as organic volatile impurities (OVIs) or residual solvents (RS).

The need to test and control for RS in pharmaceutical products was recognized in late 70's. In this time, US Pharmacopoeia (USP XX) and Great Britain Pharmacopoeia (80 + add 82) put specific tests for RS in some monographs (1). The OVI test <467> in USP was applied with no regard for the solvents used in manufacturing of pharmaceutical substances or drug products. This test was prepared for confirmation that benzene, methylene chloride, trichloroethylene, 1,4-dioxane and chloroform are below maximum acceptable limits (2).

In the early 80's, the "International Conference on Harmonization of Technical Requirements for

Registration of Pharmaceuticals for Human use" (ICH) was established. Then, in late 80's, RS were classified as impurities. Categories of residual solvents were presented in the preceding paper.

The ICH Harmonized Guidelines set up by the EU, Japan and USA for the registration of pharmaceutical products require, under ICH Topic Q3C (published in December 1997), for "testing to be performed for residual solvents when production or purification processes are known to result in the presence of such solvents". These guidelines set criteria for analytical methods used to identify and quantify these residual solvents as well provide acceptable concentration limits. USP has finally adopted the ICH Q3C guidelines set and has revised its general methods to reflect it. The new general chapter states what ICH Q3C contains, and in addition describes analytical methods for performing RS testing. Limits and different methods for determination of RS have been finally integrated in USP (1).

Methods accepted by pharmacopoeias and ICH guidelines

The first analytical method for RS, which was published in pharmacopoeias, was a loss of weight. This method could be carried out at normal pressure

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and/or under vacuum. The loss of weight is a simple and not demanding method, but apart from that it has many disadvantages, including lack of specificity, high limit of detection (about 0.1%), and also a relatively large amount of sample needed to perform the tests (about 1 – 2 g). Moreover, atmospheric humidity can significantly modify the results obtained by the loss of weight method. Nowadays, for this kind of determination, more sophisticated techniques like thermogravimetric analysis (TGA), differential thermal analysis (DTA) or differential scanning calorimetry (DSC), which will be described more precisely in later paragraphs, can be used (3).

Gas chromatography (GC), due to the volatility of organic solvents and the substantial separating capability of capillary columns, has dominated analytical methods for RS determinations. It is no wonder, that pharmacopoeias also adopted this technique for RS determination. The methods and their usage are described in general chapters and in individual monographs.

Current official methods for RS determination are described in USP XXXI in <467> chapter “Organic Volatile Impurities”. Under “Identification, control, and quantification of residual solvents” three procedures (A, B, C) for water-soluble and water-insoluble articles, are available. Procedures A and B are “useful to identify and quantify residual solvents, when the information regarding which solvents are likely to be present in the material is not available.” In cases when we have information about residues of solvents that may be expected in the tested material, only procedure C is needed for quantification of the amount of RS. All these methods exploit gas chromatography with capillary or wide-bore columns, static headspace injector, and flame ionization detector (FID). These procedures differ among themselves in column type (dimensions and film-coatings) and in chromatographic conditions. Sample preparation is also different for water-soluble and water-insoluble articles (4). The details concerning these general methods used for residual solvent determination, are presented in Tables 1 and 2. In Table 2 three different headspace conditions are shown. USP does not specify which HS conditions should be chosen, but it depends on the solvent that was chosen for preparation of the sample (water or N,N-dimethylformamide), residual solvents under analysis (high or low boiling) and analyzed material (thermally stable or unstable).

According to USP, determination of class 3 RS can be also done by loss on drying (USP <731> Chapter), as long as the total loss on drying is less

than the maximum acceptable limit for class 3 residual solvents (5000 ppm).

USP general procedures do not relate to specific solvents, but they try to compromise chromatographic and headspace conditions, in order to analyze all or the majority of organic solvents mentioned in chapter <467>. In this chapter there are also four other methods (Method I, IV, V, VI) for RS determination described. They concern determination of chloroform, 1,4-dioxane, methylene chloride and trichloroethylene, and they are suitable for both water-soluble and water-insoluble drugs. Methods I, V and VI are based on direct injection, whereas Method IV describes the static headspace injection. Method VI is general, and it refers to the individual monographs which provide necessary information about chromatographic conditions. Methods I, IV, V and VI were historically put in the USP earlier than procedures A, B, C. The main characteristics of methods I, IV, V and VI are summarized in Table 3 (4).

In European Pharmacopoeia (EP), 6th edition, under chapter 2.4.24 “Identification and control of residual solvents”, general methods for residual solvent determination can also be found. Two procedures (systems), A and B, are presented, and “System A is preferred whilst System B is employed normally for confirmation of identity.” Again, gas chromatography with headspace injection is proposed in both systems. Sample preparation relies on dissolution of 0.200 g of tested material in appropriate solvent, and diluting it to 20.0 mL with the same solvent. After that, 5.0 mL of such prepared solution is transferred to a headspace vial, filled with 1.0 mL of blank solution. For water-soluble samples, water is proposed as a solvent, for water-insoluble substances N,N-dimethylformamide is recommended, and for samples where the presence of N,N-dimethylacetamide and/or N,N-dimethylformamide is suspected, 1,3-dimethyl-2-imidazolidinone (DMI) is suggested as a solvent. In system A, chromatographic and headspace conditions are the same as in procedure A (Table 1) for water-soluble articles in USP <467> General Chapter. Also System B has its equivalent in USP: procedure B. In EP, as comprised with USP, a mass spectrometer (MS) or electron capture detector (ECD) have been additionally taken into account (apart from FID) (5).

Generally, all methods for quantitative determination of residual solvents taken from pharmacopoeias, need validation. For this reason, manufacturers try to find their own methods which would be (if possible) quicker, easier and adaptable to their specific samples and analytes.

Table 1. Tests for residual solvents determination (4)

		Procedure A	Procedure B	Procedure C
Sample preparation	Water-soluble articles	About 250 mg of the tested material dissolved in a 25 mL volumetric flask with water and then 5.0 mL of this solution transferred to head space vial filled with 1.0 mL of water		
	Water-insoluble articles	About 500 mg of the tested material dissolved in a 10 mL volumetric flask with N,N-dimethylformamide and then 1.0 mL of this solution transferred to head space vial filled with 5.0 mL of water		
Injection source		Head space sampler – operating parameters in Table 2		
Column type		30 m × 0.32 mm column coated with 1.8 µm layer of phase G43 ⁱ (<u>only for water soluble articles</u>) or a 30 m × 0.53 mm wide-bore column coated with a 3.0 µm layer of phase G43 ⁱ	30 m × 0.32 mm column with a 0.25 µm layer of phase G16 ⁱⁱ or a 30 m × 0.53 mm wide-bore column coated with a 0.25 µm layer of a phase G16 ⁱⁱ	30 m × 0.32 mm column coated with 1.8 µm layer of phase G43 ⁱ or a 30 m × 0.53 mm wide-bore column coated with a 3.0 µm layer of phase G43 ⁱ
Carrier gas		Nitrogen or helium		
Carrier gas velocity		About 35 cm/s		
Injection port temperature		140°C		
Split ratio		1:5 for water soluble articles and 1:3 for water insoluble articles (can be modified to optimize sensitivity)		1:5 (can be modified to optimize sensitivity)
Temperature program		40°C for 20 min, then raised to 240°C with rate 10°C per min and then maintained at 240°C for 20 min	50°C for 20 min, then raised to 165°C with rate 6°C per min and then maintained at 165°C for 20 min	40°C for 20 min, then raised to 240°C with rate 10°C per min and then maintained at 240°C for 20 min
Detector type		Flame ionization detector		
Detector temperature		250°C		

ⁱ 6% cyanopropylphenyl – 94% dimethylpolysiloxane, ⁱⁱ polyethylene glycol

Table 2. Headspace parameters (4)

	Headspace operating sets of parameters		
	1	2	3
Equilibration temperature (°C)	80	105	80
Equilibration time (min)	60	45	45
Transfer line temperature (°C)	85	110	105
Carrier gas	Nitrogen or helium at an appropriate pressure		
Pressurization time (s)	30		
Injection volume (mL)	1		

GC methods

GC is a natural choice, as means for RS determination, as organic solvents have relatively low boiling points and are generally thermally stable. However, different aspects of GC have to be considered, like injection systems, columns, and/or detectors. Modern analytical chemistry allows for selection of appropriate systems dedicated to specific groups of substances and samples, which results in shorter time of analysis and lower detection limits.

This richness of opportunities obliges the analysts to consider all available possibilities properly, and choose the best one, especially because of the fact that among pharmaceutical samples dedicated for RS determination, noticeable variety is present. The main concern, is how to convert a sample into a suitable form for introduction into the gas chromatograph. The choice of injection system, is determined by the sample type, the types of analytes, their quantity levels and available lab equipment.

Table 3. Tests for residual solvents determination (4)

	Method I	Method IV	Method V	Method VI
Sample preparation	Dissolved in water or other appropriate solvent to obtain concentration of about 20 mg/mL of tested material. (Additionally in Method IV 5 mL of sample solution is transferred to headspace vial.)			
Injection source	Direct injection	Headspace sampler	Direct injection	
Column type	30 m × 0.53 mm column coated with a 5 µm layer of a cross-linked phase G27 ^{III} with a 5 m × 0.53 mm guard column deactivated with phenylmethyl siloxane	30 m × 0.32 mm column coated with 3.0 µm layer of phase G43 ^{IV} with a 5 m × 0.53 mm guard column deactivated with phenylmethyl siloxane		One of nine columns listed under <467> and specified in monograph
Carrier gas	Helium			Appropriate to chosen column
Carrier gas velocity	About 35 cm/s			Appropriate to chosen column
Injection port temperature	70°C	70°C Additionally headspace vial sealed at 80°C for 60 min or as specified in individual monograph	140°C	Appropriate to chosen column
Split ratio	Not specified			
Temperature program	35°C for 5 min, then raised to 175°C with rate 8°C/min followed by raise to 260°C with rate 35°C/min and then maintained at 260°C for at least 16 min	40°C for 20 min, then increased rapidly to 240°C and then maintained at 240°C for 20 min		Appropriate to chosen column
Detector type	Flame ionization detector			
Detector temperature	260°C			Appropriate to chosen column

^{III} 5% phenyl – 95% methylpolysiloxane, ^{IV} 6% cyanopropylphenyl – 94% dimethylpolysiloxane

Direct injection

The direct injection technique, is used when the sample is introduced in a liquid form, *via* syringe into an inlet liner placed in a heated injection port and then vaporized. This technique is employed when the tested sample is soluble in low boiling organic solvents (dissolution media), and all additional sample components also evaporate in relatively low temperature. During direct injection system exploitation port liner have to be regularly inspected, as well as timely replaced, to minimize analyte–drug matrix interactions in the injection port liner, which may lead to poor peak shape, column deterioration and irreproducible results (6).

Usually, direct injection appears useful if RS are determined in a drug substance. In cases of analysis of the final products like tablets, syrups, injections etc., components of a drug matrix may not be

vaporized, or may not dissolve easily in the dissolution media applied. All these problems can be avoided by extensive sample preparation techniques. These techniques rely on extraction and pre-concentration of analytes from non-volatile matrix (7).

One can distinguish between different variants of direct injection systems, like split, splitless, on-column and programmed temperature vaporizing (PTV).

Split injection is used for volatile to semi-volatile compounds, and is one of the easiest injection techniques. With this technique, the flow of carrier gas is split between the capillary column and atmosphere *via* the “split vent” (Figure 1). It is a robust method, due to high carrier gas flows, but sensitivity is reduced because of splitting of the sample.

Splitless injection systems do not split carrier gas to atmosphere (Figure 1). This is not truly split-

less, since it is only in this state for a period of time (0.3 – 1.0 min), and then the valve is opened to a split mode. This technique is used when residual solvents remain in the sample at low concentration levels, due to increased sensitivity when compared to split injection. Greater sensitivity is achieved as most of the injected sample is transferred to the capillary column. One of the disadvantages of this technique is caused by low carrier gas flow, which forces a need to focus some peaks.

On-column and PTV injection systems belong to Large Volume Injection (LVI) techniques. Both techniques allow detection limits to achieve at part per trillion (ppt) level (9). On-column injection system is when the sample is injected onto a pre-column, and then the solvent is vented, leaving only the analytes to be transferred onto the analytical column for separation (Figure 2). In the PTV system, after the sample injection, the solvent is evaporated at a low temperature in a packed chamber, and then removed through the split vent. This leaves the solutes on the packing or inlet liner wall. When the injection port is heated, the analytes are transferred onto the column (Figure 3). This technique has many parameters that need to be optimized, therefore, it is more time consuming than on-column injection system.

Headspace

As mentioned earlier, for samples with difficult matrices that contain non-volatile or degradable substances, which can remain on GC column and make it deteriorated, or create interfering peaks from volatiles during degradation, extraction and pre-concentration should be done. However, these activities are very expensive and time consuming. That is why other sampling techniques like headspace analysis (HS) and/or solid-phase microextraction can be used. They rely on the indirect way of introducing analytes into a gas chromatograph. Moreover, in both methods, sample preparation is quicker and simpler. The headspace analysis, is an extraction technique for semi volatile and volatile compounds, and generally can be divided into two forms: static and dynamic. In static (equilibrium) headspace a single aliquot of a gas is collected over a liquid, or sometimes a solid sample, placed in a sealed and heated vial and then transferred to GC (Figures 4, 5). A gas sample is collected after the equilibration between gas and liquid (or solid) phase is reached. This method is preferred when the pharmaceutical samples are soluble in solvents, such as water, DMSO, DMA, DMF, DMI or benzyl alcohol.

Generally, static headspace analysis is the most widely used technique for RS determination in phar-

maceuticals. This fact comes from some of the advantages of this technique, mainly that only volatile substances and dissolution medium can be injected onto the column. Also HS systems are fully automated, in addition, a sample preparation is easy, and the sensitivity of analysis is sufficient for the majority of solvents mentioned in ICH guidelines. If necessary, the sensitivity can be improved by appropriate solvent (dissolution medium) selection, e.g. for polar analytes, non-polar solvents like DMSO or DMF can be used. Also inorganic salt addition, pH control, increasing the equilibrium temperature or controlling the ratio between gas and liquid phase can be fitted to improve sensitivity. The crucial point of the static headspace when solid samples are analyzed, is the establishment of an equilibrium between the solid phase and the gas phase in the sample vial, which may not be reachable due to matrix effects, such as adsorption forces (13). These problems can be solved by addition of appropriate solvents, which can displace analytes from the matrix. More details concerning the sensitivity, range and precision that can be obtain with the use of HS sampling system are presented in Table 4.

Static headspace analysis has different variants. One of the modified versions of the static headspace chromatography, is multiple headspace extraction (MHE). It relies on sampling a heated vial many times, summing the peak areas obtained from each extraction (which decreases exponentially), and determining the amount of RS in the tested sample from the external calibration curve. The sum of the amounts of the analyte removed in the individual extractions, should be equal to the total amount of analyte present in the original sample. In practice, only a limited number of extractions are performed, and the total amount of analyte is obtained by extrapolation, based on the mathematical relationships originally described by McAuliffe (14) and Suzuki (15) Primarily, this technique was dedicated for the determination of RS in solid samples (16), but it can be successfully applied to liquid samples, especially when the partition coefficient of the RS is favorable and relative to the liquid phase. According to some authors (17), this technique fell out of use, because time spent on sampling a heated headspace vial many times is more time consuming than optimization of equilibration time in classic static headspace.

A dynamic headspace often called “purge and trap” technique, joins some properties of static and multiple headspace extraction. It is based on flushing of the thermostatted sample with an inert gas, and transferring of the released volatiles onto a trap. The

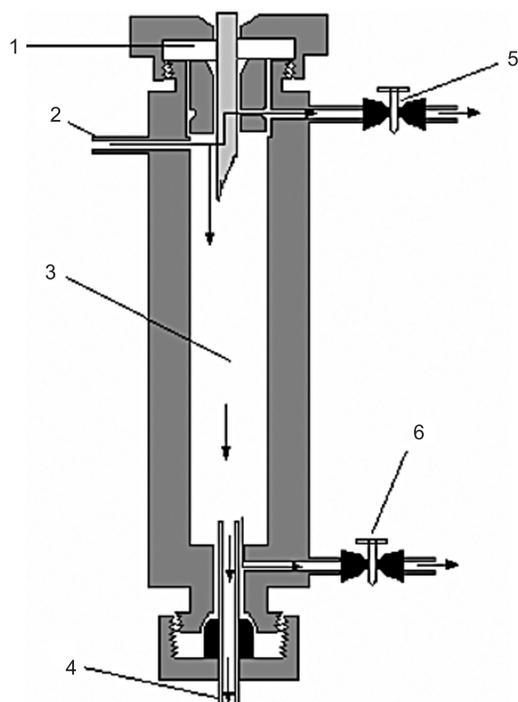


Figure 1. Diagram of the standard GC injector which can be used as split (when split valve is opened) or splitless injector (when split valve is closed); 1) septa, 2) carrier gas inlet, 3) injector cell, 4) capillary column, 5) top outlet (rinsing septa), 6) bottom outlet (splitting) (9)

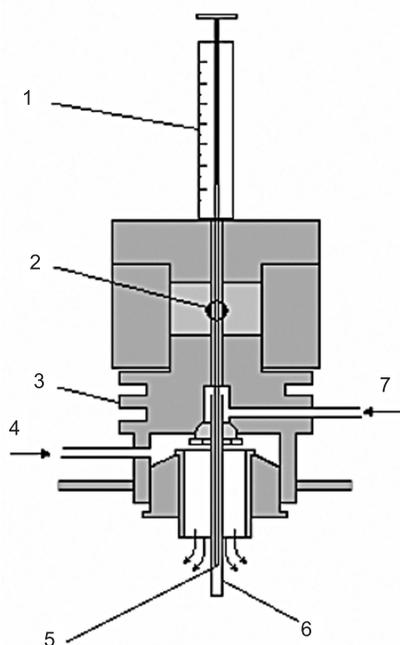


Figure 2. Diagram of the on-column injector; 1) microsyringe, 2) ball valve, 3) the main cooling system, 4) secondary cooling, 5) needle, 6) capillary column, 7) carrier gas inlet (11)

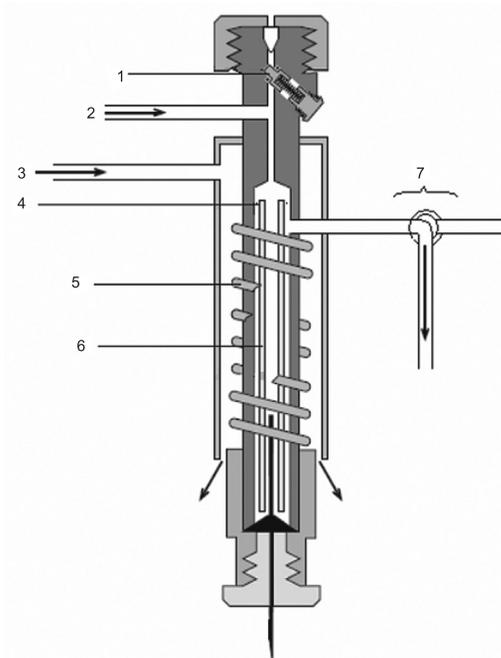


Figure 3. Diagram of the PTV injector 1) septumless sampling head, 2) carrier gas line, 3) coolant, 4) liner seal, 5) heating coil, 6) liner, 7) split/splitless solenoid (12)

trap usually consists of a column, containing a sorbent resin. A thermal desorption cycle of the trap is initiated then, and a carrier gas takes the volatiles into the gas chromatograph for further analysis. Cold trapping (cryofocusing), which follows thermal desorption, is sometimes used to increase the quality of peak shapes. A major advantage of this technique is that a thermodynamic equilibrium is not necessarily needed, and the sensitivity of the method is increased by enrichment of the analytes on the trap. Consequently, detection limits reported for dynamic headspace are lower (pg/mL) than those obtained with static headspace (ng/mL) (18). However, dynamic HS can imply the risk of higher uncertainty when compared with static HS, due to the fact that the sample amount is a quarter lower than in static version. Therefore, sample inhomogeneity may have more significant influence on the results of analysis. The static extraction procedure offers additional advantages in sample handling. It does not require very small particle size, which is necessary to put the sample into the extraction device of the dynamic headspace. On the other hand, dynamic headspace is favorable when analytes are present at low limits.

Solid-phase microextraction

Apart from the direct injection and the HS sampling systems, several other techniques dealing

with injection problems have been developed. Among them solid-phase microextraction (SPME) has to be mentioned. In SPME, the analytes are extracted into the stationary phase, which is attached to a length of fused silica fiber (18). The fiber is mounted in a syringe-like holder called an SPME fiber assembly, which protects the fiber during storage and penetration of septa in the sample vial and in the GC injector (Figure 6.). When the equilibrium between the stationary phase (fiber) and the liquid phase or its headspace phase is reached, then the analytes adsorbed on the fiber are thermally desorbed in the injector of the GC and transferred onto the column. The selectivity of the fiber can be modified by changing the phase type or thickness according to the characteristics of the analytes.

In general, two types of SPME extractions can be performed. In the first version of SPME the fiber is immersed in the liquid matrix and the analytes are directly transported to the extracting phase. It is called the "immersion" or "direct extraction" version of SPME. In the second mode, the volatile analytes after extraction to the headspace phase above the sample matrix, are then extracted to the solid phase on silica fiber. This second resolution helps to protect the fiber coating from damage by high-molecular-mass and other non-volatile interferers present in the sample matrix (19) and is usually used for pharmaceutical RS analysis. Even if SPME is not accepted as a sample preparation method by

pharmacopoeias, many authors mention it as a solventless and a valid alternative to HS-GC for RS determination in pharmaceuticals (20-22). According to some authors (17), detection limits for most frequent residual solvents analyzed by SPME, are about ten times lower than for static HS. Additional comparison of results obtained with headspace SPME is presented in Table 4.

Furthermore, in SPME technique when analytes are extracted from the headspace phase, an additional two systems can be distinguished: gas-tight SPME, where only a small volume of headspace gas is removed with the SPME fiber and a "headspace" SPME where larger volume of a head-

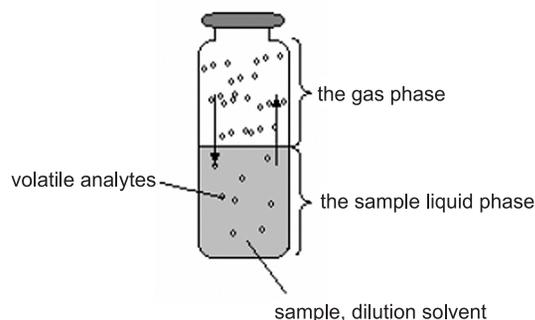


Figure 4. Phases of the headspace vial

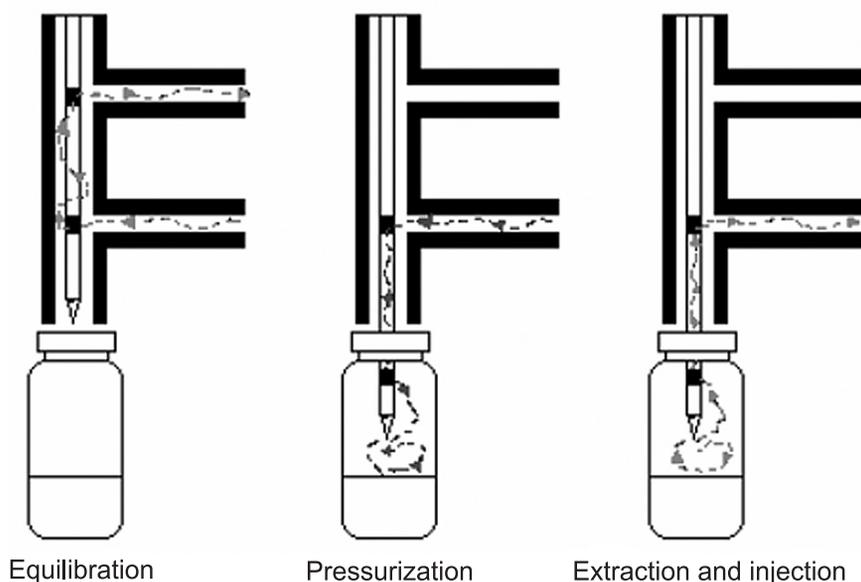


Figure 5. Diagram of static headspace balanced-pressure sampling system (13)

space gas is removed with SPME fiber. When both of them are compared, gas-tight SPME gives better sensitivities (lower LOD) and better peak shapes, especially when very volatile substances are analyzed (such as aldehydes, ketones or some hydrocarbons and alcohols). On the other hand, headspace SPME gives better precision (17).

SPME-GC system, coupled with mass spectrometer (MS) detector, is a very powerful instrument for the identification and determination of unknown solvent residues in pharmaceuticals (17).

One of disadvantages of SPME technique, is a complicated and time consuming optimization of analytical conditions. Many factors, apart from headspace conditions, like: fiber type, extraction mode, extraction temperature, extraction time, desorption time and temperature have to be considered. It has a special importance in the pharmaceutical industry, where the time and money spent on analysis are important factors. Furthermore, the SPME fiber extraction efficiency might change with the wear (number of injections) and at the same time SPME fibers sometimes show poor inter-batch reproducibility of sensitivity (22). This fact reduces the applicability of the headspace SPME in routine determinations of residual solvents in pharmaceutical products.

However, due to SPME precision and accuracy, it is after static HS and direct injection, a very popular injection technique for RS determination in pharmaceuticals especially for identification purposes when it is joined.

Single drop microextraction

Another injection technique that relies on an indirect way of introducing analytes into a gas chromatograph, is a single drop microextraction (SDME), known also under the term liquid microextraction (LME). It is often used as a simple and inexpensive alternative to the SPME technique (23) because it does not require any complicated equipment, only a typical microsyringe and a small amount of organic solvent. This relatively new technique combines classic liquid extraction and solid phase microextraction. It uses a small volume of solvent suspended as a drop at the end of the microsyringe needle in the headspace phase over the sample solution (Figure 7).

A drop size is preferred to be in the range 1 – 3 μL , what means that the surface of a liquid drop is larger than the surface of an SPME fiber and the extraction process is relatively faster (24). The extraction surface of the drop is critical for the analysis. When the drop is bigger the extraction effi-

ciency is higher, but also the stability of such a drop (loss of four drops out of ten) is lower and the reproducibility (CV 60%) decreases. There are many solvents with different polarities that can be used as extracting solvents. However, the boiling point of a candidate should prevent its evaporation during sampling (25).

A comparison of SDME and SPME in determination of RS in edible oils and pharmaceutical products, shows that the precision of both techniques is similar, but the linear range is much narrower for SDME than for the SPME procedures, and detection limits are higher (23) (Table 4). Like SPME, SDME also needs many parameters to be carefully optimized including the following: extraction solvent, size of drop, shape of needle tip, extraction time, extraction temperature, headspace volume. This process can be a time consuming and sometimes demanding task. This technique is promising in the determination of RS in pharmaceuticals, but for routine industrial use it is necessary to investigate the possibility of automation.

Within the entire analytical scheme, sample preparation is often the most time consuming and challenging step, particularly when RS are present at trace concentration levels in the presence of complex matrices. When it comes to decide which injection technique is the best, there is no one correct answer due to the variety of samples and analyses types. Experience of the analyst and the knowledge of properties of tested samples should help to choose the best solution.

In searching optimal conditions of GC analysis, apart from the injection mode, the separation and detection system should also be carefully selected.

GC separation mode

For separation step, capillary (narrow-bore) and wide-bore columns (known also as open tubular columns) are used. Thanks to their separation possibilities and small sample capacity, they practically superseded packed columns. Capillary columns consist of a long tube that can be made of metal, glass or quartz, the diameter of which may be in the range from 50 to 500 μm , and the length usually from 5 m to 200 m. The capillary is coated inside with a thick polymeric film of the stationary phase. There are numerous stationary phases. Most of them are high molecular weight, thermally stable polymers that are liquids or gums. The most common stationary phases of this type are the polysiloxanes and polyethylene glycols. Another common type of stationary phases, are small porous particles composed of polymers or zeolites (e.g., alumina, molecular

Table 4. Comparison of methods for RS determination with use of different injection systems (23, 26, 27)

Compound	Static headspace (27)			Headspace SPME (CAR-PDMS) ⁱⁱⁱ , (PDMS-DVB) ^{iv} (24)			SDME ^v (28)		
	LOD (mg/kg)	Range* (mg/kg)	Repeatability (%) (1 mg/kg)	LOD (mg/kg)	Range* (mg/kg)	Repeatability (%) (1 mg/kg)	LOD (mg/kg)	Range* (mg/kg)	Repeatability (%) (1 mg/kg)
Hexane	0.004 ⁱ	0.125 – 2, 2-5	2.5	0.002 ⁱ	0.008-0.5, 100-1000	4.7	0.30 ⁱ	0.90-1000	19
Benzene	0.009 ⁱ	0.125 – 2, 2-5	3.6	0.002 ⁱ	0.008-0.5, 100-1000	3.6	0.37 ⁱ	1.1-1000	25
Toluene	0.017 ⁱ	0.125 – 2, 2-5	5.2	0.002 ⁱ	0.008-0.5, 100-1000	5.1	0.11 ⁱ	0.33-1000	16
Trichloromethane	0.008 ⁱⁱ	0.008 – 1, 0.8-3	4.8	0.001 ⁱⁱ	0.008-0.125, 0.125-1, 1-8	2.7	0.0030 ⁱⁱ	0.0090-10	6.0
1,1,1-Trichloroethane	0.008 ⁱⁱ	0.008 – 1, 0.8-3	5	0.003 ⁱⁱ	0.004-0.06, 0.06-1, 1-8	3.7	0.0042 ⁱⁱ	0.013-5.6	8
Tetrachloromethane	0.008 ⁱⁱ	0.008 – 1, 0.8-3	3.5	0.0002 ⁱⁱ	0.002-0.016, 0.016-0.5, 0.5-2, 2-8	1.9	0.0010 ⁱⁱ	0.0030-1	7.2
Trichloroethane	0.008 ⁱⁱ	0.008 – 1, 0.8-3	2.6	0.0006 ⁱⁱ	0.002-0.008, 0.008-0.125, 0.125-1, 1-8	1.9	0.049 ⁱⁱ	0.15-25	5.7
Tetrachloroethane	0.008 ⁱⁱ	0.008 – 1, 0.8-3	1.3	0.0006 ⁱⁱ	0.0005-0.004, 0.004-0.016, 0.016-0.25, 0.25-8	3.5	0.0011 ⁱⁱ	0.0033-1	6.1

ⁱ FID detector, ⁱⁱ ECD detector, ⁱⁱⁱ fiber for hexane, benzene and toluene, ^{iv} fiber for chlorinated hydrocarbons, ^v Single drop microextraction; *, -presented ranges inclusive minimum and maximum concentrations from linearity tests (25, 28, 29)

sieves) (24). Important characteristic of the capillary columns is their small flow impedance relative to that of the packed columns. This permits very long columns to be used and thus, provides very high efficiencies or, alternatively, very short columns operated at very high mobile phase velocities, to provide very fast separations (28). The most recent are capillary columns which are very strong, very inert and can be used over a wide range of temperatures what makes them the most frequently used columns in all of GC analyses. Nowadays, the choice of capillary columns is very wide and suppliers offer many columns with different stationary phases dedicated to special analysis like residual solvents determinations. The optimal column selection for residual solvents determination, is not very complicated usually when the suppliers offer their columns together with prescribed methods for separation of analytes of interest. The most popular stationary phases used for RS determinations are presented in Table 5.

To obtain the appropriate separation, several GC methods tend to have long run times. Some of analyses involved even 60 min temperature gradients. To speed up GC separation time, several alternatives can be employed. One of them is to use hydrogen as an alternative carrier gas to helium. With its use, higher linear velocities can be achieved (29). This resolution is generally not considered due to safety reasons (30). Another alternative known as a fast gas chromatography, includes the use of shorter capillary columns, with narrower bores, and a fast temperature programming (31). For typical determination of residual solvents in a drug substance, when only a solvent or a single small set of solvents needs to be analyzed, the analysis time can further be reduced to less than 1.5 min using combined temperature and pressure programming (6). The main shortcoming of fast gas chromatography, is decreased capacity of injection that results in higher detection limits. However, the sensitivity of fast chromatography methods is sufficient to monitor residual solvents tested within the limits set by the ICH guideline (30). The fast chromatography methods can be successfully applied to in-process solvents monitoring, when a short time for analytical response is required.

Table 5. The most popular column phases used for RS determinations

Stationary phase	United States Pharmacopoeia phase name	Commercial names	Additional comments
6%-Cyanopropylphenyl-94%-dimethyl polysiloxane	Equivalent to USP phase G43	DB-624, HP-624, Rtx- 624, OVI-G43, CP-624, ZB-624, 007-624	Recommended by pharmacopoeias, low/midpolarity
6% Cyanopropyl-phenyl-methylpolysiloxane	Equivalent to USP phase G43	DB-1301, HP-1301, Rtx- 1301, OVI-G43, CP-1301, SPB-1301, 007-1301	Recommended by pharmacopoeias, low/midpolarity
Polyethylene glycol (PEG)	Close equivalent to USP phase G16	DB – WAX, HP – WAX, UPELCOWAX 10, CP-WAX 52CB, SUPEROX II, CB-WAX Stabilwax, 007-CW, Carbowax, HP-Innowax, Rtx-WAX, ZB-WAX	Recommended by pharmacopoeias, high polarity
5% Phenyl-methylpolysiloxane	Equivalent to USP Phase G27	DB-5, HP-5, Ultra-5, Rtx-5, CP-Sil 8 CB, 007-5, OV-5, SE-30, AT-5, ZB-5	Non-polar
Polydimethylsiloxane	Equivalent to USP Phase G2	DB-1, Ultra-1, SPB-1, CP-Sil 5CB, Rtx-1, BP-1, OV-1, OV-101, 007-1, CP-Sil 5 CB, ZB-1, AT-1	Non-polar
50% Trifluoropropyl-methylpolysiloxane	Close equivalent to USP Phase G6	Rtx-200, HP – 210, OV – 210, DB-210, VB – 210	Mid/high polar

Other developments in the field of fast GC also include the introduction of short (25 – 100 cm) multicapillary columns (MCC), which contain up to 1000 capillaries with the diameter size down to 10 – 100 μm (Figure 8). The efficiency of such columns depends on the dispersion of the capillary radiuses and on the way of the liquid-film loading (31). With their use, chromatographic separations can speed up ten times without loss of efficiency (32), but only isothermal analysis is possible.

For complicated separations, two capillary columns with different stationary phases, can be combined in series (tandem) to obtain tunable selectivity. Two dimensional GC (GC \times GC) chromatography coupled with stop-flow mode, can improve separation and additionally shorten analysis time. This technique is performed by programming the carrier gas pressure at the junction point where a capillary columns that exhibit differentiating selectivity for the analyzed solvents are connected. By inserting a low dead-volume valve at the column junction, and connecting it to a source of carrier gas at or above the GC inlet pressure, flow programming can be accomplished (33). When the valve is open, the carrier gas flow is stopped in the first column and is accelerated in the second column (Figure 9). Care has to be taken when components that are separated on the first column, co-elute on the second column. In such cases, the column pair can separate them if the valve is opened for a short time, when

the band of one of the problematic components has crossed the column junction and the band of the second component is still on the first column. With such pressure pulses the resolution between some components can be improved without decreasing separation between other sample components. With the use of two-dimensional gas chromatography with pressure switching techniques and fast oven temperature programming, it is possible to achieve resolution of 36 commonly analyzed RS in an analysis time of 12 minutes (35).

GC detection mode

The next step in every GC analysis is a detection process. There are many devices used for this task. In general, GC detectors are 4 to 5 orders of magnitude more sensitive than LC detectors and thus, are ideal for trace analysis (36). There are many detection methods that can be employed, including the measurement of the standard physical properties, such as thermal conductivity and light absorption to more specific properties, such as ionization potential and the heat of combustion. The requirements for a GC detector depend on the application. For residual solvents analysis, in the situation when analytes present in the sample are known, or suspected, the flame-ionization detector (FID) is recommended. The FID detector uses hydrogen and air as the combustion gases, which are mixed with the column eluent (helium, nitrogen or other appro-

ropriate gas) and burned in a small jet situated inside a cylindrical electrode. Additionally, a potential of a few hundred volts is applied between the jet and the electrode which results in collecting the electron/ion pairs obtained after the burning of carbon containing products. The FID detects all carbon containing molecules, with the exception of small molecular compounds like carbon disulfide, carbon monoxide and it can be considered as a universal detector (37). Generally, it has become the most frequently used detector in GC because of its low detection limits (2×10^{-12} g/s), wide linear dynamic range ($< 10^7$), and general reliability and utility, especially for trace organic compounds like residual solvents (38).

In situations when analytes are unknown and additional level of identification capability is needed, mass spectrometer (MS) detectors are preferable.

In general, the MS detectors capture, ionize, accelerate, deflect, and detect the ionized molecules. It happens by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio. Detection limits obtained with this kind of detector in single ion monitoring mode (SIM) can achieve $10^{-12} - 10^{-15}$ g limits (39) with linear range about 10^5 (40). It can be either universal in its scan mode or selective in ion monitoring mode (SIM).

Another detector that finds application in GC, is the electron capture detector (ECD). It is a selective and very sensitive detector (2×10^{-13} g (40) employed only when compounds with halogens or other electron capturing groups are analyzed. There are other universal or "dedicated" detectors in use, like: photoionization detector (PID), thermal conductivity detector (TCD), thermoionic emission detector (TID), flame photometric detector (PID), hall electrolytic conductivity detector, thermal energy analyzer (TEA), Fourier transform infrared detector (FT-IR) (40), nitrogen phosphorus detector (NPD).

The choice of detectors for GC is very rich, but for residual solvents analyses, the most appropriate are FID and MS detectors. This fact is confirmed by numerous publications in which these two detectors lead in frequency of use (41).

Gas chromatography, in different possible configurations of injection, separation and detection systems, is a powerful tool for residual solvent determinations. It can be categorized as the main technique for this kind of analysis and there is no sign that it can be replaced by other analytical techniques in the near future. However, in special situations alternative methods are also sometimes used.

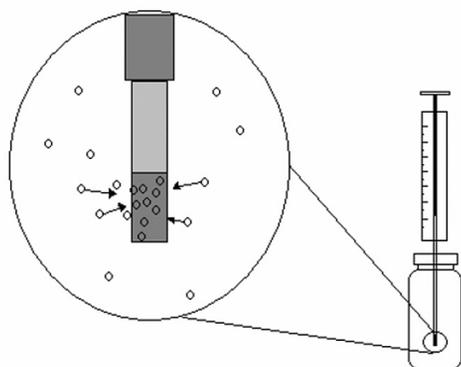


Figure 6. Diagram of SPME technique when placed into the head-space phase or liquid phase

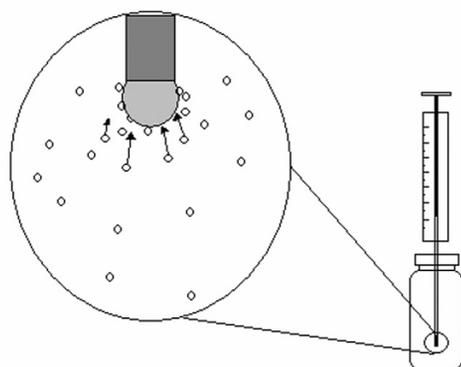


Figure 7. Diagram of the SDME technique

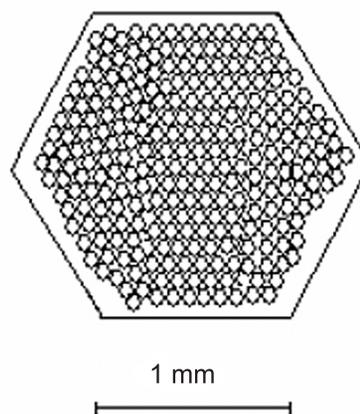


Figure 8. Diagram of cross-section of multicapillary column

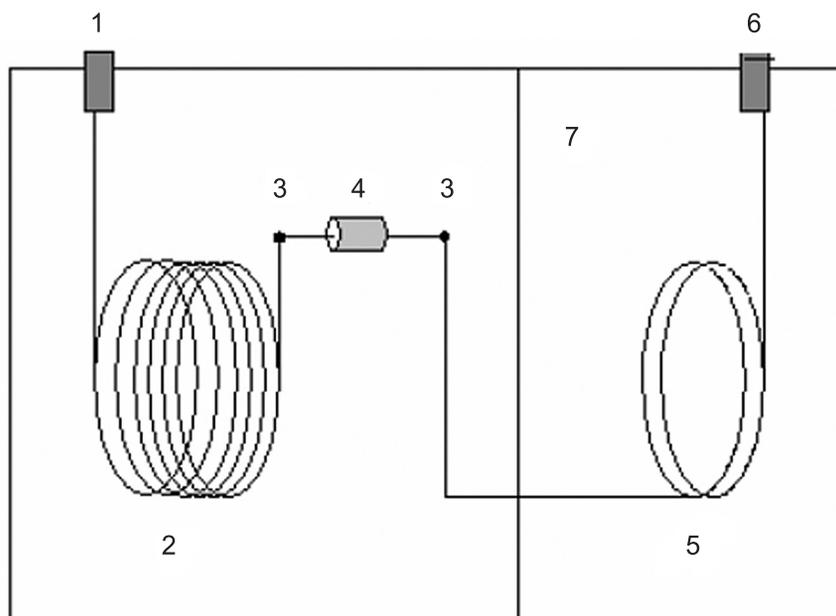


Figure 9. Diagram of GCxGC system. 1) injector, 2) first column, 3) column connectors, 4) modulator, 5) second column, 6) detector, 7) secondary column oven (optional) (34)

Other methods

Various other methods have been employed to determine RS in pharmaceutical materials. One of the oldest methods mentioned earlier, relies on measuring the loss of sample weight. This nonspecific method has its successor in a form of a thermogravimetric analysis (TGA), in which weight changes in a material are measured as a function of temperature (or time), under a controlled atmosphere. Thanks to TGA, the method is more sensitive (possible LOD 100 ppm) and requires less material (5 – 20 mg) (41). But not only TGA, but also differential thermal analysis (DTA) and differential scanning calorimetry (DSC), found application in the discussed field. With their use loss of methylene chloride (42), chloroform (43) and isopropanol (44) was monitored. Because these methods are nonspecific, when more than one solvent is present in the sample, sometimes only the general content of solvents can be determined.

Infrared spectroscopy (IR) and Fourier transformation infrared spectroscopy (FT-IR) has occasionally been used, to identify and quantify residual solvents, but their sensitivity (LOD above 100 ppm) and accuracy are rather limited when compared with GC (45). Also interferences of matrix and analytes bands are possible. With the use of IR, content of THF, dichloroethane and methylene chloride was estimated in polymer samples by the measure of characteristic solvent band (46). IR spectroscopy

was also employed for silicone oil determination in microspheres. The obtained detection limit was at 5000 ppm concentration level, while with use of $^1\text{H-NMR}$ it was decreased for the same solvent beneath 100 ppm level (47). The 100 ppm quantitation limits were also obtained for NMR determination of residual benzene, toluene, methyl ethyl ketone, ethyl ether, methylene chloride and ethyl acetate in cocaine samples (48).

None of the mentioned techniques provide the sensitivity required for the levels presented in ICH guidelines and pharmacopoeias, and for these reasons their application for RS determinations in pharmaceuticals is very rare, especially for routine analysis.

Other less popular methods like radioactivity measurement of mixed analyzed and labeled solvent (normally ^{14}C) or spectrophotometric determination of chlorinated solvents are described in the literature (5, 49).

CONCLUSIONS

The residual solvents (RS) analyses include loss on drying (LOD), thermogravimetric analysis (TGA), spectroscopic and spectrometric methods and gas chromatographic methods. The most useful analytical method of choice for identification and quantification of RS, is the gas chromatography (GC). Gas chromatographic procedures for RS can

be carried out either by direct injection method, head space (HS) analysis, solid phase microextraction (SPME) method or the new technique known as single drop microextraction (SDME). Although the direct injection is the simplest and the cheapest option, it should be excluded when samples with complex matrices are analyzed. In such cases indirect injection systems like headspace analysis, solid phase microextraction or single drop microextraction should be employed. The most popular and additionally recommended by pharmacopoeias is the use of the headspace injection system. On the other hand, SPME system, when compared with HS, offers lower detection limits. However, SPME is still less popular than HS due to the complexity of the optimization of analysis conditions and lack of intermediate precision between fibers. The SDME technique offers higher detection limits and lower precision than those obtained with HS or SPME, to be used in routine analyses. But in the future, when appropriate optimization activities would be done this extraction technique can become more popular.

For the separation process undoubtedly capillary columns dominated GC. When in-process analysis requires short time, narrower and shorter or multicapillary columns can be used. Using two-dimensional gas chromatography, complicated separations can be performed. For detection, the most appropriate seems to be the flame-ionization detector (FID), but when it comes to identify unknown solvents, also mass spectrometric detector (MS) is especially helpful.

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