

1 **Analytical procedures for the quality control of pharmaceuticals in terms of residual**
2 **solvents content – challenges and recent developments**

3
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14
15 **Abstract**

16
17 Residual solvents play an important role in the synthesis of drug substances and in product
18 formulations. At the same time they pose a problem and must be removed, because many of
19 them have toxic or environmentally hazardous properties. Therefore, constant monitoring of
20 quality control is needed. In this paper, we present an overview of regulatory and general
21 methods described by various Pharmacopoeias. Next, the most commonly used
22 methodologies for the determination of residual solvents in different pharmaceutical samples
23 are reviewed to demonstrate their limitations, which form the basis for discussion about new
24 methods. Several interesting new alternatives for sample preparation and gas chromatography
25 (GC) separation are presented using examples from recent literature. The techniques
26 described are direct injection, headspace analysis with different modifications and variations,
27 liquid extraction, single-drop microextraction, solid-phase microextraction. Various GC
28 separation techniques are compared and new solutions to improve sensitivity and efficiency
29 are presented.

30
31 **Keywords:** residual solvents; pharmaceutical samples; analytical procedures; sample
32 preparation techniques; headspace analysis; gas chromatography

35 **Abbreviations**

36

37 ANDAs – Abbreviated New Drug Applications, US Food and Drug Administration

38 API – Active Pharmaceutical Ingredient

39 BA – Benzyl Alcohol

40 B.P. – Boiling Point

41 CW – Carbowax

42 DI – Direct Injection (Immersion)

43 DMA – *N,N*-dimethylacetamide

44 DMF – *N,N*-dimethylformamide

45 DMI – 1,3-dimethyl-2-imidazolidinone

46 DMSO – Dimethylsulfoxide

47 DSC – Differential Scanning Calorimetry

48 DVB – Divinylbenzene

49 ECD – Electron Capture Detector

50 EHC – Environmental Health Criteria

51 FDA – US Food and Drug Administration

52 FET – Full Evaporation Technique

53 FID – Flame Ionization Detector

54 FTIR – Fourier Transformation Infrared Spectroscopy

55 GC – Gas Chromatography

56 GC-MS – Gas Chromatography coupled with Mass Spectrometer

57 GCxGC – Two-Dimensional Gas Chromatography

58 GMP – Good Manufacture Practice

59 HPLC – High Performance Liquid Chromatography

60 HS – Headspace Analysis

61 HS-MS – Headspace Sampler coupled with Mass Spectrometer

62 ICH – International Conference on Harmonisation of Technical Requirements for Registration of
63 Pharmaceuticals for Human Use

64 IIs – Ionic Liquids

65 IMS – Ion Mobility Spectrometry

66 IRIS – Integrated Risk Information System

67 LE – Liquid Extraction

68 LOD – Limit of Detection

69 LPME – Liquid-Phase Microextraction

70 LTM – Low Thermal Mass

71 MHE – Multiple Headspace Extraction

72 MHS-SDME – Multiple Headspace Single-Drop Microextraction

73 MLLE – Liquid Microextraction

74 MS – Mass Spectrometry

75	MTBE – Methyl <i>Tert</i> -Buthyl Ether
76	NDAs – New Drug Applications, US Food and Drug Administration
77	NIR – Near Infrared Spectroscopy
78	NMP – <i>N</i> -methyl-2-pyrrolidinone
79	PA – Polyacrylate
80	PAT – Process Analytical Technologies
81	PDE – Permitted Daily Exposure
82	PDMS – Polydimethylsiloxane
83	PTV – Programmed Temperature Vaporization inlet
84	QA/QC – Quality Assurance/Quality Control
85	SDME – Single-Drop Microextraction
86	SPME – Solid-Phase Microextraction
87	TD – Thermal Desorption
88	TGA – Thermogravimetric Analysis
89	TVT – Total Vaporization Technique
90	WHO – World Health Organization

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92

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110

111 1. Introduction

112
113 Pollution of pharmaceuticals can be any component that is not the chemical compound
114 defined as the active substance or an excipient in the drug product. Therefore, the safety of
115 pharmaceuticals does not only depend on toxicological properties of active substances and
116 excipients in the drug product, but partly also upon the pollutants that it may contain. Due to
117 the significant impact of pollutants on the pharmaceutical quality, recommendations
118 concerning contaminants were introduced by the US Food and Drug Administration (New
119 Drug Applications (NDAs) and Abbreviated New Drug Applications (ANDAs)) [1].
120 Moreover, the lack of chemical stability of the active pharmaceutical ingredients may cause
121 formation and emission of volatile compounds, which may affect the stability of drug
122 products and their physicochemical properties, causing negative or even effects [2].

123 ~~Organic solvents are routinely used in the synthesis and process chemistry of drug~~
124 ~~substances and drug products. These process solvents cannot be completely removed by~~
125 ~~practicable manufacturing practices such as freeze-drying or drying at high temperature under~~
126 ~~vacuum. Therefore, they may remain in pharmaceuticals.~~ Organic solvents do not possess
127 therapeutic benefits for patients and should be removed in order to meet the requirements of
128 product specifications, good manufacturing practices and appropriate quality - control
129 requirements [3,4]. ~~The concentration levels of residue organic solvents in drug products~~
130 ~~should be below recommended acceptable levels (safety limits). It is the responsibility of~~
131 ~~manufacturers to ensure that any solvent present in the final product does not pose a threat to~~
132 ~~patient health or to the environment [7-9].~~

133 General methods for the determination of residual solvents in pharmaceuticals are well
134 known and described by the International Conference on Harmonisation of Technical
135 Requirements for Registration of Pharmaceutical for Human Use (ICH) [5] and various
136 Pharmacopoeias [6-8]. These methods are based primarily on gas chromatography (GC) due
137 to the volatility of organic solvents and the substantial separating capability of capillary
138 columns. Over the last decade, several GC methods have been reported in the literature [9-
139 12]. Unfortunately, they do not meet expectations, mainly due to large time and effort, and,
140 generally, multi-step processes of isolation and preconcentration of analytes. Most of the GC
141 methods tend to have long run times and to be very specific for a limited number of solvents
142 (mainly solvents with similar physicochemical properties) and sample matrices. Efficient and
143 sensitive analytical methodologies need to be developed to significantly increase productivity
144 of an analytical laboratories in the pharmaceutical industry. Fig. 1 presents 'milestones' in the

145 field of development of analytical methodologies for evaluating the level of residual solvents
146 and identification as well as quantitation of specific analytes.

147

148 FIGURE 1

149

150 Determination of volatile compounds from the group of organic pollutants has many
151 analytical problems. The main source of problems in the identification of organic solvents is
152 their high volatility and hydrophobic properties, which is directly related to the difficulty in
153 sampling and their preparation for analysis. Moreover, the determination of polar residual
154 solvents in pharmaceutical preparations continues to present an analytical challenge mainly
155 because these compounds are difficult to remove from water or other polar solvents.

156 In this paper the most commonly used methodologies for the determination of residual
157 solvents in pharmaceutical samples have been reviewed and critically evaluated.
158 Recommended methods are presented and discussed. Challenges and new solutions with
159 examples from recent literature are proposed.

160

161 2. Residual solvents

162

163 Residual solvents ~~in pharmaceuticals are organic volatile chemicals that could be used or~~
164 ~~produced during the manufacturing processes of drug substances, drug products and~~
165 ~~excipients. They~~ play an important role in the production of pharmaceuticals (reaction,
166 separation, purification and drying) and in product formulations (for example: granulation,
167 coating, eye-drop formulation, spray formulation, etc.). At each of these stages, the product
168 can be potentially contaminated by organic solvents. ~~The pharmaceutical industry is one of~~
169 ~~the largest users of organic solvents per amount of the final product [18].~~ Some of them are
170 used during the synthesis of active substances or during the preparation of pharmaceutical
171 products to increase the process efficiency, improve their stability, purity, solubility and to
172 facilitate crystallization. Therefore, the solvent and its quality may be a critical parameter in
173 the synthesis process [5].

174 Toxicity is the major and unquestionable reason for control of residual solvent contents.
175 Additionally, most organic solvents are volatile, flammable and hazardous to humans and the
176 environment. They are also the main component of generated waste. Moreover, they pose a
177 risk of inducing phase transformations (e.g. transformation of orthorhombic paracetamol by
178 residual ethanol) and jeopardizing the physicochemical stability of an active substance. For



179 example, formic acid, its esters and formaldehyde, a commonly present impurities in
180 excipients, can interact with amino or hydroxyl groups from pharmaceutical compounds and
181 form amides, esters or *N*-methyl derivative [13]. Table 1 presents information on classes of
182 solvents commonly used in the pharmaceutical industry [1,5,13].

183

184 TABLE 1

185

186 Organic solvents usually used in the production process are as follows: the reaction
187 medium, reagent or catalyst of synthesis reaction and an extractant during the extraction
188 process. They can also play a role as entrainers during the process of azeotropic or extractive
189 distillation. The crystallization process is commonly used for purification of the drug
190 substance [14]. It is very important that the properties of formed crystals should be controlled
191 during the crystallization process, i.e. their size and shape which determine the quality of the
192 final product, its stability or dissolution rate. Certain products, crystallized from different
193 classes of solvents or their mixtures demonstrate a variable crystals size and morphologies, as
194 for example: ibuprofen or carboxylic acids [13]. After the crystallization process, residual
195 solvents are located in the pores of the particulate product, and then are removed in the course
196 of drying and homogenization (possibly grinding) process, or recrystallization. The
197 physicochemical properties can have a large impact on the bioavailability of the final drug
198 product [4,5,10]. Consequently, *on-line* process monitoring of solute concentrations can be
199 beneficial to control the level of supersaturation that drives nucleation and crystal growth.

200 During the formulation of the final product to a form suitable for administration, some
201 organic solvents can be used as its component to fulfill the function of diluents or solubilizers,
202 mainly in semisolid and liquid pharmaceuticals, where water cannot be used [1,9,10]. Organic
203 solvents can also contaminate the drug products during their packaging, storage and
204 transportation [13]. They may also occur as products of various reactions and processes
205 during the shelf-life of the product under its storage conditions. For example, sodium
206 benzoate used in oral liquid pharmaceutical products can potentially generate residual levels
207 of free carcinogenic benzene under heat and acidic conditions [15].

208 From the viewpoint of factories, the complete removal of residual organic solvents by
209 practical manufacturing techniques (different drying processes) is practically impossible,
210 therefore, their presence in final products is unavoidable [5,11-13]. Residual solvents are
211 significant contamination of pharmaceuticals, because they can cause toxic effects and safety
212 problems, influence on physicochemical properties of the active substance or an excipient in



213 the drug product and may affect the products formulation process [2,4,10]. As a result, they
214 may be responsible for the unpleasant odor, color change and may affect the therapeutic
215 effect, safety and stability of the final products [13]. Moreover, they may also accelerate the
216 decomposition process of the product. Therefore, constant monitoring of quality control in
217 pharmaceuticals is needed to ensure patient safety and meet regulatory expectations [3,5,11].
218 Identification of impurities in pharmaceuticals also allows to use profiles of these
219 contaminants as a ‘fingerprint’ of the manufacturer. Impurity profiles are a valuable tool for
220 detecting “counterfeit” drugs or illicit substitutions and tracking down their source, and are
221 the subject of interest for forensic laboratories [16].

222

223 3. Legislations

224

225 ~~Organic solvents play a key role in the production of pharmaceuticals, but many of them~~
226 ~~have toxic or environmentally hazardous properties. Therefore, they should be avoided unless~~
227 ~~their use can be justified on the basis of risk-benefit assessment [3-5,29].~~ In order to control
228 concentration levels of residual solvents in drug substances, products and excipients, national
229 and international guidelines were introduced [1,2,4,5-8]. In 1997, the ICH classified, in
230 guideline Q3C [5], commonly used organic solvents into four classes in terms of their level of
231 hazard to humans and the environment and to regulate the concentration level of each solvent.
232 So far, the document has been updated five times because of new toxicological data for
233 tetrahydrofuran, *N*-methylpyrrolidone and cumene [5]. Moreover, the World Health
234 Organization (WHO) [3] and other national and international health authorities and institutes
235 introduced a new term (permitted daily exposure, PDE) to define the maximum concentration
236 limits as a pharmaceutically acceptable intake of residual solvents per day [2]. The list
237 presented in the guideline is not exhaustive and one should evaluate the synthesis and
238 manufacturing processes for all possible solvents. Class 1 includes solvents (e.g. benzene,
239 carbon tetrachloride, 1,2-dichloroethane etc.) considered to be the most toxic, which using
240 should be avoided in the production of pharmaceutical products. The absolute limits in the
241 range of 2 – 8 ppm are defined for solvents that are known to be highly toxic and the limit of
242 1500 ppm is applied for trichloroethane, an environmentally hazardous chemical. Class 2 and
243 3 of residual solvents are considered a lesser risk. Class 2 solvents (e.g. acetonitrile,
244 chloroform, hexane, 2-methoxyethanol, nitromethane etc.) should be limited in their usage
245 and specifically tested for in products. Class 3 solvents (e.g. acetone, 2-butanol, ethanol, ethyl
246 acetate etc.) require only non-specific testing based on Good Manufacturing Practice (GMP).

247 Higher amounts of solvents from Class 3 can be acceptable if manufacturer will prove that the
248 content is realistic with relation to manufacturing capability and good manufacturing practice.
249 Class 4 includes solvents without any information about toxicity, for example: isooctane,
250 petroleum ether, trifluoroacetic acid etc. According to the ICH guideline, solvents from Class
251 1 must be identified and quantified, solvents from class 2 have individual limits between 50
252 and 5000 ppm, and solvents from class 3 need to be identified and quantified, when they are
253 found to be more than 0.5 % (w/w). Exposure limits in the ICH guideline are established by
254 referring to methodologies and toxicity data described in the Environmental Health Criteria
255 (EHC) monographs and the Integrated Risk Information System (IRIS) [3-5].

256 The US FDA published their guidance at the end of 1997 and the European
257 Pharmacopoeia included the guideline in the chapter entitled 'Residual solvents' [1,4,8]. Also,
258 countries from the ICH group (United States Pharmacopeia and Japanese Pharmacopoeia)
259 [6,7] and others have adopted requirements for their pharmacopoeias. The guideline set
260 criteria for analytical methods used to identify and quantify residual solvents as well as
261 provide acceptable concentration limits. However, it applies only to existing marketed drug
262 products. It cannot be applied to potential new drug substances, excipients and drug products
263 used during the clinical research stages of development.

264

265 **4. Analytical methodologies**

266

267 Manufacturers are free to choose the most appropriate validated analytical procedure for a
268 particular application. The ICH Q3C (R5) [5] guideline adopted by the regulatory bodies of
269 the European Union, Japan and USA set and define general methods for performing residual
270 solvents testing. Procedures for determination are described and detailed in a separate chapter
271 of Pharmacopoeias [6-8]. This general methods may be used:

- 272 ✓ for identification of Class 1 and 2 residual solvents when there is no information about
273 solvents which may be present in the sample;
- 274 ✓ as a limit test for Class 1 and 2 solvents when they are detected in the sample;
- 275 ✓ for quantification of Class 2 solvents when limits are greater than 0.1 % (w/w);
- 276 ✓ for quantification of Class 3 solvents when it is required.

277 It should be noticed, that a procedure applied quantitatively to control residual solvents in
278 samples must be validated [8]. Typically, they are determined by chromatographic techniques
279 due to their high sensitivity, separation efficiency and the possibility of analyzing liquid or
280 solid samples of a complex nature [9-12,15,17-19]. Direct injection (DI) of samples for GC

281 analysis is feasible and should be the method of choice in view of its simplicity and reliability
282 [9,10,18,19]. But non-volatile components or corrosive substances present in the sample can
283 cause the contamination of the GC system and the deterioration of the column and
284 consequently, frequent and time-consuming cleaning is required. Therefore, samples need a
285 separation of the volatile substances before analysis, which can be performed by using
286 headspace sampling (HS) or different extraction techniques [17,18]. HS sampling can prevent
287 from contamination, but it limits analysis to those solvents being evaporated from the HS only
288 and it requires a larger sample load [11,12]. In addition, the analysis time can be longer due to
289 sampler equilibration prior to GC separation. **Another** sample preparation techniques, for
290 example: solid-phase microextraction (SPME) and single-drop microextraction (SDME), have
291 been developed to overcome the drawbacks of those conventionally used, facilitating rapid
292 and efficient isolation and/or enrichment of analytes as well as eliminating the consumption of
293 toxic organic solvents [9,13,17,18,20-22]. Moreover, standard addition is the most
294 recommended quantitation technique to overcome the matrix effect in analyses [9,10,21]. If
295 only Class 3 solvents are present, a non specific method, such as loss on drying may be used
296 [5]. Moreover, when there is no need to specify the concentration of residual solvents in a
297 sample, but only to check their presence, other analytical techniques can be used, for example:
298 sensors, different thermal analysis techniques or spectroscopic methods [1,6-8]. ~~In Fig. 3, the
299 most popular methodologies for determining residual solvents in pharmaceutical products are
300 presented.~~

302 **FIGURE 3**

303
304 The most frequently used method for routine determination of residual solvents in
305 pharmaceutical quality assurance/quality control (QA/QC) is a static headspace sampler
306 coupled with gas chromatography [6-12,17,18]. This general method is recommended by
307 various Pharmacopeias, but at the same time its implementation is a subject of debate in the
308 pharmaceutical industry due to its limited selectivity and sensitivity [23]. For example,
309 formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, *N*-methylpyrrolidone and
310 sulfolane are not readily detected by headspace injection [5,8]. Other appropriate procedures
311 need to be developed for their control. The most important step for successful analysis is the
312 development of a selective, sensitive and stable methodology for determination of compounds
313 with different volatility and polarities. It can be seen as several trends in the determination of
314 residual solvents:

- 315 • determination of a broad spectrum of solvents in a single analytical run (multiresidue
316 methods);
- 317 • miniaturization and automation of analytical techniques by application of **solventless**
318 **and solvent-minimized sample preparation techniques**, for example: SPME, SDME;
- 319 • development of new techniques for fast screening methods, for example: HS sampler
320 coupled with mass spectrometer (MS), flow-modulation GC;
- 321 • application of advanced techniques, for example: fast GC, two-dimensional gas
322 chromatography (GC×GC);
- 323 • application of environmentally friendly sample diluents, for example: ionic liquids
324 (ILs), binary solvents, water.

325 The main advantages of these methods are shorter analysis times, minimization of harmful
326 solvents consumption, and typically, the high enrichment factor. The improved sensitivity
327 makes it possible to minimize the amount of the sample needed for the analysis. ~~Ideally, the~~
328 ~~sample preparation stage should be as simple as possible, because it does not only reduce the~~
329 ~~time required, but also decreases the possibility of introducing contaminants. Research is~~
330 ~~continuing into the improvement of existing analytical methods and the development of new~~
331 ~~ones, which would enable solvents with different physicochemical properties to be reliably~~
332 ~~and reproducibly determined at the same time in a quick, simple, cheap, effective and~~
333 ~~environmentally friendly manner.~~

334

335 **5. Methods recommended by the Pharmacopoeias**

336

337 For determination of a high content of residual solvents with lower toxicity (greater than
338 1000 ppm) a loss of weight method can be applied [6-8]. It was the first analytical method
339 published in Pharmacopoeias and is mainly dedicated to Class 3 solvents [10]. For the
340 determination of hazardous solvents (Class 1 and 2), the use of analytical methods is
341 recommended to enable determination of concentrations as low as possible [5].

342 The loss of weight method is a technique which is simple and easy to perform based on
343 measuring the weight loss of sample during the heating process and can be carried out at a
344 normal pressure or under the vacuum. However, it has many disadvantages, such as poor
345 sensitivity and specificity (multicomponent solvent mixtures cannot be analyzed), high limits
346 of detection and requires a large amount of sample for analysis (about 1 – 2 g). Moreover,
347 atmospheric humidity may affect the results of measurements [10,18]. Therefore, the current



348 trend focuses on more sophisticated techniques, which ensure the achievement of meaningful
349 results. For example, thermogravimetric analysis (TGA) and differential scanning calorimetry
350 (DSC) have been used successfully [9,10,17,18]. Also, spectroscopic and spectrometric
351 methods have proved to be a good alternative demonstrating low detection limits for toxic
352 residual solvents [24]. All of these methods used to determine residual solvents in
353 pharmaceutical products and excipients have been almost completely replaced by GC
354 methods, which have also been approved by various Pharmacopoeias. Analytical procedures
355 based on gas chromatography are the most popular and chemically specific for determination
356 of residual solvents.

357 The general method for residual solvents determination described in European
358 Pharmacopoeia, 8th edition, defines a general methodology and specifies two complementary
359 procedures (systems) [8], which are presented in Fig. 2. “System A” is recommended for
360 general use to identify unknown solvents and is equivalent to “Methods IV and V” of the U.S.
361 Pharmacopoeia for analysis of volatile organic impurities [6]. “System B” is used for
362 quantification of samples already resolved with a generic method as a confirmation of identity
363 and to solve coelutions.

364

365 **FIGURE 2**

366

367 Three procedures are defined for sample preparation depending on the type of matrix.
368 Two of them concern the nature of the solubility of analytes in water. For water-soluble
369 samples, water is recommended as a solvent and for water-insoluble substances *N,N*-
370 dimethylformamide (DMF) is suggested. For samples suspected of containing *N,N*-
371 dimethylacetamide (DMA) and/or DMF, 1,3-dimethyl-2-imidazolidinone (DMI) is proposed
372 as a solvent. After that, 5 mL of solution is transferred to vial and HS analysis is performed.
373 Three different headspace conditions are proposed and their choice depends on the solvent
374 which was selected for sample preparation. In addition, the properties of the residual solvents
375 (high or low boiling) and the type of analyzed material (thermally stable or unstable) should
376 also be taken into account. For final determination, all these methods utilized GC with
377 capillary or wide-bore columns and a flame ionization detector (FID). The procedures
378 (System A and B) vary in terms of the column type (film-coatings and dimensions) and in
379 chromatographic conditions. Moreover, in European Pharmacopoeia as a complement with
380 U.S. Pharmacopoeia, a mass spectrometer (MS) or electron capture detector (ECD) have been
381 additionally proposed as an alternative detector [6-10,18]. As was mentioned before, all



382 analytical methodologies used to quantitative determination of residual solvents must be
383 validated. For this reason, manufacturers seek to develop their own methodologies, which
384 would be faster, easier and tailored to their specific type of samples and analytes.

385

386 6. Sample preparation techniques

387

388 Because of the complex composition of pharmaceutical samples, different and low
389 concentrations of the target analytes, the sample must be adequately prepared for analysis by
390 the use of techniques for efficiently extracting the target compounds and for their cleanup
391 prior to the quantitative determination stage. ~~In addition, pharmaceutical products often
392 contain compounds with similar properties to the analytes, which further complicates the
393 separation and quantitative determination. The choice of the technique depends on the
394 properties of the target analytes, their volatility, polarity, solubility in water and organic
395 solvents.~~ Classification of sample preparation techniques commonly used for determination of
396 residual solvents is presented in Fig. 3.

397

398 FIGURE 3

399

400 At present, the literature focuses mainly on the techniques for the isolation,
401 preconcentration and final determination of analytes, all of which can affect the reliability of
402 the information obtained about a sample. Chemists tend to develop environmentally friendly
403 analytical methodologies that are consistent with the principles of 'Green Chemistry' [20].

404 ~~These ensure that:~~

- 405 ~~• the use of chemical reagents, particularly organic solvents, is eliminated or at least~~
406 ~~substantially reduced,~~
- 407 ~~• the application of highly toxic reagents is eliminated,~~
- 408 ~~• coexisting components can be removed efficiently,~~
- 409 ~~• the labour and energy consumption of processes is reduced,~~
- 410 ~~• the procedure can be carried out conveniently and quickly,~~
- 411 ~~• the cost of analysis is low,~~
- 412 ~~• a broad spectrum of target analytes can be determined in a single analytical run.~~

413 ~~The rapid development of new techniques in analytical chemistry (miniaturization,
414 automation, high-throughput performance, on-line coupling with analytical instruments etc.)~~

415 ~~has meant that the consumption of solvents in the analysis can be very substantially reduced;~~
416 ~~very often the use of solvents can be eliminated altogether if solvent free techniques are~~
417 ~~applied [40].~~

418

419 **6.1 Dissolution and liquid extraction**

420

421 The traditional technique of sample preparation for residual solvent determination is direct
422 injection in which sample is dissolved in or extracted with suitable solvent. Good sample
423 diluent should have a high capability for dissolving a large number of samples, high boiling
424 point and good stability. For water-soluble samples and analytes, water is recommended as a
425 diluent [6-8]. Water has the advantage of having no solvent peak when the FID is used.
426 Moreover, it is a clean, stable and inexpensive solvent, but at the same time causes a sample
427 backflash as a result of the large expansion volume of water causing poor injection
428 reproducibility and poor method precision. To overcome this problem a polar organic solvent
429 can be added to the water. A mixture 3:1 (v/v) of water and methanol was proposed as a
430 diluent for dissolution of pantoprazole sodium in the form of tablets [19]. DI-GC-MS was
431 used for analysis. The obtained limit of detection for dimethyl sulfate (genotoxic reagent used
432 during the synthesis) was $1 \mu\text{g mL}^{-1}$. Matrix effects as interfering peaks from the drug and the
433 diluent were not observed. For other types of pharmaceutical samples dimethylsulfoxide
434 (DMSO), DMF, DMA, benzyl alcohol (BA), hexane and ethylene glycol have been applied as
435 sample diluents [9-11,18]. Using high boiling point solvents has the advantage that the diluent
436 solvent peak will elute later, thus not interfering with the earlier eluting analyte peaks. In
437 cases where a solvent with a low boiling point is used as diluent, only residual solvents having
438 a high boiling point can be determined, so that the peaks do not overlap and their separation is
439 possible [17,20]. Particular attention should be paid when benzyl alcohol is used as a diluent
440 in combination with the DI technique. Interactions inside the GC injection port between
441 solvent and matrix components could cause a number of problems. Benzene was reported to
442 be the product of any interaction involving drug salts and benzyl alcohol inside a heated
443 injection port [10]. Temperature in the injection port must be high enough to ensure complete
444 vaporization of the solvents but low enough to avoid problems of sample reactivity or
445 decomposition.

446 Direct injection to the GC column of a sample dissolved in an appropriate diluent is useful
447 if residual solvents are determined in a drug substance [19,25,26]. If products such as tablets,
448 gels, syrups are analysed, components of matrix may not be vaporized, or may not dissolve in



449 the dissolution media applied. All these problems can be avoided by extensive sample
450 preparation techniques. To overcome the matrix effects and to isolate trace analytes, liquid
451 extraction (LE) can be applied. LE is one of the most common and also one of the oldest
452 extraction techniques. The solvents are usually toluene and methyl *tert*-butyl ether (MTBE)
453 [18,20]. Though fairly simple and cheap, this technique has a number of drawbacks: it
454 requires relatively large quantities of often toxic solvents, there is a risk of emulsions forming
455 during stirring, and there is the problem of how to dispose of the post-extraction solvents. To
456 achieve the desired preconcentration coefficient, the excess solvent usually has to be
457 evaporated; extract cleanup is often also necessary. To minimize these disadvantages
458 numerous improvements have been made to this method, most of which have involved
459 miniaturizing the process to reduce the amounts of solvents consumed. For example, only 3
460 mL of MTBE was applied as a solvent for extraction of dimethyl sulfate from active
461 pharmaceutical ingredient (API) intermediate [27]. To reduce the process time and more rapid
462 phase separation after mechanically shaking the mixture was centrifuged at 13000 rpm for 10
463 min. Approximately 1.5 mL of organic phase was obtained and analyzed by GC-MS. The
464 limit of detection was 0.05 $\mu\text{g mL}^{-1}$ and the linearity range was 0.16 – 9.72 $\mu\text{g mL}^{-1}$.

466 6.2 Headspace analysis

467
468 Headspace (HS) analysis is a sampling method for determination of residual solvents in
469 pharmaceuticals which is preferred and approved by ICH and various Pharmacopoeias. It is a
470 more suitable technique which avoids many drawbacks of direct injection [12,17,21,28].
471 Pharmaceutical samples usually contain non-volatile or degradable compounds that can
472 accumulate in the injector liner or the GC column, reduce their lifetime, co-elute with analytes
473 or create interfering peaks from volatiles during thermal degradation. As a result, it can cause
474 deterioration in method performance (recovery, sensitivity, precision, etc.) [9]. In most cases,
475 samples require the separation of volatile residual solvents before analysis. This can be
476 performed by HS analysis. It is an alternative technique, but is rather limited in terms of
477 optimization possibilities with respect to its selectivity [17]. The analysis is conducted when a
478 volume of gas above the pharmaceutical sample is collected and analyzed by GC. HS is
479 desirable because it minimizes potential contamination of the system by avoiding introduction
480 of a large quantity of the sample. It can be performed in two forms: in a static mode and a
481 dynamic mode (in the literature it is also referred to as purge and trap analysis) [18]. These



482 techniques have been extensively reviewed in the literature [9,10,16-18,28,29] and are briefly
483 described in Table 2.

484

485 TABLE 2

486

487 The main advantage of the static mode of HS is easy operation and automation, whereas
488 the dynamic mode of HS has the general advantages of low detection limits and smaller
489 sample volumes required for analysis. While static HS commonly offers straightforward
490 initial sample preparation, analysts often notice differences in instrument response during
491 analysis of complex pharmaceutical samples, depending on their matrices [29]. In the static
492 HS procedure, the sample (liquid or sometimes solid) is placed into a sealed vial and heated
493 until a thermodynamic equilibrium (between the sample and the gas phase) is achieved. Then,
494 a volume of headspace gas is collected and transferred to the gas chromatograph for analysis.
495 Thus, only volatile components are introduced into the GC, resulting in a longer lifetime of
496 the column and the system. The equilibrium should be reached within the shortest possible
497 time and the temperature should be non-destructive [9,10,18,29-31]. This method is mainly
498 dedicated for pharmaceutical samples soluble (or extractable) in solvents. Moreover, the
499 addition of inorganic salt, pH control, an increase in the equilibrium temperature or control of
500 the phase ratio can be used to improve sensitivity.

501 In a dynamic HS analysis, a stream of inert gas (usually high purity nitrogen) passes
502 through the sample or sweeps over the surface of sample to ensure a maximum surface
503 contact between the phases. Consequently, the solubility of volatile components in the liquid
504 decreases, thus the removal of residual solvents is faster and more efficient. Volatiles from the
505 sample matrix are transported to a trap where analytes are accumulated prior to analysis. The
506 trap is generally a short column containing a sorbent, such as Chromosorb[®], Porapak[®], XAD[®]
507 resins or Tenax[®], which is the most used sorbent because of its thermal stability, in spite of its
508 limited specific surface area [9,10,17,18]. After that, a thermal desorption cycle of the trap is
509 initiated and the carrier gas transports the analytes into GC for further analysis. Cold trapping
510 (cryofocusing) followed by thermal vaporization is another technique of dynamic mode
511 headspace analysis and is sometimes applied to increase the quality of peak shapes [17]. By
512 freezing out the excess of water vapour in a cold trap, contamination of the chromatographic
513 column by water can be avoided. Dynamic headspace analysis is mainly dedicated for the
514 determination of solvents at very low concentration levels. Because the thermodynamic
515 equilibrium is not necessarily needed and the adsorption of analytes on the trap increased the

15

516 method sensitivity, lower detection limits are obtained. Moreover, the sample volume is not
517 restricted and solid samples, which are insoluble or cannot be heated, can be analyzed.
518 However, this may result in the risk of higher measurement uncertainty. The method
519 efficiency can be enhanced by increasing the temperature or by salting out [32].

520 Static HS is one of the most popular technique for residual solvents analysis in
521 pharmaceuticals due to full automation, good precision, accuracy and it is preferred for
522 samples soluble and uniformly distributed in water or organic solvents (dissolution medium)
523 [12,18,28,29-31,33-50]. The matrix and sample dissolving solvents can significantly affect the
524 sensitivity of the HS method due to variable partition coefficients of analytes in different
525 matrices [35]. It was demonstrated, that detection sensitivity can be increased by four times
526 when sample is dissolved in a specific solvent [30]. Water is the most appropriate for water
527 soluble samples [6-8,36,41,43,44], but many drug substances and drug products have low
528 water solubilities, which would limit the sample load [11,12]. Moreover, applying water as a
529 dissolution medium can also lead to lower method precision in comparison with organic
530 solvents, like DMF [28]. In order to improve the dissolution of the samples, mixtures of water
531 with solvents (for example: DMA, DMF, DMSO or sodium phosphate buffer) were proposed
532 [12,19,28,34]. Mixtures are able to solubilise the sample and prove to be the most suitable
533 solutions to obtain good recoveries and to increase method sensitivity
534 [11,12,18,26,30,33,49,50]. It should be noticed, that the HS equilibration temperature cannot
535 be higher than the boiling point of sample diluent, because a large amount of sample may be
536 vaporized, resulting in a hazardously high vial pressure and flood of the sample diluent and
537 analytes to the gas chromatograph [9,17,18]. In the case of water and water-organic mixtures
538 application, the equilibration temperature should be lower than 100 °C [12,36]. However,
539 many organic solvents may not be fully vaporized at this temperature due to higher boiling
540 points. In order to obtain a good phase distribution and HS equilibration efficiency a longer
541 equilibration time is needed, for example 30 – 90 minutes [28,29,30,33,35]. This significantly
542 extends the time of analysis, which is contrary to the principles of 'Green Chemistry' [20].
543 Furthermore, a high equilibration temperature can be problematic in antibiotics analysis,
544 because many of them are water insoluble and temperature sensitive [29]. Antibiotics are
545 complex in nature and several other volatile impurity peaks in the chromatogram are
546 expected, which leads to separation and identification difficulties [33].

547 Organic solvents have higher boiling points than water and provide higher method
548 sensitivity due to better solvent recoveries [11,30,32,33,34,35,37-40,42,46,48]. On the basis
549 of literature data it can be concluded that the following solvents are most frequently used for



550 the analysis of pharmaceuticals: BA (b.p. 204 °C); DMA (b.p. 166 °C); DMF (b.p. 153 °C);
551 DMI (b.p. 105 °C); DMSO (b.p. 189 °C) [11]. However, BA, DMF and DMA do not exhibit
552 stability at higher temperatures and are susceptible to degradation under the influence of
553 ultrasonic wave energy during sample preparation. The products of these processes may
554 interfere with the analyses of residual solvents [30]. Thus, the use of sonication to dissolve
555 greater quantity of pharmaceutical samples in a matrix medium should be avoided [50]. In
556 order to elude interferences by amines, the HS unit equipped with a sample loop, needle
557 assembly and transfer line made of Silcosteel® was proposed [48]. In addition, residual
558 solvents with very low vapour pressures, such as ethylene glycol, 2-ethoxyethanol, 2-
559 methoxyethanol, formamide, *N*-methylpyrrolidone and sulfolane cannot be readily analysed
560 using the HS method [28]. Other procedures are needed to control these solvents in
561 pharmaceutical samples, especially that they should be limited according to the ICH guideline
562 [5] and the U.S. Pharmacopoeia [6].

563 In the case of solid samples, the establishment of an equilibrium between the solid phase
564 and the gas phase poses a challenge, due to matrix effects involving the difficulty in
565 homogeneous mixing of residual solvents into the solid samples, such as polymer resins or
566 hard gelatin capsules, which cannot be dissolved in water and common organic solvents [35].
567 Moreover, preparation of a sample solution requires a large amount of pharmaceutical sample
568 to obtain a concentrated sample solution. For example, 0.5 g of sample needs to be dissolved
569 in a matrix medium to obtain 5 mL of solution. It can be a serious problem in analysis of
570 expensive products, products made for animal studies (such as toxicity testing for pre-clinical
571 work) with a small lot size and their intermediate products [17,30]. Recently, immiscible
572 binary solvents (mixture of 5 mL of decane and 1 mL of HCl) have been proposed as a
573 dissolution medium in the static HS method for determination of residual ethanol in
574 hydroalcoholic sealed hard gelatin capsules [35]. A mixture of ethanol and water is commonly
575 used to seal hard shell capsules by liquid encapsulated and microspray sealing technology.
576 What is important, residual ethanol occurs between the caps and bodies of capsule shells and
577 cannot be measured when capsules remain intact. Therefore, an appropriate capsule dissolving
578 and disintegrating solvent is needed. Organic solvents and water cannot completely dissolve
579 and uniformly distribute the hard gelatin capsule shells which are a mixture of amino acids
580 derived from collagen. For this reason, 0.1 M HCl was chosen to completely disintegrate the
581 capsules. Moreover, additional peaks on the chromatogram were not observed after the
582 sonication of 0.1 M HCl solution. It was noticed, that the ethanol headspace concentrations
583 increased four times when an aliphatic hydrocarbon solvent (**decane**) was added into the

584 sample solution, in comparison with commonly used organic solvents, such as: *N*-methyl-2-
585 pyrrolidinone (NMP), DMA, DMF and DMSO. ~~Ethanol responses in different organic~~
586 ~~solvents: *N*-methyl-2-pyrrolidinone (NMP), DMA, DMF, DMSO and decane are presented in~~
587 ~~Fig. 6.~~

588

589 ~~FIGURE 6~~

590

591 Ethanol is a polar protic and hydrophilic compound, thus cannot form hydrogen bonds
592 with decane in contrast to other solvents. Moreover, decane was not miscible with the capsule
593 sample solution of 0.1 M HCl, which further improved method sensitivity [35].

594 ~~Ionic liquids~~ IIs have attracted scientific attention due to a unique combination of
595 physicochemical properties and were proposed as dissolution medium for determination of
596 residual solvents in pharmaceuticals [20,51-56]. Especially, low vapor pressure at ambient
597 temperatures makes them an interesting ‘green’ alternative for many applications, for which
598 the volatility of traditional organic solvents (matrix medium) causes problems [29,52]. In
599 addition, IIs have a wide liquid range, high thermal, chemical stability and extraordinary
600 dissolubility so that the organic solvents are readily dissolved in them and the effect of matrix
601 medium in chromatogram can be avoided, even at higher headspace equilibrium temperatures
602 [53]. Thus, IIs appear to be an ideal matrix medium for HS analysis of pharmaceuticals and
603 several examples from recent literature are presented in Table 3.

604

605 TABLE 3

606

607 Based on the literature data, it can be concluded, that selection of the most appropriate
608 ionic liquid for HS analysis of pharmaceutical samples still remained at “trying-comparing-
609 screening” step, because of the relatively low availability of physical property data [51-56].
610 More advanced properties, such as solvation and polarity, measured by various techniques are
611 needed. For example, calculated decomposition temperature of 1-butyl-3-methylimidazolium
612 hexafluorophosphate is 370 °C, but during the experiment it started to decompose at 180 °C
613 [53]. Until more precise data on the properties of ionic liquids are available, the study of
614 development of ionic liquids as a matrix medium for sample preparation will continue based
615 mainly on empirical experimentation [55]. Another problem with the application of IIs is their
616 purity related to volatile remnants from the synthesis. At higher headspace equilibrium
617 temperatures, within the range of 160 to 200 °C, many interfering peaks on chromatograms

618 were observed. This may be also associated with premature thermal decomposition of IIs.
619 Moreover, the influence of background signals from the ionic liquid matrix on the separation
620 efficiency was examined [56]. Six commonly used IIs in HS analysis were tested under
621 different conditions: involving no treatment, sparging the liquid with heat, vacuum and
622 purging the headspace vial with nitrogen. These three different methods were applied for each
623 ionic liquid to reduce the amount of volatile species detected above them. The headspace
624 above untreated IIs was found to contain a significant number of impurities, which were
625 solvents (such as acetone, methanol, methylene chloride) and reagents (tetradecene,
626 imidazole) used in the manufacture of IIs. The calculated solvent concentrations were in the
627 range of 3 to 8700 ng mL⁻¹ of vapor. The background detected above the sparged IIs was the
628 cleanest, even simpler than that detected above most conventional solvents. The residual
629 solvent concentrations were less than 10 ppm. Based on the results, it can be concluded that
630 IIs routinely need to be purified prior to use to be useful as a matrix medium for HS analysis.
631 Sparging with high purity nitrogen is effective but inconvenient. Higher purity IIs need to be
632 available or simpler methods of purification need to be developed. Moreover, they must also
633 effectively dissolve analytes and matrix components such as derivatives of cellulose or fatty
634 acid salts, which still pose a challenge [29,56]. In order to overcome the drawbacks of IIs,
635 liquid paraffin has been recently proposed as a new matrix medium for the determination of
636 high boiling point residual solvents such as: DMF, DMA, DMSO and BA [57]. Liquid
637 paraffin in high purity is easy to obtain, relatively cheap and proved to be suitable for routine
638 analysis of particular sample types. The obtained limits of detection were below 1 µg/vial for
639 each compound, which indicates a drastically improved sensitivity compared to the
640 Pharmacopoeia method, which has not ensured their determination at 1/20 of respective
641 official limit concentrations, as is prescribed in the European Pharmacopoeia [8]. Moreover,
642 interfering peaks from the matrix medium were not observed on chromatograms at HS
643 temperatures below 100 °C, but the application range of liquid paraffin is rather small
644 regarding to general residual solvents analysis due to limited analyte or sample compatibility.

645 Static HS analysis can be carried out in different variants. One of the modified versions, in
646 which the equilibrium time and partition coefficients are not known, is the multiple headspace
647 extraction (MHE) [9,18]. It relies on gas extraction repeated many times from a sample,
648 followed by summing the peak areas from each step. The total concentration of the residual
649 solvent in the sample, which decreases exponentially, is determined based on the external
650 calibration curve [10,18]. This technique is mainly used for determination of solvents in solid
651 samples, but it can be also applied to liquid samples, particularly when the partition



652 coefficient of residual solvents is favorable and relative to the liquid phase. The method
653 requires more time to carry it out than to optimize the classic HS version, which makes it
654 unpopular. Another interesting development of static HS is based on evaporation of all
655 volatile compounds from a very small amount of sample at a sufficiently high equilibration
656 temperature. As a result, all volatiles are in the gas phase and there is no longer an
657 equilibrium, thus matrix effects are eliminated and the concentration in the gas phase is
658 linearly related to the sample size [17,28,29]. However, in practice matrix constituents are not
659 always completely volatile and may remain in the vial interacting with analytes. Therefore, it
660 is recommended that a recovery test should be performed. [58]. In the case when a sealed vial
661 containing the sample (a few milligrams or microliters) is heated to 20 °C above the melting
662 point of the matrix or the desolvation temperature, the method is named full evaporation
663 technique (FET). When the vial is heated to 20 °C above the boiling point of the matrix, the
664 method is named total vaporization technique (TVT) [59]. In this concept, all compounds
665 (including analytes, sample matrix and dilution medium) are transferred to the gaseous phase
666 of the vial. Therefore, since there is no condensed phase left in the vial, the choice of the
667 dissolution medium no longer influences the sensitivity [9,18,58,59]. It should be noted, when
668 the full evaporation is established further increasing the equilibration temperature will not
669 increase the sensitivity. In addition, the evaporation technique can be performed directly from
670 the powdered solid sample, overcoming matrix effects, or a dissolved sample in an
671 appropriate dissolution medium. The volume of the sample introduced for a TVT is limited
672 (between 10 μ L and 20 μ L) [59] by the increase of pressure in the vial at the high
673 equilibration temperature, which is one of its benefits and further development is expected in
674 this area. Especially, it can be quite interesting in analysis of expensive products.

675 In order to overcome the complex matrix interferences and improve the accuracy and
676 precision of GC analysis, the internal standard can be added [20,22,49]. The internal standard
677 efficiently compensates for the variables occurring during sample extraction as well as
678 injection, especially when a complex sample preparation procedure is involved [60]. A
679 standard addition method may be used, provided that the standard will not change
680 significantly the thermodynamic properties of the phases [10]. In FET and TVT sampling, the
681 addition of an internal standard is necessary to overcome the uncertainty of the dilution effect
682 induced by small variations of the vial volume [59]. Moreover, in determination of broad
683 spectrum of solvents in a single analytical run, several internal standards can be applied. But
684 at the same time it involves a greater number of parameters to be optimized and controlled.
685 The development of such a complex analytical methodology requires an appropriate



686 optimization procedure. The use of chemometric tools is very helpful and they have been
687 increasingly applied to processes optimization, especially over the past few years [21,61].
688 Recently, the experimental design and the multiple responses optimization techniques have
689 been successfully used in the development of analytical methodology for the determination of
690 31 residual solvents in metronidazole and betamethasone samples [61]. Chemometric tools
691 proved to be effective in achieving the fast and satisfactory optimization of chromatographic
692 conditions.

693 Many factors of HS analysis have a significant influence on the quantitative determination
694 of residual solvents and their optimization can be a critical to the development of an accurate
695 methodology. For these reasons, new solutions for sample preparation are needed.

696

697 **6.3 ~~Liquid-phase microextraction~~ Single-drop microextraction**

698

699 ~~Liquid-phase microextraction (LPME) Single-drop microextraction (SDME)~~ is an attempt
700 to solve the problems of classical LE by minimizing the consumption of solvents. It requires
701 only tiny amounts of organic solvents, of the order of a few microlitres, which eliminates the
702 need for extract cleanup prior to qualitative and quantitative determination. ~~The method is~~
703 ~~straightforward, quick and inexpensive. It is based on the partition of analytes between the~~
704 ~~sample solution and the small quantity of organic solvent.~~ There are two types of performing
705 of SDME sampling: direct mode and headspace analysis (HS-SDME), which is similar to
706 traditional HS sampling where the volatile compounds are isolated from the vapours above
707 the sample sealed in the vial, thus avoiding interferences from the matrix [20]. A HS-SDME
708 technique coupled with the GC method was proposed in 2006 for extraction and
709 determination of residual solvents in pharmaceutical products of hydroxycarbamide [22].
710 ~~While the extraction medium is in the form of a drop, this type of LPME technique is named~~
711 ~~single drop microextraction (SDME) and thereby makes it practically a solvent free method.~~
712 ~~Analyte isolation and preconcentration takes place in a single step. Around 1—3 μ L of~~
713 ~~solvent is drawn into a microsyringe (5—10 μ L) for the extraction of residual solvents this~~
714 ~~is usually DMSO or 1-octanol. Then the needle penetrates the membrane (rubber septum in~~
715 ~~the screw cap) of the vial containing the sample. In order to form a droplet, the syringe is~~
716 ~~fixed in such a way that the needle tip with the solvent drop is situated in accordance with the~~
717 ~~requirements of headspace analysis. When the extraction is completed, the microdroplet is~~
718 ~~drawn back into the syringe needle and injected into a GC for further analysis.~~



719 SDME is easy to apply and does not require sophisticated equipment. But at the same time
720 it requires the optimization of many parameters affecting performance of extraction, including
721 the following: extraction solvent, drop volume, shape of needle tip, extraction time,
722 thermostating temperature, sample amount and headspace volume. This process can be a
723 time-consuming and challenging task at times. A proper choice of the extraction medium is
724 fundamental for obtaining an optimal extraction procedure. There are many solvents with
725 different polarities that can be applied (for the extraction of residual solvents this is usually
726 DMSO or 1-octanol) but the relatively high vapour pressure and a low boiling point of some
727 solvents limited their use in SDME [18]. The amount of analyte extracted to the drop of
728 organic solvent is strictly related to its volume. With larger drops, analyte preconcentration is
729 better and extraction is more efficient. It should be noted that a larger drop, which is injected
730 into the chromatographic system, may cause band broadening in capillary GC. Moreover,
731 drops larger than 3 μL are less stable and the reproducibility of results with such drops is
732 poorer [17,18,22]. On the other hand, too small of drop volume may affect the precision of
733 sampling and insufficient isolation of analytes from the matrix. Moreover, magnetic stirring
734 and addition of inorganic salt can accelerate extraction efficiency, thus reducing the time
735 needed for reaching of thermodynamic equilibrium between the solid (or liquid) and gaseous
736 phases. The stirring time and speed must be selected such that the solvent drop does not
737 become detached from the needle. In order to perform microextraction directly from the solid
738 drug samples and eliminate dissolving step, a multiple headspace single-drop microextraction
739 (MHS-SDME) was proposed [21]. This method combines the SDME and MHE techniques,
740 thereby eliminating possible memory effects as a fresh drop of solvent is used for each
741 extraction. The obtained results indicate that MHS-SDME coupled with GC is very promising
742 and useful in the determination of residual solvents in solid drug products with high precision.

744 6.4 Solid-phase microextraction

745
746 Solid-phase microextraction (SPME), similarly to dynamic headspace analysis, has the
747 advantage of concentrating the analytes, thus lower limits of detection are achieved. It is a
748 sensitive, universal and solvent-free technique. In addition, SPME is simply applied in sample
749 preparation and can be automated routinely [62]. It is based on the adsorption of analytes on a
750 fibre coated with a suitable stationary phase, exposed from a microsyringe [9,17,18]. The
751 sensitivity of this technique depends primarily on the partition coefficient between the sample
752 and the fibre stationary phase. Commonly, fused silica fibres are used [20]. The stationary



753 phase is placed in contact with the sample matrix for predetermined time to establish a
754 concentration equilibrium of volatile analytes. A longer exposure of fibres does not lead to
755 accumulation of additional analytes. After that, adsorbed analytes are usually transferred to
756 the injection port of GC, where their thermal desorption and subsequent determination takes
757 place. The efficacy of preconcentration mainly depends on the type of stationary phase and its
758 thickness [62]. Moreover, the following parameters of the process are also important and can
759 influence the extraction efficiency: sample amount, extraction temperature and vial volume,
760 addition of inorganic salt, the pH of the solution and sample stirring. The materials used for
761 coating fibres include: polydimethylsiloxane (PDMS), polyacrylate (PA) and also mixtures of
762 polydimethylsiloxane and polydivinylbenzene (PDMS-DVB) and Carbowax and
763 polydivinylbenzene (CW-DVB) [63-66].

764 Depending on where the fibre is situated in relation to the sample, SPME can be divided
765 into direct immersion (DI-SPME) and headspace (HS-SPME) types. ~~In the first version,~~
766 ~~analytes are transported directly from the liquid sample solution to the extracting phase. In the~~
767 ~~second mode, volatile analytes are extracted after their transfer to the headspace phase above~~
768 ~~the sample. Thus, the fibre coating is protected from damage by high molecular mass and~~
769 ~~non-volatile interferences present in the sample.~~ HS-SPME coupled with GC is usually used
770 for determination of residual solvents in pharmaceutical samples. The advantage of this
771 method is that the limited capacity of the adsorbent precludes column overloading. In
772 addition, although it is not accepted by various pharmacopoeias, it provides a promising
773 alternative to HS-GC due to the simplicity of execution and greater sensitivity.

774 Furthermore, the HS mode of SPME can be performed in additional two systems, as
775 regards different volumes of headspace gas removed with the fibre. If only a small volume of
776 headspace gas is removed from the sample matrix, the technique is named gas-tight SPME
777 [23]. When a larger volume of headspace gas is removed with SPME fibre, the technique is
778 named “headspace” SPME. Both of these techniques were applied and compared with the
779 static HS analysis [67]. The obtained results of detection limits are presented in Table 4.

780

781 TABLE 4

782

783 Gas-tight SPME was found to be the most sensitive method, especially when very volatile
784 substances are determined (such as alcohols, aldehydes, ketones or some hydrocarbons).
785 However, the HS-SPME exhibited better method precision. Of all developed versions of the
786 SPME method, HS-SPME is the only practically feasible variation that can be employed,

23



787 because the extraction must be performed from a relatively concentrated drug solution, which
788 eliminates the use of immersion, “in-tube” SPME or stir-bar techniques [17,20,67].

789 Both SPME and SDME are alternatives to traditional extraction methods. Their
790 superiority over classical LE emerges from their rapidity, their consumption of minimal
791 amounts of organic solvent and the possibility of determining compounds present in low
792 concentrations. Moreover, the method is difficult to optimize and selectivity is poor in the
793 case of residual solvents extracted from samples with a complex matrix composition (such as
794 antibiotics). In addition, the extraction efficiency of SPME may change with the growing
795 number of injections, which limits its application in routine analysis of pharmaceutical
796 products.

797 There are many widely used novel and improved techniques that require the use of
798 minimal amounts of solvent, if any at all. But it is hard to state definitively which of them is
799 the most appropriate for extracting residual solvents from pharmaceutical samples, because
800 each one has its specific advantages and disadvantages. The summary of characteristics of
801 sample preparation techniques used in procedures for determining residual solvents in
802 pharmaceuticals is presented in Table 5.

803

804 TABLE 5

805

806 7. Detection, identification and quantitation of analytes

807

808 The most commonly used technique for the determination of residual solvents is
809 conventional GC, but high performance liquid chromatography (HPLC) is also practicable
810 [18,45]. GC is a natural choice because organic solvents have relatively low boiling points
811 and are generally thermally stable. For GC separation step, capillary (narrow-bore) and wide-
812 bore columns are applied. The most commonly used stationary phases of capillary columns
813 are based on the polysiloxanes and polyethylene glycols. While they have been applied
814 coelution of solvents with similar physicochemical properties was reported [28]. Therefore,
815 they are used alternatively to verify identity. To achieve the satisfactory separation, the GC
816 methods tend to have long run times because of the use of long capillary columns (up to 100
817 m) and slow temperature gradients (up to 60 minutes). Moreover, an additional time is
818 required to cool the column from the final temperature of the temperature programme to its
819 initial conditions. Nevertheless, in analyses of specific samples, most of the chromatographic
820 run time is not useful since in most pharmaceuticals only a few solvents are present.

821 In most cases, chromatographic techniques are used in combination with suitable
822 detectors. For analysis, when residual solvents present in the sample are known or suspected,
823 an universal FID is recommended. For detection of halogenated residual solvents, ECD can be
824 applied. In situations when solvents are unknown and an additional level of identification
825 capability is needed, MS is preferable. The choice of detectors for GC analysis is very rich,
826 but for residual solvents determinations, FID and MS detectors are the most appropriate,
827 which is confirmed by literature data [11,12,19,21,22,23,25-27,28,30,33,34,35,37-39,40-
828 48,50,51-53,57-59,60,65,66].

829 Information about examples of analytical procedures for determining residual solvents in
830 different types of pharmaceutical samples are presented in Table 6.

831

832 TABLE 6

833

834 Based on the review of literature data presented in Table 6, it can be concluded that no
835 single universal methodology is applicable to all kinds of solvent samples, which constitutes a
836 further challenge to analysts. Most of them meet the requirements of legal regulations but at
837 the same time they do not meet the expectations of the pharmaceutical industry, because of
838 the high cost of analyses, need for specialized equipment, problems with optimization,
839 complexity of multi-step procedures and thus increased time consumption. Still the most
840 frequently used method for the determination of residual solvents is static HS coupled with
841 GC. Currently, in order to decrease the analysis time significantly, while maintaining
842 complete separation, instrument modification is necessary.

843

844 8. Recent developments in the field of determination of solvent residues

845

846 In order to improve the efficiency of analyses, method sensitivity and reduce the GC
847 separation time, several alternatives were proposed in the recent literature, including:

- 848 • the use of shorter capillary columns with narrower bores (stationary phase layer about
849 0.1 – 1 μm) and fast temperature programming, known as a fast gas chromatography
850 (fast GC) [44,47,48];
- 851 • the use of two capillary columns with different stationary phases and separation
852 mechanisms, proposed as: parallel dual-column system connected with ‘Y’ splitter
853 [43], flow-modulation gas chromatography [68] and two-dimensional gas
854 chromatography (GCxGC) [69];

- 855 • the use of hydrogen as an alternative carrier gas to helium, thus higher linear velocities
856 can be achieved [18];
- 857 • the use of specially developed technologies of heating of capillary columns allowing
858 very high temperature programme rates (up to 1800 °C/min), for example: low thermal
859 mass (LTM) oven [46] and the EZ Flash GC technology (uses resistive heating) [48];
- 860 • the use of higher sample injection split ratio (within the range of 1:5-20, in some cases
861 up to 1:100) [11,48];
- 862 • the use of the programmed temperature vaporizer inlet (PTV) to inject the samples
863 into the GC column [36,60];
- 864 • the use of the methodology without sample pretreatment step, for example: the thermal
865 desorption (TD) technique coupled with GC-MS [70], where residual solvents
866 desorbed from sample by heating were cryofocused at the head of the column prior to
867 GC analysis;
- 868 • the use of the nonseparative method for quantitative analysis, for example: the HS-MS
869 method, based on direct coupling of headspace sampler with a mass spectrometer
870 without chromatographic separation [44].

871 One of alternatives of instrument modifications to improve sensitivity, keeping the simple
872 headspace instrumentation, is the use of the PTV inlet to inject the samples into the GC
873 column. This injector is equipped with a heating and cooling system. By using liners packed
874 with selective adsorption material (mostly Tenax), the analytes can be trapped in the liner.
875 Thus, no sample pretreatment is required and analysis can be performed directly from a solid
876 pharmaceutical sample. In addition, the analytical procedure is simpler and can be automated.
877 Moreover, the creation of artifacts or errors associated with the sample preparation step is
878 minimized. A traditional HS autosampler in combination with fast GC equipped with a PTV
879 and MS detector was applied for the determination of Class 1 residual solvents in drug
880 samples [36]. Different injection techniques were compared: classical split-hot injection,
881 classical splitless-hot injection and solvent vent injection. All experiments were carried out in
882 the PTV inlet. ~~The obtained window of the chromatograms (scan mode) of the most abundant
883 extracted ion for three residual solvents: 1,1-dichloroethene (a), 1,1,1-trichloroethane (b) and
884 benzene (c) with the three injection modes is presented in Fig. 7.~~

885
886 **FIGURE 7**
887



888 In all three cases, the solvent vent injection technique gave better results. Important
889 benefits of using this type of injection instead of classical-hot injection, such as better peak
890 shapes and better signal-to-noise ratios, were found. Thus limits of detection at the low ppt
891 level were obtained. It should be also noted, that the method showed good precision and
892 accuracy.

893 Another alternative of instrument modifications, in order to reduce analysis time up to
894 only 3 minutes, is a nonseparative method based on direct coupling of the HS sampler with
895 the mass spectrometer (HS-MS) [44]. First, identification of 20 residual solvents presented in
896 the samples was performed using HS-fast GC/MS. Then, information obtained from low-
897 resolution chromatograms visualized by using contour plots with time and mass/charge ratio
898 axes, and established specific zones for each studied solvent. Quantitative analysis of 27
899 different pharmaceutical products was performed without chromatographic separation. The
900 proposed methodology proved to be sensitive and sufficient to determine residual solvents
901 according to the ICH requirements. Moreover, no treatment of the sample was required.

902 An interesting development for fast determination of volatile impurities is the use of an
903 electronic nose technology. E-noses are based on an array of nonselective or selective (based
904 on functional group chemistry) sensors coupled to a pneumatic sampling system [17,71]. The
905 whole set of signals given by the sensor array provides a fingerprint of the analyzed vapor. To
906 compare different fingerprints, a multivariate data analysis is used. Even if the sensitivity of
907 these devices is much weaker than the GC methodologies, their development is important for
908 impurities monitoring in process analytical technologies (PAT) [2,4,17]. For determination of
909 residual volatile impurities after the cleaning of manufacturing equipment the ion mobility
910 spectrometry (IMS) is used [72]. The separation is based on the gas phase ion mobility at
911 atmospheric pressure, which is related to the geometry (structure and size) of the ions. Thus,
912 IMS can be highly specific and selective.

913

914 **9. Conclusions**

915

916 **Organic solvents routinely used in the manufacture of pharmaceuticals pose a problem**
917 **and must be removed. Nowadays, pharmaceutical companies try to exchange toxic solvents**
918 **by more friendly ones with similar properties or look for some new innovations. Substances**
919 **such as water, supercritical fluids and IIs are taken into consideration as a new alternatives.**
920 **However, implementation of new technologies into practice is still in development stage due**



921 to the lack of complete understanding of the basic principles and properties, and the high costs
922 of specialized equipment.

923 Static HS coupled with GC is the most frequently used methodology for the determination
924 of residual solvents due to the full automation, good precision, accuracy and is preferred for
925 samples soluble and uniformly distributed in dissolution medium. Several interesting new
926 alternatives have been developed in the last few years to support HS analysis. All of them
927 improve the method sensitivity through increasing the instrument response factor, decreasing
928 solution-vapor partition coefficient or increasing the activity coefficient. This can be achieved
929 by the instrument modifications, modified versions of HS and the use of new dissolution
930 medium, such as: mixtures of water and organic solvents or mixtures of different organic
931 solvents, binary solvents and IIs. Instrumental methods include use of the PTV inlet or direct
932 coupling of HS with MS. Research is continuing into the improvement of existing methods
933 and the development of new ones, which would allow determination in a quick, simple,
934 cheap, effective and environmentally friendly manner. This can be also achieved by
935 automation of microextraction techniques and coupling with GC. Currently, automated
936 sampling systems of SPME and SDME are available from major instrument manufacturers.
937 They offer high sensitivity and reproducibility, without many of problems or expense of HS
938 sampling system.

939 One of the most important trends in the development of methods and low-cost tools for
940 QC/QA of pharmaceuticals, according to the Process Analytical Technology (PAT) concept,
941 is the introduction of different types of sensors and techniques, such as: IMS techniques,
942 electronic nose technology and NIR-based chemical/physical sensors. They provide *on-line*
943 monitoring of volatile impurities during the manufacturing process, thus analysis time is
944 significantly reduced. Moreover, they are automated, efficient, cost-effective and do not
945 require large sample volumes for analysis. Further studies and developments are expected in
946 this area.

947

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949

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953

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1133 **List of figure captions and table headers**

1134

- 1135 1) Fig. 1 ‘Milestones’ in the field of development of analytical methodologies for evaluation the level of
1136 residual solvents and identification as well as quantitation of specific analytes
- 1137 2) Table 1 Examples of classes of solvents commonly used in the pharmaceutical industry [1,5,13]
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