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# Evaluation of the European Pharmacopoeia method for control of residual solvents in some antibiotics

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ABSTRACT

Residual solvents (RS) are volatile organic chemicals that are used or produced during the manufacturing process of drug substances or excipients. The European Pharmacopoeia (Ph. Eur.) limits the amount of RS in pharmaceuticals, considering the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for RS. According to the Ph. Eur. general method, water insoluble samples may be analyzed using DMF as dilution solvent at high equilibration temperatures such as 105 °C. This could be problematic in the case of antibiotics, many of which are water insoluble and temperature sensitive. Moreover, antibiotics are complex in nature and beside RS, one can expect several other volatile impurity peaks in the chromatogram. In this study, the Ph. Eur. method for RS analysis was evaluated for selected groups of antibiotics. An alternative dilution medium was proposed (DMSO–water), which offers optimum sensitivity while working at lower equilibration temperatures such as 80 °C. The optimized method was investigated for precision, accuracy, linearity and detection limits.



## 1. Introduction

Residual solvents (RS) are volatile organic chemicals (VOCs) that are used or produced during the manufacturing process of drug substances or excipients. As they have no therapeutic value and many of them are known to be toxic, RS need to be removed at the end of the manufacturing process. Although it is difficult to remove the RS completely with the common techniques in practical manufacturing processes, they need to be minimized to a level of safety. The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use classifies regularly used RS into three different classes based on their toxicity: Class-1 (solvents to be avoided), Class-2 (solvents to be limited), Class-3 (solvents with low toxic potential). According to ICH guidelines, Class-1 solvents must be identified and quantified, Class-2 solvents have individual limits and Class-3 solvents (when found to be more than 0.5%) need to be identified and quantified [1]. The European Pharmacopoeia (Ph. Eur.) limits the amount of RS in pharmaceuticals, considering the ICH guidelines for RS. The Ph. Eur. describes two different methods for qualitative and quantitative analysis of RS: System-A and System-B (Table 1) [2]. Both systems use static headspace gas chromatography (sHS-GC) with flame ionization detection (FID).

Three different sample preparation procedures are proposed based on the sample solubility—I: water for the water soluble samples; II: DMF (*N*,*N*-dimethylformamide) for the water insoluble samples; III: DMI (1,3-dimethyl-2-imidazolidinone) for the control of DMF and DMA (*N*,*N*-dimethylacetamide) in water insoluble samples. In the case of water soluble samples where water insoluble RS are present, the reference RS solutions in water are prepared using DMSO (dimethylsulfoxide) as bridging solvent. The headspace parameters proposed for each sample preparation procedure are shown in Table 2. Mass spectrometry and electron capture detectors are proposed as alternatives for FID in the analysis of chlorinated RS of Class-1. This is due to the poor sensitivity of FID towards chlorinated solvents. This method is intended for pharmaceuticals in general, but for some drug substances adaptations are necessary as already mentioned by Otero et al. [3].

Antibiotics are among the most frequently prescribed medications in modern medicine. RS in antibiotics are concerned today not only because of the safety, but also because of the type and the amount of residual solvent may influence physicochemical properties such as: particle size, crystalline structure [4], wettability [5,6], stability and dissolution properties [7] of the drug product. Moreover, RS may play a key role in the modification of odor as well [8]. This implies that quality control of antibiotics should

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**Table 1**
Overview of System-A and -B according to the Ph. Eur. method for identification and control of residual solvents

| | Parameter | System-A | System-B |
|---|---|---|---|
| 1 | Analytical column | A 30 m fused-silica capillary or wide-bore column with 0.25 or 0.53 μm i.d. | A 30 m fused-silica capillary or wide-bore column with 0.25 or 0.53 μm i.d. |
| | Internal coating | Cross-linked 6% polycyanopropylphenylsiloxane and 94% polydimethylsiloxane | Macrogol 20000 R (polyethylene glycol 20000) |
| | Film thickness | 1.8–3 μm | 0.25 μm |
| | Temperature | 40 °C for 20 min, 10 °C/min to reach 240 °C and 240 °C for 20 min | 50 °C for 20 min, 6 °C/min to reach 165 °C and 165 °C for 20 min |
| 2 | Carrier gas | Nitrogen (99.95%, v/v) or helium (99.995%, v/v) | Nitrogen (99.95%, v/v) or helium (99.995%, v/v) |
| | Linear velocity | 35 cm/s | 35 cm/s |
| | Split ratio | 1:5 | 1:5 |
| 3 | Detector | FID (MS or ECD may be used in the case of chlorinated solvent from Class-1) | FID (MS or ECD may be used in the case of chlorinated solvent from Class-1) |
| | Temperature | 250 °C | 250 °C |

i.d.: internal diameter; FID: flame ionization detector; MS: mass spectrometer; ECD: electron capture detector.

include accurate information on identity and quantity of any RS present.

Antibiotics can be considered as a complex group of pharmaceuticals. The RS analysis of such a group brings some potential challenges: many antibiotics are water insoluble and most of the antibiotics suffer from thermal instability. One can expect several other peaks than RS-related peaks in the chromatogram, which can lead to separation and identification difficulties.

Following the Ph. Eur. method for the RS analysis, antibiotics may be analyzed using DMF as a dilution medium with an equilibration program of 105 °C for 60 min. Three major problems are encountered when the general method is used for the analysis of RS in antibiotics:

1. *Sensitivity problems*: organic dilution media such as DMF offer higher partition coefficient values for most of the RS leading to less headspace sensitivity. In regular practice, achieving the required detection limits for all the RS is not always possible. This can only be partly solved by increasing the required sample amount for analysis.
2. *Stability problems*: many of the antibiotics may undergo degradation during the equilibration program (105 °C for 60 min) possibly leading to volatile degradation products. Moreover, the proposed dilution medium itself has been found to be unstable at temperatures higher than 100 °C [9] and produces artifact peaks when HCl salts are present [10].
3. *Selectivity problems*: as mentioned above, several other volatile impurities can be expected in the chromatogram together with the RS peaks. This will turn retention time-based identification questionable.

A solution for the equilibration temperature associated problems is changing the dilution medium. This dilution medium should offer lower partition coefficient values for most of the RS at low equilibration temperatures to give higher concentration in the headspace. Several alternative organic dilution media are published in the literature, which may include: DMSO, DMA, DMI, benzyl alcohol (BA) and *n*-octanol [9,11–15]. Any of these dilution media would offer less sensitivity at 80 °C than working at 105 °C. A way out can be the combination of organic dilution media with water (mixed aqueous dilution medium), which has been shown to enhance the sensitivity for most of the RS at low equilibration temperatures. Otero et al. reported the use of DMF in combination with water (2:3, v/v) as a dilution medium to achieve better sensitivity and recovery for the RS at low equilibration temperatures [3]. Such mixed aqueous dilution media have been reported since 1976, but are only employed now and then in RS analysis [16–19]. In our previous study (submitted for publication) we have investigated the mixed dilution media such as DMSO–water, DMF–water and DMA–water and reported the influence of the total liquid volume, water percentage and their interaction on the sensitivity of the regularly used VOCs. Considerable increase in the sensitivity was observed with the mixed aqueous dilution media over the pure organic dilution media for all the VOCs investigated. Moreover, mixed aqueous dilution media have produced similar validation data as that of the pure organic dilution media.

In this study, different dilution media were investigated at 80 °C equilibration temperature using different groups of antibiotics, regularly investigated in our laboratory. The dilution media included are DMSO, DMF, DMA, DMSO–water, DMF–water and DMA–water. Using the dilution media that offered better sensitivity, all the antibiotic samples were investigated according to the Ph. Eur. method requirements using HS-GC-MS and HS-GC-FID.

**Table 2**
Headspace parameters according to the Ph. Eur. method for identification and control of residual solvents

| Parameters | Sample preparation procedure | | |
|---|---|---|---|
| | I | II | III |
| Equilibration temperature (°C) | 80 | 105 | 80 |
| Equilibration time (min) | 60 | 60 | 60 |
| Transferline temperature (°C) | 85 | 110 | 105 |
| Pressurization time (s) | 30 | 30 | 30 |
| Injection volume (ml) | 1 | 1 | 1 |

I: water soluble samples; II: water insoluble samples; III: for the control of DMF and DMA.

## 2. Experimental

### 2.1. Reagents and samples

The purity of all the reference VOCs used was more than 99% by GC. Acetone, methanol, ethanol, acetonitrile and dichloromethane were obtained from Fisher Chemicals (Loughborough, England); *p*-xylene and ethylbenzene from Acros Organics (Geel, Belgium); benzene and *m*-xylene from Merck (Darmstadt, Germany); toluene, *o*-xylene and 1-propanol from BDH (Poole, England) and carbon tetrachloride, chloroform and methyl isobutyl ketone (MIBK) from Riedel-de Haën (Seelze, Germany). Organic dilution solvents, DMF (99.9%), DMA (99.9%) and DMSO (99.9%), were obtained from Fisher. DMSO was bought in 100 ml bottles as it was giving additional peaks on long standing, once the bottle was opened. Distilled water was produced in the laboratory. The 20 ml headspace vials and the aluminum crimp caps were obtained from Filter Service (Eupen, Belgium).