Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 113–119

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

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

Yarramraju Sitaramaraju, Adil Riadi, Ward D'Autry, Kris Wolfs, Jos Hoogmartens, Ann Van Schepdael, Erwin Adams*

Laboratory for Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, K.U.Leuven, Leuven, Belgium

ARTICLE INFO

Article history:
Received 4 April 2008
Accepted 12 May 2008
Available online 21 May 2008

Keywords:
Residual solvents
European Pharmacopoeia
Antibiotics
Headspace
GC

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

* Corresponding author. Tel.: +32 16323444; fax: +32 16323448.
E-mail address: Erwin.Adams@pharm.kuleuven.be (E. Adams).

0731-7085/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jpba.2008.05.015

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).

Table 3
Standard solutions used for this investigation and the concentration levels obtained by serial dilution

	For benzene and CCl_4 (mg)	For other RS (mg)
Quantity weighed in 50.0 ml of DMF (S_1)/DMSO (S_2)/DMA (S_3)	200	250
Conc. level 1	0.0008	0.001
Conc. level 2	0.004	0.005
Conc. level 3	0.008	0.010
Conc. level 4	0.016	0.020
Conc. level 5	0.040	0.050
Conc. level 6	0.080	0.100
Conc. level 7	0.160	0.200
Conc. level 8	0.400	0.500

2.2. Antibiotic samples

A group of 70 commercial bulk antibiotic samples that were available in our laboratory was selected for this investigation. This group includes: erythromycin (23), doxycycline (12), gentamicin (12), colistin (9), cefalexin (3), cefradine (3), cefadroxil (1), gramicidin (3), tetracycline (1), ampicillin trihydrate (2) and amoxicillin trihydrate (2) samples. These samples were from different origin.

2.3. Standard solutions

Two hundred and fifty milligrams of the reference VOCs (see Section 2.1) belonging to three different classes according to ICH and one unclassified RS were weighed carefully into three 50.0 ml volumetric flasks, which were half filled one with DMF (S_1), one with DMSO (S_2) and an other with DMA (S_3). These mixtures were later made up to 50.0 ml with the respective solvent. S_1, S_2 and S_3 were further diluted to the desired concentrations by serial dilution (Table 3). All the dilution media were purged with nitrogen for 4 h to remove volatile impurities.

2.4. Sample preparation

The total liquid volume used in the HS vial was 5.0 ml. In the case of organic dilution media, 1.0 ml of organic dilution medium containing reference compounds/sample was added to a HS vial and 4.0 ml of the same dilution medium was added before the vial was sealed. In the case of mixed aqueous dilution media, 1.0 ml of organic dilution medium containing reference compounds/sample was added to a HS vial and 4.0 ml of water was added before the vial was sealed.

2.5. Instrumentation

2.5.1. HS-GC-FID

The GC-FID instrument used was a DELSI 200 capillary gas chromatograph (Delsi Nermag, Argenteuil, France), which was connected with a DANI 8650 static headspace autosampler (DANI, Milan, Italy). The headspace sampler was equipped with a 1 ml injection loop.

2.5.2. HS-GC-MS

The GC instrument used was an Autosystem XL capillary gas chromatograph (PerkinElmer, Foster City, CA, USA) coupled to a Turbomass mass spectrometer (PerkinElmer). The headspace used was a Turbomatrix HS40XL (PerkinElmer). The data from the MS were collected and integrated by TURBOMASS software (PerkinElmer).

Both systems were connected with a chromatographic column from the same manufacturer and same batch. The chromatographic column used was an AT-Aquawax (Alltech, Deerfield, IL, USA), 30 m in length and 0.53 mm in internal diameter. It is coated with a 0.5 μm film of bonded polyethylene glycol (PEG). This bonded PEG offers more stability towards repeated injections of water. The carrier gas used in this study was helium (Messer, Machelen, Belgium). The instrumental parameters are mentioned in Table 4. The GC parameters were adapted from System-B of the Ph. Eur.

Table 4
HS-GC-FID and HS-GC-MS parameters used in this study

Parameter	Optimized settings
1. GC	
Oven temperature	50 °C for 15 min, increased at 40 °C/min to 180 °C, held for 10 min
Injection port temperature	140 °C
Carrier gas	Helium 5.6
Linear velocity	35 cm/s (approx. 4.0 ml/min)
Split flow	20 ml/min
2. Headspace	
Equilibration temperature	80 °C
Equilibration time	45 min
Needle temperature	105 °C
Transferline temperature	120 °C
Carrier gas pressure	180 kPa
Pressurization time	30 s
Injection time	0.04 min
Needle withdrawal time	0.3 min
3. FID	
Temperature	250 °C
4. MS	
Ion source temperature	250 °C
Ionization mode	Electron ionization
Ionization energy	−70 eV
Scan mode	Total ion recording
Scan range	*m*/*z* 16–350
Scan time	1.0 s
Inter-scan delay	0.5 s

3. Results and discussion

3.1. Chromatographic separation

To investigate the chromatographic separation compliance with the Ph. Eur. method requirements, a mixture of dichloromethane and acetonitrile in DMF was injected in both HS-FC-FID and HS-GC-MS. The resolution was found to be 3.6, which passes the Ph. Eur. system suitability requirement for System-B (minimum 1.0). The separation obtained with the AT-Aquawax column was found to be better than that of the separation shown in the Ph. Eur. (Fig. 1). However, dichloromethane and ethanol could not be resolved (Fig. 1, peaks 4 and 5).

3.2. Headspace parameters optimization

With a fixed equilibration temperature of 80 °C, different equilibration times such as 5, 10, 15, 30, 45, 60 and 90 min were investigated. The obtained peak areas were plotted against the equilibration time. In the case of organic dilution media, 1.0 ml of 100 μg/ml standard solution S_1 and 4.0 ml of DMF were added to the HS vials. In the case of mixed aqueous dilution media, 1.0 ml of 100 μg/ml standard solution S_1 and 4.0 ml of water were added to the HS vials. In no case further significant increase in peak area was observed for any RS at equilibration times longer than 30 min. Although 30 min was enough to achieve an equilibrium state, it was observed that the repeatability was better at longer equilibrium times. Hence, an equilibration time of 45 min was chosen for further investigations. As all the selected organic dilution media are known to behave similarly in reaching the equilibrium state, DMSO and

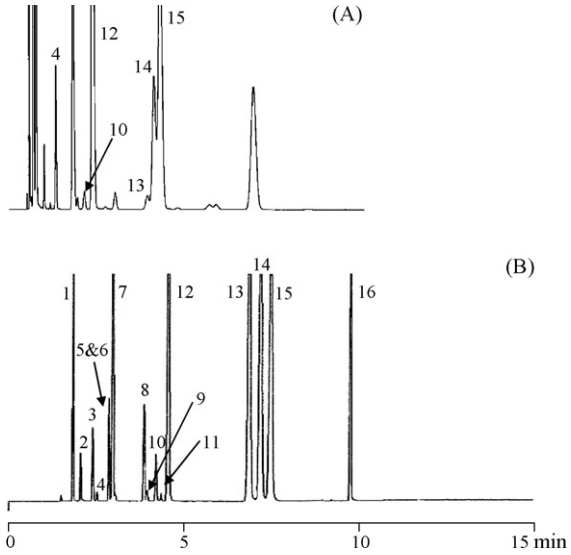

Fig. 1. Chromatograms obtained with reference solvents: (A) chromatogram according to Ph. Eur. System-B and (B) chromatogram obtained in this study using AT-Aquawax column. 1, DMS (dimethyl sulfide, not discussed in this study); 2, acetone; 3, carbon tetrachloride; 4, methanol; 5, dichloromethane; 6, ethanol; 7, benzene; 8, MIBK; 9, acetonitrile; 10, chloroform; 11, 1-propanol; 12, toluene; 13, *p*-xylene; 14, *m*-xylene; 15, *o*-xylene; 16, ethylbenzene.

DMA were not investigated in this stage. Initially the manifold and transferline temperatures were maintained at 85 °C, as described in Ph. Eur. method. At such temperatures carryover of xylene was observed. Working at increased temperatures with 105 °C for the manifold and 120 °C for the transferline showed no detectable carryover of any RS investigated.

3.3. Dilution media comparison

The dilution media were compared using the optimized equilibration program (80 °C for 45 min) and the other parameters mentioned in Table 5. The concentration level investigated was 100 μg/vial. Sample preparation was as mentioned in Section 2.4. Each experiment was performed in triplicate. The total liquid volume in the vial was maintained at 5.0 ml. Huge difference in sensitivity was found between the pure organic dilution media and the organic dilution media mixed with water. The increments ranged from 1.3 to 78 times depending on the RS (Table 5). Water miscible RS showed less increase in sensitivity than water insoluble RS. The sensitivity difference between the organic dilution media (DMSO, DMF and DMA) was quite high. DMSO showed better sensitivity for all the RS except alcohols. For alcohols DMF showed similar or better sensitivity followed by DMA. These observations are in agreement with the results reported in the literature [9]. The sensitivity difference between the organic dilution media mixed with water (DMSO–water, DMF–water and DMA–water) was also considerable, but less as compared to that of the organic dilution media. Among all, DMSO–water showed better headspace sensitivity for most of the RS. Hence, DMSO–water was chosen as dilution medium for further experiments.

3.4. Qualitative analysis of antibiotic samples

Both HS-GC-MS and HS-GC-FID were used in this stage. All the samples were investigated using DMSO–water as dilution medium. Here, the main aim was to detect as many as possible volatile compounds that are present in the samples. Hence, maximum amount of sample that can be dissolved in 1.0 ml of DMSO was added and additionally 4.0 ml of water was brought in to the HS vial. After addition of water, some samples precipitated when more than 250 mg/vial was used. Hence, a sample concentration of 250 mg/vial sample was used for qualitative analysis. Typical HS-GC-FID chromatograms obtained with erythromycin, amoxicillin, colistin sulfate are shown in Fig. 2.

All the antibiotic samples, except colistin and some of the gentamicin sulfate samples, showed several peaks in the chromatogram. The majority of these peaks was eluted during the first 5 min of the chromatogram. As can be seen from Fig. 2A and B, several of the early eluted peaks show retention times close to those of the RS. Identification purely based on chromatographic retention times was found to be difficult. Several of the peaks could be misinterpreted as RS peaks. Unless an additional dimension of identification is applied, it was practically not possible to establish a confirmative identification for such samples. Hence, all the samples were screened with HS-GC-MS to discriminate the RS peaks.

With the MS, RS peaks were identified by comparing their mass spectra with the standard spectra in the database of mass spectra library (National Institute of Standards and Technology). It was observed that about 80% of the samples investigated contained several peaks other than RS. When the peak purity of the identified RS in each sample was investigated, none of the RS peaks was found to be coeluted with any of the other impurity peaks. Hence, in the cases such as colistin (Fig. 2C), where there were no detectable peaks other than RS, retention time-based identification was found to be sufficient. However, with the majority of the samples where several non-RS peaks were present, MS was

Table 5
Sensitivity difference between the different dilution media investigated (equilibration at 80 °C for 45 min)*

	RS	DMSO	DMA	DMF	DMSO + water	DMA + water	DMF + water
1	Benzene	1.00	0.65	0.38	21.5	17.6	16.6
2	CCl_4	1.00	0.61	0.54	19.7	20.1	19.0
3	Dichloromethane	1.00	0.94	0.72	12.4	10.9	10.5
4	Chloroform	1.00	0.65	0.30	55.3	42.6	41.7
5	Toluene	1.00	0.59	0.39	39.3	31.2	33.0
6	*o*-Xylene	1.00	0.59	0.44	78.3	60.5	66.0
7	*m*-Xylene	1.00	0.56	0.38	70.0	56.8	57.7
8	*p*-Xylene	1.00	0.56	0.37	69.3	56.2	57.3
9	Ethylbenzene	1.00	0.56	0.39	70.3	57.3	61.5
10	Methanol	1.00	1.79	2.10	2.03	2.37	2.19
11	Acetonitrile	1.00	1.03	1.02	3.05	2.80	2.74
12	Acetone	1.00	0.50	0.71	1.39	0.78	1.13
13	Ethanol	1.00	1.23	1.39	2.05	2.32	1.92
14	1-Propanol	1.00	0.93	1.30	4.80	3.84	4.23
15	MIBK	1.00	0.55	0.39	11.8	8.65	8.98

*Peak areas normalized to the peak area obtained in DMSO.

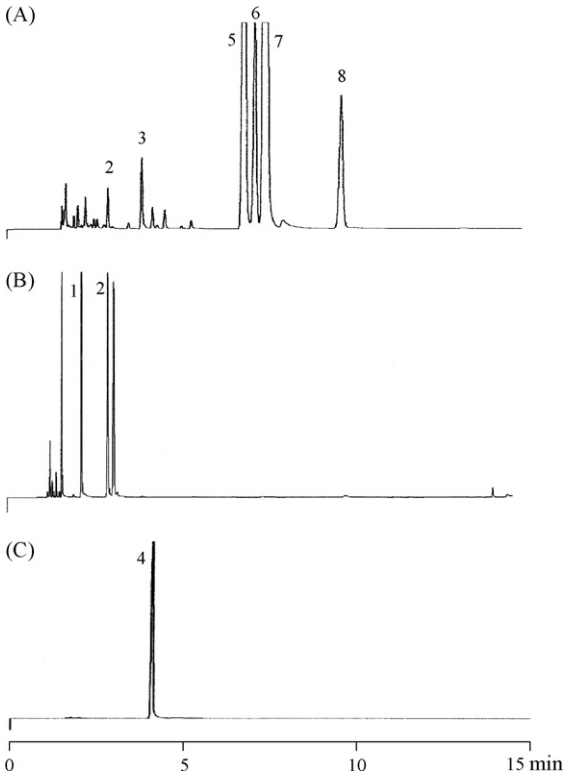

Fig. 2. FID chromatograms obtained with 200 mg of sample in 1.0 ml of DMSO + 4.0 ml of water (A, erythromycin; B, ampicillin; C, colistin). 1, Acetone; 2, dichloromethane; 3, MIBK; 4, chloroform; 5, *p*-xylene; 6, *m*-xylene; 7, *o*-xylene; 8, ethylbenzene. The instrumental parameters used were those from Table 5.

found to be necessary for accurate identification. Besides the RS peaks, a lot of impurity peaks were also identified by using the standard mass spectra library search. Antibiotics belonging to the same group showed similar impurity peaks. Some of the identified impurity peaks were found to be aldehydes and esters. Among the aldehydes, acetaldehyde and propanal and among the esters propyl acetate were found in many of the samples. Considering the results obtained by Barbarin et al., many of the impurity peaks obtained in our study could be generated due to the presence of RS. Barbarin et al. reported that the acetaldehyde presence would be due to ethanol and the propanal due to propanol [8].

To see whether these impurity peaks can also be detected using the Ph. Eur. proposed dilution medium, erythromycin samples were also injected using DMF and an equilibration temperature of 105 °C for 45 min. The chromatograms obtained for an erythromycin sample using the Ph. Eur. conditions versus the optimized conditions (DMSO–water at 80 °C for 30 min) are compared in Fig. 3. As can be seen from Fig. 3, some RS peaks and many of the other impurity peaks could not be detected by using the Ph. Eur. method. The RS found in all the samples investigated are shown in Table 6.

3.5. Quantitative analysis of antibiotic samples

The optimized method (Table 4) was validated by HS-GC-FID for linearity, precision, accuracy and limit of detection.

3.5.1. Linearity

The linearity was investigated in a concentration range from 0.8 to 500 μg/vial. Eight different concentration levels were investigated (Table 3). All the RS investigated were found to show a linear relationship with the concentration. The R^2 values for all the RS were found to be more than 0.999. The data are presented in Table 7.

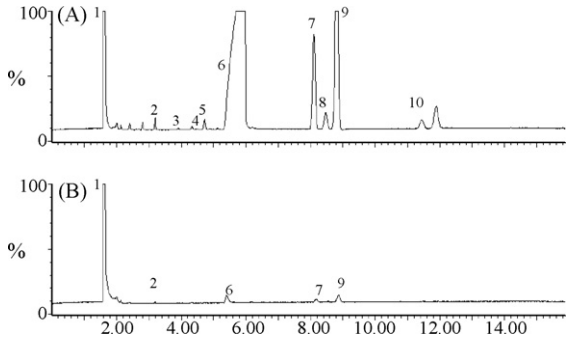

Fig. 3. Total ion chromatograms obtained with 200 mg of an erythromycin sample. A: in 1.0 ml of DMSO + 4.0 ml of water at 80 °C for 45 min; B: in 6.0 ml of DMF as in the Ph. Eur. method at 105 °C for 60 min. 1, System peak; 2, dichloromethane; 3, MIBK; 4, chloroform; 5, toluene; 6, water; 7, *p*-xylene; 8, *m*-xylene; 9, *o*-xylene; 10, ethylbenzene. Unlabelled peaks are not in the RS list.

3.5.2. Repeatability

Each point investigated in the linearity study was done in triplicate, except the concentration level of 100 μg/vial where six vials were analyzed. Relative standard deviations of peak areas were taken as a measure of repeatability. At quantities around and more than 50 μg/vial, less than 2% R.S.D. on the peak area was achieved. Quantities of 1, 5 and 20 μg/vial showed less than 4% R.S.D. As an example, repeatability values for 100 μg/vial concentrations are shown in Table 7.

The same 100 μg/vial concentration level was investigated for 3 days on the same instrument by the same analyst. Each day six

Table 6
List of the residual solvents found in the antibiotic samples investigated

Sample group	RS found	ICH class
Erythromycin (23)	Benzene (2)	1
	Ethanol (4)	3
	Dichloromethane (5)	2
	Chloroform (1)	2
	Toluene (9)	2
	MIBK (10)	3
	Xylene (10)	2
Gentamicin (12)	Dichloromethane (1)	2
Doxycycline (12)	Acetone (1)	3
	Methanol (1)	2
	Ethanol (12)	3
	Toluene (3)	2
	Ethyl acetate (3)	3
Gramicidin (3)	–	
Cefradine (3)	Acetone (3)	3
	Benzene (1)	1
	Propanol (2)	3
	MIBK (1)	3
Colistin (9)	Ethanol (1)	3
	Ethyl acetate (1)	3
Cefadroxil (1)	–	
Tetracycline (1)	Acetone (1)	3
	1-Butanol (1)	3
Ampicillin (2)	Benzene (2)	1
	Dichloromethane (2)	2
	Ethanol (1)	3
Amoxicillin (2)	Acetone (2)	3
	Benzene (2)	1
	Ethanol (2)	3
	Dichloromethane (2)	2
Cefalexin (3)	–	

Table 7
Validation data for the residual solvents investigated ($S_{y,x}$: standard error, precision was calculated at 100 μg/vial; LOD: limit of detection; s/n: signal-to-noise ratio; *A*: at 10 μg/vial and *B*: at 100 μg/vial)

RS	R^2	Equation	$S_{y,x}$	R.S.D. (%)	LOD (ng/vial)	Recovery	
						A	*B*
Benzene	0.999	$722x - 678$	679	0.8	6	99.8	100.6
CCl_4	0.999	$1423x + 680$	3,050	1.2	50	100.3	100.8
Dichloromethane	0.999	$10{,}785x - 6977$	18,329	1.7	40	102.5	101.8
Chloroform	0.999	$1938x - 413$	1,993	0.8	50	99.5	101.1
Toluene	0.999	$935x + 39$	1,637	0.7	6	100.2	99.2
o-Xylene	0.999	$13{,}429x - 176$	11,778	0.5	10	98.2	98.9
m-Xylene	0.999	$13{,}420x + 16{,}024$	23,852	0.6	10	99.4	99.0
p-Xylene	0.999	$13{,}162x + 12{,}270$	16,829	0.5	10	100.8	99.2
Ethylbenzene	0.999	$13{,}103x + 3496$	8,918	0.8	15	101.0	99.8
Methanol	0.999	$11{,}120x + 5191$	13,689	1.6	140	102.9	101.6
Acetonitrile	0.999	$13{,}429x - 176$	11,778	1.6	100	97.9	100.7
Acetone	0.999	$13{,}420x + 16{,}024$	23,852	1.3	40	99.9	100.5
Ethanol	0.999	$13{,}162x + 12{,}270$	16,829	1.8	120	101.2	102.0
1-Propanol	0.999	$13{,}103x + 3496$	8,918	1.1	100	103.3	102.7
MIBK	0.999	$11{,}120x + 5191$	13,689	1.2	40	99.7	100.4

injections were performed. The between-day repeatability was calculated as overall relative standard deviations, which were found to be less than 4% (for $n = 18$, 6 injections/day × 3 days).

3.5.3. Recovery

The recovery values were determined at two concentration levels. A gentamicin sample with no RS or impurity peaks in the chromatogram was spiked with reference RS and analyzed. Sample preparation was done by dissolving in a HS vial 50 mg of sample in 1.0 ml of standard solution S_2 corresponding to the concentration level 3 or 6 (Table 3), and then 4.0 ml of water was added. The recovery values obtained are given in Table 7. The recovery values were found to be within 97.9 and 103.3%.

3.5.4. Detection limits

The limit of detection is defined as the lowest amount of analyte in a sample which can be detected, which is accepted to correspond to a signal-to-noise ratio of 3. The detection limits are listed in Table 7. The detection limits obtained are sufficient for sample quantities around 10 mg/vial to meet the ICH limit for any residual solvent.

3.5.5. Quantitative results

Using an erythromycin sample containing dichloromethane, toluene and MIBK, both the standard addition and external calibration methods were tried to quantify the RS. In all cases, 50.0 mg/vial of sample were used. In the case of external calibration, the calibration curve from the method validation was used. For the standard addition, four sets of vials were prepared. Each set consisted of three vials with the same content to check the repeatability. Set 1 contained the sample in dilution medium, set 2 also contained 1.0 μg/vial of reference analytes, set 3 also contained the sample + 5.0 μg/vial of reference analytes and set 4 also contained the sample + 10.0 μg/vial of reference analytes. The obtained peak areas for each analyte from the four sets were plotted against the concentration of the reference analyte added. From the equation $y = ax + b$ (y: peak area, x: amount of reference analyte added, a: slope, b: intercept of the regression line), the concentration of the analyte in the sample was calculated by dividing b by a (i.e. amount of analyte present in the vials from set 1).

The results from both methods are presented in Table 8. As can be seen, the difference between both methods is very small. Hence, all the RS present in the samples were estimated using the external calibration method. Sample weights ranging from 10.0 to 50.0 mg/vial concentrations were used. The obtained concentrations were below the recommended limits by the ICH guidelines except for two samples. The failed samples contained benzene (12 ppm) and chloroform (720 ppm).

Table 8
Quantification results obtained for an erythromycin sample at a concentration of 50.0 mg/vial using external calibration and standard addition method

RS	External calibration (μg/vial)	Standard addition (μg/vial)
Dichloromethane	6.4 μg	6.3 μg
Toluene	2.6 μg	2.5 μg
MIBK	3.5 μg	3.4 μg

4. Conclusions

The Ph. Eur. method for RS analysis was evaluated for a selected group of antibiotics. Correct identification based on retention time only was not always possible. Application of MS was found mandatory. Increased needle and transferline temperature (105 and 120 °C, respectively) could reduce the possibility of carryover of high boiling point analytes. To enhance the sensitivity, an alternative dilution medium, a mixture of organic solvent with water was proposed. With the proposed dilution medium ICH limits can now be achieved with more reasonable sample quantities. Equilibration conditions were reduced to 80 °C for 45 min without sacrificing the sensitivity, which is a great advantage for temperature sensitive and water insoluble antibiotics.

References

[1] International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Q_3C: Impurities: Guidelines for Residual Solvents, Step 4, July 1997.
[2] European Pharmacopoeia 6th edition (2.4.24), Identification and Control of Residual Solvents, Strasbourg, France, 2006.
[3] R. Otero, G. Carrera, J.F. Dulsat, J.L. Fabregas, J. Claramunt, J. Chromatogr. A 1057 (2004) 193–201.
[4] S. Nojavan, A. Ghassempour, Y. Bashour, M.K. Darbandi, S.H. Ahmadi, J. Pharm. Biomed. Anal. 36 (2005) 983–988.
[5] G.A.M. Hermann, STP Pharma Pratiques 1 (1991) 258–265.
[6] C. Witchi, E. Doelker, Eur. J. Pharm. Biopharm. 43 (1997) 142–215.
[7] I. Devotta, M.V. Badiger, P.R. Rajamohanan, S. Ganapathy, R.A. Mashelkar, Chem. Eng. Sci. 50 (1995) 2557–2569.
[8] N. Barbarin, B. Rollmann, B. Tilquin, Int. J. Pharm. 178 (1999) 203–212.
[9] K. Urakami, A. Higashi, K. Umemoto, M. Godo, J. Chromatogr. A 1057 (2004) 203–210.
[10] C.R. Lee, N. van Dau, A.M. Krstulovic, Int. J. Pharm. 195 (2000) 159–169.
[11] T. Natishan, Y. Wu, J. Chromatogr. A 800 (1998) 275–281.
[12] C.B. Hymer, Pharm. Res. 23 (2003) 337–344.
[13] K. Fliszar, J.M. Wiggins, C.M. Pignoli, G.P. Martin, Z. Li, J. Chromatogr. A 1027 (2004) 83–91.

[14] S. Klick, A. Skold, J. Pharm. Biomed. Anal. 36 (2004) 401–409.
[15] C. Camarasu, C. Madichie, R. Williams, Trend Anal. Chem. 25 (2006) 768–777.
[16] R.J. Steichen, Anal. Chem. 48 (1976) 1398–1402.
[17] H. Hachenberg, A.P. Schmid, Gas Chromatographic Headspace Analysis, Heyden & Son, London, 1977.
[18] B.V. Ioffe, A.G. Vitenberg, Headspace Analysis and Related Methods in Gas Chromatography, John Wiley & Sons, New York, 1984.
[19] B. Kolb, L.S. Ettre, Static Headspace-Gas Chromatography: Theory and Practice, Wiley-VCH, Weinheim, 1997.