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# Headspace gas chromatographic analysis of residual solvents in pharmaceuticals: comparison of two matrix media

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Dimethylformamide (DMF) and a deep eutectic solvent choline chloride-ethylene glycol (ChCl-Eg) were investigated as potential matrix media for static headspace gas chromatographic (SHS-GC) determination of residual solvents in pharmaceuticals. Sample equilibration temperature, equilibration time and injection time were optimized. In the case of DMF 140°C equilibration temperature was applied. For ChCl-Eg equilibration temperature could not exceed 80°C as ChCl-Eg started to degrade at elevated temperatures. The higher equilibration temperature of DMF solutions favoured a transition of the analytes to the headspace and consequently resulted in lower detection limits of the analytes. Thus DMF has been considered a more suitable matrix medium than ChCl-Eg and was applied for the SHS-GC determination of residual solvents in pharmaceuticals.

**Keywords:** pharmaceuticals, residual solvents, static headspace gas chromatography

## INTRODUCTION

Residual solvents in pharmaceuticals are considered as volatile organic chemicals that are used or produced during the manufacture of pharmaceutical ingredients, excipients and drug products. Residual solvents may also contaminate products during packaging, storage and transportation [1]. Because residual solvents have no therapeutic benefits but many of them have toxic or environmentally hazardous properties, it must be ensured that they are either not present in products or are only present below recommended acceptable levels [2].

The International Council for Harmonisation of Technical Requirements for Pharmaceutical and Human Use (ICH) has issued limits for the levels of residual solvents in pharmaceutical products [3, 4]. Residual solvents are classified in three categories. Class 1 solvents are known human carcinogens or are strongly suspected carcinogens and/or environmental hazards and should be avoided. Class 2 solvents are not genotoxic impurities, but with a level of toxicity that must be limited in drug products to the indicated concentration. Class 3 solvents have the lowest risk and are limited to 5000 ppm [1, 5].

Residual solvent analysis can be performed with a large array of analytical techniques but the most frequently used and selective analytical technique is gas chromatography (GC). However, pharmaceutic samples often contain thermolabile or nonvolatile substances. Those substances can

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contaminate the chromatographic system or their peaks in the chromatogram can interfere with peaks of the analyte. Those problems can be solved by combining gas chromatographic analysis with headspace sampling as in the case of headspace sampling only a volatile portion of the sample is subjected to analysis [1, 6].

Several headspace techniques such as static headspace (SHS) [7–11], dynamic headspace [12] and headspace solid phase microextraction [5, 13] can be employed. Static headspace analysis is probably the most widely used technique for residual solvent analysis in pharmaceuticals as it is inexpensive, easy to perform and automate, can be successfully used routinely in control labs. In the static headspace procedure, a sample is placed into a sealed vial and is heated until a thermodynamic equilibrium between a sample and a gas phase is reached. After, a determined volume of the gas phase is injected into the gas chromatograph for analysis [14].

For quantification, it is necessary to achieve that the distribution of the analyte between the calibration solution and the headspace is the same as between the sample and the headspace. The sample must therefore be dissolved or dispersed in a matrix medium that is the same as the solvent used for calibration solutions. The nature of the matrix medium is of primary importance as has an essential influence on the amount of the analytes in the headspace and thus on the determination sensitivity. For residual solvents determination in pharmaceuticals, water [8], dimethylsulfoxide [9, 15], N,N-dimethylacetamide [16], N,N-dimethylformamide (DMF) [15], benzyl alcohol [17], liquid paraffin [18] and ionic liquids [3, 11] were suggested as matrix media. Recently, for residual solvents determination deep eutectic solvents (DESs) have been proposed [7, 8, 19].

DESs are gaining an increasing interest as they are eco-friendly, biodegradable, inexpensive and easy to prepare [20]. They are composed of a hydrogen bond donor and of a hydrogen bond acceptor. Generally, DESs have a low vapour pressure, a relatively wide liquid range and a much lower melting point than that of any of its individual components [21]. Those features make DESs very attractive as matrix media for application in SHS-GC.

The goal of this study was to compare two solvents as matrix media – conventional DMF and recently proposed deep eutectic solvent choline chloride-ethylene glycol (ChCl-Eg) – and to develop a simple and sensitive static headspace gas chromatographic (SHS-GC) procedure for the determination of residual solvents in pharmaceuticals. Five solvents – methanol, ethanol, dichloromethane (DCM), acetone and acetonitrile (ACN) – often used for the synthesis and purification of drug substances were employed as target analytes.

## EXPERIMENTAL

### Reagents and samples

Methanol (99%), ethanol (96%), acetonitrile (99.8%), choline chloride (98%) and ethylene glycol (99%) were purchased from Alfa Aesar (Germany). Dichloromethane (99.8%) was purchased from Merck (Germany). Acetone (99.9%) and N,N-dimethylformamide (99.8%) were purchased from Roth (Germany).

The pharmaceuticals investigated were 'Paracetamolis Sanitas' (Sanitas, PharmaSwiss Czech Republic), 'Paracetamol' (Borisovskiy zavod medicinskikh preparatov, Belarus), 'Omeprazol Sandoz' (Sandoz, Netherlands), 'Omeprazole' (Lekpharm, Belarus), Drotaverine hydrochloride 'No-Spa' (UAB Sanofi-Aventis, Lithuania), 'Drotaverine' (Borisovskiy zavod medicinskikh preparatov, Belarus), 'Valerijonas Forte' (Sopharma® Pharmaceuticals, Bulgaria), 'Valerianae radix' (Belmedpreparaty, RUE, Belarus), Amoxiciline 'Ospamox' (Sandoz, Sloenia) and 'Amoxicillin' (Farmlend, Belarus).

Standard solutions of individual analytes (methanol, ethanol, DCM, acetone and ACN), were prepared in DMF (10 g $l^{-1}$ each) and in ChCl-Eg (10 g $kg^{-1}$ each). Working mixed solutions of the analytes were prepared from standard solutions of individual analytes in DMF or in ChCl-Eg and diluted with a corresponding solvent to a required concentration.

### ChCl-Eg preparation

ChCl-Eg was prepared by the heating method [8]. In brief, ChCl was mixed with ethylene glycol in the molar ratio 1:2 and the mixture was heated in a glass vial at 80°C temperature in a water bath under stirring until the formation of a homogeneous liquid.

### Sample preparation

0.2 g of a grinded sample was placed into a 20 ml headspace vial and 1 ml of DMF was added. The vial was hermetically capped, irradiated with ultrasonic waves for 10 min and subjected for headspace gas chromatographic analysis.

### Instrumentation and conditions

Headspace gas chromatographic analysis was performed on a PerkinElmer Clarus 580 series gas chromatograph (PerkinElmer, USA) equipped with a flame ionisation detector (temperature 250°C, hydrogen flow 40 ml $min^{-1}$, air flow 400 ml $min^{-1}$, auxiliary gas (helium) flow 30 ml $min^{-1}$). The GC system was equipped with the Elite 200 capillary column (30 m × 0.25 mm id, 0.25 µm film thickness) (PerkinElmer, USA). Headspace extraction and sample introduction was performed on a PerkinElmer Headspace Sampler Turbomatrix 16 (PerkinElmer, USA) equipped with a balanced pressure system. Twenty millilitre headspace vials were used in all experiments. A headspace vial was positioned in the HS autosampler and equilibrated at selected temperature. The needle temperature and the transition line temperature was by 10°C higher than the headspace vial equilibration temperature. The settings of the headspace sampler were 1 min for pressurization and 0.07 min for injection. Helium was employed as a carrier gas with 16.7 psi column head pressure. The injector temperature was held at 110°C. The GC oven temperature was programmed as follows: 40°C for 1 min from 40 to 50°C at 10°C $min^{-1}$ and from 50°C to 200°C at 40°C/min.

## RESULTS AND DISCUSSION

### Operation conditions

Five common solvents used in the fabrication of the pharmaceuticals were selected as analytes: methanol, ethanol, dichloromethane, acetone and acetonitrile. The solvents according to the ICH classification belong to Class 2 and Class 3, their concentration limits are indicated in Table 1. Class 1 solvents were not considered as because of their high toxicity they are avoided and normally are not present in pharmaceuticals.

The nature of the matrix medium is essential for determination sensitivity as it is one of the main parameters that the concentration of the analyte in the headspace depends on. For the determination of residual solvents in pharmaceuticals by the SHS-GC method we tested two solvents as a matrix media – a traditional solvent dimethylformamide and a deep eutectic solvent choline chloride-ethylene glycol.

Table 1. **Concentration limits of residual solvents in pharmaceuticals**

| Solvent | Class | Concentration limits, ppm [4] |
|---|---|---|
| Methanol | 2 | 3000 |
| Ethanol | 3 | 5000 |
| DCM | 2 | 600 |
| Acetone | 3 | 5000 |
| ACN | 2 | 410 |

For the SHS sample heating temperature is one of the main parameters to be optimized. Vapour pressure increases with temperature, thus partition of the analytes between a sample phase and a gas phase will decrease and more of the compound will pass into the headspace. Moreover, the time needed to achieve the equilibrium between a sample and a gas phase at higher temperatures is smaller and the analysis is faster.

Different equilibration temperatures (60–140°C) were tested for DMF. Higher temperatures were not used as the equilibration temperature should not exceed the boiling point of the matrix medium DMF (152°C). Under these conditions, the content of the analytes in the headspace permanently increased (Fig. 1). Based on the results, for DMF 140°C equilibration temperature was chosen as optimal.

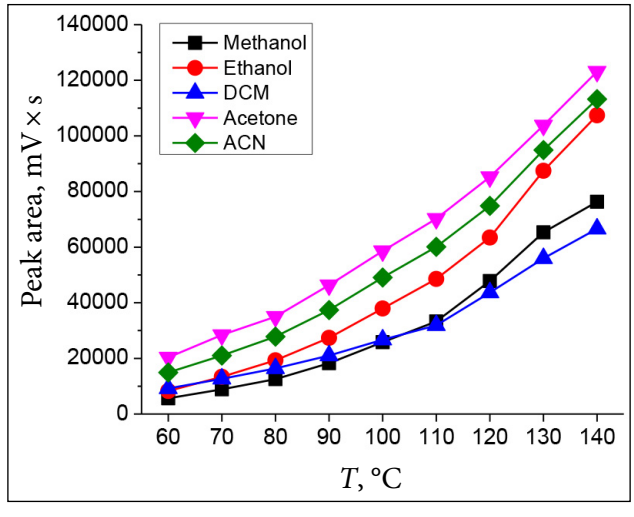


**Fig. 1.** Influence of SHS equilibration temperature on peak areas of 1 ml 0.1 g $l^{-1}$ analytes solution in DMF (coloured online). Equilibration time 20 min, injection time 0.03 min

Deep eutectic solvents are considered especially suitable for SHS, as due to their low vapour pressure their concentration in the headspace is very low and thus analytes are favoured to enter the headspace. Moreover, a high boiling point of DESs allows higher equilibration temperatures.

Unfortunately, the preliminary experiment showed that above 80°C ChCl-Eg starts to decompose and peaks of decomposition products appear in the chromatogram close to the peaks of the residual solvents of interest (Fig. 2). Because of that 80°C equilibration temperature was considered as optimum for ChCl-Eg.

For the optimisation of equilibration time, 1 ml of 0.1 g $l^{-1}$ DMF solution of the analytes was heated at 140°C for 2–20 min. The results presented in Fig. 3a demonstrate that the peak areas levelled at 8 min. This time was considered as optimum and selected for further work.

For the analytes solution in ChCl-Eg (1 g of 0.1 g $kg^{-1}$) the sample was heated at 80°C up to 60 min.

Longer equilibration time in comparison with DMF solutions was examined because of the lower equilibration temperature applied and because of the bigger viscosity of the matrix medium. For comparison, at 20°C viscosity of DMF is 0.92 mPa s and that of ChCl-Eg is 48.95 mP s [22]. The results demonstrated (Fig. 3b) that the equilibration time was 40 min.

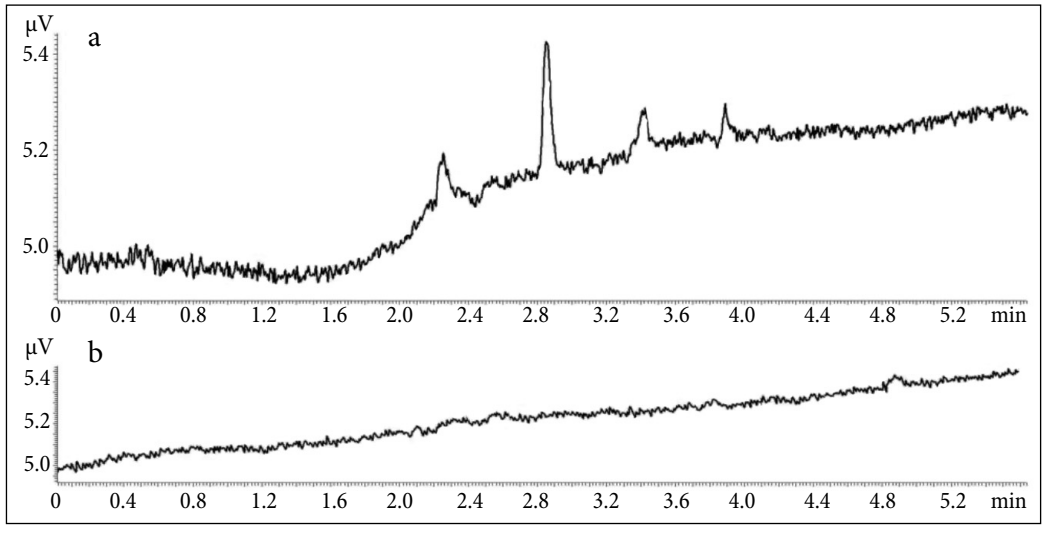


**Fig. 2.** Headspace chromatograms of ChCl-Eg equilibrated at 100°C (a) and at 80°C (b). Equilibration time 10 min, injection time 0.03 min. For SHS-GC conditions see Experimental

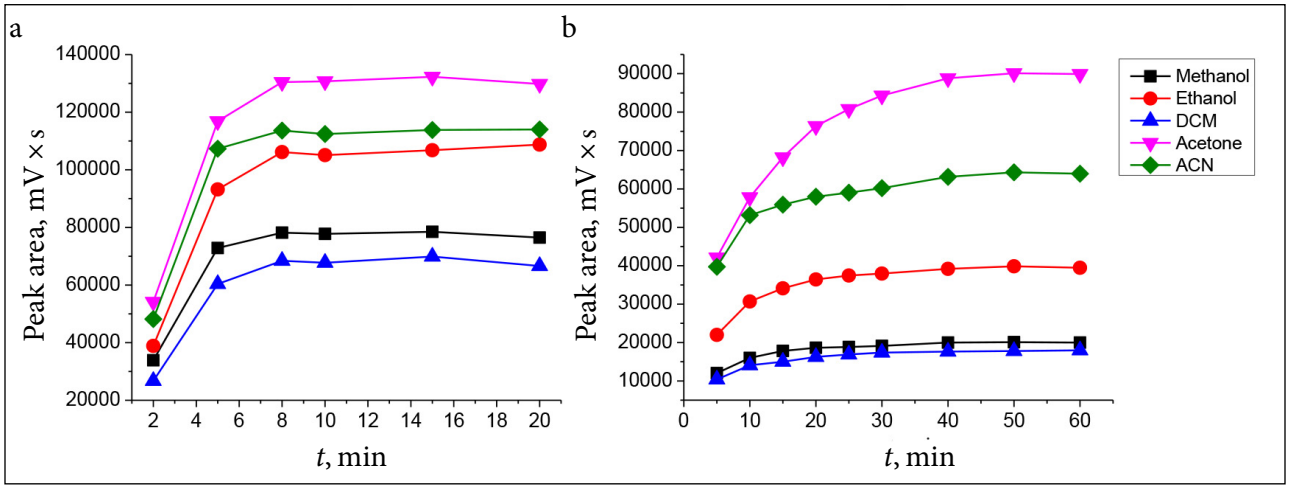


**Fig. 3.** Influence of SHS equilibration time on peak areas of: (a) 1 ml 0.1 g $l^{-1}$ analytes solution in DMF. Equilibration temperature 140°C, injection time 0.03 min; (b) 1 g 0.1 g $kg^{-1}$ analytes solution in ChCl-Eg (coloured online). Equilibration temperature 80°C, injection time 0.03 min

The volume of the gas phase injected to GC has also been optimized. The more gas phase injected, the bigger peak should be observed. On the other hand, with the increase of injected gas phase volume, peaks can start to broaden and tail. The equipment used was supplied by pressure balanced sampling that allows a direct control of the time width of the vapour plug entering the GC column. Injection times from 0.01 to 0.12 min have been examined. The results presented in Fig. 4a demonstrate that for DMF solutions peak areas proportionally increased up to 0.09 min injection time with the peak area correlation coefficients bigger than 0.99. For ChCl-Eg solutions peak areas proportionally increased up to 0.12 min injection time with the peak area correlation coefficients bigger than 0.98 (Fig. 4b). However, as it is demonstrated for acetone and acetonitrile, peak efficiencies decreased with the increase of the injection volume (Fig. 5). Thus the optimum injection time should be selected with respect to the sample. In the case of a good separation of the residual solvent peak, long injection time can be applied. Contrarily, for better separation efficiency shorter injection time should be advantageous. For further work, 0.09 min injection time was considered to be optimal.

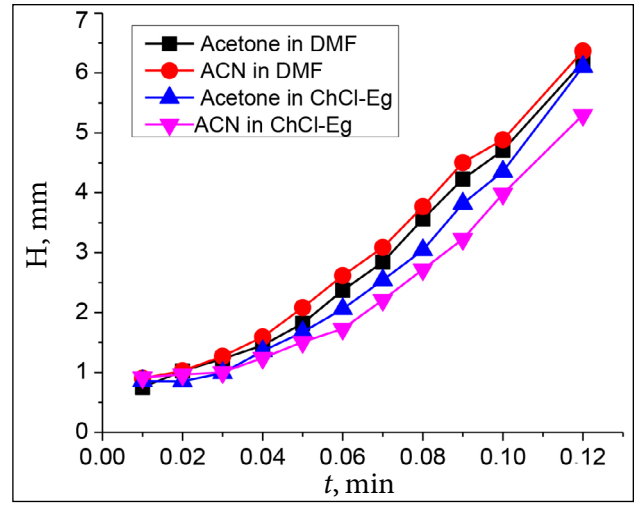


**Fig. 5.** Theoretical plate height (H) dependence on the injection time at optimal equilibration temperatures and times (coloured online). Analytes concentration in DMF 0.1 g $l^{-1}$, analytes concentration in ChCl-Eg 0.1 g $kg^{-1}$

## Quality parameters

Quality parameters for both analysed matrix media were determined under the optimized conditions. The calibration curves were drawn with 10 calibration points with three-replicate injections and for all the analytes were linear up to 1 g $l^{-1}$ inDMF and up to 1 g $kg^{-1}$ in ChCl-Eg. Correlation coefficients, relative standard deviations (RSDs) and limits of detection (LOD) calculated as three times the baseline noise are presented in Table 2. The relative standard deviations were determined by five-replication analysis at two different concentrations of the analytes and did not exceed 6%.

Detection limits for all the analytes were up to 4 times lower when DMF was used as a matrix medium. This fact could be explained by higher equilibration temperature applied when DMF was used as a diluent. Another advantage of DMF over ChCl-Eg is shorter equilibration time (8 min over 40 min). Additionally, ChCl-Eg solutions are more difficult to handle because of their higher viscosity. Based on that, DMF was chosen as a matrix medium for

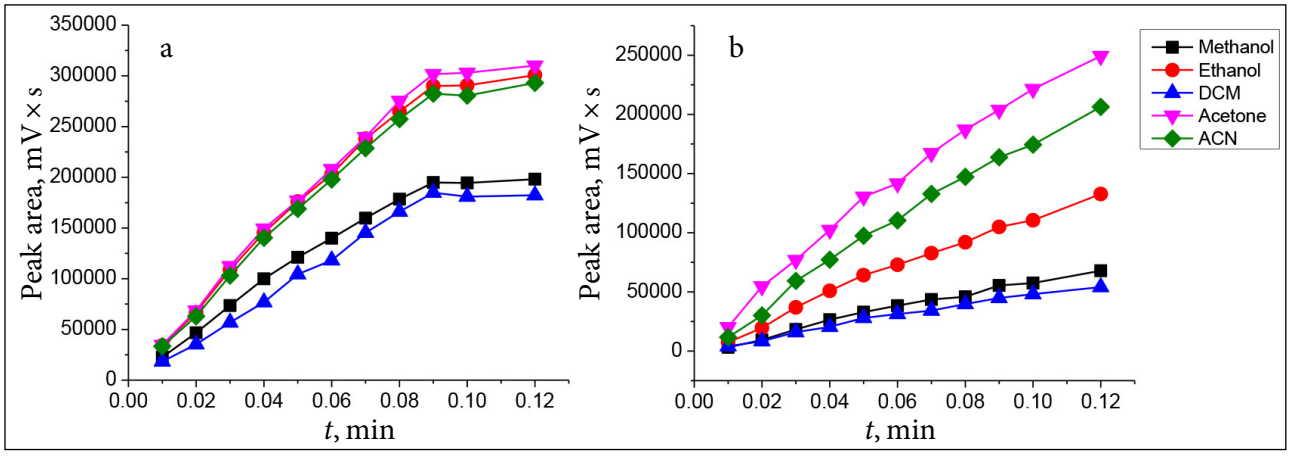


**Fig. 4.** Influence of SHS injection time on peak areas of: (a) 1 ml 0.1 g $l^{-1}$ analytes solution in DMF. Equilibration temperature 140°C, equilibration time 8 min; (b) 1 g 0.1 g $kg^{-1}$ analytes solution in ChCl-Eg (coloured online). Equilibration temperature 80°C, equilibration time 40 min

Table 2. **Analytical figures of merit**

| Analyte | In DMF | | | | In ChCl-Eg | | | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | LOD, μg $l^{-1}$ | RSD, % ($n = 5$) | | $R^2$ | LOD, μg $l^{-1}$ | RSD, % ($n = 5$) | |
| | | | 10 mg $l^{-1}$ | 100 mg $l^{-1}$ | | | 10 mg $kg^{-1}$ | 100 mg $kg^{-1}$ |
| Methanol | 0.9997 | 17 | 4.8 | 4.6 | 0.9995 | 68 | 5.9 | 1.0 |
| Ethanol | 0.9995 | 9 | 4.2 | 4.1 | 0.9992 | 25 | 4.8 | 1.5 |
| DCM | 0.9997 | 22 | 5.4 | 4.0 | 0.9931 | 72 | 5.9 | 1.2 |
| Acetone | 0.9999 | 7 | 5.4 | 2.2 | 0.9985 | 18 | 5.1 | 1.7 |
| ACN | 0.9996 | 12 | 5.0 | 4.2 | 0.9983 | 37 | 6.0 | 2.2 |

residual solvents determination in real pharmaceutical samples.

## Real sample analysis

In order to evaluate if the matrix of the sample had an influence on the determination of the analytes, two pharmaceuticals – Ospamox and Omeprazole (Bl) – were analysed using the calibration curve method and the multiple standard addition method.

0.2 g of a grinded sample was placed into a 20 ml headspace vial and 1 ml of DMF was added. The vial was hermetically capped, irradiated with ultrasonic waves for 10 min and subjected for headspace gas chromatographic analysis.

In the multiple standard addition method, three different volumes of additions of 1 g $l^{-1}$ solution of the analytes were spiked to three portions of the grinded sample (0.2 each) and the analysis was carried out as described above.

The results obtained by the calibration curve method and the multiple standard addition method differed less than by 10%. Thus, as the multiple standard addition method requires extra time for making the additions and measurements, the calibration curve method was applied to quantify the analytes in other pharmaceuticals. The results presented in Table 3 demonstrate that one of the tested drugs, Valerianae radix, exceeded the permissible (5000 ppm) ethanol concentration. In other pharmaceuticals the concentrations of all analytes did not exceed the permissible levels. On the other hand, residual solvent concentrations in pharmaceutical products with the same active substance but from different manufacturers may vary significantly. This is illustrated by the chromatograms of Valerianae radix and Valerijonas Forte presented in Fig. 6. Valerijonas Forte is almost free of solvent impurities and

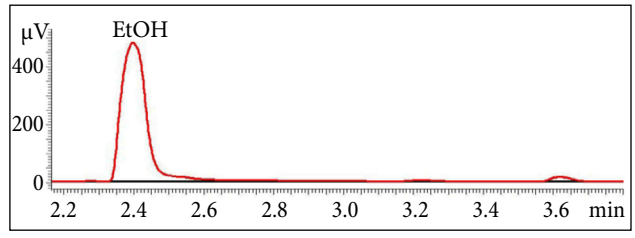


**Fig. 6.** Valerijonas Forte (black) and Valerianae radix (red) headspace gas chromatograms (coloured online). For SHS-GC conditions see Experimental

Table 3. **Concentration of the analytes in pharmaceuticals, ppm**

| Pharmaceutical | Methanol | Ethanol | DCM | Acetone | ACN |
|---|---|---|---|---|---|
| Ospamox | 155 | – | 227 | 16 | 23 |
| Amoxicillin | 85 | 19 | – | 60 | 109 |
| Omeprazol Sandoz | 90 | 37 | 250 | 30 | – |
| Omeprazole | 94 | 67 | – | – | – |
| Omeprazole-Akrikhin | 139 | 72 | 401 | – | – |
| Paracetamolis Sanitas | – | – | 58 | – | 37 |
| Paracetamol | – | 151 | 42 | – | 40 |
| No-Spa | – | 492 | – | – | – |
| Drotaverine | – | 469 | – | – | – |
| Valerijonas Forte | – | 18 | – | – | – |
| Valerianae radix | – | 8607 | – | – | 84 |

the chromatogram of Valerianae radix shows a high peak in ethanol.

## CONCLUSIONS

Two potential matrix media – a traditional solvent DMF and a deep eutectic solvent ChCl-Eg – for SHS-GC determination of residual solvents in pharmaceuticals have been investigated. It was expected that due to the high boiling point of the DES, high equilibration temperature could be applied. However, ChCl-Eg tends to degrade at elevated temperatures and 80°C was the maximal temperature that did not result in the degradation of ChCl-Eg, meanwhile for DMF solutions 140°C equilibration temperature could be applied. The higher equilibration temperature of DMF solutions favoured a transition of the analytes to the headspace and consequently resulted in lower detection limits. Thus DMF has been considered a more suitable matrix medium than ChCl-Eg and was applied for residual solvents determination in 11 pharmaceuticals. It was determined that in all the pharmaceuticals investigated except one, residual solvents concentrations did not exceed allowable concentration limits. Only Valerianae radix was found to exceed the permissible ethanol concentration.

To further increase determination sensitivity, thermostable DESs could be promising. Investigations are ongoing.

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## TIRPIKLIŲ LIKUČIŲ FARMACINIUOSE PREPARATUOSE NUSTATYMAS VIRŠERDVĖS DUJŲ CHROMATOGRAFIJOS METODU: DVIEJŲ MATRICOS TERPIŲ PALYGINIMAS

*S a n t r a u k a*

Dimetilformamidas (DMF) ir eutektinis tirpiklis cholino chloridas-etilenglikolis (ChCl-Eg) buvo ištirti kaip potencialios matricos terpės atliekant statinį viršerdvės dujų chromatografinį (SHS-GC) tirpiklių likučių nustatymą farmaciniuose preparatuose. Buvo optimizuota mėginio termostatavimo temperatūra ir trukmė bei įleidimo trukmė. Naudojant DMF mėginys buvo termostatuojamas 140 °C temperatūroje, o ChCl-Eg termostatavimo temperatūra negalėjo viršyti 80 °C, nes aukštesnėse temperatūrose ChCl-Eg ėmė skilti. Aukštesnė termostatavimo temperatūra naudojant DMF sudarė palankesnes sąlygas analitėms pereiti į viršerdvę ir nulėmė mažesnes analičių aptikimo ribas. Nuspręsta, kad dimetilformamidas yra tinkamesnė matricos terpė nei ChCl-Eg. DMF buvo naudojamas tirpiklių likučiams nustatyti vaistuose SHS-GC metodu.