Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 834–840

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Static headspace gas chromatography of (semi-)volatile drugs in pharmaceuticals for topical use

Yarramraju Sitaramaraju, Ariane van Hul, Kris Wolfs, Ann Van Schepdael, Jos Hoogmartens, Erwin Adams*

Laboratorium voor Farmaceutische Analyse, Faculteit Farmaceutische Wetenschappen, Katholieke Universiteit Leuven, O & N 2, Herestraat 49 (PB 923), B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 17 January 2008
Received in revised form 2 April 2008
Accepted 2 April 2008
Available online 11 April 2008

Keywords:
GC
Static headspace
(Semi-)volatiles
DMSO–water
Pharmaceuticals

ABSTRACT

An analytical method that allows simultaneous analysis of some (semi-)volatile drugs and additives in pharmaceuticals for topical use was developed using classical static headspace as sampling technique combined with gas chromatography (HS-GC). The capillary column used, RSL-200, showed good selectivity towards all the analytes in the samples investigated. Among the different dilution media investigated, dimethyl sulfoxide in combination with sodium chloride solution showed better sensitivity. Using the optimized headspace sample dilution medium, better sensitivities for all the analytes were achieved at low thermostatting temperature (85 °C). The optimized HS-GC method with flame ionization detection showed good repeatability, linearity and accuracy.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Pharmaceuticals for topical use are a diverse and extensive class of pharmaceuticals that cover a large variety of indications. Most pharmaceutical products for topical use are composed of a wide variety of compounds that considerably differ in molecular weight, polarity and volatility. Ointments and creams certainly belong to the more complex pharmaceutical dosage forms, as they consist of emulsions, containing at least three or four, often however considerably more excipients with very different properties. As the determination of the analyte of interest is often impeded by interference due to the presence of matrix components, quantitative and qualitative analysis of complex samples like these is not evident. Quality control authorities however, require the development of analytical methods which enable the simultaneous investigation of components present in restricted amounts.

Conventional analytical methods that are commonly applied for the analysis of ointments and creams are reversed-phase high performance liquid chromatography (HPLC), thin layer chromatography (TLC) and direct injection gas chromatography (GC). All of these chromatographic methods require various and extensive clean-up steps prior to the analysis to isolate the desired analytes from interfering substances and to concentrate dilute analytes up to detectable levels. Many sample preparation methods still account for the major inaccuracies and time consumption of the total analytical process. Although HPLC and TLC methods without preceding sample clean-up have been described, these techniques are only applicable to a limited number of oil in water (O/W) creams [1–3].

The use of chromatographic techniques for the assay of pharmaceuticals for topical use without complex sample preparation has important consequences for the ease of interpretation of the analytical result. Co-injection of excipients often results in complex chromatographic patterns that are difficult to interpret. This is due to the high backgrounds of unresolved peaks produced by the presence of large amounts of matrix components. Furthermore, exposure of stationary phases to high quantities of cream base components should be avoided to prevent early column wear.

For the analysis of a variety of volatile compounds present in pharmaceutical preparations, these time-consuming and costly procedures could be avoided by using static headspace (HS) in combination with GC. HS analysis is done by analyzing a portion of the upper gas phase being in equilibrium with the liquid phase in a closed vial. It is a well demonstrated and established analytical tool in the field of volatile organic chemicals in pharmaceuticals and other related areas [4–12]. Using static headspace, volatile or (semi-)volatile analytes can be injected selectively into GC, leaving the non-volatile compounds in the headspace vial. Because only volatile molecules are being transferred to the chromatographic system, the headspace approach leads to an overall improved

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

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.04.006

Table 1
List of reference compounds used in this study and the related data

Substance	Purity	Mw (g/mol)	Boiling point (°C)	Source	Solubility in water (mg/l)
α-Pinene	≥97%	136.23	155	Acros Organics	0.0025
Eucalyptol	≥99%	154.25	177	Acros Organics	3.50
Linalool	≥97%	154.25	194–197	Acros Organics	1.59
Camphor	≥95%	152.23	209	PCB	1.60
Menthol	≥99%	156.26	212	Merck	0.51
Carvacrol	≥97%	150.22	237–238	Fluka	1.25
Thymol	≥99.5	150.22	232	Riedel de Haën	0.90
Eugenol	≥99%	164.20	255	Acros Organics	2.46

analytical performance. Time, money and ease of operation are the main reasons why using headspace analysis would mean an improvement in the assay of complex pharmaceutical preparations. Concerning the (semi-)volatiles included in this study, qualitative investigations using HS-GC and solid phase microextraction HS-GC are vastly published in fields other than pharmaceuticals. In pharmaceuticals relatively few studies were published and they used mostly solid phase microextraction (SPME)/single drop microextraction (SDME) HS-GC [13–19]. No validated HS-GC methods were found in literature that allow both qualitative and quantitative analysis for such analytes in matrices like pharmaceuticals for topical use.

This study demonstrates a HS-GC method using flame ionization detection for the analysis of a selected group of (semi-)volatile compounds used in pharmaceutical preparations: α-pinene, eucalyptol, camphor, linalool, menthol, carvacrol, thymol and eugenol. A mass spectrometer was also used in this study to discriminate the peaks of interest and to evaluate the matrix effects. Some of the included (semi-)volatiles are also of safety concern. As examples: laryngospasms have even been reported in young children after topical application of pharmaceuticals containing menthol or eucalyptol; eugenol is listed as a suspected carcinogenic by the IARC (International Agency for Research on Cancer); and thymol is an eye, skin and respiratory irritant [20–25]. A simple and sensitive analytical method for detecting this group of compounds would thus not only be useful for quantification purposes to ensure the quality of pharmaceuticals, but would also offer the possibility for targeted screening of substances with potential sensitization or irritant properties.

2. Experimental

2.1. Chemicals and reagents

Dilution solvents were of ≥99% purity and were purchased from the following sources: dimethyl sulfoxide (DMSO) from Merck (Darmstadt, Germany), *N*,*N*-dimethylformamide (DMF) from Fisher Scientific (Leicestershire, United Kingdom) and paraffin from Riedel-de-Haën AG (Seelze-Hannover, Germany). DMSO was bought in 100 ml bottles as it can generate additional peaks on standing once the bottle is opened. The DMSO was purged with nitrogen for 3 h before use. Purified water was produced in the laboratory by the use of a Compact Milli-Q system from Millipore (Milford, MA, USA). Sodium chloride (99.5% purity) was obtained from BDH (Poole, England).

The reference compounds were purchased from the following sources: linalool, α-pinene, eucalyptol and eugenol from Acros Organics (Geel, Belgium), carvacrol from Fluka (Buchs, Switzerland), thymol from Riedel-de-Haën AG (Seelze-Hannover, Germany), camphor from PCB (Brussels, Belgium) and menthol from Merck (Darmstadt, Germany). The related data for the reference compounds used are presented in Table 1.

Table 2
List of the samples investigated in this study

Samples	Composition
Preparation 1	Menthol, camphor, eucalyptol, oleorosine capsicum PB V, ung. cetylicum.
Preparation 2	Camphor, oleorosine capsicum PB V, menthol, propylene glycol, lavand. aetherol, ung. cetylicum.
Formulation 1	Veratrol, resorcinol, chlorali hydras, menthol, geranii essent, citri cedrae essent., acid. salicylic., tartrazin., isopropanol, aqua.
Formulation 2	Benzoas benzyl., cinnamas benzyl., vanillinum, camphor, rosmarini aetherol., eucalyptol, polysorbatum, carbomer, natr. hydroxyd., aqua purificata.
Formulation 3	Xylometazolini hydrochlorid., natr.dihydrogenophosphas, dinatr. phosphas, natr. chlorid., benzalkon. chlorid., natr.edetas, levomenthol, eucalyptol, sorbitol-cremophor, aqua.
Formulation 4	Menthae arvensis ess., eucalyptol, cajeputi ess., juniperi ess., gaultheriae ess., menthol, eugenol.
Formulation 5	Eucalyptol, α-pinene, camphora, mentholum, guajacolum, lavand. aetherol., thymi aetherol., monostearin., cera emulsificans, aqua purificata.

Compounds investigated in this study are underlined.

2.2. Samples

Five commercial formulations and two in-house preparations from pharmacists in Belgium were included in this study. The samples also contained several other compounds than the compounds of interest as listed in Table 2.

2.3. Reference solutions

All the compounds of interest were weighed accurately and dissolved in DMSO. Using the same solvent, the stock solution was further diluted to the different concentration levels mentioned in Table 3.

Table 3
Different concentration levels diluted from the stock solution

	Concentration level (μg/ml)							
	I	II	III	IV	V	VI	VII	VIII
α-Pinene	2	4	6	8	10	12	16	20
Eucalyptol	2	4	6	8	10	12	16	20
Linalool	50	100	150	200	250	300	400	500
Camphor	50	100	150	200	250	300	400	500
Menthol	50	100	150	200	250	300	400	500
Carvacrol	200	400	600	800	1000	1200	1600	2000
Thymol	200	400	600	800	1000	1200	1600	2000
Eugenol	200	400	600	800	1000	1200	1600	2000

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

	Parameter	Optimized settings
1	GC	
	Oven temperature	50 °C for 5 min, increased at 10 °C/min to 200 °C, held for 10 min
	Injection port temperature	250 °C
	Detector temperature	300 °C
	Carrier gas	Helium 5.6 at 2.0 ml/min
	Split ratio	1:4
2	Headspace	
	Thermostatting temperature	85 °C
	Thermostatting time	90 min
	Needle temperature	210 °C
	Transferline temperature	220 °C
	Carrier gas pressure	180 kPa
	Pressurization time	0.5 min
	Injection time	0.04 min
	Needle withdrawal time	0.3 min
3	FID	
	Temperature	300 °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 16–450
	Scan time	1.0 s
	Inter-scan delay	0.5 s

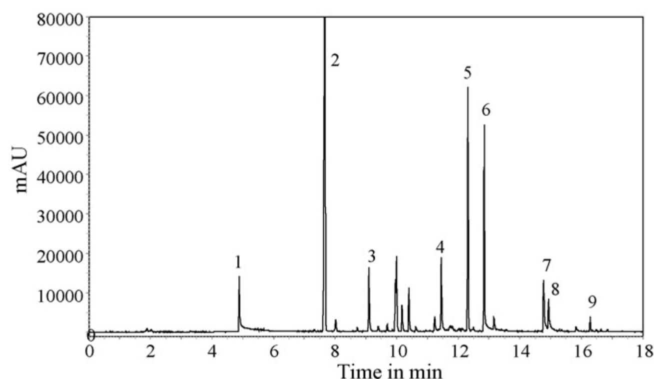

Fig. 1. Chromatogram obtained with reference solution I in DMSO. 1: DMSO, 2: α-pinene, 3: eucalyptol, 4: linalool, 5: camphor, 6: menthol, 7: carvacrol, 8: thymol and 9: eugenol. *Unlabelled peaks are related to volatile impurities from the reference compounds.

2.4. Instrumentation

The GC-FID instrument used to perform the study was a DELSI 200 capillary gas chromatograph (Delsi Nermag, Argenteuil, France), connected to a static headspace autosampler, Turbomatrix HS40XL headspace autosampler (Perkin-Elmer, California, USA). This sampler applies the principle of time-controlled injection. The analytical column used was a bonded polydimethylpolydiphenyl-siloxane coated (0.25 μm film thickness) capillary column (RSL 200, 30 m × 0.32 mm i.d., Bio-Rad, Belgium). This column tolerates temperatures up to 330 °C. The chromatographic data were collected and integrated using a HP integrator. The optimized parameters are listed in Table 4.

The carrier gas used was helium of 5.6 grade purity and was supplied by Messer (Machelen, Belgium). The 20 ml headspace vials and the aluminum crimp caps were obtained from Filter Service (Eupen, Belgium). The 1.000 ml micropipets were obtained from Biohit (Helsinki, Finland).

3. Results and discussion

3.1. Chromatographic separation

Temperatures exceeding 200 °C were found to be necessary for the HS needle, transfer line and the GC injection port to reduce the peak tailing for carvacrol, thymol and eugenol. In general HS-GC experiments are performed at an average split ratios of 1:5. As the high boiling point compounds are expected to show poor headspace sensitivity, the GC injector split ratio was reduced to 1:2 (4.0 ml to the column and 8.0 ml to exit). By using the optimized HS-GC-FID parameters (Table 4), all the peaks of interest were sufficiently separated. The solvent peak (corresponding to DMSO) was eluted first and was well separated from the peaks corresponding to the reference compounds (Fig. 1). Although the peaks corresponding to carvacrol and thymol were not baseline separated, the observed separation was found sufficient to continue qualitative and quantitative analysis. With the optimized HS-GC parameters no detectable carry over was observed for the peaks of interest.

3.2. Sample dilution medium

Selecting a proper dilution medium in HS-GC analysis is critical. An ideal headspace dilution medium should be able to dissolve a wide variety of compounds and offer low partition coefficient values for the analytes of interest. The lower the partition coefficient the better the transfer of analytes from the sample liquid to the gas phase in a HS vial, which will result in better sensitivity of the determination. In comparison, water offers lower partition coefficient values for most of the analytes than any organic dilution medium [4–12]. However, in the situations where water insoluble samples have to be analyzed, usage of organic dilution media is unavoidable. As the selected samples for this study are water insoluble and have different solubility properties, various organic HS dilution media such as DMF, DMSO and liquid paraffin were considered. Initially, the solubility of the samples and later the sensitivity obtained with the different dilution media were investigated. Solubility was investigated using 200 mg of sample per milliliter of solvent. DMSO offered the best solubility. As the sample quantities required in later stages were not more than 100 mg/ml, experiments with more than 200 mg/ml were not investigated. In the cases where the sample was not soluble, a uniform homogenate could be obtained that went into solution during thermostatting. The sensitivity was examined by injecting amounts of the reference compounds, corresponding to concentration level IV, Table 3, in 5.0 ml of the different dilution media. The thermostatting conditions were set at 105 °C for 60 min. As a result of both solubility and sensitivity advantages, DMSO was selected for further experiments.

3.3. Dilution media modification

3.3.1. Combination with water

Although DMSO was shown to offer better sensitivity than the other dilution media investigated, the sensitivity was not sufficient for analysis of samples containing carvacrol, eugenol and thymol. When the injected sample amount was increased to counter the sensitivity problem for the above mentioned analytes, the other analytes of interest present in the sample caused overloading of the column. As combinations of organic dilution media with water were proven to enhance the transfer of the analytes into the gas phase [4–6], the influence of DMSO–water as a dilution medium on the sensitivity was investigated. A HS vial with 2.0 ml of DMSO containing the analytes (corresponding to concentration level IV,

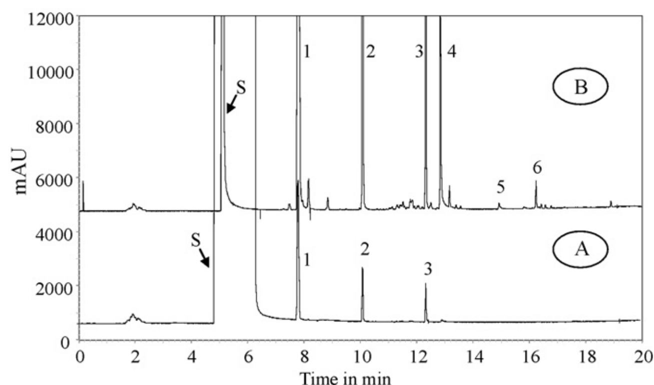

Fig. 2. Chromatograms obtained with reference analytes in DMSO (A) and DMSO + water (B). Thermostatting program used: 60 min at 105 °C (A) and 60 min at 85 °C (B). S: DMSO, 1: α-pinene, 2: eucalyptol, 3: camphor, 4: menthol, 5: thymol, 6: eugenol.

Table 3) and 3.0 ml of water was injected in triplicate. The presence of water in the HS vials restricts the thermostatting temperatures to temperatures below 90 °C. This limitation is applied to avoid over-pressurization in the vial due to evaporation of water. Hence, the thermostatting temperature was set at 85 °C with a thermostatting time of 60 min (Fig. 2).

In comparison to DMSO at a thermostatting temperature of 105 °C, DMSO–water at 85 °C gave better sensitivity for both high boiling point analytes (such as carvacrol, eugenol and thymol) and low boiling point analytes. The increased sensitivity was due to the fact that adding water to DMSO lead to increased interactions between the solvent molecules and increased activity coefficient. The activity coefficient is inversely proportional to the partition coefficient and the vapor pressure [4–6]. Hence, the increased activity coefficient resulted in lower partition coefficients for all the analytes in the dilution medium and better transfer of analytes from liquid to headspace.

3.3.2. Changing ionic strength

An additional possibility to enhance the sensitivity is by increasing the ionic strength of the dilution medium. A 1 M NaCl solution was prepared for the latter purpose. To 2.0 ml of DMSO containing analytes (corresponding to concentration level IV, Table 3), increasing volumes of 1 M NaCl were added (3.0, 4.0, 6.0 and 8.0 ml) and analyzed at a thermostatting program of 85 °C for 60 min. Volumes of more than 8.0 ml 1 M NaCl were not investigated to avoid precipitation of the samples.

The peak areas for all the analytes increased by replacing the water with 1 M NaCl. The percentage of increment in peak area varied for the different compounds. Eugenol and α-pinene showed about 15%; eucalyptol, linalool, camphor and menthol showed about 35% and thymol and carvacrol showed up to 55% increment in the peak area. An increase in added volume of 1 M NaCl from 3.0 to 8.0 ml also showed an increase in sensitivity for all the analytes. The analytes with a high boiling point showed more increase in peak area than those with a low boiling point (Fig. 3).

3.4. Thermostatting time

Different thermostatting times at 85 °C were investigated, namely 30, 45, 60, 90 and 120 min. Sample preparation was done using 2.0 ml of reference solution containing the analytes in DMSO corresponding to concentration level IV and 8.0 ml of 1 M NaCl. The other HS-GC-FID parameters were set as mentioned in Table 4. All the analytes except thymol, carvacrol and eugenol reached the equilibrium state within 60 min. Thymol, carvacrol and eugenol needed more than 60 min to reach equilibrium. Overall, a thermostatting time of 90 min was found to be sufficient for achieving the equilibrium between the liquid phase and the gas phase for all the analytes of interest. As an example, the influence of thermostatting time on the peak areas of camphor, menthol, thymol, eugenol and eucalyptol is shown in Fig. 4.

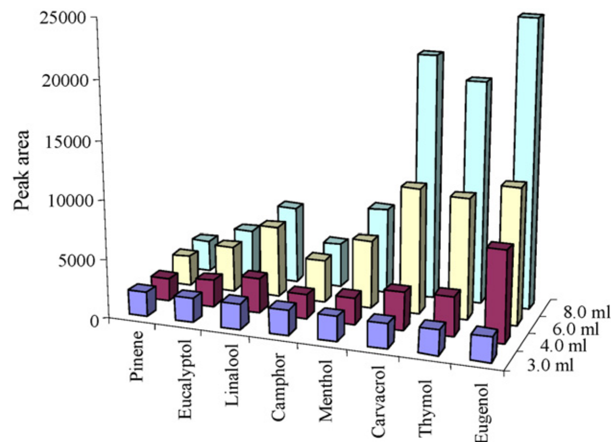

Fig. 3. Influence of increasing volume of 1 M NaCl added in the vial.

3.5. Method validation

Using 2.0 ml of DMSO containing the analytes and 8.0 ml of 1 M NaCl in a HS vial, the developed HS-GC-FID method was validated using the optimized parameters from Table 2.

3.5.1. Precision

Precision was evaluated at concentration levels II, IV and VI (Table 3). For each concentration six headspace vials were prepared and injected. α-Pinene, linalool, thymol and eugenol showed high R.S.D. values ranging from 2 to 7%. The other analytes showed R.S.D. values between 0.2 and 2.5%. The results are presented in Fig. 5.

3.5.2. Linearity

Eight different concentration levels as mentioned in Table 3 were investigated. All injections were done in triplicate and the

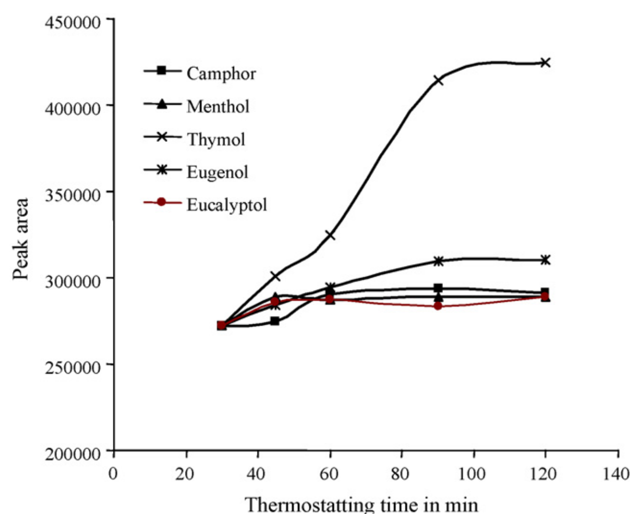

Fig. 4. Influence of thermostatting time on the peak areas of some analytes used in this study at 85 °C.

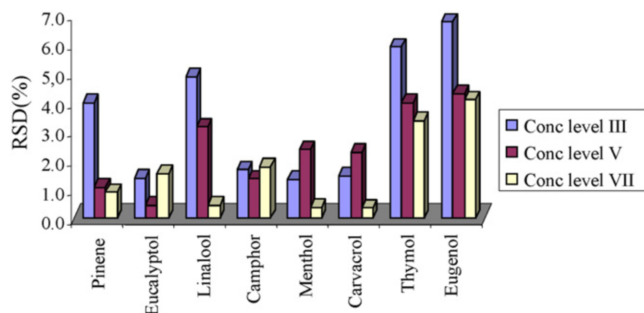

Fig. 5. Precision data of the analytes investigated using the parameters mentioned in Table 4.

average peak areas were used for the calculations. The method showed a linear response towards all the analytes investigated. The R^2 values for the analytes were found to be at least 0.998. The data are presented in Table 5.

3.5.3. Recovery

Recovery was investigated by spiking in-house preparation I with known concentrations of analytes. Three concentration levels (levels II, IV and VI) were examined to evaluate the recovery. Average recovery values are presented in Table 5.

3.5.4. Detection limits

The detection limit of an analytical procedure is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified as an exact value. It is generally accepted that a detectable peak should have a signal-to-noise ratio of at least 3. The solution with concentration level I was further diluted until the signal-to-noise ratio reached 3. The results are also presented in Table 5.

3.6. Interference peaks from DMSO

The advantageous increment in sensitivity was also found to be problematic, as it also caused increased impurity peaks in the chromatogram. Most of the impurity peaks eluted after the DMSO peak. The retention times of these peaks were sometimes interfering with those of the analytes of interest in this study. The presence of impurities was also observed with DMSO from freshly opened bottles. Therefore, DMSO was purged for several hours with high purity nitrogen gas by insertion of a clean and inert polymer tube into the liquid. During purging, samples were taken periodically and analyzed using the optimized HS-GC-FID parameters. When the chromatograms obtained at different time points were compared, a two hours purging was found to be sufficient to reduce the impurity peaks to a negligible level. The chromatograms obtained with purging times of 60 and 120 min are shown in Fig. 6. As a precaution, DMSO bottles were purged with nitrogen for about 3 hours before use.

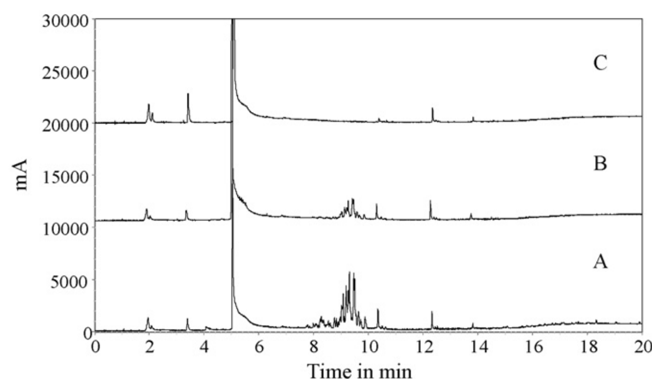

Fig. 6. Chromatograms obtained with 2.0 ml of DMSO + 3.0 ml 1 M NaCl. (A) DMSO from a freshly opened bottle, (B) DMSO purged for 60 min and (C) DMSO purged for 120 min.

3.7. Qualitative analysis

This stage was performed both on HS-GC-FID and HS-GC-MS. Samples (100 mg) were weighed carefully in a headspace vial and dissolved in 2.0 ml of DMSO, 8.0 ml of 1 M NaCl was added, after which the vials were immediately sealed. As mentioned earlier, one sample (formulation 2) was not completely soluble in DMSO. Therefore, weighed quantity was vortexed for 1 min after adding DMSO and vortexed for an additional minute after the 1 M NaCl solution was added. These homogenates went into solution once exposed to the thermostatting temperature.

All the samples contained several other compounds than those of interest. This led to matrix effects: additional peaks other than the peaks of interest showed up in the chromatograms. As the RSL-200 column provided sufficient selectivity for the investigated samples, the presence of additional peaks did not interfere with the interpretation of the peaks of the analytes. As examples, typical chromatograms obtained for formulation 3 and preparation 2 are shown in Figs. 7 and 8. Initially the peaks of interest were located by comparing the retention times with those of the reference compounds. Later, the peak purity of each identified peak was investigated by inspecting their mass spectra. All the peaks were found to be without any detectable interference from other peaks. Hence, the retention time based approach was used for estimating the content.

3.8. Content

As all the sample matrices are rather complex and no blank matrices were available, the standard addition method was used

Table 5
Linearity, detection limits and related data for the compounds investigated

Analyte	Conc. range (μg/vial)	R^2 (>)	Linearity equation	Standard error	LOD (μg/vial)	Average recovery (%)
α-Pinene	2–20	0.999	$Y = 22374X - 7075$	2374	0.01	104
Eucalyptol	2–20	0.998	$Y = 3330X - 300$	864	0.04	101
Linalool	50–500	0.998	$Y = 426X - 8005$	1934	0.5	96
Camphor	50–500	0.999	$Y = 414X - 3994$	2467	0.5	107
Menthol	50–500	0.999	$Y = 397X - 14964$	1862	2.5	99
Carvacrol	200–2000	0.999	$Y = 29X + 679$	663	6	98
Thymol	200–2000	0.998	$Y = 20X - 1747$	517	8	98
Eugenol	200–2000	0.998	$Y = 10X + 226$	220	20	97

X: concentration in μg/vial, Y: peak area.

Table 6
Contents of (semi-)volatile compounds in the different samples investigated

Sample	Compounds of interest	Labelled quantity (mg/100 mg)	Observed quantity (%)	R.S.D. (%)
Preparation 1	Eucalyptol	4.0	21.8	2.9
	Camphor	3.0	97.7	3.1
	Menthol	1.2	94.9	3.2
Preparation 2	Camphor	5.0	97.8	4.1
	Menthol	1.0	108.0	3.4
Formulation 1	Menthol	0.26	96.1	2.7
Formulation 2	Eucalyptol	5	102.6	3.3
	Camphor	12.5	99.7	3.6
Formulation 3	Eucalyptol	Not specified	0.03 mg/100 mg	–
	Menthol	Not specified	0.03 mg/100 mg	–
Formulation 4	Eucalyptol	2.7	94.8	2.7
	Menthol	2.9	97.2	3.2
	Eugenol	0.009	88.9	5.8
Formulation 5	Pinene	1.1	110.0	2.6
	Eucalyptol	1.5	106.0	2.3
	Camphor	1.5	100.0	2.6
	Menthol	0.5	112.0	2.8

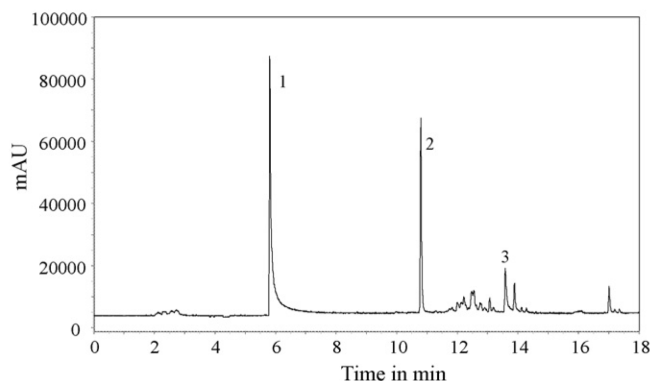

Fig. 7. Chromatogram obtained with formulation 3. 1: DMSO, 2: eucalyptol, 3: menthol, unlabelled peaks are related to the sample matrix.

to determine the content. Depending on the analyte concentration in the sample, sample quantities varying from 10 to 100 mg/vial were used for the quantification. The procedure of standard addition consists of adding a known amount of a reference analyte to a sample containing an unknown amount of the same analyte. The obtained peak areas were plotted against the amount of reference analyte added. The results are presented in Table 6.

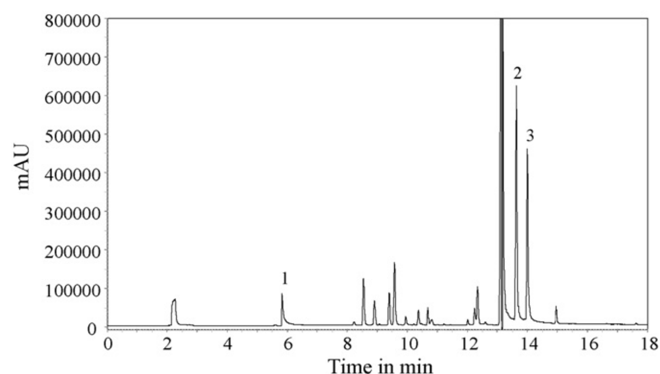

Fig. 8. Chromatogram obtained with preparation 2. 1: DMSO, 2: Camphor, 3: menthol, unlabelled peaks are related to the sample matrix.

4. Conclusions

An analytical method utilizing HS-GC-FID was developed for the analysis of some (semi-)volatile compounds used in topical pharmaceuticals. DMSO–1 M NaCl as a headspace sample dilution medium offered better sensitivity for all the analytes at a thermostatting temperature of 85 °C than working with organic dilution media at 105 °C. Although, FID was found sufficient to quantify the compounds, mass spectrometric investigation was found mandatory while developing the method and during the qualitative analysis. When examining new formulations, it is also advisable to check for interferences by mass spectrometry.

Acknowledgement

E. Adams is a post-doctoral fellow of the Fund for Scientific Research—Flanders (Belgium).

References

[1] O.A. Lake, A. Hulshoff, A.W.M. Indemans, Pharmaceutisch Weekblad Scient. Ed. 4 (1982) 43–48.
[2] F.J. Van De Vaart, A.W.M. Indemans, A. Hulshoff, O.A. Lake, Chromatographia 16 (1982) 247–250.
[3] O.A. Lake, A. Hulshoff, F.J. Van De Vaart, A.W.M. Indemans, Pharmaceutisch Weekblad Scient. Ed. 5 (1983) 15–21.
[4] H. Hachenberg, A.P. Schmid, Gas Chromatographic Headspace Analysis, Heyden & Son, London, 1977.
[5] B.V. Ioffe, A.G. Vitenberg, Headspace Analysis and Related Methods in Gas Chromatography, John Wiley & Sons, New York, 1984.
[6] B. Kolb, L.S. Ettre, Static Headspace-Gas Chroamtography: Theory and Practice, Wiley-VCH, Weinheim, 1997.
[7] J. Drozd, J. Novák, J. Chromatogr. 165 (1979) 141–165.
[8] A.J. Nunez, L.F. González, J. Chromatogr. 300 (1984) 127–162.
[9] C. Witschi, E. Doelker, Eur. J. Pharm. Biopharm. 43 (1997) 215–242.
[10] N.H. Snow, G.C. Slack, Tr. Anal. Chem. 21 (2002) 608–617.
[11] C. B'Hymer, Pharm. Res. 20 (2003) 337–344.
[12] C. Camarasu, C. Madichie, R. Williams, Tr. Anal. Chem. 25 (2006) 768–777.
[13] J. Schuberth, Anal. Chem. 68 (1996) 1317–1320.
[14] C. Deng, Y. Mao, N. Yao, X. Zhang, Anal. Chim. Acta 575 (2006) 120–125.
[15] L. Dong, J. Wang, C. Deng, X. Shen, J. Sep. Sci. 30 (2007) 86–89.
[16] C. Kohlert, G. Abel, E. Schmid, M. Veit, J. Chromatogr. B 767 (2002) 11–18.
[17] D.Y.H. Yeung, T. Lee, G. Grant, J. Pharm. Biomed. Anal. 30 (2003) 1469–1477.
[18] S. Abu-Lafi, I. Odeh, H. Dewik, Md. Qabajah, L.O. Hanuš, V.M. Dembitsky, Bioresour. Technol. 99 (2008) 3914–3918.
[19] J. Romero, P. López, C. Rubio, R. Batlle, C. Nerín, J. Chromatogr. A 1166 (2007) 24–29.
[20] A.C. De Groot, P.J. Frosch, Contact Dermat. 36 (1997) 57–86.

[21] S. Budavari (Ed.), The Merck Index, 13th Edition, Whitehouse Station, New Jersey, 2001.
[22] U.R. Juergens, U. Dethlefsen, G. Steinkamp, A. Gillissen, R. Repges, H. Vetter, Respir. Med. 97 (2003) 250–256.
[23] F. Santos, R. Rao, Phytother. Res. 14 (2000) 240–244.
[24] M. Šegvić Klarić, I. Kosalec, J. Mastelić, E. Piecková, S. Pepeljnak, Lett. Appl. Microbiol. 44 (2007) 36–42.
[25] A. Nostro, A.S. Roccaro, G. Bisignano, A. Marino, M.A. Cannatelli, F.C. Pizzimenti, P.L. Cioni, F. Procopio, A.R. Blanco, Med. Microbiol. 56 (2007) 519–523.