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Quality control of liquid herbal drug preparations: ethanol content and test on methanol and 2-propanol

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Abstract

In the quality control of liquid herbal drug preparations, i.e. tinctures and liquid extracts, the ethanol content is determined and the test on methanol and 2-propanol is performed. Capillary headspace GC/MS methods for both analyses were developed and fully validated. These specific, selective, accurate and precise methods are a fast and fully automated alternative for the laborious methods of the European Pharmacopoeia, since they need no or only simple sample preparation.
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Keywords: Liquid herbal drug preparations; Tinctures; Liquid extracts; Ethanol content; Methanol; 2-Propanol; Quality control

1. Introduction

The European Pharmacopoeia 4th edition defines herbal drug preparations (monograph 01/2002: 1434) as preparations obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal drugs, tinctures, extracts, essential oils, expressed juices and processed exudates.

Extracts and tinctures obtained from herbal drugs have to comply, respectively, with the monographs on extracts (01/2002: 0765) and tinctures (01/2002: 0792). Both alcoholic liquid extracts and tinctures have to comply with the tests on the ethanol content (Ph Eur. 2.9.10; the ethanol content complies with that prescribed) and on methanol and 2-propanol (Ph Eur. 2.9.11; not more than 0.05% v/v of methanol and not more than 0.05% v/v of 2-propanol, unless otherwise prescribed). In this edition of the European Pharmacopoeia the ethanol content in pharmaceutical preparations is determined by means of the pycnometer or hydrometer methods after distillation. The test on methanol and 2-propanol is performed by gas chromatography using a packed glass column after distillation. Since these methods are laborious and time consuming, and because packed glass columns are not commonly used anymore with modern GC equipment, two similar capillary headspace GC/MS methods for both determinations in liquid herbal drug prepara-

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tions were developed and fully validated. The differences between this method and the capillary GC methods published so far on the determination of ethanol or the determination on residual solvents are the concentration to be determined and the matrix to be analyzed. Existing literature describes the determination of residual solvents, i.e. traces of solvents, among which ethanol, methanol and 2-propanol in solid drugs or polymers. Articles published on analysis of biological fluids only describe the determination of the ethanol content. Here we aim to analyze liquid herbal preparations for their compliance to the European Pharmacopoeia in which we want to simultaneously quantify a substantial amount of ethanol (45–80%) and detect traces of methanol and 2-propanol.

2. Experimental

2.1. Solvents, standards and standard solutions

2.1.1. Solvents

Distilled water (RiOs) proceeding from a millipore water purification system (Millipore, Brussels, Belgium) was used. Ethanol absolute of pro analysi grade was purchased from Merck (Darmstadt, Germany).

2.1.2. Standards

1-Propanol of HPLC quality, used as internal standard, was purchased from Filterservice (Acros, Eupen, Belgium). Methanol (Uvasol), 2-propanol (Uvasol) and the ethanol/water—1.0 mg/ml (= 0.1267% v/v)—solution, were from Merck (Darmstadt, Germany).

Standard solutions were prepared as follows:

(1) The internal standard solution (IS solution, 0.1%) contains 100 μl 1-propanol in 100.0 ml water.

(2) The standard ethanol/water solutions with concentrations of 0.0048, 0.0095, and 0.0143% were prepared by pipetting, respectively, 75, 150 and 225 μl of the ethanol/water—1.0 mg/ml—solution into 10 ml head-space vials. After adding 100 μl of the IS-solution to each vial distilled water was added up to a total volume of 2.0 ml.

(3) The methanol/2-propanol standard solution (concentration: 0.05%) was prepared by pipetting 50 μl of the methanol standard and 50 μl of the 2-propanol standard into a 100.0 ml volumetric flask and filling it up with 50% ethanol solution. Two hundred microlitre of this solution was brought into a 10 ml head-space vial and after adding 100 μl of the IS-solution, distilled water was added up to a total volume of 2.0 ml.

2.2. Equipment

The analysis was performed on a thick layer Rtx 1 (30 m × 0.32 mm × 5 μm) GC column coupled with a Hydroguard FS (5 m × 0.18 mm), both from Restek (Interscience, Louvain-la-Neuve, Belgium). The apparatus used was a Trace/Voyager GC/MS (EI ionization) instrument equipped with a Combipal liquid/headspace injector (Interscience, Louvain-la-Neuve, Belgium).

2.3. Methods

Samples for both analyses were prepared in a very similar way. The first step in the determination of the ethanol content is the dilution of the test solution with distilled water to obtain a solution with a concentration between 0.0634 and 0.1901% (v/v). One hundred and fifty microlitre of this solution was brought into a 10 ml head-space vial. Unlike the determination of the ethanol content, undiluted samples are used in the test on methanol/2-propanol. Two hundred microlitre of the undiluted test solution was pipetted into a 10 ml headspace vial. To each vial 100 μl of the IS solution was added. After adding distilled water up to a total volume of 2.0 ml, the vials were closed with a suitable crimp cap. In this way the test solutions for the ethanol content had an ethanol concentration between 0.0048 and 0.0143% (v/v). The concentration of methanol and 2-propanol in the test solution were 0.005% (v/v).

Following head-space conditions were used: syringe: 2.5 ml, sample volume: 500 μl, syringe T: 90.0 °C, incubation T: 85.0 °C, incubation time: 20.00 min. The GC settings were: inlet T: 200 °C, split flow: 50 ml/min, split ratio: 1:50, flow: 1.0 ml/

min (constant flow). The temperature gradient applied started at 40 °C (hold 1.55 min) going with 3.0 °C/min up to 65 °C (hold 0 min) and then with 30 °C/min up to 175 °C (hold 6.5 min).

Each solution was injected twice in the SIM mode (scanning on typical ions for EtOH: *m/z* 31 + 45 and for MeOH/2-Prop: 0.0–7.0 min: *m/z* 31, 7.0–11.5 min: *m/z* 45, 11.5–15.0 min: *m/z* 31) and once in the TIC mode (1–60 amu) to confirm the identification of the peaks due to ethanol, methanol and 2-propanol, based on the retention time with a mass spectrum.

2.4. Validation

Both methods were validated according to the ICH guidelines on the validation of analytical methods [1,2], i.e. the method for the ethanol content was treated as an assay whereas the method on methanol/2-propanol as a limit test. All results were expressed as area ratio; n represents the number of values. For the statistical analysis excel 2000 (Microsoft Office) was used. A 5% level of significance was selected.

2.4.1. Ethanol content

2.4.1.1. Linearity and accuracy. Samples containing 50, 75, 100 and 150% of the aimed test concentration, i.e. 0.0048, 0.0071, 0.0095, and 0.0143% ethanol in water, were prepared according to the above described sample preparation procedure using the standard ethanol solution (1 mg/ml). At each level samples were prepared in triplicate, each sample was injected twice and analyzed according to the method previously described. For assessing the linearity the least squares line and the correlation coefficient were calculated. The calibration curve obtained was tested on the slope ($a \neq 0$) and intercept ($b = 0$) by means of Student's t-tests. In order to check the goodness (or lack) of fit of the linear model a lack-of-fit (LOF) test [3] was performed and the residuals were graphically inspected. For each sample the recovery % was calculated. By means of a Student's t-tests the mean recovery % was checked to be equal to 100%.

2.4.1.2. Precision. The repeatability and the inter-day intermediate precision were determined by analyzing six samples containing a final concentration of 0.0095% (100%) according to the above described method on three different days. The standard deviation and coefficient of variation were calculated for each day. In order to check whether the results obtained on the three different days were not significantly different, the results were analyzed by means of an ANOVA single factor. Within and between days variation coefficients were calculated [4].

2.4.2. Test on methanol and 2-propanol

2.4.2.1. Precision. The repeatability and the inter-day intermediate precision were determined by analyzing six samples of the methanol/2-propanol standard solution (concentration test solution: 0.005% of methanol and 0.005% of 2-propanol) were prepared and analyzed according to the above described method on three different days. The standard deviation and coefficient of variation were calculated for each day. In order to check whether the results obtained on the three different days are not significantly different, the results were analyzed by means of an ANOVA single factor. Within and between days variation coefficients were calculated [4].

3. Results and discussion

Since methods described in the European Pharmacopoeia to determine the ethanol content and perform the test on methanol and 2-propanol are laborious and time consuming, and because packed glass columns, described for the latter limit test, are not commonly used anymore with modern GC equipment the aim of this work was to develop capillary static headspace [5,6] GC/MS methods for both determinations in liquid herbal drug preparations. Most of the publications on the determination of the ethanol content [7–9], or more generally on the quantification of volatile organic compounds [10–12], have been developed for biological fluids. Moreover, methods developed to determine residual solvents such as

Table 1
Overview of the validation data of the assay on ethanol and the limit tests on methanol and 2-propanol

Parameter	Assay on ethanol	Limit on methanol	Limit on 2-propanol
Linearity			
Correlation coefficient	0.998		
Slope ± standard error	8.811 ± 0.086		
Intercept ± standard error	− 0.032 ± 0.011		
Confidence interval (95%)	− 0.055 to − 0.009		
F_{LOF} (F_{crit} = 4.46)	1.9		
Concentration range(% v/v)	0.0048–0.0143		
Number of standards (triplo)	4		
Accuracy			
Mean recovery (%)	100.1		
R.S.D. (%)	1.8		
Concentration range test solution (% v/v)	0.0048–0.0143		
Number of levels	4		
Number of replicates	3		
Precision			
Repeatability			
Concentration test solution (% v/v)	0.0095	0.005	0.005
Number of replicates	6	6	6
R.S.D. (%) (day 1/day 2/day 3)	3.58/1.22/3.59	3.51/1.46/5.71	2.29/2.39/1.70
Intermediate precision			
Concentration test solution (% v/v)	0.0095	0.005	0.005
Number of days	3	3	3
Number of replicates	6	6	6
R.S.D. between groups	3.01	4.00	2.38
F_{calc} (F_{crit} = 3.682)	0.001	1.003	2.348

ethanol, methanol and 2-propanol in solid drugs [13–24] are not appropriate to simultaneously quantify a substantial amount of ethanol (45–80%) and detect traces of methanol and 2-propanol in liquid extracts or tinctures.

Two major problems faced during the development of the capillary headspace method for both determinations were the separation of the solvents to be determined and the high amount of water of the samples. The difficulty in analyzing these samples is resolving the peak of 2-propanol from the major ethanol peak. Using a macrogol 20.000 (50 m × 0.25 mm × 0.2 μm) we did not succeed to overcome this problem, i.e. the peak of the trace of 2-propanol eluted in the tailing of the huge peak of ethanol. An additional problem with this type of columns is the strong bleeding of the column when applying temperature programming. In order to get a good separation of methanol, ethanol and 2-propanol a 100% dimethylpolysiloxane (30 m × 0.32 mm) GC-column with a thick film (5 μm) was used. For our purpose two types of 100% dimethylpolysiloxane columns could have been suitable: a very long column, i.e. 100 m with a common film thickness (0.2 μm) or a short, thick film (30 m, 5 μm) GC-column. For economical reasons with respect to the price of the columns and the time of analysis the GC-column with the thick film was preferred. The samples to be analyzed contain a high amount of water. When injected on the GC-column this water is retained in part on the column (thick film) and the alcohols to be determined dissolve in this retained water. The temperature gradient thus applied to overcome this problem yields accurate and repeatable results. Kocijan et al. [25] described a headspace GC/MS method using a PLOT-column for the determination of solvent residues in drugs. Samples contain-

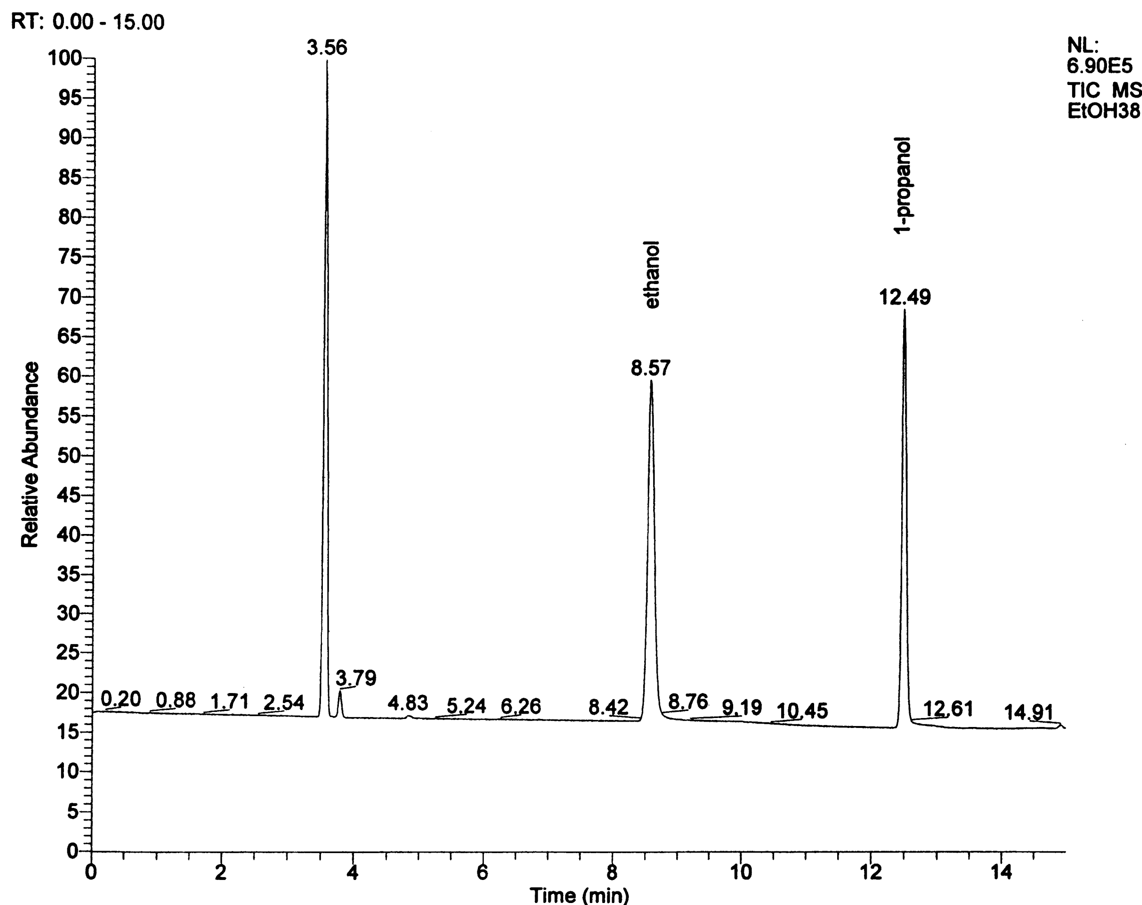

Fig. 1. A chromatogram of the standard solution used for the determination of the ethanol content.

ing water can be injected on such columns, but these columns show a high bleeding and consequently can dirten the MS detector and finally shorten its life time considerably. In order to protect the mass detector from water vapours a Hydroguard FS (5 m × 0.18 mm) column was coupled to the thick layer Rtx 1 (30 m × 0.32 mm × 5 μm) GC column.

In the proposed capillary headspace GC/MS method appropriately diluted samples were incubated in the headspace oven at 85 °C during 20 min for the determination of the ethanol content. Five hundred microlitre of the resulting gas phase above the sample was injected into the GC/MS instrument. A temperature gradient going in several stages from 40 to 175 °C was applied. The MS spectra were recorded in the TIC scan mode to identify the peak due to ethanol, whereas the SIM (m/z 31 + 45) scan mode was used to quantify ethanol. A calibration using three standard solutions was performed for each sequence of samples. 1-Propanol, the internal standard, was added to the samples before the determination is carried out.

Both methods were fully validated. All validation data were presented in Table 1.

Solutions of different concentrations of ethanol in water were used in the validation of this method. The calibration curve was linear over a range of 0.0048–0.0143% ethanol. Graphical inspection of the residuals, the LOF test ($F_{calculated}$: 1.9 < $F_{0.05,k-2,N-k}$: 4.46) and the correlation coefficient of 0.998 proved the method to be linear. The slope, 8.811 ± 0.086, was significantly different from 0.

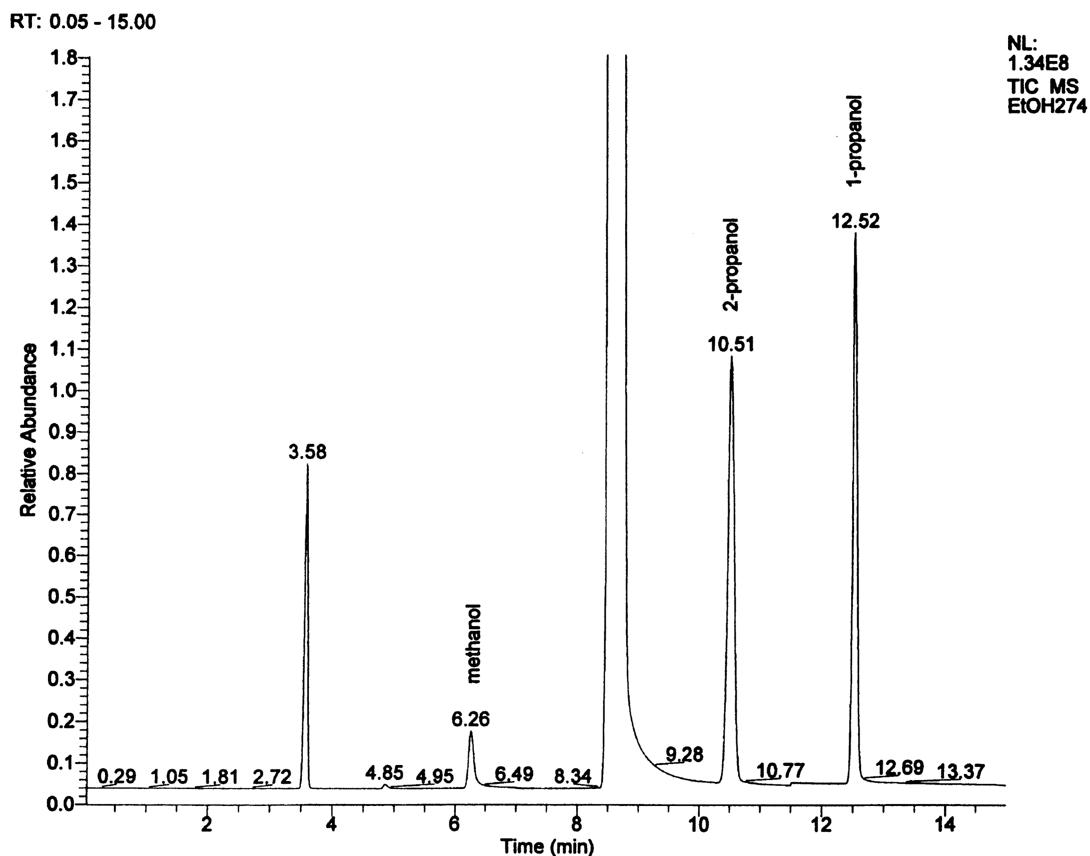

Fig. 2. Test on methanol and 2-propanol: a chromatogram of the standard solution.

The t-test on the intercept (-0.032 ± 0.011) revealed that point (0, 0) did not fall within the calibration curve. Therefore, a calibration curve was run for every sequence of samples. The accuracy of the method was proven to be 100.1% with an R.S.D.% of 1.8%. Replicate tests ($n = 6$) indicated a good precision, represented here by the repeatability and the intermediate precision, of the method (R.S.D.% = 3.0%). A chromatogram of the standard solution is shown in Fig. 1. Peaks of ethanol and 1-propanol were indicated on the chromatogram, the other peaks visible in the chromatogram are due to O_2, CO and CO_2.

In the limit test on methanol and 2-propanol undiluted samples were analyzed in the same way as was described in the determination of the ethanol content. The chromatograms recorded in the SIM scan mode (0.0–7.0 min: m/z 31, 7.0–11.5 min: m/z 45, 11.5–15 min: m/z 31) were used for quantification purposes. Standard solutions containing 0.05% methanol and 2-propanol were run for each sequence of samples (concentration in test solution: 0.005%). 1-Propanol, the internal standard, was added to the samples before the determination was carried out. The repeatability and intermediate precision, investigated by analyzing solutions containing 0.05% methanol and 2-propanol, were acceptable, i.e. an R.S.D.% of 4.0% for methanol and 2.4% for 2-propanol. A chromatogram of the standard solution is shown in Fig. 2. Peaks of methanol, 2-propanol and 1-propanol were indicated on the chromatogram, the other peaks visible in the chromatogram are due to ethanol, O_2, CO and CO_2.

Samples of liquid extracts and tinctures are analyzed in duplicate. In order to check whether

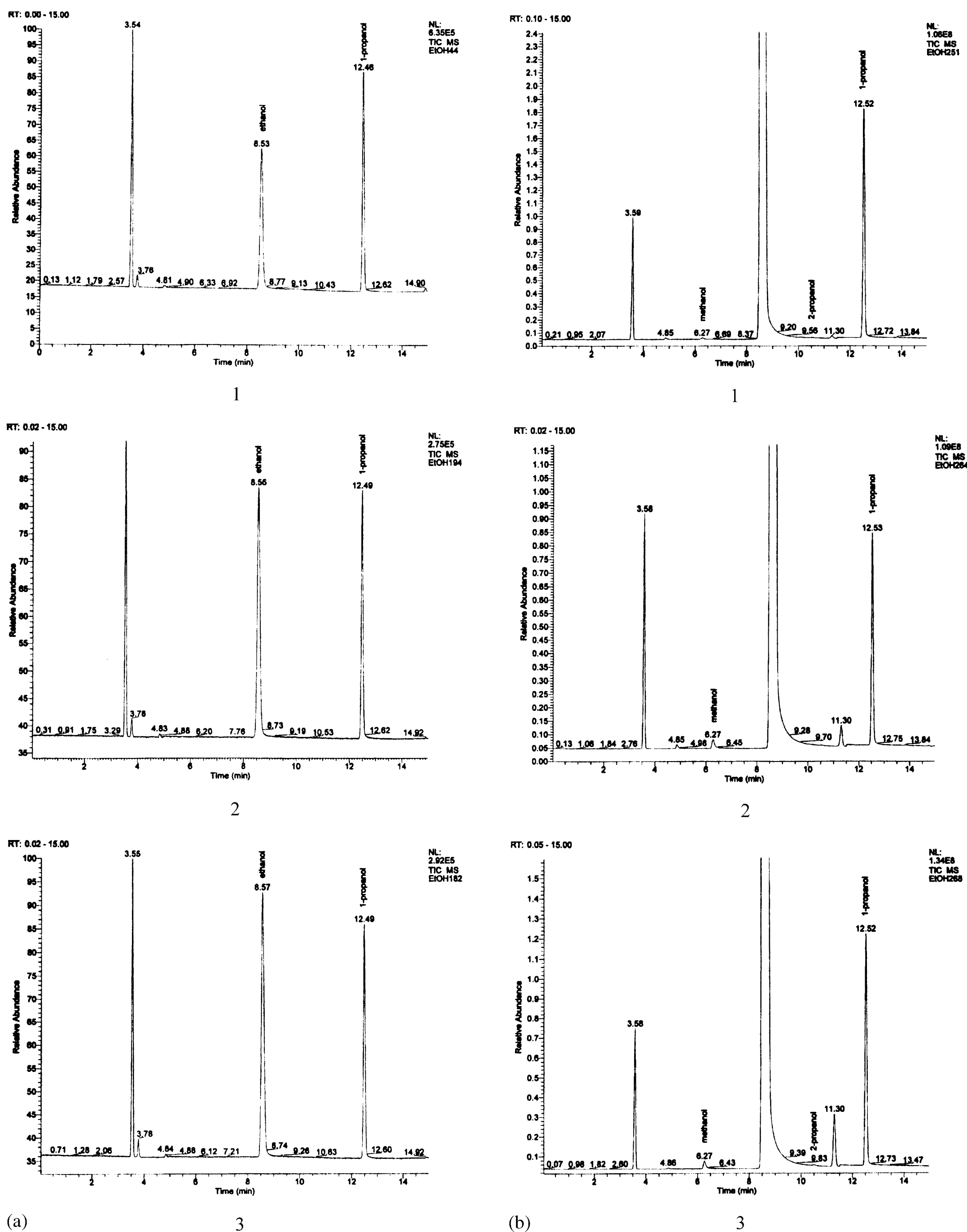

Fig. 3. Chromatograms of the determination of the ethanol content (a) and of the test on methanol and 2-propanol (b) of three samples, i.e. (1) *Ballota* liquid extract, (2) *Salvia* liquid extract and (3) *Eucalyptus* tincture.

Table 2
Recovery data for the assay on ethanol on a mixture of liquid extracts

Test solution	Determined content of ethanol in test solution (μl/150 μl)	Added amount of ethanol (μl/75 μl)	Ethanol content of the extract (μl/75 μl)	% recovery
1	0.1674	0.0905	0.0712	101.3
2	0.1740	0.0905	0.0712	108.2
3	0.1674	0.0905	0.0712	101.3
4	0.1734	0.0905	0.0712	107.6
Mean				104.6
Standard error				3.8
R.S.D. (%)				3.65
$t_{calculated}$				2.421
t_{table}				3.182

Table 3
Comparison of the results obtained by the European Pharmacopoeia methods (certificate of analysis) and by the proposed headspace GC/MS methods

Sample	Ethanol content ($n = 2$)		Test on methanol ($n = 2$)		Test on 2-propanol ($n = 2$)	
	Certificate	HS GC/MS	Certificate	HS GC/MS	Certificate	HS GC/MS
Ballota liquid extract	23.3%	23.45% 24.28%	< 0.05%	< 0.05%	< 0.05%	< 0.05%
Salvia liquid extract	54.2%	52.51% 52.62%	< 0.05%	< 0.05%	< 0.05%	< 0.05%
Eucalyptus tincture	75.51%	76.32% 73.76%	< 0.05%	< 0.05%	< 0.05%	< 0.05%

possible interference of other matrix components occur, standard addition experiments using standard solutions of ethanol are run for each new type of liquid extract or tincture. In Table 2 the results of a recovery experiment on a mixture of equal parts of three liquid extracts, i.e. *Ballota*, *Crataegus* and *Passiflora* liquid extracts, containing 47.43% ($n = 4$, VC: 1.93%). Instead of pipetting 150 μl of the extract, 75 μl of the extract was taken and 75 μl ethanol reference solution was added. The analysis was carried out four times. By means of a Student's t-tests the obtained mean recovery % of 104.6% ($n = 4$, VC: 3.65%) was proven to be equal to 100%. The accuracy of the determinations can also be proven by comparing the results obtained with the headspace GC/MS methods with the results obtained by the current Pharmacopoeia methods as performed by suppliers of liquid extracts and tinctures. A few examples are listed in Table 3. The chromatograms of the ethanol assay and the test on methanol and 2-propanol on three samples are shown in Fig. 3 a and b, respectively.

These fully automated, rapid, selective and sensitive methods are significantly faster than those reported in the current European Pharmacopoeia since they need no or only simple sample preparation.

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