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DEVELOPMENT AND VALIDATION OF GC-FID METHOD FOR THE DETERMINATION OF ETHANOL RESIDUE IN MARJORAM OINTMENT

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Abstract: Our study describes the procedure development and validation of the GC-FID method for the determination of ethanol content in marjoram ointment. At the validation procedure linearity ranged from 1×10^{-4} % to 1×10^{-1} %, limit of detection was 5×10^{-5} %, relative standard deviation for precision RSD = 1.07%, relative standard deviation for intermediate precision RSD = 1.43% and good recoveries (average 97,47%) were obtained. It is suggested after the analysis of ethanol content in marjoram ointments from different producers that the method is intended for routine standardization assays of ethanol. From the data of validation criteria it is suggested that the method is suitable for the determination and for the quality identification of ethanol residue in pharmaceutical products.

Keywords: ethanol, solvent residues, marjoram ointment, gas chromatography

Ethanol is an organic solvent with characteristic physicochemical properties and pharmacological activity suitable for pharmaceutical production and is used also as disinfectant (1-4) and as an aid in marjoram ointment pharmaceutical processing (4).

In the pharmacopoeial monographs and other reports the quality and quantity determination of the residual solvents are typically determined using gas chromatography with a proper detection or used headspace techniques (2-9).

According to pharmacopoeias and ICH requirements, the classification and allowed limits for solvents concentration, which may remain in active substances, as well as in pharmaceutical semi- and final products after processing, are clearly described.

The aim of the study was to develop and validate gas chromatographic (GC-FID) method for the determination of ethanol residue in marjoram ointments from different producers.

There were no similar data in the literature what suggests that ethanol residue in ointments still remains a problem to assess.

EXPERIMENTAL

Apparatus

All GC experiments were performed using an Agilent Technologies 6890N Network GC System (USA), coupled with a flame ionization detector (FID), autosampler (Agilent 7683 Series Injector) and hydrogen generator Agilent S184-3503, equipped with – Rtx-1301 (Crossbond 6% cyanopropylphenyl, 94% dimethylpolysiloxane, Restek) capillary column (30 m × 0.32 mm i.d., 1,5 μm film thickness).

Computer HP Vectra, with installed software ChemStation was used to collect chromatographic data.

Chemicals and reagents

Standard substances

The following compounds were used during experiments*:* ethanol 96% density d=0,7893 g/cm^3 (Chempur, Piekary Śląskie, Poland), marjoram herb – (Kamis S.A, Egypt), Vaseline – (Pharma-Cosmetic, Kraków, Poland).

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Preparations

Ethanol content was determined in marjoram ointments from: Aflofarm, serial No. 01AF1008, Hasco-Lek S.A. serial No. 060908, Pharmaceutical Laboratory „Avena", serial No. 021008, and Elissa, serial No. 010908. All ointments were Polish products taken from pharmacies and were chosen randomly.

Standard solutions preparation

Standard solutions were prepared by adding 1.267 mL of ethanol 96% and filled up with water to the volume of 100 mL to prepare 1% ethanol stock solution. Then after adequate dilutions, standard solutions were prepared: 1×10^{-1}, 5×10^{-2}, 1×10^{-2}, 5×10^{-3}, 1×10^{-3}, 1×10^{-4}, 5×10^{-5} and 2.5×10^{-5} percent.

Preparation of ointment solutions

Ethanol extraction from marjoram ointment

One gram of marjoram ointment was weighed and water was added up to 100 mL in a tightly closed volumetric flask. The extraction procedure was performed in water bath at 40°C for 15 min.

Table 1. Validation of the method.

Parameter	Results
Retention time (t_R), [min] n = 6	x_m = 1.35 RSD = 0.0084%
Number of theoretical plates n = 6	x_m = 12367.89
Peak symmetry n = 6	x_m = 0.31
Limit of detection, [%]	5×10^{-5}
Limit of quantitation, [%]	1×10^{-4}
Linearity range, [%]	$1 \times 10^{-4} - 1 \times 10^{-1}$
Regression coefficients P = a c + b ± S_e[a]	a = 12362.76 b = -8.941 ± 31.91
Standard deviation of the regression coefficients	S_a = 356.15 S_b = 16.34
Correlation coefficient, r	r = 0.99834
Precision n = 6	x_m = 0.28 RSD = 1.07%
Intermediate precision n = 6	x_m = 0.35 RSD = 1.43%
Recovery, [%] n = 9	x_m = 97.47 RSD = 1.88%

[a] P = peak area; c = concentration; a and b = regression coefficients, S_e = standard error of the estimate, S_a = standard deviation of the regression coefficient a, S_b = standard deviation of the regression coefficient b, RSD = relative standard deviation, x_m = the mean

Table 2. Ethanol content [%] in marjoram ointments from different producers.

Sample no.		Ethanol content [%]			
		Aflofarm	Avena	Hasco-Lek	Elissa
1	x_m	0.39	1.15	1.07	0.27
	RSD %	1.54	0.52	0.19	0.26
2	x_m	0.35	1.02	0.89	0.28
	RSD %	1.14	0.59	0.22	0.36
3	x_m	0.34	1.24	1.07	0.30
	RSD %	1.47	0.81	0.06	1.33

x_m – the mean, RSD – relative standard deviation

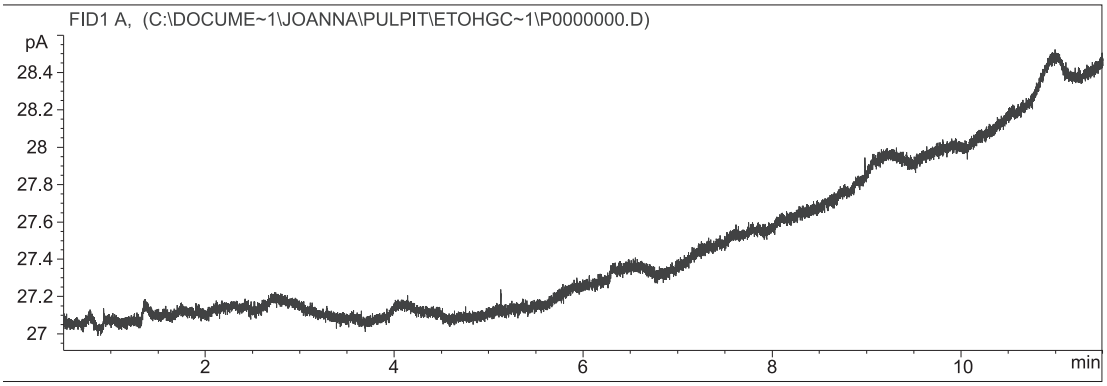

Figure 1. Chromatogram of placebo solution.

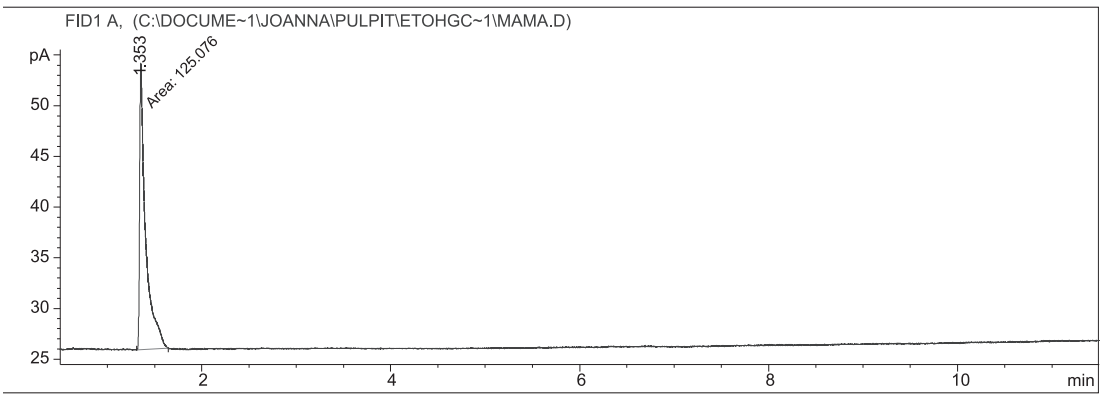

Figure 2. Chromatogram of sample solution.

After shaking well the extract was cooled at 4°C for 2 h. The extraction procedure was repeated three times for each marjoram ointments. One µL of each extract was taken for further studies.

Extraction and preparation of placebo

Twenty milligrams mg of marjoram herb, 1000 mg of vaseline were weighed and water was added to 100 mL in a tightly closed volumetric flask. The extraction procedure was performed in water bath at 40°C for 15 min. After shaking well, the extract was cooled at 4°C for 2 h. The extraction procedure was repeated three times. One µL of each extract was taken for further studies.

Analysis

In order to establish chromatographic conditions, duration of analysis, mobile phase temperature, pressure etc. were taken into account. As a result, the following chromatographic parameters were established: front inlet splitless mode, 150°C, helium flow at 25.2 mL/min, injection volume 1 µL, oven temperature: 35°C for 5 min, increased at 10°C/min to 100°C and 110°C for 2 min. Temperature of the FID detector was 200°C.

Helium at a flow rate of 8 mL/min was used as a carrier gas. Synthetic air (flow rate of 400 mL/min), hydrogen (30 mL/min) and nitrogen as a make-up gas (25 mL/min) were fed to the FID

detector. All the gases used in these studies were of N5.0 purity. Analysis time was 11.5 min.

Validation of the method

The procedure was validated following ICH guidelines and the following validation parameters were determined (10): specificity, linearity, limits of detection and determination, and recovery .

Chromatographic system condition

Chromatographic condition was determined as repeatability by measuring six replicates of standard solution 1×10^{-2} percent of ethanol (compared peak areas and retention times), peak symmetry and number of theoretical plates (Table 1).

Specificity

Specificity and selectivity of the method were checked. To determine an effect of matrix components on the determination results comparative analysis was carried out for the blank sample, placebo extracts, standard solutions of ethanol 1×10^{-1}, 1×10^{-2} and 1×10^{-3} percent, as well as the extracts of marjoram ointments. On the basis of the chromatograms obtained, no significant influence of other compounds on quantification was observed (Figs. 1, 2).

Linearity

Linearity was verified by analyzing eight standard solutions of ethanol in the range from 1×10^{-1} to 1×10^{-4} percent. For each concentration of standard solution there were three injections. The relationship between the ethanol concentration in the sample and the corresponding peak area (detector response) was calculated using the linear regression method (Statistica 8.0 StatSoft). Using the calibration curve equation, the concentrations corresponding to each value of the peak area have been calculated and compared with the expected values (Table 1).

Limit of detection and limit of determination

Limits of detection and determination were obtained experimentally, after the analysis of standard solutions 1×10^{-4}, 5×10^{-5} and 2.5×10^{-5} percent of ethanol. Limit of determination was calculated at the base of signal/noise = 3 and the limit of detection at the base signal/noise = 6 (Table 1).

Precision

The precision is defined as the degree of consistence between measurements repeated many times. The precision of the method was assessed as the degree of consistence between concentrations of ethanol in ointment extracts (n = 6). Intermediate precision was assessed as described previously and measured in the next day (Table 1).

Accuracy

The accuracy of the method was defined in terms of ethanol recovery. Therefore, specified amounts from 80% to 120% of ethanol were determined. Three model mixtures were prepared for the study by weighing of 20 mg marjoram herb and 1000 mg of vaseline, 8.0 mL, 10.0 mL and 12.0 mL of ethanol standard solution 1×10^{-1} percent were added, respectively, and diluted with water to 100 mL. After extraction, chromatographic analyses were done. The accuracy of the method was described by the results of recovery % obtained on the basis of determined content of ethanol to the amount added to the model mixtures (Table 1)

Quantitative analysis

Determination of ethanol residue in marjoram ointments from different producers was carried out under chromatographic conditions described previously. The ethanol content was calculated from the parameters of calibration curve based on the registered peak area (Table 2).

RESULTS AND DISCUSSION

In this paper the validation of the GC-FID method for the determination of ethanol residue in marjoram ointments was carried out. Only one publication on ethanol residue in tablets by HS-GC in the available literature was found. No papers concerning the determination of ethanol residue in ointments have been found.

When analyzing extracts of placebo and marjoram ointments no effects of the matrix on the obtained results were found, thus indicating a good selectivity of the method (Figs. 1, 2).

From the data it is suggested that GC-FID method is suitable and acceptable for chromatographic conditions, the number of theoretical plates was high (12367.89) and symmetry of the ethanol peaks was good (0.31). The problem of ethanol water solutions peak symmetry is widely known (11). The detected signals were only from ethanol.

The method demonstrated a wide linearity range from 1×10^{-4} to 1×10^{-1} percent (Table 1). A wide linearity range guarantees that good results are obtained even if individual product series differ in ethanol content.

The recovery and precision of the method were satisfactory for quantitative analyses and good

repeatability of the results has been achieved. The RSD values were 1.07% and 1.43%, which indicated good precision and intermediate precision. The obtained high recovery value was 97.47%.

Good precision and accuracy of the method were confirmed by determining the concentration of ethanol in marjoram ointments from different producers (Table 2.)

Peak area *versus* concentrations of ethanol was linear, the resulting regression had a good slope and correlation coefficient was r = 0.99833.

The limit of detection was 5×10^{-5} percent, while the limit of determination was 1×10^{-4} percent. There were satisfactory results for ethanol residue analyses.

The validation of the GC-FID method along with statistical analysis indicate high sensitivity under established conditions. The detailed results of validation are presented in Table 1.

The developed GC-FID method enables easy qualitative and quantitative analyses of ethanol residue in marjoram ointments. Therefore, the method can be used for routine standardization assays of pharmaceuticals containing ethanol residue. The validation data show that the results are accurate and precise so the method can be widely applied. It seems that the developed gas chromatographic method is especially suitable for determination of ethanol residue.

REFERENCES

1. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Q3C: Impurities: Guideline for Residual Solvents, 1997.
2. European Pharmacopoeia 6th ed., Limit Tests 2.4.24, Identification and Control of Residual Solvents, Council of Europe, Strasburg, 2007.
3. The United States Pharmacopoeia, 30th ed., Chemical Tests, Organic Volatile Impurities, The United States Pharmacopeial Convention Inc., Rockville 2007.
4. Polish Pharmacopeia VII, Urząd Rejestracji Produktów Leczniczych Wyrobów Medycznych i Produktów Biobójczych, Warszawa 2006.
5. B'Hymer C.: Pharm. Res. 20, 337 (2003).
6. Sitaramaraju Y., Riadi A., D'Autry W., Wolfs K., Hoogmartens J., Van Schepdael A., Adams E.: J. Pharm. Biomed. Anal. 48, 113 (2008).
7. Michulec M., Konieczka P., Namieśnik J.: J. Qual. Assur. 12, 257 (2007).
8. Krzek J.: Acta Pol. Pharm. 43, 265 (1986).
9. Krzek J., Bielska A.: Farm. Pol. 45, 534 (1988).
10. ICH-Q2 (R1) Validation and Analytical Procedures: Text and Methodology, International Conference on Harmonisation, Geneva, November 2005.
11. Agilent Technologies. www.chem.agilent.com.

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