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ARTICLES

Determination of Impurities in Validol Tablets by Gas–Liquid Chromatography

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Abstract—A procedure is developed for determining impurities in Validol tablets by gas–liquid chromatography (GLC). The reliability of the results obtained is confirmed by the analysis of model mixtures containing the active and all auxiliary substances of the tablets. The proposed procedure is introduced into the standardized documentation on the manufactured tablets. The presence of impurities in Validol tablets due to the technology of the synthesis of the Validol is inevitable. Therefore, the routine control of production is very important to ensure its quality.

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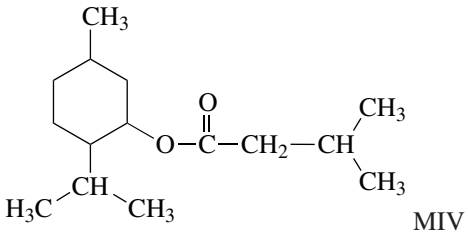
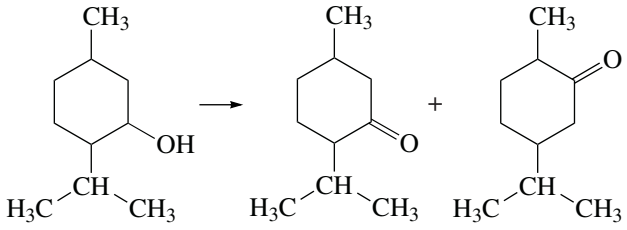
Concentrated H_2SO_4 is used as a catalyst of esterification in the synthesis of one of the main components of the validol (V) substance, menthyl isovalerate (MIV).



MIV

According to the studies of production engineers, under these conditions, a number of side substances, including a mixture of isomeric products of menthol (M) destruction, menthones (MH), are formed.



M MN-1 MN-2

Contrary to M and MIV, these and other side substances possess no useful pharmacological properties and are undesirable impurities in the ready-made product. Therefore, their reliable determination is important for ensuring the quality of both the validol substance and Validol tablets.

The goal of this work was to develop a procedure for analyzing Validol tablets for the index "foreign impurities" and to determine its performance characteristics.

EXPERIMENTAL

Reagents. The following substances were used to prepare solutions: validol and menthol of pharmaceutical grade checked by the quality control department of the plant and met all requirements of the standardized documentation (SD) and diethyl ether for narcosis. The standard sample of MIV was obtained in the laboratory by the rectification of validol. Its purity (99.9%) was confirmed by GLC. The remaining reagents were of analytical grade or better.

Apparatus. A Kristall 2000M (SKB Chromatek, Russia) gas chromatograph with a flame-ionization detector (FID-1) and a fused-silica capillary column (50 m × 0.3 mm) with an OV-101 stationary phase were used. In the course of analysis, the column temperature was programmed from 100 to 240°C at a rate of 5 K/min. The overall time of analysis was 35 min; the temperature of the injector and detector was 250°C. The flow rate of the carrier gas (nitrogen) was 20 mL/min, and the split ratio was 1 : 20. The flow rates of hydrogen and air were 20 and 200 mL/min, respectively. The volume of a sample was 1.0 μL.

Preparation of the model mixture of impurities. A 30-mL portion of conc. H_2SO_4 and 5 g of menthol were mixed in a 100-mL beaker for 10 min. The orange mixture obtained was transferred into another 150-mL beaker, which contained 20 g of ice, and the mixture was stirred until ice completely dissolved plus another 5 min. After 10 min, the green organic supernatant

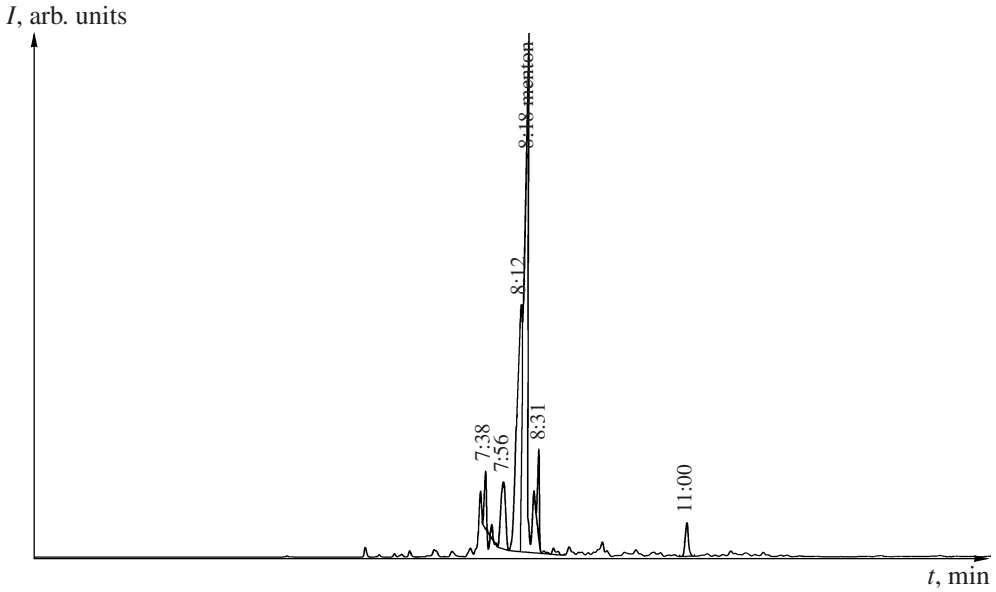


Fig. 1. Chromatogram of a model mixture.

(about 3 mL) was decanted into a 50-mL beaker with 20 mL of water. Granulated NaOH was added to the obtained two-phase system in small portions along with stirring, and the pH of the organic layer was controlled after adding each portion of the alkali using an indicator paper. When the pH of the lower aqueous layer became neutral, it was removed with a pipette, and the remaining organic layer was dried over anhydrous Na_2SO_4. About 2.0 mL of an oily yellowish brown liquid was obtained. The chromatogram of the mixture obtained is presented in Fig. 1.

Preparation of test solutions for verifying the results. The accuracy of the procedure for determining impurities in "Validol 0.06 g" tablets was verified using model solutions that contained validol, impurities, and auxiliary substances in amounts extractable with ether into a test solution. Model solutions were prepared from 150 tablets (120 g). The mixture obtained was transferred into a 0.75-mL Erlenmeyer flask, 200 mL of ether was added, and the mixture was shaken for 15 min. After standing, the solution obtained was filtered through a red-ribbon paper filter into a 500-mL Erlenmeyer flask, and the solvent was evaporated on a water bath at 50–60°C to a volume of 6–7 mL (ether was almost completely removed).

Accurate samples of impurities in the range from 2.0 to 15.0 wt %, which corresponded to the range 0.7 to 5.4% found by the chromatogram, were added to accurate samples of validol. Seventeen test solutions were prepared, three chromatograms were recorded for each of them.

Preparation of test solutions of tablets. A 6.0-g portion of carefully triturated tablets was placed in a 250-mL Erlenmeyer flask with a ground stopper, 50 mL of ether was added, and the mixture was shaken for 15 min. The suspension obtained was filtered through a red-ribbon paper filter into a 100-mL Erlenmeyer flask, and the filtrate was evaporated on a water bath at 50–60°C to a volume of ~1 mL.

Calculation of results. The concentration of foreign substances in tablets (%) was calculated by the method of internal normalization [1, 2] using the equation

$$X = (\Sigma S_{total} - S_{val})/\Sigma S_{total} \times 100\%, \quad (1)$$

where ΣS_{total} is the sum of all areas in the chromatogram excluding the peak areas of ether and its impurities (recorded approximately in 1.5 min after the elution of the ether peak), mV s; S_{val} is the sum of peak areas of M and MIV in the chromatogram, mV s.

The concentration of impurities should be no higher than 4.0%.

RESULTS AND DISCUSSION

Determination of the coefficient of detector response with respect to the weight concentration of impurities. It was assumed that the percentage of impurities in validol found by the method of internal normalization is proportional to the weight percent concentration of these impurities in validol, but is not equal to it. To determine the coefficient of proportionality, we analyzed four samples of the validol substance used in the production of tablets and four model solutions prepared by mixing accurate samples of validol and the synthesized impurities. For each sample, three chromatograms were recorded. The response coefficient K_{resp} was calculated by the following equation:

$$K_{resp} = (X - X_{initial})/X_{added}, \quad (2)$$

Table 1. Calculation of the coefficient of detector response with respect to the weight concentration of impurities

Ordinal number	Sample	X_{added}, wt %	S_{total}, mV s	S_{val}, mV s	S_{imp}, mV s	X, %	$X - X_{initial}$, %	K_{resp}
1	Validol	0	432900	428600	4300	0.993		
		0	519860	514610	5250	1.010		
		0	460760	456110	4650	1.009		
2	The same	0	560640	554730	5910	1.054		
		0	569110	562790	6320	1.111		
		0	615400	608760	6640	1.079		
		0	652330	644800	7530	1.154		
3	″	0	537280	531240	6040	1.124		
		0	575460	568880	6580	1.143		
		0	651330	644800	6530	1.003		
4	″	0	536490	531240	5250	0.979		
		0	574550	568880	5670	0.987		
5	Validol + impurities	7.594	564760	542980	21780	3.857	2.803	0.369
		7.594	492860	473240	19620	3.981	2.927	0.385
		7.594	461190	443430	17760	3.851	2.797	0.368
6	The same	10.838	432140	411330	20810	4.816	3.762	0.347
		10.838	429700	408440	21260	4.948	3.894	0.359
		10.838	597330	568020	29310	4.907	3.853	0.356
7	″	3.971	561210	547830	13380	2.384	1.330	0.335
		3.971	539680	527180	12500	2.316	1.262	0.318
		3.971	504010	492365	11645	2.310	1.257	0.316
8	″	1.880	515400	506780	8620	1.672	0.619	0.329
		1.880	533400	524370	9030	1.693	0.639	0.340
		1.880	457590	450110	7480	1.635	0.581	0.309
	Average							0.344
	S							0.024

Table 2. Determination of impurities in Validol tablets (%; $n = 9$; $P = 0.95$; according to the SD, the norm is at most 4%)

Nos. of samples	X_{aver}	RSD, %	$s_{X_{aver}}$	ΔX_{aver}	ε, %
1	1.07	1.0	0.0033	0.0078	0.73
2	1.04	4.0	0.0133	0.0350	3.36
3	1.00	3.0	0.0100	0.0236	2.36

where X is the concentration of impurities (in %) in the validol substance to which impurities were added (found from chromatogram); $X_{initial}$ is the concentration of impurities (in %) in the initial validol substance (found from the chromatogram); and X_{added} is the amount of impurities added to the validol substance (in wt %).

The results are presented in Table 1. The average value of K_{resp} found from all results was 0.344. The repeatability of the results was satisfactory (RSD = 6.5%). The value of K_{resp} was used to calculate the relative error of determining impurities in test solutions by the added–found method. To simplify the analysis, this value was omitted in the procedure intended for routine

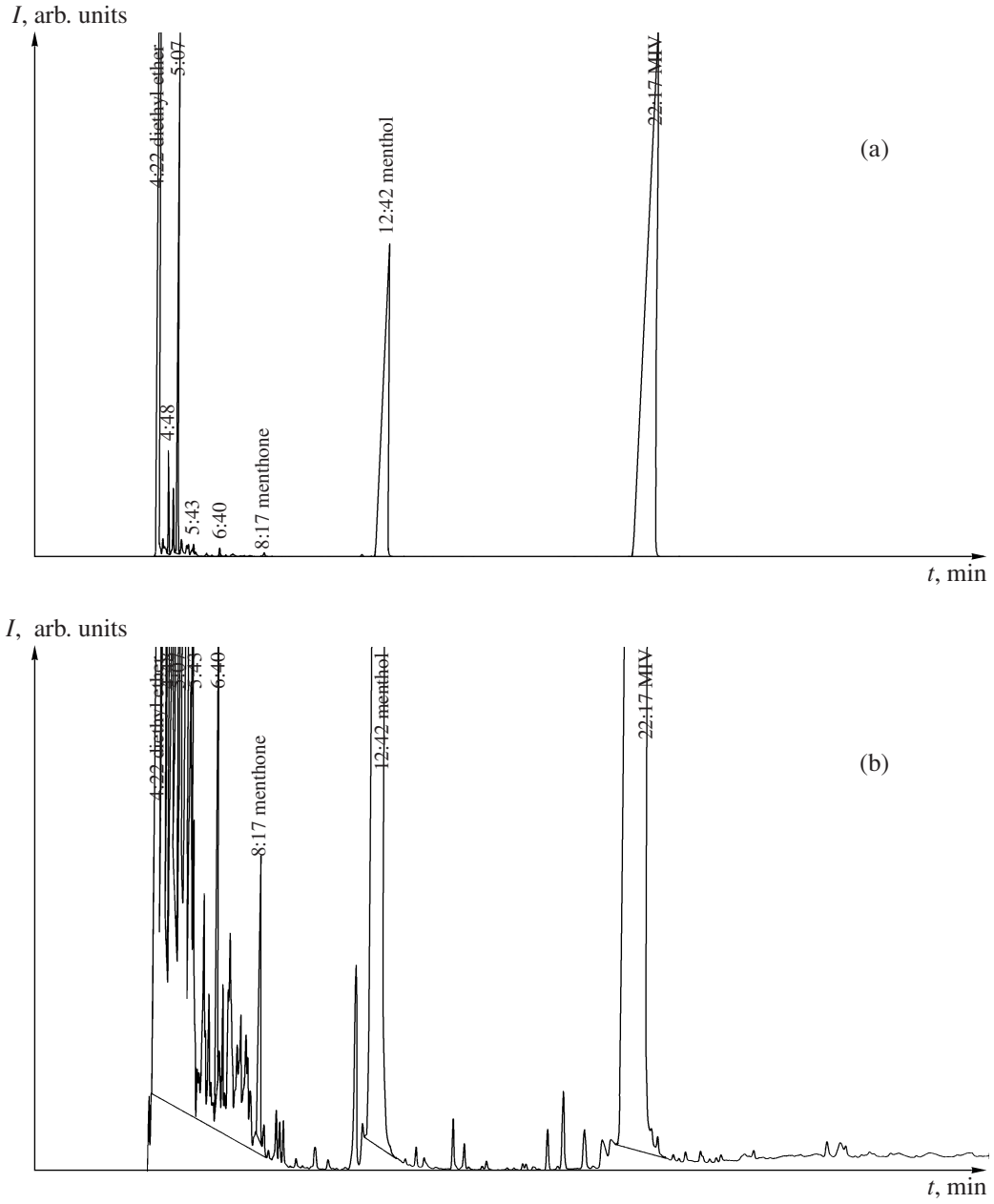


Fig. 2. (a, b) Chromatograms of a test solution for the quantitative analysis of Validol tablets (a and b refer to different batches of tablets).

control. This is permissible, because the quality of production is ensured by the fact that the concentration of impurities is not higher than its level specified in the SD, rather than by the low concentration of impurities itself.

Performance characteristics of the procedure were determined in the analysis of test solutions that contained the validol substance, all auxiliary substances of tablets extractable by diethyl ether, and accurate samples of added impurities. The following results were obtained: $s_{max} = 0.193\%$, $s^2_{max} = 0.0372$.

The average value of the relative error of analysis $e_{raver} = -0.361\%$ is lower than the corresponding confidence level $\Delta e_r = 0.745\%$; therefore, the procedure has no systematic error.

Detection and determination limits of the procedure. The level of noises of the baseline in the peak-free portion of the test solution chromatogram was calculated using a data processing software. The N value was found to be 3.62×10^{-4} V. This value of P comprises 0.002% of the MIV peak height; therefore, the values $3P = 0.006\%$ and $10P = 0.02\%$ can be taken as the theoretical detection and determination limits,

respectively. The analysis of the actually recorded chromatograms has demonstrated that the peaks with heights of at least 0.01 V, which comprised 0.06% of the MIV peak height, were reliably detected. This value can be considered as the actual determination limit of the procedure.

Analysis of tablets. Three samples of commercially produced tablets were analyzed using the proposed procedure. The chromatograms recorded and results are presented in Fig. 2 and Table 2, respectively. All samples meet the requirements of the SD on the concentration of impurities; the results of replicate analysis are well reproducible. The procedure was introduced into the draft of new SD for Validol tablets.

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