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ARTICLES

Quantitative Analysis of Pentalgin Tablets by Gradient and Isocratic High-Performance Liquid Chromatography

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Abstract—The retention of paracetamol, propyphenazone, caffeine, phenobarbital, and codeine phosphate, which are the components of the new medicine Pentalgin, was studied by reversed-phase high-performance liquid chromatography on a column (150 × 3.9 mm) filled with the Symmetry C18 sorbent (5.0 μm) in the gradient elution mode and on a column (150 × 3.9 mm) filled with the Nova-Pak CN HP sorbent (4.0 μm) as a function of the profile and composition of the gradient and as a function of the concentrations of acetonitrile and KH_2PO_4 and the pH of the mobile phase, respectively, with detection at 212 nm. The optimum composition of the mobile phase was selected. The time of separation was 16 and 11 min for the gradient and isocratic elution modes, respectively. The procedures were used for the analysis of a preproduction sample of the tablets. The procedures provide accurate and reproducible results of analysis; however, the isocratic version is preferable for mass production control as a technically simpler technique.

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Pentalgin tablets are a new five-component analgesic, antipyretic, and antiphlogistic medicine containing paracetamol, propyphenazone, caffeine, phenobarbital, and codeine phosphate. The new medicine differs from Pentalgin ICN tablets, which were produced previously, by the replacement of analgin with more efficient and less toxic propyphenazone. The chromatographic properties of analgin and propyphenazone are different; therefore, simultaneously with the development of technology, a new procedure for the quantitative determination was developed. Solving this problem, we considered two main techniques: analysis by the gradient and isocratic versions of high-performance liquid chromatography (HPLC) [1]. Previously, we developed procedures for the analysis of the medicines Pentalgin ICN [2] and Pentalgin N [3] by reversed-phase HPLC on the Nova-Pak C18 sorbent in the gradient elution mode and on the Nova-Pak CN HP sorbent in the isocratic mode. The analysis of the eight-component medicine Spasmoveralgin Neo in the isocratic mode with the use of a sorbent with modifying nitrile groups was described in [4]. This medicine also contains paracetamol, caffeine, phenobarbital, and codeine phosphate, along with other components; therefore, we suggested that Pentalgin tablets can be analyzed in the isocratic mode.

The aim of this work was to develop gradient and isocratic procedures for the quantitative analysis of Pentalgin tablets and to compare their performance characteristics.

EXPERIMENTAL

Reagents. For preparing eluents and for dissolving reference and test samples, we used acetonitrile of high-purity grade for chromatography sort 0 (Kriokhrom, Russia) and ultrapure water with specific resistance of 18.2 MΩ/cm obtained on a Direct Q device (Millipore). As reference samples of determined medicines, we used pharmaceutical substances checked by the quality control department of the plant and complying with all requirements of the regulations. All other reagents were of at least analytical grade.

Instruments. Chromatographic analysis was performed on a Waters Alliance 2695 chromatograph with a Waters 2996 diode array detector. The certified dead volume of the chromatograph was below 0.650 mL. A column with the Symmetry C18 reversed-phase sorbent with the average particle size of 5.0 μm (Waters) was used for gradient elution, and a column with the Nova-Pak CN HP with modifying nitrile groups and the average particle size of 4.0 μm (Waters) was used for isocratic elution. The length of the columns was 150 mm; their inner diameter was 3.9 mm.

Preparation of solutions. For the determination, 20 tablets of the analyzed medicine are weighed on an analytical balance and the average mass of a tablet in grams is determined with an accuracy of 0.0001 g. About 0.160 g (weighed portion) of ground tablets is transferred into a 100-mL volumetric flask, dissolved in 20 mL of a mixture (1 : 1 in volume) of acetonitrile and 0.025 M KH_2PO_4 with pH 3.0, and stirred for 10 min.

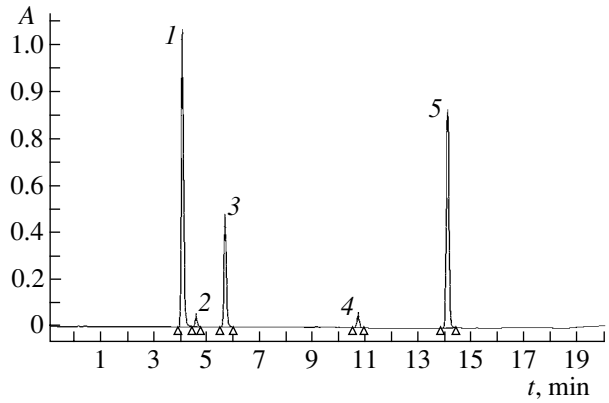

Fig. 1. Chromatogram of a solution of a reference sample for the analysis of Pentalgin tablets on a column (150 × 3.9 mm) filled with Symmetry C18 (5 μm) in the gradient elution mode; gradient from 0.025 M KH_2PO_4 (pH 3.0) to CH_3CN : 0.025 M KH_2PO_4 (pH 3.0) = 1 : 1 within 16 min; flow rate of the mobile phase 1.0 mL/min; detection wavelength 212 nm; (*1*) paracetamol, (*2*) codeine, (*3*) caffeine, (*4*) phenobarbital, and (*5*) propyphenazone.

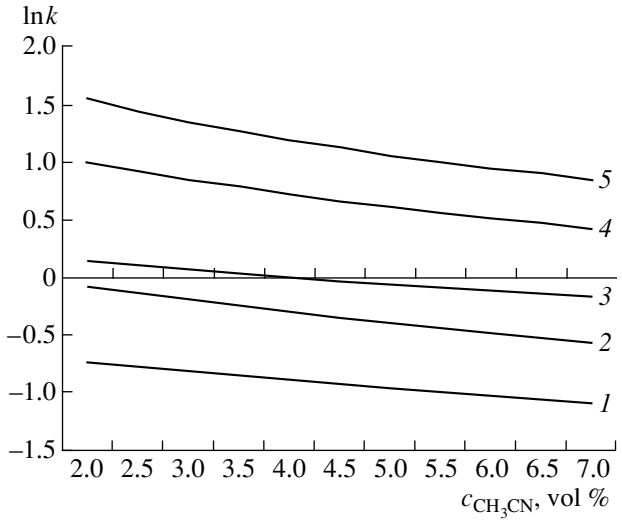

Fig. 2. Dependence of the retention of the components of Pentalgin tablets on the concentration of acetonitrile in a 0.0075 M KH_2PO_4 solution (pH 4.8); column (150 × 3.9 mm) filled with Nova-Pak CN HP (4.0 μm); flow rate of the mobile phase 1.0 mL/min; (*1*) paracetamol, (*2*) caffeine, (*3*) phenobarbital, (*4*) codeine, and (*5*) propyphenazone.

The resulting solution is diluted with a 0.025 M KH_2PO_4 solution with pH 3.0 to the mark. Simultaneously a solution of a reference sample containing 0.06 g of paracetamol, 0.05 g of propyphenazone, 0.01 g of caffeine, 0.0020 g of phenobarbital, and 0.0016 g of codeine phosphate was prepared. All solutions were filtered through a hydrophilic membrane filter with a pore size of 0.45 μm (Teflon filters stable in water–acetonitrile solutions are preferable).

Determination and calculation of results. The test solution and the solution of the reference sample are chromatographed obtaining at least three chromatograms for each solution. The peak areas of the analytes are calculated, and the amount of each component in the analyzed tablets is determined by the formula

$$X = \frac{S_t m_{ref} m_{av}}{S_{ref} m_p},$$

where S_t and S_{ref} are the average values of the peak areas of the analytes in the chromatograms of the test solution and the solution of the reference sample, respectively; m_{ref} is the mass of the analyte in the solution of the reference sample; m_{av} is the average of a tablet in grams; and m_p is the mass of the portion of ground tablets taken for the preparation of the test solution.

In all cases, the volume of injected samples and the flow rate of the mobile phase were 5.0 μL and 1.0 mL/min, respectively. The analytes were detected at 212 nm. The composition of the eluents is given in the figure captions.

RESULTS AND DISCUSSION

Preliminary studies demonstrated that on the C8 and C18 sorbents the capacity factor of phenobarbital is more than 10 times larger than the capacity factor of paracetamol, and the retention of propyphenazone is even stronger. Therefore, columns with the C8 and C18 sorbents can be used only with the gradient elution mode. For the gradient mode on a column with the Symmetry C18 sorbent, the initial (0 vol %) and final (50 vol %) concentrations of acetonitrile in the mobile phase and the slope of the gradient were selected empirically to provide the satisfactory resolution of the critical pair of peaks (paracetamol–codeine) and a reasonable time of analysis related to the elution of the last component, propyphenazone. The pH of a 0.025 M KH_2PO_4 solution, which is a constituent of the mobile phase, was decreased to 3.0 (initial solution of the salt has pH 4.8) for improving the shape of the codeine peak, which substantially increases the accuracy of the calculation of its area.

The chromatogram is presented in Fig. 1, chromatographic parameters are listed in Table 1, and the results of the analysis of tablets (pilot laboratory batch) are listed in Table 2.

The sorbent with modifying nitrile groups is more polar; therefore, the retention of compounds on this sorbent is weaker, and it can be used in the isocratic mode. In the search for the optimum conditions of the separation the components of the medicine in the isocratic mode on a column with the Nova-Pak CN HP sorbent, we studied the dependences of their retention on the concentration of CH_3CN and KH_2PO_4 in the mobile phase and on the pH of the mobile phase. The

Table 1. Chromatographic parameters of the separation of the components of Pentalgin tablets

Component	Sorbent	Mobile phase	t_R, min	N	$\alpha(t_0 = 1.477)$	R_S
Paracetamol	Symmetry C18 (5.0 μm)	Gradient from 0.025 M KH_2PO_4 (pH 3.0) to CH_3CN : 0.025 M KH_2PO_4 (pH 3.0) = 1 : 1 within 16 min	5.032	(1262)*		
Codeine phosphate			5.555	(4924)	1.15	1.4
Caffeine			6.658	(3036)	1.27	3.1
Phenobarbital			11.680	(12574)	1.97	10.5
Propyphenazone			15.040	(11271)	1.33	6.0
Paracetamol	Nova-Pak CN HP (4.0 μm)	0.075 M KH_2PO_4 (pH 4.8) containing 2 vol % acetonitrile	2.183	180		
Caffeine			2.833	740	1.92	2.1
Phenobarbital			3.173	424	1.25	0.8
Codeine phosphate			5.441	607	2.34	5.2
Propyphenazone			8.308	136	1.72	2.2
Paracetamol	Nova-Pak CN HP (4.0 μm)	0.075 M KH_2PO_4 (pH 4.8) containing 5 vol % acetonitrile	2.037	249		
Caffeine			2.461	558	1.76	1.5
Phenobarbital			2.869	387	1.41	1.2
Codeine phosphate			4.703	421	2.32	2.4
Propyphenazone			5.691	172	1.31	1.4

* Hereafter the number of theoretical plates is given in parentheses.

concentration of KH_2PO_4 and pH substantially affect only the retention of codeine: a decrease in pH and an increase in the concentration of the salt in the mobile phase lead to a decrease in retention. CH_3CN affects the elution of all components (Fig. 2), and the critical pair in this case is caffeine and phenobarbital. Figure 3 presents a chromatogram of a solution of the reference sample obtained at a concentration of 2 vol % CH_3CN in the mobile phase. It is seen that the peaks of caffeine and phenobarbital are not completely resolved. Upon increasing the concentration of CH_3CN to 5 vol %, the resolution of these peaks is improved, because the retention of caffeine decreases stronger than the retention of phenobarbital (Fig. 4). The calculation of the resolution of adjacent peaks according to the United States Pharmacopoeia, 25th Ed. (USP 25) [4] demonstrated that, for the "critical pair" paracetamol–codeine, this value is at least 1.8. The asymmetry coefficient for the broadest peak (propyphenazone) is below 1.8. These characteristics are satisfactory for quantitative

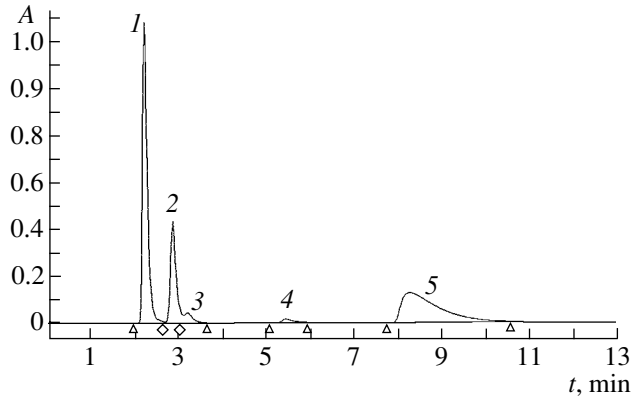

Fig. 3. Chromatogram of a solution of a reference sample for the analysis of Pentalgin tablets in the isocratic elution mode with a 0.0075 M KH_2PO_4 solution (pH 4.8) containing 2 vol % acetonitrile; detection wavelength 212 nm; the other conditions of chromatography are given in the caption to Fig. 2; (*1*) paracetamol, (*2*) caffeine, (*3*) phenobarbital, (*4*) codeine, and (*5*) propyphenazone.

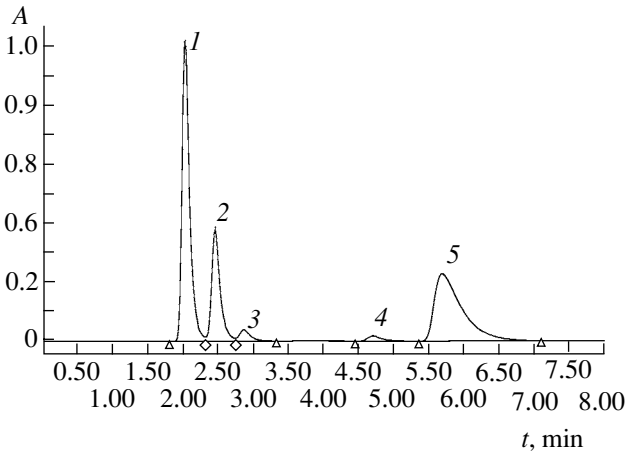

Fig. 4. Chromatogram of a solution of a reference sample for the analysis of Pentalgin tablets in the isocratic elution mode with a 0.0075 M KH_2PO_4 solution (pH 4.8) containing 5 vol % acetonitrile; the other conditions are given in the caption to Fig. 3.

Table 2. Results of the analysis of Pentalgin tablets (pilot laboratory batch, 2004) using the (a) gradient and (b) isocratic HPLC techniques ($n = 3$; $P = 0.95$)

Components	Standards according to regulations, g	$X_{av} \pm \Delta X$	s, g	ε, %
Paracetamol	0.2850–0.3150	a) 0.300 ± 0.002	0.0008	0.73
		b) 0.306 ± 0.001	0.0006	0.42
Propyphenazone	0.2375–0.2625	a) 0.258 ± 0.003	0.0010	1.01
		b) 0.253 ± 0.002	0.0009	0.87
Caffeine	0.04675–0.05325	a) 0.0507 ± 0.0004	0.00018	0.85
		b) 0.0523 ± 0.0001	0.00004	0.17
Phenobarbital	0.0090–0.0110	a) 0.0101 ± 0.0001	0.00003	0.89
		b) 0.0105 ± 0.0003	0.00013	3.23
Codeine phosphate	0.0072–0.0088	a) 0.0082 ± 0.0001	0.00003	1.12
		b) 0.0082 ± 0.0001	0.00003	1.10

analysis. The permissible values of these parameters are regulated in particular sections of USP 25 for particular medicines (commonly no lower than 1.0 and no higher than 2.0, respectively). The results of the analysis of tablets with the mobile phase containing 5 vol % acetonitrile are presented in Table 2. The sample complies with the requirements of regulations in the Quantitative Determination index. Within the permissible error in analysis, the results obtained by the gradient and isocratic techniques coincide. The peak areas of the components at replicate injections are highly reproducible (RSD ≤ 1%). Both procedures are characterized by accuracy and high reproducibility. For routine analyses, the isocratic elution mode is preferable because it is simpler in implementation.

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