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ARTICLES

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

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Abstract—Two procedures were proposed for determining analgin (dipyrone), paracetamol, caffeine, phenobarbital, and codeine in the multicomponent drug Pentalgin ICN with the use of HPLC in gradient and isocratic modes. In the gradient version, columns packed with Nucleosil 100 C18 or Nova-Pak C18 adsorbents were used; at the initial stage, an acetonitrile–water mixture was used as an eluant, and a 0.025 M KH_2PO_4 solution was introduced as a mobile phase constituent after the emergence of all peaks other than that of codeine or immediately after the emergence of the analgin peak. In the isocratic version, a Nova-Pak CN HP column was used; a 1% KH_2PO_4 solution containing 5 vol % acetonitrile was an eluant. The detector wavelength was 212 nm. The separation time was shorter than 10 min (mobile-phase flow rate of 1 mL/min). Model solutions containing all active components and additives of the tablets were analyzed, and the performance characteristics of both procedures were calculated. The procedures ensure reliable analytical results; however, the isocratic version is technically simpler and more preferable for product control in commercial manufacture.

The quantitative analysis of multicomponent preparations containing substances with strongly different chromatographic properties on reversed-phase columns with C18-type adsorbents is difficult to perform in an isocratic mode. Pentalgin ICN belongs to these preparations; analgin (dipyrone), paracetamol, caffeine, codeine phosphate, and phenobarbital are the constituents of these tablets. We experimentally found that, on 150 × 4.0 mm Nucleosil 100 C18 (Elsico) and 150 × 3.9 mm Nova-Pak C18 (Waters) columns, the capacity factor of phenobarbital was more than ten times higher than those of the other components. It is unreasonable to analyze these tablets with the use of the specified columns in an isocratic mode because of long-time separation. Moreover, reliable results cannot be obtained in determining a relatively small amount of phenobarbital (0.01 g per tablet) by a broad and low peak.

The above problem can be solved using the following two techniques:

(1) The use of gradient elution, which exhibits a number of advantages [1], considerably shortens the total analysis time, improves the separation of the entire mixture and peak shapes, decreases tails, and increases the sensitivity of determining of a number of mixture components eluted from the column after the other.

(2) Operations in an isocratic mode but with the use of chromatographic columns with more polar adsorbents of other types, for example, with grafted nitrile groups, can be performed. These adsorbents provide an opportunity to operate in a reversed-phase mode; however, they exhibit closer retention values of separated substances, as compared with C18 adsorbents.

Larionova *et al.* [2] exemplified an analysis of a multicomponent preparation in an isocratic mode. They reported a procedure for the quantitative analysis of Spazmoveralgin Neo tablets of complex composition containing bromisoval (0.250 g), paracetamol (0.150 g), caffeine (0.050 g), papaverine hydrochloride (0.030 g), phenobarbital (0.020 g), codeine phosphate (0.008 g), ephedrine hydrochloride, and atropine sulfate (the amounts of the two last-named components in a table are not specified in the publication) by reversed-phase HPLC on a column packed with an adsorbent with attached nitrile groups using acetonitrile–0.05 M phosphate buffer solution in a ratio of 15 : 85 as a mobile phase. Larionova *et al.* [2] simultaneously determined bromisoval, codeine phosphate (concentration ratio of 31 : 1), paracetamol, caffeine, phenobarbital, and papaverine hydrochloride. It is our opinion that the main disadvantages of the above procedure are related to the special features of isocratic elution and consist in the inadequate resolution of the peaks of substances eluted first from the column (seven substances were eluted over a range of capacity factors k' from 0.4 to 1.4) and in a relatively high retention of the peak of papaverine, which was eluted last ($k' = 7.0$). The inadequate separation of analyte peaks adversely affects the accuracy of the quantitative determination of the analytes.

In spite of the above disadvantages, we hypothesized that a column with an adsorbent with attached nitrile groups can also be used for the isocratic analysis of Pentalgin ICN tablets because they also contain

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paracetamol, caffeine, codeine phosphate, and phenobarbital. According to Larionova *et al.* [2], paracetamol, caffeine, codeine phosphate, and phenobarbital exhibit relatively low capacity factors and can be easily separated in an isocratic mode on a column with grafted nitrile groups. Analgin, which is a constituent of Pentalgin ICN tablets in addition to these four substances, complicates separation and quantitative determination in an isocratic mode. However, we assumed that the presence of analgin does not exclude the possibility of this analysis.

The aim of this work was to develop procedures for the HPLC analysis of Pentalgin ICN tablets in gradient and isocratic versions and to compare the characteristics of these procedures.

EXPERIMENTAL

Reagents. To prepare eluants and dissolve standard and test preparations, grade 0 high-purity acetonitrile for chromatography (Kriokhrom, Russia) and ultrapure water with a resistivity of 18.2 MΩ/cm obtained using a Direct Q system (Millipore) were used. Pharmaceutical substances, which were tested by a quality control department and suited to all of the regulatory requirements, were used as the reference standards of test drugs. All of the other chemicals used were of analytical grade or better.

Instrumentation. 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 smaller than 0.650 mL. Columns with the reversed-phase Nucleosil 100 C18 adsorbent with an average particle size of 5 μm (Elsico) or the Nova-Pak C18 adsorbent with an average particle size of 4.0 μm (Waters) were used for gradient elution, and a column with the Nova-Pak CN HP adsorbent with grafted nitrile groups with a particle size of 4.0 μm (Waters) was used for isocratic elution. The column length was 150 mm, and the internal diameter was 4.0 (Elsico) or 3.9 mm (Waters).

Preparation of solutions. To perform analysis, 20 test tablets were weighed on an analytical balance, and the average weight of a tablet in grams was found to within 0.0001 g. About 0.16 g (an accurately weighed portion) of ground tablets and 0.12 g of sodium sulfite (a stabilizer for analgin) were dissolved in 15 mL of a water–acetonitrile mixture (2 : 1) in a 100-mL volumetric flask with stirring for 10 min. The resulting solution was diluted with water to the mark. Simultaneously, a reference solution of a standard sample containing 0.12 g of sodium sulfite, 0.06 g of analgin, 0.06 g of paracetamol, 0.01 g of caffeine, 0.0020 g of phenobarbital, and 0.0016 g of codeine phosphate was prepared. All of the solutions were filtered though a hydrophilic membrane filter with a pore size of 0.45 μm (fluoroplastic filters, which are stable in aqueous acetonitrile solutions, are preferable).

Determination procedure. The test and reference solutions were chromatographed to obtain no less than three chromatograms for each solution. The peak areas of the test components were calculated, and the amounts of these components in the analyzed tablets were found from the equation

$$X = \frac{S_a m_{st} m_s}{S_{st} m_a},$$

where S_a and S_{st} are the average peak areas of an analyte in the chromatograms of the test solution and the reference solution, respectively, and m_{st}, m_s, and m_a are the weight of the analyte standard in the reference solution, the average weight of a tablet, and the weight of a sample of ground tablets taken for preparing test solutions, respectively (in grams).

In all cases, the injected sample volume and the mobile-phase flow rate were 5.0 μL and 1.0 mL/min, respectively. The analytes were detected at 212 nm. The compositions of eluants are specified in the figure captions.

RESULTS AND DISCUSSION

In preliminary experiments, we found that codeine was not eluted from Nucleosil 100 C18, Nova-Pak C18, and Nova-Pak CN HP adsorbents with CH_3CN–water mixtures; KH_2PO_4 should be added to the mobile phase for the elution of codeine. This property of codeine can be used for improving the peak separation of codeine and other components in both gradient and isocratic procedures. In accordance with this chromatographic property of codeine, in the gradient procedure on columns with Nucleosil 100 C18 and Nova-Pak C18 adsorbents, a CH_3–water region was introduced at the initial stage of a gradient, whereas a 0.025 M KH_2PO_4 solution was added to the mobile phase after the emergence of all peaks other than the peak of codeine. Moreover, a 0.025 M KH_2PO_4 solution can be introduced into the mobile phase earlier, namely, immediately after the emergence of the peak of analgin. The resulting chromatograms differed in the order of emergence of the peaks of codeine and phenobarbital: in the former case, the penultimate and ultimate peaks in a chromatogram were due to phenobarbital and codeine, respectively, and vice versa in the latter case. As can be seen in Figs. 1–3, this methodological procedure provided the complete resolution of the peaks of analgin and codeine. In this case, the peak of codeine was symmetrical and afforded better quantitative results.

In the optimization of conditions for the separation of drug components on a column with the Nova-Pak CN HP adsorbent in an isocratic mode, we used the effect of KH_2PO_4 for better separation of the peaks of an analgin degradation product and codeine. In this case, we found that, as the concentration of KH_2PO_4 in the eluant was increased, the retention of codeine

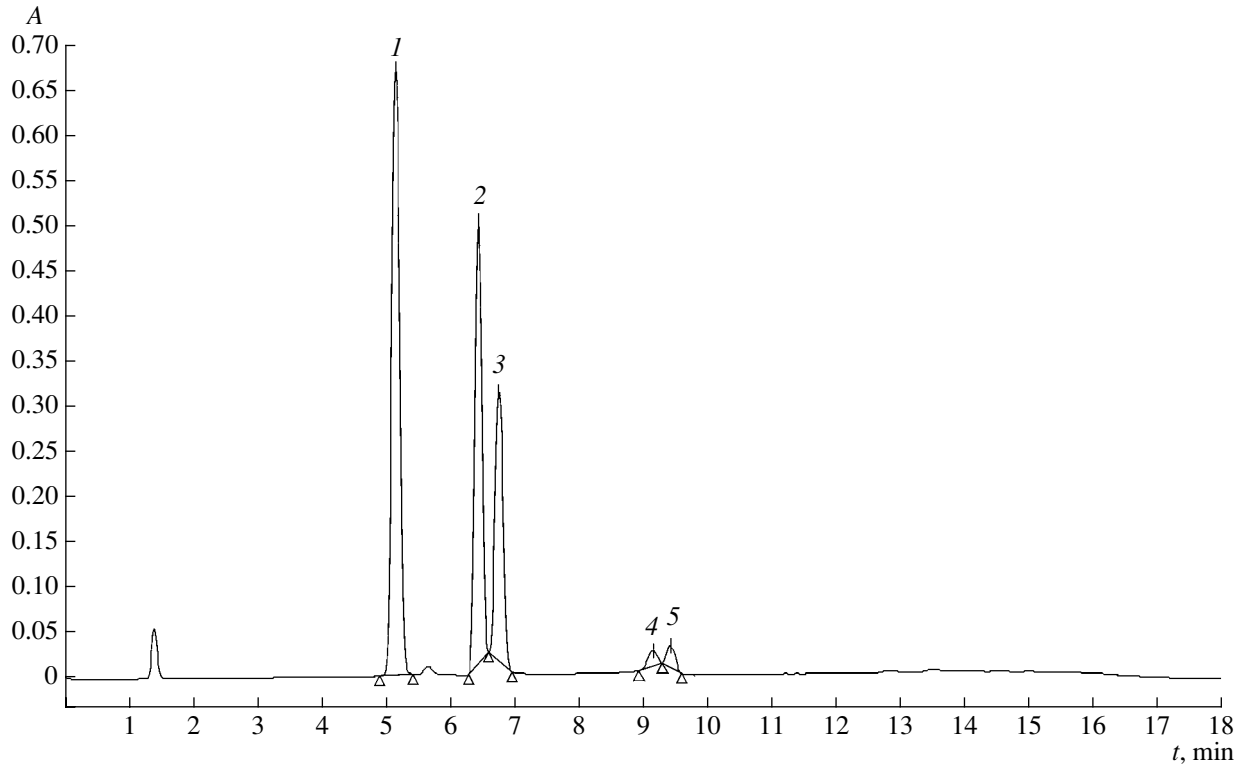

Fig. 1. Chromatogram of the test solution of Pentalgin ICN tablets with a mobile phase containing a phosphate buffer solution from the onset of analysis. Gradient: from a 0.025 M KH_2PO_4 to a CH_3CN–0.025 M KH_2PO_4 mixture (1 : 1) in 12 min. Mobile-phase flow rate: 1 mL/min. Column (150 × 4.0 mm): Nucleosil 100 C18 (5 μm). Detection: 212 nm. Peaks: (*1*) paracetamol, (*2*) analgin, (*3*) caffeine, (*4*) unidentified substance, and (*5*) phenobarbital. Codeine was eluted between the peaks of analgin and caffeine (cannot be seen in the figure).

decreased, whereas the retention of analgin increased. A comparison of chromatograms shown in Figs. 4 and 5 demonstrates that a target-oriented change in the concentration of KH_2PO_4 in the eluant allowed us to improve simultaneously the separation of the peaks of codeine and analgin degradation product, as well as analgin and paracetamol. A calculation of the resolution of neighboring peaks in accordance with the *United States Pharmacopeia*, 25th edition (USP 25) [3], demonstrated that this value was no lower than 1.3 for the critical pair caffeine–phenobarbital. The coefficient of asymmetry for the most broadened peak of codeine was no higher than 1.2. These characteristics are satisfactory for the purpose of quantitative analysis. Thus, as a rule, the permissible values of these parameters in particular items of USP 25 for specific drugs are no lower than 1.0 and no higher than 2.0, respectively. At the same time, we believe that the chromatographic column with the Nova-Pak CN HP adsorbent (4.0 μm) used in this work does not outperform a wide variety of columns of this type. We intend to purchase and test a 150 × 4.6 mm with the Zorbax SB CN adsorbent with spherical particles 3.5 μm in diameter; we hope that this column will provide even better separation.

To determine the reliability of analysis in accordance with both of the procedures, we analyzed model mixtures containing all of the active principles and additives of the drug preparation. The total number of model mixtures was 34 (17 for both of the two procedures), and the analytical range in these mixtures was ±200% of the range corresponding to regulatory requirements. Table 1 summarizes the results. According to these results, a systematic error in determining all components was found for the gradient procedure (the average relative error is greater in magnitude than the confidence interval of this error). We assumed that the error was due to the continuous operation of the instrument in a gradient mode for a long time: more than 12 h was required for the analysis of 17 solutions (51 injections). It is likely that the column should be washed successively with CH_3CN and the mobile phase at regular intervals (after 9–12 injections) in order to obtain more accurate results. In the isocratic procedure, the found concentrations of the components were closer to the added amounts. Taking into account that, in all cases, the relative error was several times smaller than the component concentration range allowed by regulatory requirements, both of the procedures can be considered adequate in terms of the reliability of results.

Table 2 summarizes the results of analysis of tablets (commercial set). These results are consistent within the limits of the permissible error of analysis. For

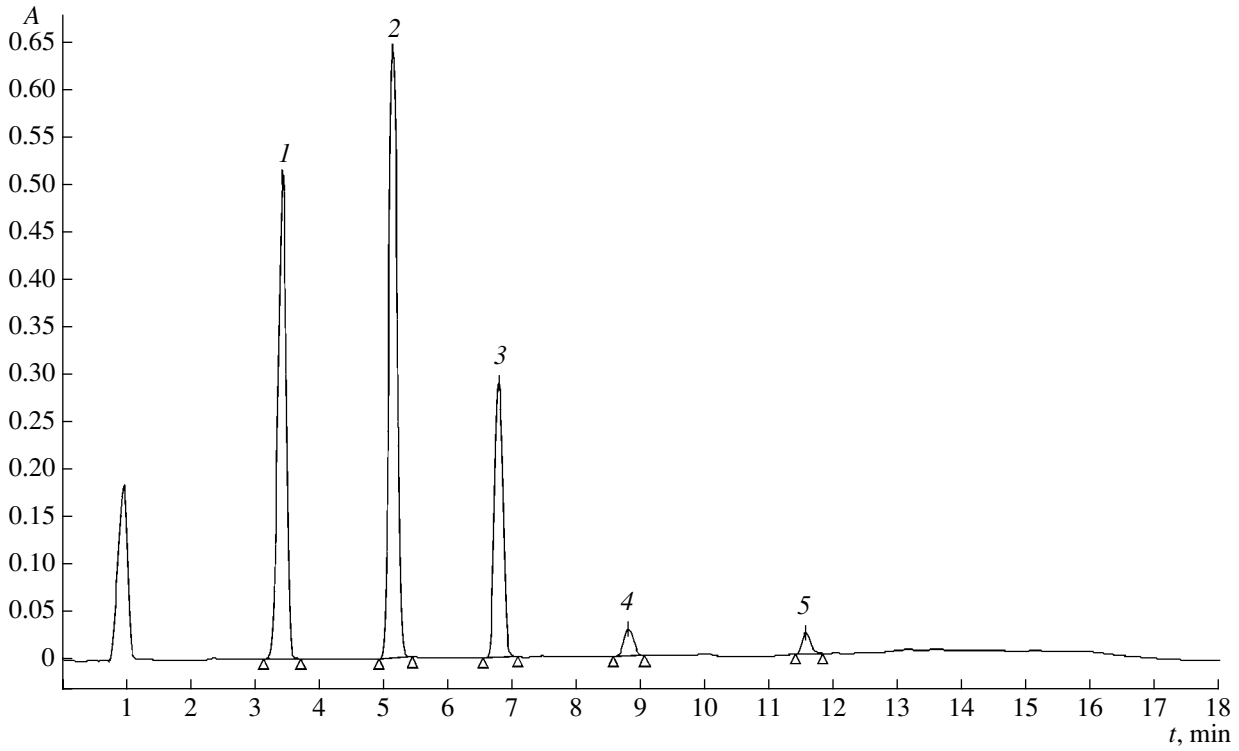

Fig. 2. Chromatogram of the test solution of Pentalgin ICN tablets with a mobile phase containing a phosphate buffer solution since the ninth minute of analysis. Gradient: from water to a CH_3CN–0.025 M KH_2PO_4 mixture (1 : 1) in 12 min. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Column (150 × 4.0 mm): Nucleosil 100 C18 (5 μm). Peaks: (*1*) analgin, (*2*) paracetamol, (*3*) caffeine, (*4*) phenobarbital, and (*5*) codeine.

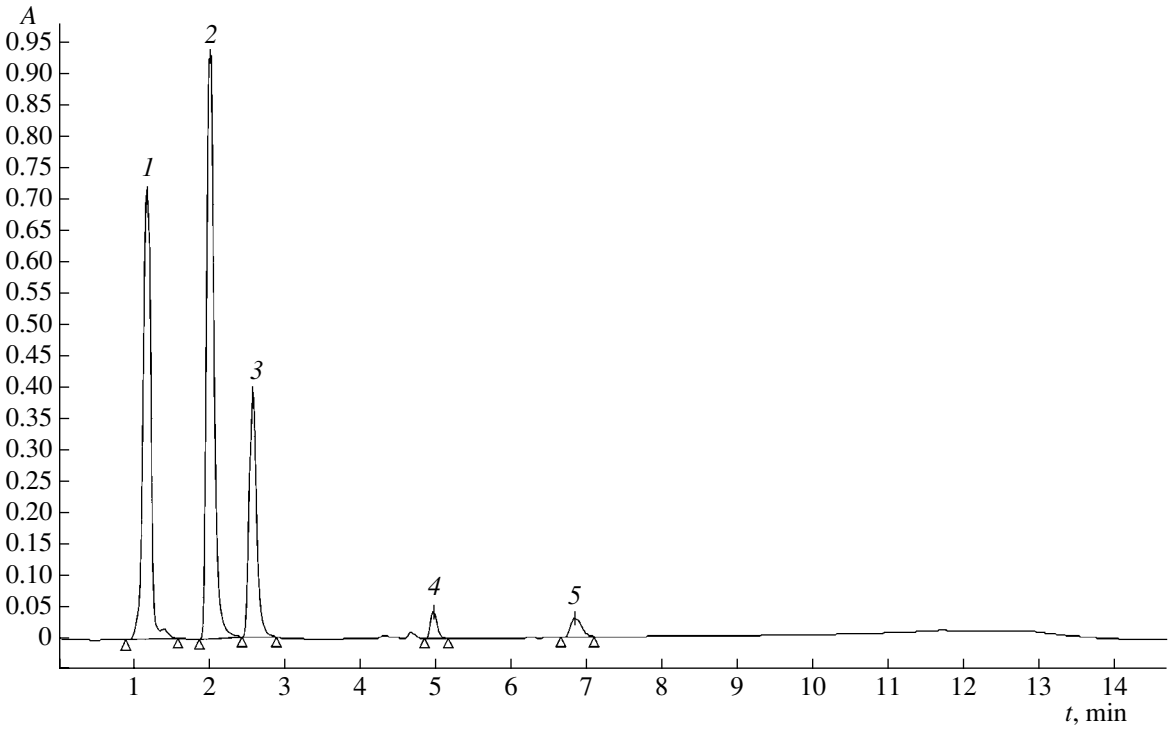

Fig. 3. Chromatogram of the test solution of Pentalgin ICN tablets with a mobile phase containing a phosphate buffer solution since the second minute of analysis. Gradient: from a CH_3CN–water mixture (15 : 85) to a CH_3CN–0.025 M KH_2PO_4 (pH 3.0; H_3PO_4) mixture (45 : 55) in 10 min. Column (150 × 3.9 mm): Nova-Pak C18 (4 μm). Peaks: (*1*) analgin, (*2*) paracetamol, (*3*) caffeine, (*4*) codeine, and (*5*) phenobarbital.

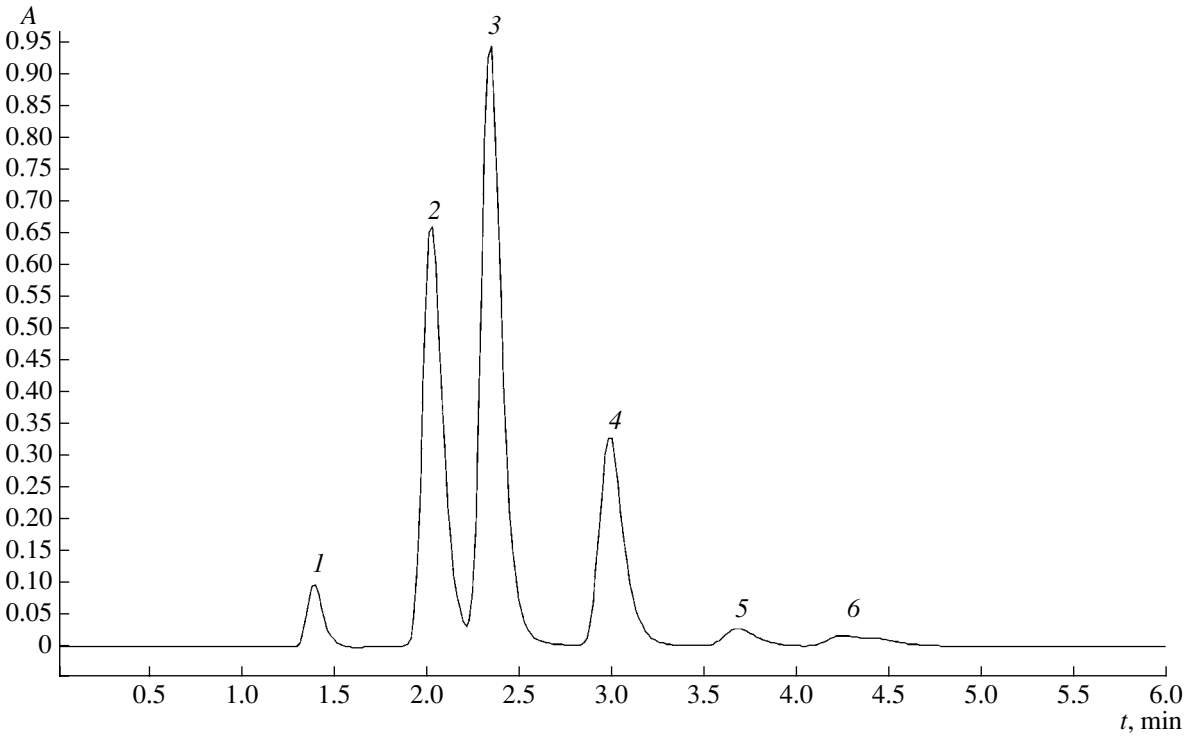

Fig. 4. Chromatogram of the test solution of tablets on a column packed with the Nova-Pak CN HP adsorbent in an isocratic mode. Mobile phase: a 0.025 M KH_2PO_4 solution containing 5 vol % CH_3CN. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Peaks: (*1*) analgin, (*2*) paracetamol, (*3*) caffeine, (*4*) phenobarbital, (*5*) codeine, and (*6*) product of analgin degradation.

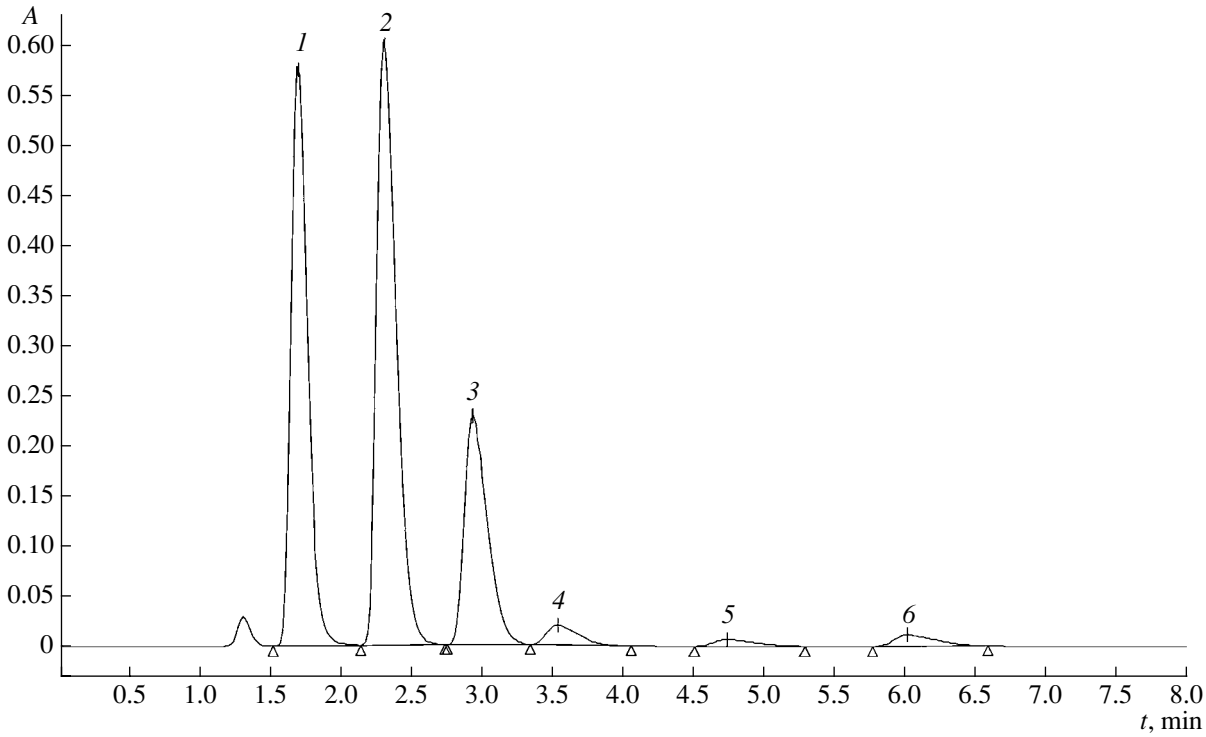

Fig. 5. Chromatogram of the test solution of tablets on a column packed with the Nova-Pak CN HP adsorbent in an isocratic mode. Mobile phase: a 0.1% KH_2PO_4 solution containing 5 vol % CH_3CN. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Peaks: (*1*) analgin, (*2*) paracetamol, (*3*) caffeine, (*4*) phenobarbital, (*5*) product of analgin degradation, and (*6*) codeine.

Table 1. Comparison of the reliabilities of the results of the HPLC analysis of Pentalgin ICN tablets in the gradient and isocratic elution modes

Component	Allowable concentration range, % of the average	Gradient elution mode			Isocratic elution mode		
		ε_a, %	$\Delta_{x\,a}$, % (n = 17, P = 0.95)	systematic error	ε_a, %	$\Delta_{x\,a}$, % (n = 17, P = 0.95)	systematic error
Analgin	±5.0	–0.905	0.669	Yes	0.113	0.192	No
Paracetamol	±5.0	–1.356	0.409	Yes	0.042	0.136	No
Caffeine	±7.5	–1.516	0.402	Yes	0.406	0.168	Yes
Codeine phosphate	±10.0	–1.847	0.465	Yes	0.171	0.462	No
Phenobarbital	±10.0	–1.988	0.420	Yes	0.273	0.160	Yes

Notes: ε_a is the average value of the relative error; $\Delta_{x\,a}$ is the confidence interval of the average value of the relative error.

Table 2. HPLC analysis of Pentalgin ICN tablets (commercial set, 2004) with (a) gradient and (b) isocratic elution modes

Component	Amount per table, g (n = 9; P = 0.95)				
	regulatory requirements	X_a	s, g	ΔX	ε, %
Analgin	0.2850–0.3150	(a) 0.2933	0.0010	0.0007	0.24
		(b) 0.2944	0.0029	0.0024	0.82
Paracetamol	0.2850–0.3150	(a) 0.2946	0.0003	0.0002	0.07
		(b) 0.2961	0.0004	0.0002	0.07
Caffeine	0.04675–0.05325	(a) 0.05043	0.00045	0.00035	0.69
		(b) 0.04965	0.00062	0.00050	1.01
Phenobarbital	0.0090–0.0110	(a) 0.00975	0.00017	0.00014	1.44
		(b) 0.01005	0.00013	0.00009	0.90
Codeine phosphate	0.0072–0.0880	(a) 0.00825	0.00032	0.00026	3.15
		(b) 0.00818	0.00006	0.00005	0.61

codeine phosphate, the results of the gradient procedure were less reproducible because of the partial interference of the peak of an analgin degradation product. This interference can occur in the long-term operation of a column because of a partial loss of column efficiency.

Thus, the procedures developed allow us to determine the quantitative composition of multicomponent preparations in a single experiment with satisfactory performance characteristics. In general, the isocratic procedure is more preferable for the given preparation, because it can be implemented on simple instruments; the time taken to obtain a chromatogram is shorter by a factor of about 2, and the consumption of acetonitrile is lower by a factor of 10.

REFERENCES

1. Hadden, N. *et al.*, *Basic Liquid Chromatography*, Walnut Creek, CA: Varian Aerograph, 1971. Translated under the title *Osnovy zhidkostnoi khromatografii*, Moscow: Mir, 1973.
2. Larionova, S.G., Dement'eva, N.N., Nechaeva, E.B., Nazarenko, P.V., and Nesterova, G.A., *Farmatsiya*, 2002, vol. 51, p. 16.
3. *The United States Pharmacopeia*, 25th ed., Rockville: United States Pharmacopeial Convention, 2001, p. 1991.