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ARTICLES

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

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Abstract—Two procedures were proposed for the quantitative analysis of the drug Pentalgin N with the use of HPLC in gradient and isocratic modes. Analgin (dipyrone), caffeine, naproxen, phenobarbital, codeine, an analgin degradation product, and sodium sulfite (added to the test solution to stabilize analgin) were separated on a column (150 × 3.9 mm) packed with Nova-Pak C18 (4.0 μm) with elution with a 0.00625 M KH_2PO_4 solution with an acetonitrile concentration gradient from 10 to 60 vol % in 10 min or on a column (150 × 3.9 mm) packed with Nova-Pak CN HP (4.0 μm) with elution with a 0.0110 M KH_2PO_4 solution (pH 5.8) containing 5 vol % acetonitrile. The wavelength of the diode-array detector was 212 nm. Model solutions containing all of the active principles and additives of the tablets were analyzed, and the performance characteristics of both procedures were calculated. Both procedures afford reliable analytical results; however, the isocratic version is technically simpler and more preferable for product control in commercial production.

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In preparing for the commercial production of the five-component drug Pentalgin N, which contains analgin (dipyrone), naproxen, caffeine, phenobarbital, and codeine, procedures for the quantitative determination of the components were developed simultaneously with the development of the production process.

Procedures for the quantitative analysis of multicomponent preparations using isocratic HPLC have been described [1, 2]. The advantages of these procedures consist in the applicability of simpler and inexpensive instruments and in less stringent requirements imposed on the quality of water and organic solvents to be used for preparing a mobile phase. At the same time, the use of gradient elution for quantitative analysis is of practical and scientific interest. In the gradient mode, substances with capacity factors differing by a factor of thousands can be efficiently separated in several minutes, peak shapes can be improved, and the sensitivity of the determination can be enhanced [3].

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

EXPERIMENTAL

Reagents. To prepare mobile phases and dissolve standard test pharmaceuticals, grade 0 high-purity acetonitrile for chromatography (Kriokhrom, Russia) and ultrapure water with a resistivity of 18.2 MΩ/cm prepared using a Direct Q system (Millipore) were used; chemically pure KH_2PO_4 was used. The pH values of the mobile phases were adjusted by adding chemically pure H_3PO_4 or a 10% aqueous solution of chemically pure KOH. Pharmaceutical substances, which were tested by a quality control department and subjected to all of the regulatory requirements, were used as drug standards. To inhibit degradation, Na_2SO_3 of analytical grade was added to the solutions of analgin, which is unstable in aqueous solutions.

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 smaller than 0.650 mL. A column packed with the reversed-phase Nova-Pak C_{18} adsorbent with an average particle size of 4.0 μm (Waters) was used for gradient elution, and a column packed with the Nova-Pak CN HP adsorbent with grafted nitrile groups with an average particle size of 4.0 μm (Waters) was used for isocratic elution. The column length was 150 mm, and the internal diameter was 3.9 mm.

Preparation of solutions. To perform analysis, 20 test tablets were weighed on an analytical balance and the average weight of a tablet was found. An accurately weighed portion (0.145 g) of powdered tablets and a weighed portion of sodium sulfite (stabilizer for analgin) (0.18 or 0.500 g for isocratic or gradient mode, respectively) were dissolved in 15 mL of a water–acetonitrile mixture (1 : 1) in a 100-mL volumetric flask with stirring for 10 min. The resulting solution was diluted with water to the mark. Simultaneously, a refer-

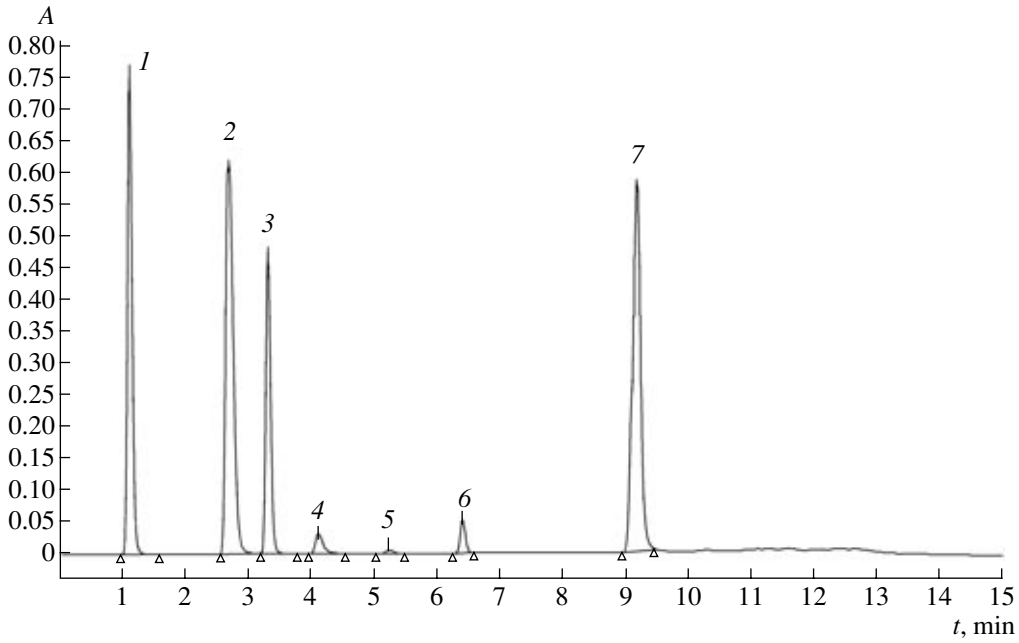

Fig. 1. Chromatogram of the reference solution of standard samples for analyzing Pentalgin N tablets on a column (150 × 3.9 mm) packed with Nova-Pak C18 (4.0 μm) in a gradient mode. The concentration of KH_2PO_4 in the eluant was 0.00625 M. Gradient: from 10 to 60 vol % acetonitrile in 10 min. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Peaks: (*1*) sodium sulfite, (*2*) analgin, (*3*) caffeine, (*4*) codeine, (*5*) product of analgin degradation, (*6*) phenobarbital, and (*7*) naproxen.

ence solution of standard samples was prepared. This solution contained Na_2SO_3 in the same amount as the test solution, 0.06 g of analgin, 0.02 g of naproxen, 0.01 g of caffeine, 0.0020 g of phenobarbital, and 0.002 g of codeine phosphate. The solutions were filtered though a hydrophilic membrane filter with a pore size of 0.45 μm (fluoroplastic filters stable in aqueous acetonitrile solutions are preferable).

Determination procedure; calculation of the results. The test solution and the reference solution were chromatographed to obtain no less than three chromatograms for each solution. The peak areas of the test components were calculated, and the amount (X) of each particular component in the analyzed tablets was found from the equation

$$X = \frac{S_t m_{st} m_a}{S_{st} m_t},$$

where S_t and S_{st} are the average peak areas of an analyte in the chromatograms of the test solution and the reference solution, respectively; m_{st}, m_a, and m_s 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 the test solution, 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 figure captions.

RESULTS AND DISCUSSION

To optimize conditions for the separation and quantitative determination of the drug components, we studied the retention of these components eluted with water–acetonitrile mobile phases. We found that codeine was not eluted from 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. The retention time depends on the salt concentration: an increase in the concentration of KH_2PO_4 decreased the retention. In contrast, the retention of analgin increased as the concentration of KH_2PO_4 in the mobile phase was increased. These chromatographic retention properties of codeine and analgin can be used for improving the peak resolution of analgin, codeine, and other components in both gradient and isocratic procedures. In the gradient procedure on a column packed with the Nova-Pak C18 adsorbent, the peaks of analgin, codeine, and caffeine were separated at a KH_2PO_4 concentration of 0.00625 M in the mobile phase (Fig. 1). The initial (10 vol %) and final (60 vol %) concentrations of CH_3CN in the mobile phase and the slope of the gradient were chosen empirically in order to reach the satisfactory resolution of a critical pair of peaks (analgin–caffeine) and an appropriate analysis time. In this mode, the peak of an analgin degradation product was completely separated from the neighboring peaks of codeine and phenobarbital. An increase in the concentration of KH_2PO_4 in the mobile phase impaired separation: at a concentration of

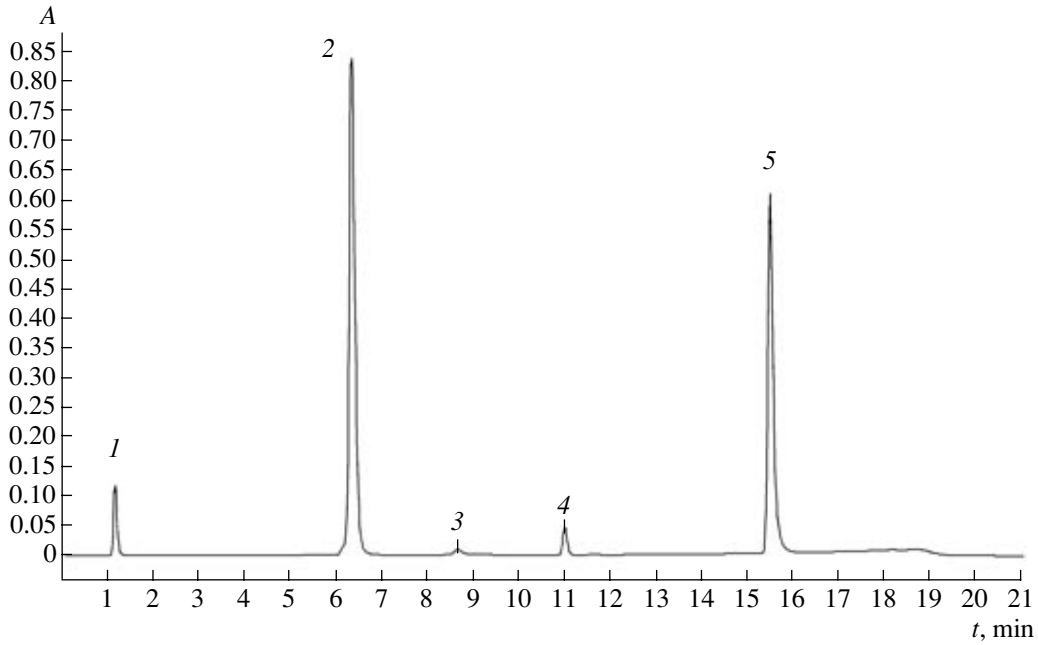

Fig. 2. Chromatogram of the reference solution of standard samples for analyzing Pentalgin N tablets on a column (150 × 3.9 mm) (4.0 μm) in a gradient mode. Concentration gradient: from 0.025 M KH_2PO_4 to a CH_3CN–0.025 M KH_2PO_4 mixture (1 : 1) in 16 min. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Peaks: (*1*) sodium sulfite, (*2*) analgin + caffeine + codeine, (*3*) product of analgin degradation, (*4*) phenobarbital, and (*5*) naproxen.

0.025 M, analgin, caffeine, and phenobarbital were eluted simultaneously, even though the slope of the gradient decreased (Fig. 2).

When optimizing the conditions for the separation of drug components on a column packed with the Nova-Pak CN HP adsorbent in an isocratic mode, we found that the concentration of KH_2PO_4 in the mobile phase also affected the retention of naproxen: the retention increased with concentration. The pH of the mobile phase had an even greater effect: an increase in pH decreased the retention of naproxen. Figure 3 shows a chromatogram obtained with the use of 0.0074 M KH_2PO_4 (pH 4.8). In this case, column efficiency with respect to the naproxen peak was low, and the results were inapplicable to the quantitative determination of this component. An optimum resolution of neighboring peaks and satisfactory peak shapes were obtained with the use of 0.0110 M KH_2PO_4 (pH 5.8) (Fig. 4). In this case, the peak of the analgin degradation product was also completely separated (eluted at the tail of the peak of naproxen). A calculation of the resolution of neighboring peaks in accordance with the United States Pharmacopeia, 25th edition (USP 25) [4], demonstrated that this value was no lower than 1.3 for the critical pair naproxen–analgin degradation product. The asymmetry coefficient for the most broadened peak of codeine was no higher than 1.7. These characteristics are satisfactory for the purpose of quantitative analysis. Thus, the permissible values of these parameters are regulated in particular items of USP 25 for specific drugs (as a rule, they are no lower than 1.0 and no higher than 2.0, respectively).

To stabilize analgin, which is unstable in an aqueous acetonitrile solution, Na_2SO_3 was added. In preliminary experiments, we found that the addition of Na_2SO_3 (1.2–1.8 mg/mL) decreased the rate of analgin degradation to 2% of the initial concentration in 40 min, so that four reproducible parallel chromatograms of a single solution could be obtained using an isocratic procedure (the duration of a single separation was 9 min). In this case, a further increase in the concentration of Na_2SO_3 is undesirable, because it impairs the peak resolution of phenobarbital and naproxen. The mechanism of this phenomenon is unclear and should be studied in more detail. In the reproduction of a gradient procedure, in which peak resolution is not a limiting factor, the concentration of Na_2SO_3 can be increased to 5.0 mg/mL, at which the peak of analgin decreased by 2% in 2 h. Thus, in this case, not only can three or four reproducible chromatograms be obtained for quantitative analysis (the duration of a single separation run was 15 min), but other studies can be performed (for example, the determination of the reproducibility of peak areas for testing the applicability of a chromatographic system).

To determine the reliability of analysis in both 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 either of the two procedures), and the analytical range in these mixtures was ±200% of the range speci-

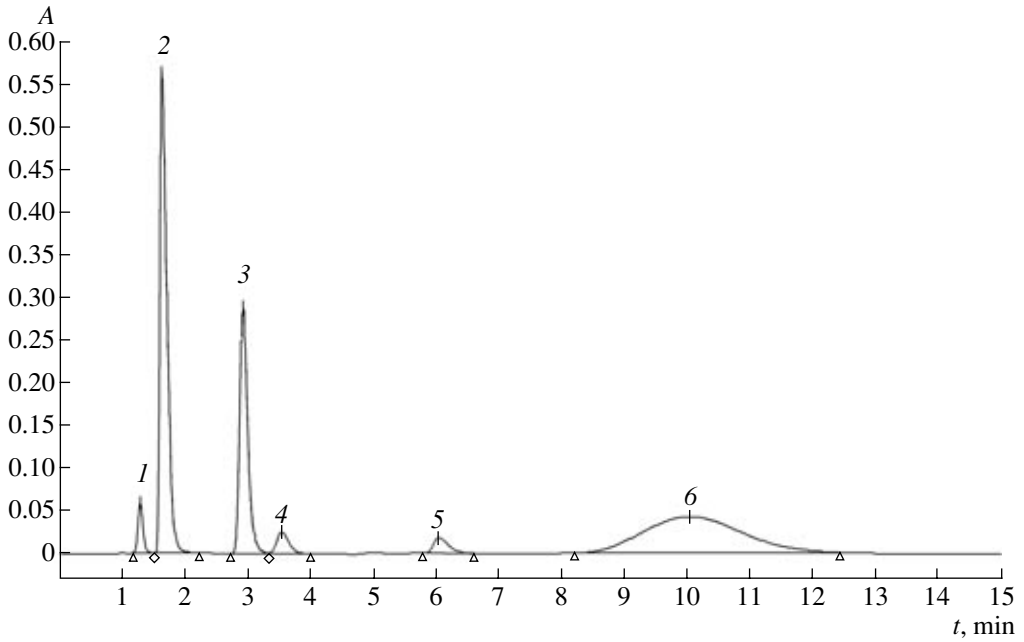

Fig. 3. Chromatogram of the reference solution of standard samples for analyzing Pentalgin N tablets on a column (150 × 3.9 mm) packed with Nova-Pak CN HP (4.0 μm) with elution with a 0.0074 M KH_2PO_4 solution (pH 4.8) containing 5 vol % acetonitrile. Mobile-phase flow rate: 1 mL/min. Peaks: (*1*) sodium sulfite, (*2*) analgin, (*3*) caffeine, (*4*) phenobarbital, (*5*) codeine, and (*6*) naproxen.

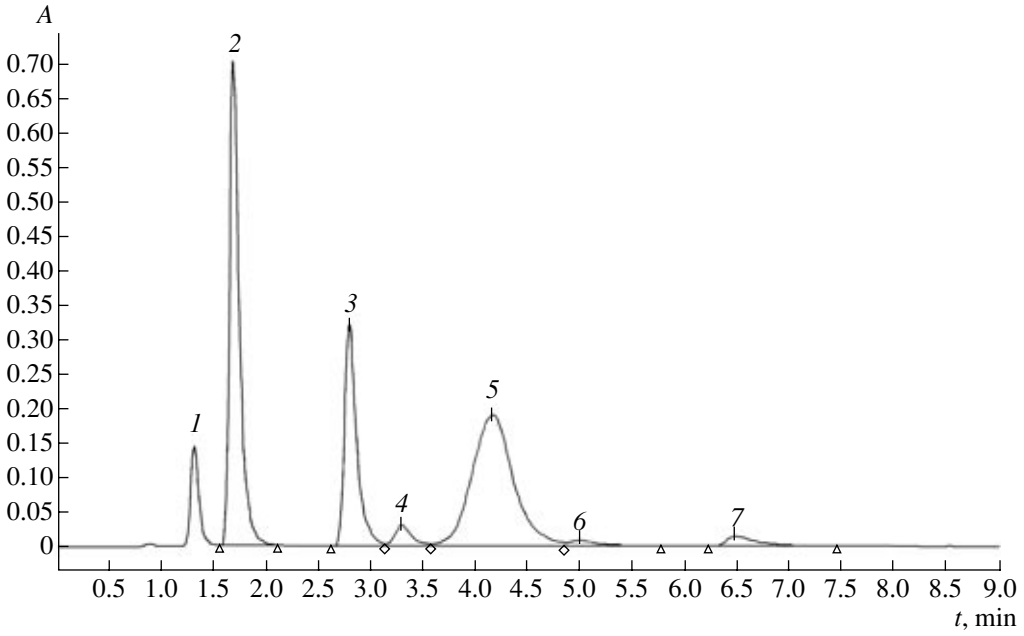

Fig. 4. Chromatogram of the reference solution of standard samples for analyzing Pentalgin N tablets on a column (150 × 3.9 mm) packed with Nova-Pak CN HP (4.0 μm) with elution with a 0.011 M KH_2PO_4 solution (pH 5.8) containing 5 vol % acetonitrile. Mobile-phase flow rate: 1 mL/min. Detection: 212 nm. Peaks: (*1*) sodium sulfite, (*2*) analgin, (*3*) caffeine, (*4*) phenobarbital, (*5*) naproxen, (*6*) product of analgin degradation, and (*7*) codeine.

fied by the regulatory requirements. Table 1 summarizes the results. According to these results, the relative error in all cases was several times smaller than the component concentration range allowed by the regulatory requirements. The systematic error found for some components was insignificant because the average value of the relative error was only slightly higher than the confidence interval of this error in these cases.

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

Component	Allowable concentration range, % of the average	Procedure					
		gradient			isocratic		
		e_{av}, %	$\Delta_{x\,av}$, % ($n = 51$; $P = 0.95$)	systematic error	e_{av}, %	$\Delta_{x\,av}$, % ($n = 51$; $P = 0.95$)	systematic error
Analgin	±5.0	0.092	0.187	No	–0.022	0.192	No
Naproxen	±5.0	–0.271	0.196	Yes	–0.202	0.171	Yes
Caffeine	±7.5	–0.114	0.252	No	–0.213	0.168	Yes
Codeine	±10.0	–0.373	0.258	Yes	–0.231	0.325	No
Phenobarbital	±10.0	–0.087	0.225	No	0.390	0.202	Yes

Note: e_{av} is the average value of the relative error; $\Delta_{x\,av}$ is the confidence interval of the average value of the relative error.

Table 2. Results of the HPLC analysis of Pentalgin N tablets (experimental laboratory work, 2003) with the use of (a) gradient and (b) isocratic elution modes ($n = 3$; $P = 0.95$)

Component	Standard according to regulatory requirements, g	X_{av}, g	s, g	ΔX, g	ε, %
Analgin	0.2850–0.3150	a) 0.2931	0.0018	0.0043	1.47
		b) 0.2902	0.0031	0.0077	2.65
Naproxen	0.0925–0.1075	a) 0.0999	0.0004	0.0009	0.90
		b) 0.0985	0.0004	0.0009	0.91
Caffeine	0.04675–0.05325	a) 0.05113	0.00022	0.00056	1.10
		b) 0.05080	0.00018	0.00043	0.85
Phenobarbital	0.0090–0.0110	a) 0.01004	0.00004	0.00009	0.90
		b) 0.00985	0.00004	0.00009	0.91
Codeine	0.0072–0.0088	a) 0.00810	0.00004	0.00009	1.11
		b) 0.00802	0.00009	0.00021	2.62

Thus, both procedures can be considered adequate in terms of the reliability of the results.

Table 2 summarizes the results of analyzing tablets (experimental laboratory work). The sample meets the regulatory requirements in terms of quantitative determination. The results obtained by the gradient and isocratic procedures are consistent within the limits of allowable analytical error.

Thus, both of the developed procedures allowed us to determine the quantitative composition of the multicomponent preparation Pentalgin N in a single step with satisfactory performance characteristics.

REFERENCES

1. Vergeichik, E.N. and Onegova, N.S., *Farmatsiya,* 2001, vol. 50, p. 24.
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. Schoenmakers, P.J., *Optimization of Chromatographic Selectivity*, Amsterdam: Elsevier, 1986. Translated under the title *Optimizatsiya selektivnosti v khromatografii*, Moscow: Mir, 1989.
4. *The United States Pharmacopeia*, 25th ed., Rockville: U. S. Pharmacopeial Convention, 2001, p. 1991.