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ARTICLES

Effect of Column Loading on the Accuracy of Analyzing Pentalgin N Tablets by Gradient High-Performance Liquid Chromatography

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Abstract—Two approaches for the high-performance liquid chromatographic analysis of multicomponent medical preparations were experimentally studied by an example of Pentalgin N tablets. One of them was the simultaneous determination of all components of the preparation; another was the independent analysis of concentrated and diluted solutions for determining the presentpresent in small and large amounts, respectively. The performance characteristics of the studied procedures were determined in the analysis of model solutions containing all active and adjuvant substances of the tablets. In the simultaneous determination of all components, the relative error was smaller because of the more sustained performance of the chromatographic column in the absence of its overload with the sample observed in the independent analysis.

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The concentration of active substances in multicomponent medical preparations varies in dependence of their pharmacological properties. This is important in the analysis of such preparations by high-performance liquid chromatography (HPLC). If the concentration of components and, consequently, the peak sizes in chromatograms differ greatly from each other, an increase in the detection linearity range and in the negative effect of the background noise and secondary peaks (for example, systematic peaks in gradient elution) is observed. The accuracy of the calculations of the peak areas and the analysis results reduces in both cases. Therefore, a number of scientists propose to determine independently components containing in the preparation in different concentrations. For example, a procedure was proposed for the analysis of Caffetin tablets containing paracetamol, propyphenazone, caffeine, and codeine phosphate [1]. The concentration of codeine phosphate was 21–25 times less than the concentration of propyphenazone and paracetamol, and the analysis was performed in a two-stage procedure. Paracetamol, propyphenazone, and caffeine were determined at first stage, and codeine phosphate was determined at the second stage after its extraction preconcentration with chloroform. According to the data reported by the authors, the recovery of codeine from tablets was 89–93%. Multicomponent preparations can be analyzed using a one-stage procedure and with more accurate results. For example, in the analysis of Pentalgin ICN tablets (the concentration of paracetamol and analgin is 37.5 times higher than the concentration of codeine phosphate) by gradient HPLC, the average relative error of the determination of codeine phosphate was less than 2.0%, while with the use of the isocratic mode of the procedure, the error was 0.2% [2]. For Pentalgin N tablets with the same difference in the analgin and codeine concentrations, the corresponding values were 0.4 and 0.2% [3].

There are no data in the literature on the comparison of performance characteristics of the simultaneous and independent determination of components of the same preparation. Such a study is of scientific and practical interest and is a goal of this work.

EXPERIMENTAL

Reagents. To prepare the mobile phase and dissolve standard and analyzed medical preparations, we used acetonitrile for gradient chromatography (Merck, Germany) and ultrapure water with a resistivity of 18.2 MΩ/cm obtained using a Direct Q system (Millipore). Pharmaceutical substances, which were tested by the quality control department of a producer and suited to all the regulatory requirements, were used as the reference samples of the tested drugs. All other used reagents were of analytical grade or better.

Apparatus. We used a 2695 Waters Alliance chromatograph with a 2996 Waters diode-array detector. The certified dead volume of the chromatograph was less than 0.650 mL. A 3.9 × 150 mm separation column was used with a 3.9 × 20 mm precolumn; both were

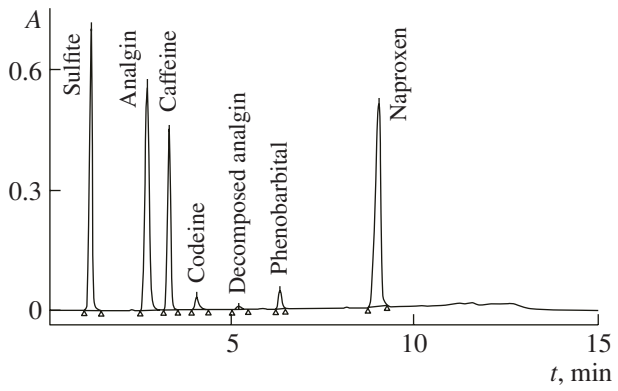


Fig. 1. Chromatograms of the test solution obtained by the procedure using a single dilution of the test solution.

packed with a reversed-phase Nova-Pak C18 adsorbent with a particle size of 4.0 μm (Waters).

Preparation of solutions. For a single-stage analysis, about 0.145 g (accurately weighed portion) of powdered tablets and about 0.500 g of Na_2SO_3 were mixed in a 100-mL volumetric flask with 15 mL of the mixture of CH_3CN–water (1 : 2) for 5 min. The obtained solution was diluted up to the mark with water and mixed. Alongside with that, a reference solution of standard samples was prepared, each 100 mL of which contained 0.500 g of Na_2SO_3 and about 0.060 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 (accurately weighed portions).

In the analysis using two dilutions, about 0.365 g (accurately weighed portion) of powdered tablets and about 0.200 g of Na_2SO_3 were mixed in a 100-mL volumetric flask with 15 mL of the mixture of CH_3CN–water (1 : 2) for 5 min. The obtained solution was diluted up to the mark with water and mixed (solution A for the determination of caffeine, codeine phosphate, and phenobarbital). Ten milliliters of solution A was placed in a 50-mL volumetric flask with 0.200 g of Na_2SO_3, where 2.0 mL of CH_3CN was added. The mixture was diluted up to the mark with water and mixed (solution B for the determination of analgin and naproxen). To prepare a reference solution of standard samples, 0.4 g of Na_2SO_3 and about 0.06 g of analgin, 0.02 g of naproxen, 0.05 g of caffeine, 0.002 g of phenobarbital, and 0.002 g of codeine phosphate (accurately weighed portions) were dissolved in the mixture of CH_3CN–water (1 : 19) in a 200-mL volumetric flask. All the solutions were filtered through a hydrophilic membrane fluoroplastic filter with a pore size of 0.45 μm, which is stable in water–acetonitrile solutions.

To estimate the performance characteristics of the procedures, model solutions of all the active substances in the range of ±20% of their average concentrations using accurately weighed portions and the solution of all the adjuvant substances (placebo) were prepared. The model solutions were prepared in 100-mL volumetric flasks in a CH_3CN–water (1 : 19) mixture, adding the corresponding amount of Na_2SO_3 in each solution.

Determination procedure and calculation of results. The test solutions and the reference solutions of standard samples were successively analyzed. The concentration of each analyte in the analyzed tablets was found using their peak areas according to the formula

$$X = (S_1 \times a_0 \times b)/(S_0 \times a_1),$$

where S_1 and S_0 were the average peak areas of the analyte in the chromatograms of the test solution and the reference solutions of standard samples, respectively, and a_0, b, and a_1 were the weight of the pharmaceutical standard of the analyte in the reference solution, the average weight of a tablet, and a weight of the portion of powdered tablets used to prepare the test solution (in grams), respectively.

In the simultaneous determination of the components, the injected volume was 5.0 μL, while in the two-dilution procedure, it was 20.0 μL (after the second dilution). In the former case, the analytes were detected at 212 nm; in the latter case, at 220 (caffeine, codeine, and phenobarbital) and 245 nm (analgin and naproxen).

The analysis was performed in a gradient mode with the mobile phase flow rate of 1.0 mL/min according to the following mode [the eluents were the mixtures of CH_3CN, 0.025 M KH_2PO_4 solution, and water in the ratio of (eluant A) 10 : 25 : 65 (vol) and (eluant B) 60 : 25 : 15 (vol)]:

Time, min	Eluent A, %	Eluent B, %
0	100.0	0.0
10	0	100.0
11	0	100.0
12	100.0	0.0
16	100.0	0.0

RESULTS AND DISCUSSION

The obtained chromatograms are presented in Figs. 1 and 2. The characteristic of the chromatograms obtained by the procedure with two dilutions is that the peak of naproxen has a flatter ascending limb than the descending one, that is, the asymmetry factor $T < 1$. The asymmetry factor T, or tailing factor according to the United States Pharmacopeia [4], characterizes the peak shape. It is calculated by formula

$$T = 2f/W,$$

where f is the distance from the ascending limb of the peak to the perpendicular line dropped from the peak maximum to the base line measured at 1/20 of the peak

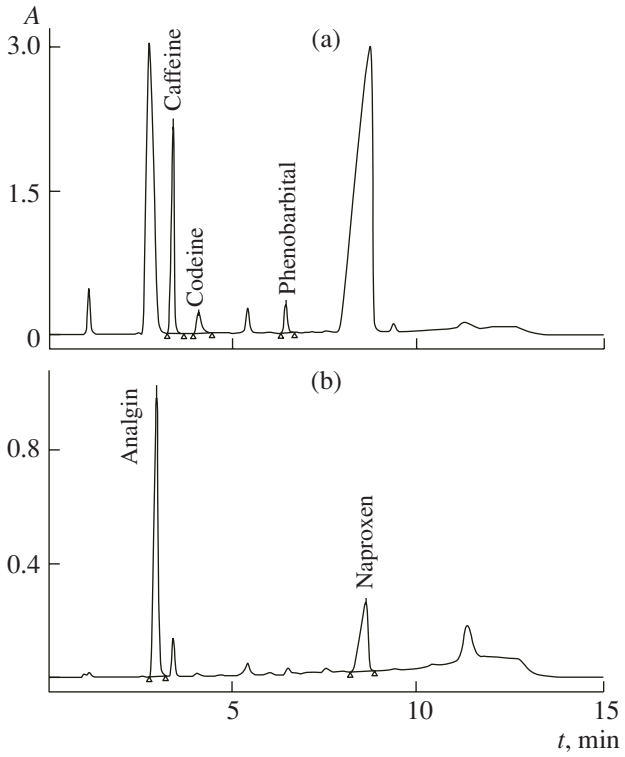
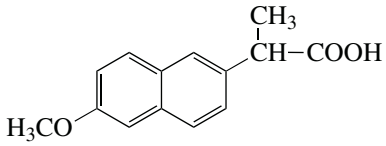


Fig. 2. Chromatograms of (a) the conc. solution (determination of codeine, caffeine, and phenobarbital) and (b) the diluted solution (determination of analgin and naproxen) obtained by the procedure using a twofold dilution of the test solution.

height and W is the peak width between the tangent lines to the ascending and descending limbs of the peak measured at the base line.

The shape of a chromatographic peak is connected to the mechanism of mass-transfer processes in the adsorbent layer and with the loading of the column, that is, a mass injected during a single analysis of a sample. In reversed-phase chromatography, the tail in the descending limb ($T > 1$) is characteristic for basic substances. The tail is more pronounced for the adsorbents with high residual silanol activity, and it arises with an increase in the loading of the column.

Naproxen is an acid ($pK_a = 4.8$ [5]). To provide the optimal retention characteristics in the analysis of the five-component preparation, pH of the mobile phase used in this procedure was maintained close to that value.



The combination of the data on the peak shape of naproxen with the data obtained earlier [5] allows the conclusion that peaks with $T < 1$ are characteristic for

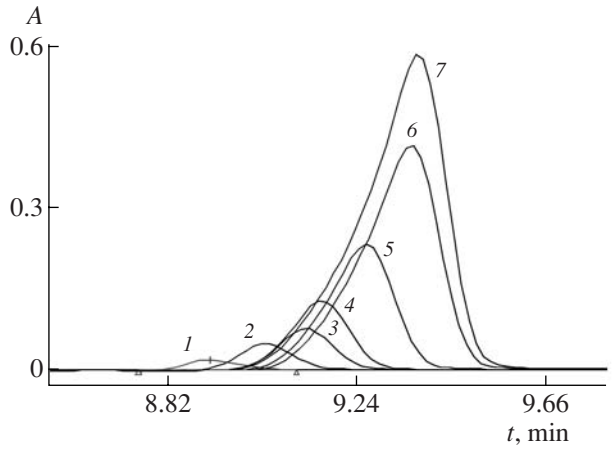


Fig. 3. Dependence of the peak shape of naproxen on the sample weight injected in the column, μg: (*1*) 0.2, (*2*) 0.4, (*3*) 0.6, (*4*) 1.0, (*5*) 2.0, (*6*) 4.0, and (*7*) 6.0.

naproxen; the T value decreasing with dissociation and with an increase in the column load. From the comparison of Figs. 1 and 3 follows that in the procedure with two dilutions, the peak of naproxen in the chromatogram of the diluted solution is also more asymmetric, which is due to that the column load is 10 times higher in the analysis of the concentrated solution and 2 times higher for the diluted solution (10 and 2 μg, respectively). To confirm this fact, a 0.2 mg/mL naproxen solution in a 1 : 1 mixture of CH_3CN and water was analyzed. The column load (m) was varied by changing the volume of injected sample. The obtained results are presented in Fig. 2 and Table 1. To simplify the calculations, the f and W parameters were measured at the base line, which resulted in the T_{cond} value that also characterized the peak tail. It is seen that at $m \le 1$ μg, the peak of naproxen was almost symmetric and the T_{cond} was close to 1. With a higher column load, the ascending limb of the peak became flatter and T_{cond} decreased; therefore, the column overloading could be judged by the shape of the peak of naproxen. Moreover, considerable changes in the symmetry of the peak, observed in the load range of 1–2 μg, indicated the change in the adsorption mechanism, that is, the overloading of the adsorption layer, which was also confirmed by the increase in the retention time of naproxen (Table 2). It can be assumed that the overload of the adsorbent negatively affects the stability of the chromatographic process and, therefore, the reproducibility of the peak area and the analysis, which was confirmed by the results of the analysis of model solutions.

For each procedure, 17 model solutions were prepared containing all the active and adjuvant substances of the tablets; for each solution, three chromatograms were obtained. The results are presented in Table 3. The performance characteristics of the procedure with two dilutions of the test solution were considerably worse.

Table 1. Loading of the chromatographic column in the procedure of simultaneous determination and with two dilutions of the test solution

Components	Amount per tablet, mg	Amount in the injected sample, μg		
		twofold dilution		simultaneous determination
		conc. solution	diluted solution	
Analgin	300	30	6	3
Naproxen	100	10	2	1
Caffeine	50	5	1	0.5
Codeine phosphate	10	1	0.2	0.1
Phenobarbital	10	1	0.2	0.1
Total	470	47	9.4	4.7
Recommended load, μg			6.0	

Table 2. Dependence of the peak area and shape and the retention time of naproxen on the volume of sample injected in the column

V_{inj}, μL	S, mV s	t_r, min	m, μg	T_{cond}
1	147278	8.913	0.2	1.107
2	406308	9.034	0.4	1.050
3	654776	9.125	0.6	1.034
5	1180503	9.160	1.0	0.971
10	2462257	9.258	2.0	0.919
20	5031770	9.357	4.0	0.869
30	7609091	9.372	6.0	0.828

Table 3. Comparative analysis of the reliability of the results for Pentalgin N tablets obtained by the procedure using the simultaneous determination and the procedure with two dilutions of the test solution ($n = 51$, $P = 0.95$)

Components	Range of acceptable concentrations according to regulatory requirements, % of average	Procedure					
		simultaneous determination			twofold dilution		
		$e_{r\,av}$,%	$e_{r\,max}$,%	Δe_r, %	$e_{r\,av}$,%	$e_{r\,max}$,%	Δe_r, %
Analgin	±5.0	0.092	–1.64	0.187	0.420	2.69	0.257
Naproxen	±5.0	–0.271	1.83	0.196	–1.059	–5.63	0.587
Caffeine	±7.5	–0.114	–2.11	0.252	–3.484	–7.99	0.726
Codeine	±10.0	–0.373	–2.10	0.258	–2.822	–7.07	0.707
Phenobarbital	±10.0	–0.087	1.95	0.225	–4.006	–7.32	0.698

Note: e_r is a relative error.

In this case, the average value of relative error ($e_{r\,av}$ exceeded the confidence range of this value Δe_r for all the components; that is, the systematic error was observed. In all cases, the error was comparable to the concentration ranges acceptable according to the regulatory requirements, while the maximum values of the relative error for naproxen and caffeine exceeded these ranges. Therefore, the procedure with two dilutions of the test solution cannot be recommended for the serial analysis of the preparation, because with the found values of the relative error, the reliability of the results cannot be assured. In the simultaneous determination, the relative errors for all the components were several times lower than the ranges of their acceptable concentrations.

In the procedure with two dilutions of the test solution, the peak areas for codeine and phenobarbital were an order of magnitude large than that for the procedure

with the simultaneous determination. However, the accuracy of their determination in the latter case was considerably higher. Therefore, it is unreasonable to increase the sample injected in the column. The overload of the column in the serial analysis negatively affects the reliability of the determination results of all components of the preparation including those contained in relatively small amounts.

REFERENCES

1. Vergeichik, E.N. and Onegova, N.S., *Farmatsiya*, 2001, vol. 50, p. 24.
2. Golubitskii, G.B., Budko, E.V., and Ivanov, V.M., *Zh. Anal. Khim.*, 2005, vol. 60, no. 10, p. 1080 [*J. Anal. Chem.* (Engl. Transl.), vol. 60, no. 10, p. 961].
3. Golubitskii, G.B., Budko, E.V., and Ivanov, V.M., *Zh. Anal. Khim.*, 2006, vol. 61, no. 1, p. 74 [*J. Anal. Chem.* (Engl. Transl.), vol. 61, no. 1, p. 61].
4. *The United States Pharmacopeia,* Rockville: United States Pharmacopeia and Convention, 1994, 23th ed.
5. Golubitskii, G.B., Budko, E.V., and Ivanov, V.M., *Zh. Anal. Khim.,* 2005, vol. 60, no. 12, p. 1267 [*J. Anal. Chem.* (Engl. Transl.), vol. 60, no. 12, p. 1129].