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ANALYSIS

DETERMINATION OF DICLOFENAC SODIUM AND PAPAVERINE HYDROCHLORIDE IN TABLETS BY HPLC METHOD

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Abstract: A HPLC method for simultaneous determination of diclofenac sodium and papaverine hydrochloride in tablets was developed and validated. The determination was performed with a Zorbax SB – C18 column, mobile phase: methanol – water (60:40, v/v), flow rate: 1 mL· min^{-1} and UV detection at 278 nm.

Keywords: diclofenac sodium, papaverine hydrochloride, tablets, HPLC determination, method validation

Diclofenac sodium belongs to a group of nonsteroidal antiflammatory agents and papaverine hydrochloride is used as a spasmolytic drug. The preparations consisting of both drugs offer the advantage of parallel activity and in consequence may cause a greater analgetic effect. In this case, the composed tablets have been prepared and reported to the patent (1). In consequence, simple and rapid simultaneous determination of these active agents and auxiliary substances in tablets mass has been needed.

In the literature there are many HPLC methods for determination of diclofenac sodium (2-6) in which different columns are used, such as C_{18}, an Inertil ODS-3, Shim-pack CLC-C_8, Zorbax C_8 column, Macherey – Nagel Nitrile columns and as the mobile phases are used for example: methanol – water – acetic acid (80 : 20 : 0.5); 0.1 M ammonium acetate buffer – methanol (15 : 85, v/v); acetonitrile – methanol – pH 3.0 triethylamine buffer (22: 36 : 42); acetonitrile: 50 mM disodium hydrogen orthophosphate (pH adjusted to 3.5 with orthophosphoric acid; water – methanol (85 : 15, v/v) containing 0.01 M each of sodium acetate and glacial acetic acid and pH adjusted to 4.6.

For determination of papaverine hydrochloride also HPLC techniques are described (7-11) in which as the stationary phase C_{18} column or ODS column were applied, whereas acetonitrile – 0.02 mol/L sodium dihydrogen phosphate (0.2% triethylamine, phosphoric acid, at pH 3) (25:75); water – methanol – acetonitrile solvent system containing sodium lauryl sulfate ion – pair reagent; 0.02 M potassium dihydrogen phosphate, pH 3.5 acetonitrile (55 : 45); acetonitrile – 5 mM aqueous heptane sulfonic acid sodium salt (50 : 50, v/v) and adjusted to apparent pH 4 using acetic acid; methanol: 25 mmol/L potassium dihydrophosphate (90 : 10) as the mobile phases were reported.

In the literature there is no information about simultaneous determination of diclofenac sodium and papaverine hydrochloride by HPLC technique, therefore, in this paper a new method for an assay of both these substances in tablets with addition of different excipients was proposed and validated (12, 13).

EXPERIMENTAL

Materials

Diclofenac sodium (D) was produced by Caesar and Loretz, GmbH, Hilden, Germany, papaverine hydrochloride (P) was purchased from Galfarm PPH, Cefarm Lublin, Poland, polyvinylpyrrolidone (PVP), mannitol (M), potato starch (PS), hydroxypropylmethylcellulose (HPMC), microcrystaline cellulose (MC), magnesium stearate (MS), trisodium citrate dihydrate, citric acid monohydrate, methanol and water were the products of Merck, Germany. All other reagents used were of analytical grade (pure for analysis).

One tablet (T1) was consisted of 50 mg D, 20 mg P, 70 mg PVP, 70 mg M, 90 mg PS and another tablet (T2) comprised of 50 mg D, 20 mg P, 70 mg PVP, 112 mg M, 30 mg HPMC, 15 mg MC, 3 mg MS.

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Methods

Chromatography

The HPLC chromatograph Series 200 was produced by Perkin Elmer, USA and equipped with autosampler, pump, UV/VIS detector, vacuum degasser and chromatography interface 600 series LINK. Zorbax SB – C 18 column 150 × 4.6 mm was used. The detection wavelength was at 278 nm. The mobile phase consisted of methanol – water (60 : 40, v/v). The flow rate was 1 mL·min^{-1}.

Procedures

Preparation of the calibration curve for diclofenac sodium and papaverine hydrochloride.

Accurately weighed 100 mg of D and 100 mg of P were transferred into a 50 mL volumetric flask using 30 mL of methanol. After dissolving the substances the flask was completed to volume with methanol. Accurate volumes of this solutions were mixed with methanol to obtain seven solutions to final concentrations of active substances in the ranges from 0.05 to 0.6 mg/mL. These solutions were mixed with citric buffer at pH 6.5 at the ratio 1 : 1 (v/v) and filtered through the Sartorius (Germany) membrane filters with the size of pore 0.20 µm. Next, 10 µL portion of the solution was injected on the column by autosampler and chromatogram was developed for a period of 15 min. One of the chromatograms for each substance is presented in Figure 1.

The areas of the peaks as the function of the amount of D and P injected on the column are presented in Figure 2. These data enabled to evaluate the ratios f_D and f_P for calculated amount of the injected substances M_D and M_P from the following expressions:

$$M_D = f_D \cdot A\ (\mu g), \text{ and } M_P = f_P \cdot A\ (\mu g),$$

where $f_D = 2.05245 \cdot 10^{-7}$ and $f_P = 4.45326 \cdot 10^{-7}$ are the ratios calculated from the quotient of standard amount of the substances injected on the column and the area peaks obtained from chromatograms for calibration curve, A is the area peak (µV·s).

The accuracy and the linearity of the method was determined for 4 concentrations of the active substances in the range 50% to 120% with respect to the contents of D and P in two kinds of tablets T1 and T2 assumed as 100%.

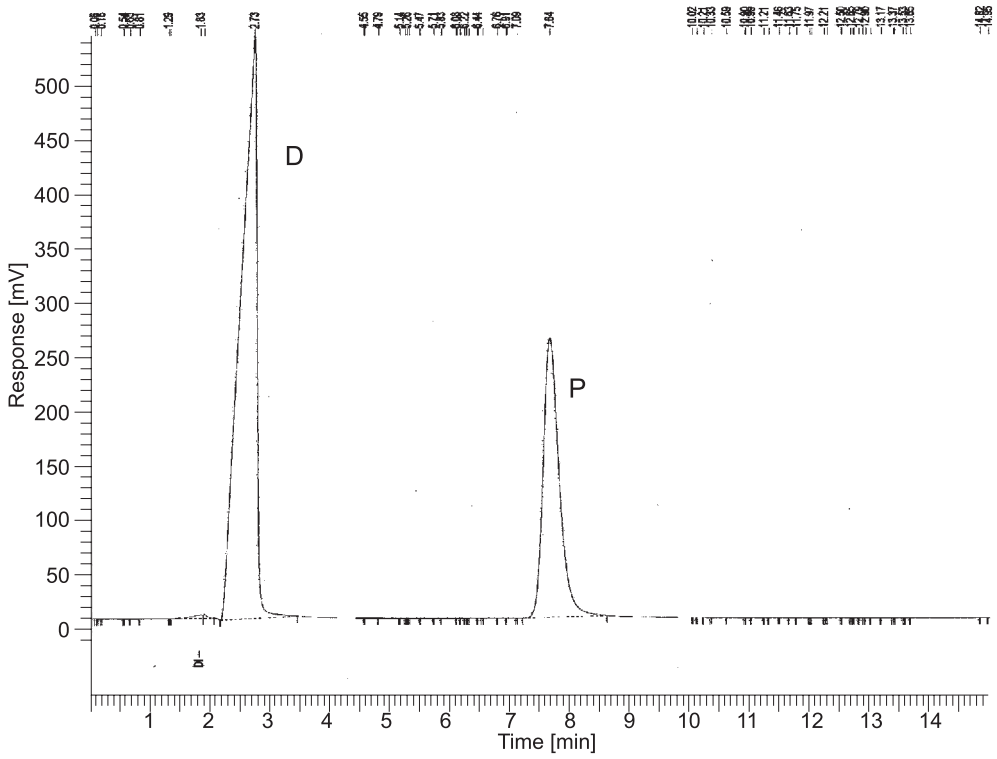

Figure 1. The chromatogram of the D (t_R = 2.73 min) and P (t_R = 7.64 min) standard solution at the concentration of 0.4 mg/mL of D and P.

Table 1. HPLC determination of D and P in T1.

No.	% of content	Mass of tablet [mg]	D			P		
			Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)
1.		151	25.17	25.80	102.5	10.07	10.48	104.1
2.		148	24.67	24.60	99.7	9.87	9.50	96.3
3.	50%	154	25.67	24.46	95.3	10.27	10.09	98.2
4.		147	24.50	23.54	96.1	9.80	9.39	95.8
5.		153	25.50	25.17	98.7	10.20	10.13	99.3
6.		150	25.00	24.05	96.2	10.00	9.51	95.1
1.		241	40.17	39.84	99.6	16.07	16.44	102.3
2.		240	40.00	41.52	103.8	16.00	15.90	99.4
3.	80%	245	40.83	42.01	102.9	16.33	16.61	101.7
4.		242	40.33	39.24	97.3	16.13	15.32	95.0
5.		238	39.67	39.31	99.1	15.87	15.58	98.2
6.		239	39.83	38.24	96.0	15.93	15.47	97.1
1.		300	50.00	49.60	99.2	20.00	19.58	97.9
2.		298	49.67	47.34	95.3	19.87	19.57	98.5
3.	100%	295	49.17	48.63	98.9	19.67	19.53	99.3
4.		309	51.50	52.27	101.5	20.60	20.35	98.8
5.		306	51.00	52.43	102.8	20.40	21.26	104.2
6.		303	50.50	52.17	103.3	20.20	21.01	104.0
1.		362	60.33	62.68	103.9	24.13	24.52	101.6
2.		367	61.17	61.48	100.5	24.47	24.20	98.9
3.	120%	365	60.83	59.80	98.3	24.33	23.21	95.4
4.		359	59.83	58.57	97.9	23.93	23.16	96.8
5.		360	60.00	57.24	95.4	24.00	23.04	96.0
6.		368	61.33	60.16	98.1	24.53	24.00	101.9
			S_x = 13.107			S_x = 5.24		
			S_y = 13.121		$\bar{x}$ = 99.26	S_y = 5.219		$\bar{x}$ = 98.99
			S = 2.8459			S = 2.9019		
			$S_{\bar{x}}$ = 0.5809			$S_{\bar{x}}$ = 0.5923		
			RSD = 2.867%			RSD = 2.93%		
			$\mu_{0.05}$ = 99.26 ± 1.2118			$\mu_{0.05}$ = 98.99 ± 1.2356		

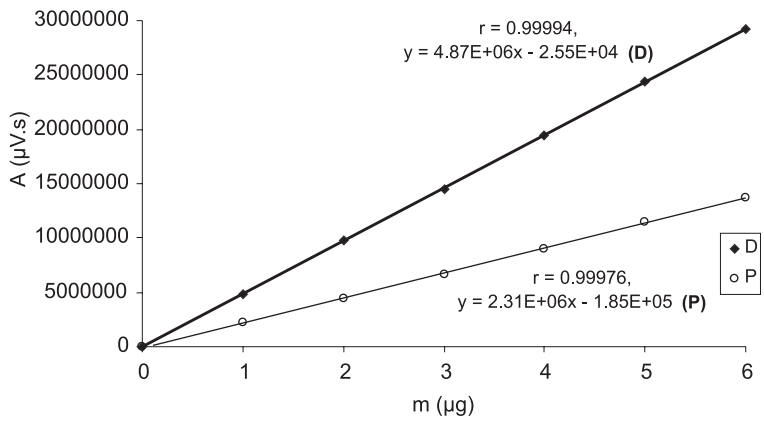

Figure 2. The calibration curves of D and P from the standard solutions.

Table 1. HPLC determination of D and P in T2.

No.	% of content	Mass of tablet [mg]	D			P		
			Added (mg)	Found (mg)	Recovery (%)	Added (mg)	Found (mg)	Recovery (%)
1.		150	25.00	24.95	99.8	10.00	9.58	95.8
2.		149	24.83	25.15	101.3	9.93	10.25	103.2
3.	50%	151	25.17	24.29	96.5	10.07	9.84	97.7
4.		147	24.50	23.96	97.8	9.80	9.72	99.2
5.		152	25.33	24.87	98.2	10.13	9.73	96.1
6.		148	24.67	25.48	103.3	9.87	9.43	95.5
1.		242	40.33	41.82	103.7	16.13	16.76	103.9
2.		240	40.00	40.72	101.8	16.00	16.69	104.3
3.	80%	244	40.67	40.06	98.5	16.27	15.49	95.2
4.		237	39.50	39.22	99.3	15.80	15.71	99.4
5.		238	39.67	38.24	96.4	15.87	15.44	97.3
6.		245	40.83	42.01	102.9	16.33	15.58	95.4
1.		301	50.17	49.92	99.5	20.07	21.01	104.7
2.		297	49.50	50.44	101.9	19.80	20.22	102.1
3.	100%	305	50.83	51.95	102.2	20.33	20.13	99.0
4.		299	49.83	51.37	103.1	19.93	19.35	97.1
5.		302	50.33	49.73	98.8	20.13	19.43	96.5
6.		300	50.00	49.60	99.2	20.00	20.76	103.8
1.		366	61.00	59.66	97.8	24.40	23.33	95.6
2.		358	59.67	57.70	96.7	23.87	22.96	96.2
3.	120%	360	60.00	59.88	99.8	24.00	24.79	103.3
4.		362	60.33	61.17	101.4	24.13	23.60	97.8
5.		359	59.83	59.95	100.2	23.93	23.14	96.7
6.		364	60.67	62.67	103.3	24.27	24.10	99.3
			S_x = 13.039			S_x = 5.214		
			S_y = 13.100		$\overline{x}$ = 100.14	S_y = 5.174		$\overline{x}$ = 98.96
			S = 2.3084			S = 3.3133		
			$S_{\overline{x}}$ = 0.4712			$S_{\overline{x}}$ = 0.6763		
			RSD = 2.305%			RSD = 3.348%		
			$\mu_{0.05}$ = 100.14 ± 0.9829			$\mu_{0.05}$ = 98.96 ± 1.4108		

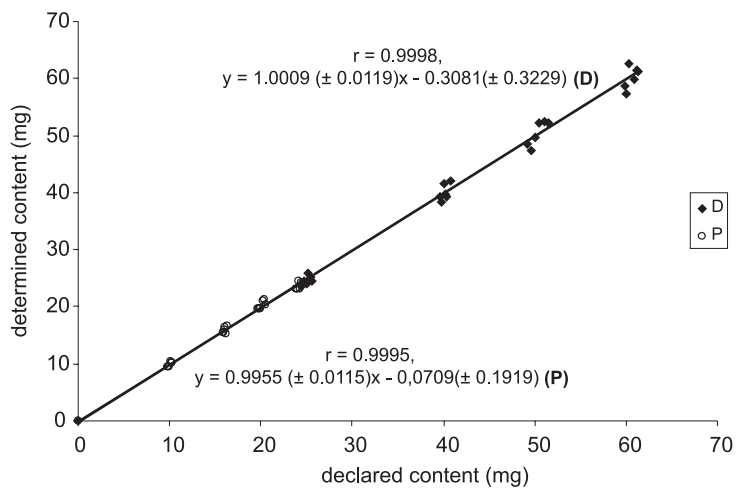

Figure 3. The determined vs. declared amount of D and P assayed in T1.

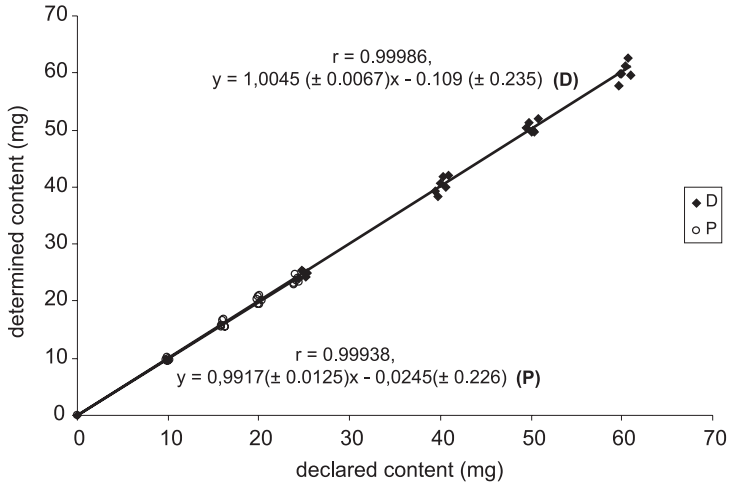

Figure 4. The determined vs. declared amount of D and P assayed in T2.

Accurately weighed portions of the powdered tablets T1 or T2 equivalent of 50%, 80%, 100% and 120% of declared single doses of D and P were placed in 50 mL volumetric flasks, dissolved in methanol and the flasks were completed to volume with methanol. The solution was filtered by the Sartorius filter at the size of pore 0.20 µm and the solution was mixed with citric buffer pH 6.5 at ratio (1:1, v/v). The solution was injected in the column by the autosampler in the amount of 10 µL.

The declared and determined amount of the substances in T1 and T2 and statistical data are collected in Tables 1 and 2. The results are shown in Figures 3 and 4.

RESULTS AND DISCUSSION

The HPLC method was found selective towards diclofenac sodium and papaverine hydrochloride in tablets mass containing the excipients such as PVP, M, PS, HPMC, MC and MS, using methanol – water (60:40, v/v) as the mobile phase. Figure 1 shows one of the chromatograms from the standard solution at 4 µg content of D and P. The areas of the peaks are 19719319 for D and 9218277 for P, the heights 1043713 for D and 499865 for P and the retention times 2.73 min for D and 7.64 min for P, respectively. Under the conditions studied, the linear relationship between the area under the peaks and the amount of D and P within the range 0.05 to 0.6 mg/mL was obtained. For the calibration curves, the correlation coefficients (r) equal 0.99994 for D and 0.99976 for P tend to be 1 which confirms the accuracy of the method.

The limit of quantitation of D and P by HPLC was less than 0.5 µg of the substances injected on the column.

The content of the active substances in the powder mass tablets containing from 50% to 120% of D and P, calculated from 300 mg portion of powder mixture, equivalent to about 50 mg of D and 20 mg of P (100%), were assayed by using the procedure given above. The results presented in Table 1 and 2 show that the average recovery ($\overline{x}$) and the standard deviation (SD) for D is 99.26% (± 2.85%) in T1, 100.14% (± 2.31%) in T2 and for P is 98.99% (± 2.9%) in T1, 98.96% (± 3.31%) in T2, 95%. Confidence intervals (µ) from 0.9829% to 1.4108% and relative standard deviaton (RSD) within the range 2.305% to 3.348%, confirm good precision of the method and the results are satisfactory.

As presented in Figure 3 and 4, the determined and declared content of D and P have the linear dependence what confirms the linearity of the method and the regression coefficients (a) from equation y = ax + b tend to be or equal 1 and (b) are not significant, so the method is not charged with systematic error. The correlation coefficients (r) equal 0.9998 for D in T1, 0.99986 in T2 and 0.9995 for P in T1, 0.99938 in T2 confirm the accuracy of the method.

The HPLC method can be recommended for simultaneous determination of diclofenac sodium and papaverine hydrochloride in tablets, because it is simple, rapid and accurate.

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