DRUGS, COSMETICS, FORENSIC SCIENCES

Application of Liquid Chromatography to the Simultaneous Determination of Acetylsalicylic Acid, Caffeine, Codeine, Paracetamol, Pyridoxine, and Thiamine in Pharmaceutical Preparations

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This paper describes a rapid reversed-phase liquid chromatographic method, with UV detection, for the simultaneous determination of acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine, and thiamine in pharmaceutical preparations. A reversed-phase C_{18} Nucleosil column is used. The mobile phase consists of 2 successive eluants: water (5 min) and acetonitrile–water (75 + 25, v/v; 9 min), both adjusted to pH 2.1 with phosphoric acid. Before determination acetylsalicylic acid is completely converted to salicylic acid by alkaline hydrolysis. Salicylic acid, caffeine, paracetamol, pyridoxine, and thiamine are all detected at 285 nm, whereas codeine is detected at 240 nm. Calibration curves were linear for salicylic acid, caffeine, paracetamol, and pyridoxine in the range of 50–500 mg/L, and for codeine and thiamine in the range of 50–1000 mg/L. The method was applied to the analysis of 13 fortified commercial pharmaceutical preparations. Recoveries ranged from 92.6 to 105.5%, with relative standard deviations of 1.1–5.8%.

Combinations of analgesics as active principles in commercial pharmaceutical preparations usually contain 2 or more of the most common, i.e., acetylsalicylic acid (ASA), salicylamide, paracetamol (PCT), and codeine (CO), together with central nervous system stimulants, e.g., caffeine (CF).

Gas chromatography (1, 2) and liquid chromatography (LC; 3–5) have been used for the determination of these analgesics. However, the LC methods developed for this purpose deal usually with only 2 or 3 compounds (5–7). UV detection is usually used (8–12), sometimes with precolumn derivatization (13).

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Some vitamins of the B-group, e.g., thiamine (TH) and pyridoxine (PY), are found along with analgesics or central nervous system stimulants in pharmaceutical preparations. LC provides numerous methods for the separation and determination of vitamins of the B-group in different matrixes: foods (14–16), medical foods (17), infant formula (18), and pharmaceuticals (19, 20).

Nevertheless, only one method has been published for the simultaneous determination of both water-soluble vitamins and analgesics or central nervous system stimulants (9). The method allows the determination of only 2 of the above mentioned analgesics and 1 vitamin B in a pharmaceutical preparation.

In this paper, we describe an LC method for the simultaneous determination of 6 active principles in pharmaceuticals. Three of them are analgesics: ASA, CO, and PCT; 2 are water-soluble vitamins: PY and TH; and one is a central nervous system stimulant: CF.

The method described is sensitive, rapid, and reliable and provides accurate results in analyses of pharmaceutical preparations.

Experimental

Reagents

(**a**) *Stock solutions*.—Stock solutions at 1.000 g/L for salicylic acid (SA; Fluka, Madrid, Spain), CF (Panreac, Barcelona, Spain), PY hydrochloride (Fluka), and PCT (Fluka), and at 10.000 g/L for CO (Abelló, Madrid, Spain) and TH hydrochloride (Fluka) were prepared by dissolution of the appropriate amounts in water (LC grade). All chemicals were analytical grade, and ultrapure water was used. Stock solutions of vitamins were stored at 4°C. Working solutions were prepared daily by suitable dilution.

(**b**) *Mobile phases*.—Methanol (Fluka), acetonitrile (Fluka), and ultrapure water were used. Phosphoric acid (Panreac, Madrid, Spain) was added to adjust the pH of the mobile phases after the acetonitrile and water were mixed. Ultrapure water was obtained from a Milli-Q Plus system (Millipore, Madrid, Spain; LC grade).

Table 1. LC parameters obtained under the operating conditions[a] of the developed method

Analyte	Retention time, $t_{R(min)}$	Capacity factor, k'[b]	Asymmetry factor, SF[c]	Resolution, R_s[d]
Thiamine	4.297	0.480	3.2	
				7.93
Pyridoxine	7.085	1.438	2.6	
				19.01
Paracetamol	11.251	2.978	1.25	
				3.50
Codeine	11.690	3.315	1.9	
				3.70
Caffeine	12.150	3.301	3	
				10.74
Salicylic acid	13.363	3.375	2.64	

[a] Flow rate of mobile phase = 1 mL/min; column temperature = 35°C.

[b] $k' = (t_R - t_0)/t_0$, where t_R = retention time of each compound, and t_0 = retention time of the eluant (unretained compound).

[c] SF = ratio of the 2 half-widths at 10% peak height.

[d] $R_s = 2\Delta Z/(W_a + W_b)$, where ΔZ = distance between the maxima of 2 consecutive peaks, and W_a and W_b = peak widths.

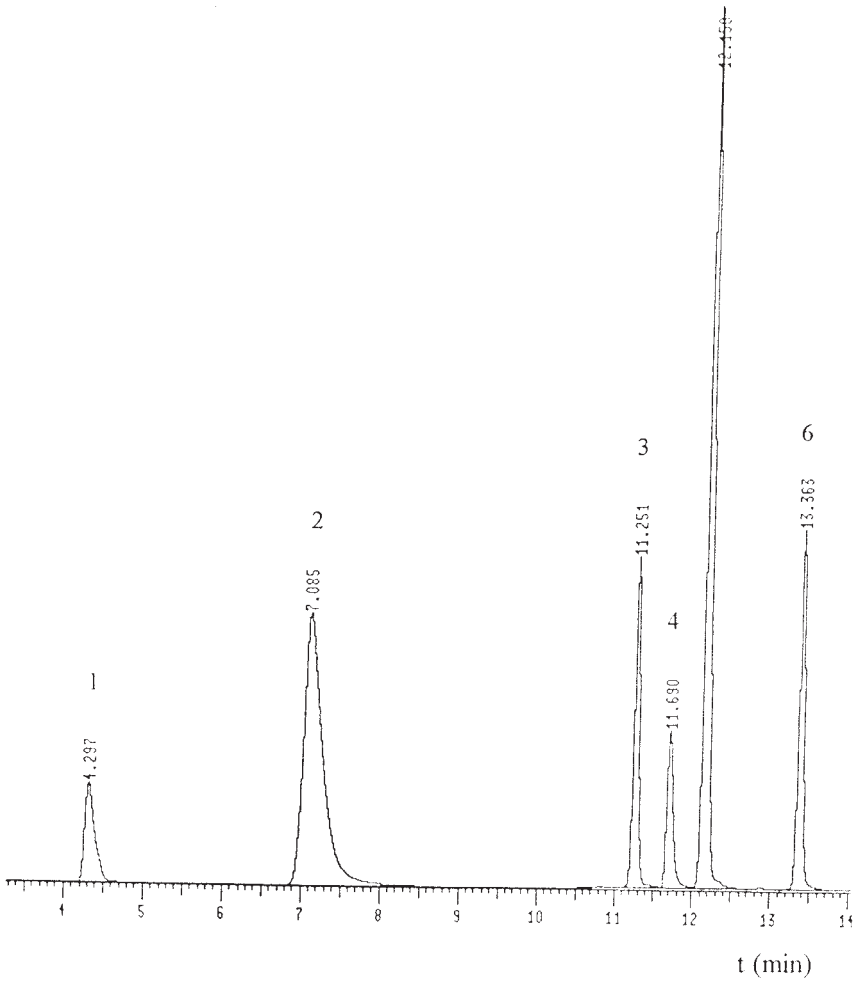

Figure 1. Liquid chromatogram obtained by the developed method and showing the separation of the 6 compounds. Conditions: detector 285 nm; concentration of each compound 200 mg/L. Peaks: 1 = TH; 2 = PY; 3 = PCT; 4 = CO; 5 = CF; and 6 = SA.

Table 2. Statistical parameters of calibration curves obtained for the developed method

Analyte	Intercept	Slope	SD_a[a]	SD_b[b]	r[c]	F[d]
Salicylic acid	–1.04	4.85	9.08	0.022	0.9998	1.27
Caffeine	59.08	11.84	25.54	0.091	0.9994	6.40
Codeine	89.48	6.15	19.34	0.037	0.9996	1.59
Paracetamol	–5.29	4.41	9.08	0.032	0.9995	2.37
Pyridoxine	–40.13	14.20	11.77	0.041	0.9999	2.23
Thiamine	–8.37	2.66	4.38	0.008	0.9999	1.68

[a] Standard deviation of the intercept.
[b] Standard deviation of the slope.
[c] Correlation coefficient.
[d] *F* ratio.

Apparatus

(**a**) *Liquid chromatograph*.—Model HP 1050 (Hewlett-Packard Co., Arondale, PA).

(**b**) *LC column*.—Nucleosil C_{18} stainless steel, 250 × 4.6 mm, 5 μm particle size (No. 2515; Scharlau Science, Barcelona, Spain).

(**c**) *Absorbance detector*.—Hewlett-Packard diode-array detector HP 1040 M series II with variable wavelength range of 200–600 nm. Spectra of the eluates and absorbance measurements at 285 and 240 nm were obtained at time intervals of 0.640 s.

Treatment of Samples

The contents of capsules are quantitatively transferred, and tablets are crushed to a fine powder for dissolution in water by sonication. The solutions are then filtered through a 0.45 μm pore size Millipore filter, and the filtrates are diluted to appropriate volume with LC grade water. To obtain the complete transformation of ASA to SA, alkaline hydrolysis is performed for pharmaceuticals containing ASA by treating the sample with 1M NaOH solution and heating at 60°C for 30 min. Suitable dilutions are made in all cases before the sample solutions are injected.

LC Conditions

The following procedure is used for all samples: water (pH 2.1) as the mobile phase for 5 min and then acetonitrile–water (75 + 25, v/v) for 9 min, at a flow rate of 1 mL/min. The detection wavelength is set at 285 nm for SA, CF, PCT, PY, and TH and at 240 nm for CO. The working column temperature is 35°C. The sample volume injected is 10 μL. The chromatographic parameters obtained under these conditions are summarized in Table 1.

Table 3. Analytical parameters calculated for the developed method

		RSD, %[b]		DL, mg/L[c]	
Compound	LDR, mg/L[a]	Analyte concentration, 100 mg/L	Analyte concentration, 400 mg/L	Test I[d]	Test II[e]
Salicylic acid	50–500	1.65	1.47	27	5.1
Caffeine	50–500	5.84	2.84	13	4.9
Pyridoxine	50–500	2.12	1.87	27	10.5
Thiamine	50–1000	3.24	4.21[f]	19	7.6
Codeine	50–1000	1.11	1.25[f]	20	7.7
Paracetamol	50–500	3.51	2.33	17	6.6

[a] LDR = linear dynamic range.
[b] RSD = relative standard deviation.
[c] DL = detection limit.
[d] From ref. 21.
[e] From ref. 22: $DL = \frac{S_{y/x}}{b}\sqrt{\frac{n-2}{n-1}}$; b = slope; and $S_{y/x}$ = standard deviation of y-residuals.
[f] Analyte concentration = 800 mg/L.

Table 4. Recovery[a] of salicylic acid, caffeine, pyridoxine, thiamine, codeine, and paracetamol from synthetic mixtures

Level of each analyte added, mg/L	Salicylic acid		Caffeine		Pyridoxine		Thiamine		Codeine		Paracetamol	
	Analyte found, mg/L	Avg. rec. ± SD, %[b]	Analyte found, mg/L	Avg. rec. ± SD, %	Analyte found, mg/L	Avg. rec. ± SD, %	Analyte found, mg/L	Avg. rec. ± SD, %	Analyte found, mg/L	Avg. rec. ± SD, %	Analyte found, mg/L	Avg. rec. ± SD, %
50	50.0	100 ± 2	46.5	93 ± 1	50.3	100.6 ± 0.3	49.3	96 ± 2	45.1	90 ± 4	46.7	93.5 ± 0.1
200	199.8	99.9 ± 0.5	205.4	102.7 ± 0.2	198.9	99 ± 1	395.7 [c]	99 ± 2	402.7[c]	100.7 ± 0.3	201.6	101 ± 3
500	500.4	100.1 ± 0.2	494.4	98.9 ± 0.1	500.7	100.2 ± 0.7	1001.4[d]	100.3 ± 0.3	988.2[d]	99 ± 1	498.2	99.7 ± 0.7

[a] Each value is the mean of 3 replicate determinations.
[b] SD = standard deviation.
[c] Analyte added at 400 mg/L.
[d] Analyte added at 1000 mg/L.

LC Procedure

A 10 μL aliquot of solution containing the analytes in their linear dynamic concentration ranges are injected into the liquid chromatograph: 50–500 mg/L for SA, CF, PCT, and PY and 50–1000 mg/L for CO and TH. A flow rate of 1 mL/min and working column temperature of 35°C are used. The compounds are separated on a reversed-phase C_{18} Nucleosil column, with a mobile phase consisting of the 2 successive eluants described above, both adjusted to pH 2.1 with phosphoric acid before the water and acetonitrile are mixed. After all the compounds are separated, the water is passed through the column for 4 min. Absorbance peak areas are measured in all cases.

Results and Discussion

Temperature and Flow Rate

In the proposed LC method, the temperature variation and the flow rate for the resolution of the system did not have any significant influence on the analytical signal. However, a temperature of 35°C was used because it allowed a liquid phase with a lower viscosity, and a flow rate of 1 mL/min, which was appropriate for the working pressure of the chromatographic equipment, was used to shorten the time required to perform the analysis.

Order of Elution

Preliminary studies with several eluant systems were conducted to select the most effective eluant for the separation of the 6 analytes of the system. With some eluants, water at pH 5.0 and acetonitrile–water from (25 + 75, v/v) to (75 + 25, v/v), also at pH 5, no resolution was observed because of large overlap of the signals (for example, the CO and PY peaks). Moreover, asymmetric and very wide peaks were obtained for water-soluble vitamins.

With a more acidic eluant, acetonitrile–water (75 + 25, v/v), pH 2.1, thinner peaks as well as a good separation were achieved for CO with respect to water-soluble vitamins, but PY and TH were not satisfactorily separated. With only water at pH 2.1, PY and TH were separated satisfactorily, but the other 4 compounds, with lower polarity, were not eluted. It could be expected that the use of a less polar mobile phase after the elution of the water-soluble vitamins would separate them. Methanol–water (35 + 65, v/v) was tried, but it failed to produce a good separation for CO and PCT.

Finally, acetonitrile–water (75 + 25, v/v), pH 2.1, gave a satisfactory resolution of the 4 compounds. Therefore, the mobile phase selected for the most efficient separation consisted of 2 eluants: the first eluant was water (LC) adjusted to pH 2.1 with phosphoric acid. It allowed the separation of the vitamins, which were retained more weakly in the apolar stationary phase because of their ionic nature. The second eluant was acetonitrile–water (75 + 25, v/v), also adjusted to the same pH with phosphoric acid, for the separation of the 4 remaining components of the system. When the elution time for the first eluant was increased from 3 to 5 min, the signal from PY was greatly improved, producing only 1 peak (elution times of <5 min gave 2 close peaks). This variable, however, had no in-

Table 5. Results[a] for the determination of acetylsalicyclic acid, caffeine, codeine, and paracetamol in commercial pharmaceutical preparations

	Acetylsalicylic acid		Caffeine		Codeine		Paracetamol	
Pharmaceutical preparation	Label claim, mg/unit	Avg. found ± SD, mg/unit[b]	Label claim, mg/unit	Avg. found ± SD, mg/unit	Label claim, mg/unit	Avg. found ± SD, mg/unit	Label claim, mg/unit	Avg. found ± SD, mg/unit
Analgilasa	—	—	30.0	27 ± 2	10.0	10 ± 1	500	458 ± 1
Dolmen	500	486 ± 3	—	—	10.0	10.5 ± 0.2	—	—
Dolvirán	400	387 ± 3	50.0	46 ± 1	9.6	9.4 ± 0.2	—	—
Fiorinal	200	197 ± 3	40.0	31.1 ± 0.4	14.7	14.6 ± 0.2	300	274 ± 4
Rinomicine	—	—	30.0	27.7 ± 0.7	—	—	150	138 ± 1

[a] Each value is the mean of 3 replicate determinations.
[b] SD = standard deviation.

fluence on the resolution of the other compounds, including TH. With the second eluant, 9 min was shown to be appropriate to obtain satisfactory resolution (Table 1). A typical chromatogram is shown in Figure 1.

Before the next injection the column was reequilibrated by passing ultrapure water through the column for 4 min. This was necessary to return the column to conditions appropriate for separation of the water-soluble vitamins. Only after this procedure were the peaks of the vitamins resolved.

Calibration and Analytical Parameters

Standard calibration graphs for the analytes were constructed by plotting peak areas produced by injection of standard solutions in the following concentration ranges: 50–500 mg/L for SA, CF, PCT, and PY, and 50–1000 mg/L for CO and TH. Calibration curves were analyzed by regression analysis, and the correlation coefficient (r), slope, and y-intercept for each run were calculated (Table 2). This process was repeated 3 times for statistical analysis. The detection limits (DL) for each compound, calculated according to the criteria of Miller and Miller (21) and Cuadros et al. (22), as well as the linear dynamic ranges (LDR) and relative standard deviation (RSD) at 2 analyte levels are shown in Table 3.

Applications

The proposed method was used in the simultaneous determination of the analytes in both synthetic mixtures and commercial pharmaceutical formulations.

Synthetic mixtures.—Solutions containing the 6 active principles at 3 different levels were prepared and analyzed by the proposed method. Three determinations were performed in all cases. Results found were completely satisfactory (Table 4).

Commercial pharmaceutical preparations.—Thirteen commercial pharmaceuticals were analyzed by the developed

Table 6. Results[a] for the determination of codeine, pyridoxine, and thiamine in commercial pharmaceutical preparations

	Codeine		Pyridoxine		Thiamine (NO_3^- or HCl)	
Pharmaceutical preparation	Label claim, mg/unit	Avg. found ± SD, mg/unit[b]	Label claim, mg/unit	Avg. found ± SD, mg/unit	Label claim, mg/unit	Avg. found ± SD, mg/unit
Benadom	—	—	300	277 ± 3	—	—
Codeisán	28.7	26.1 ± 0.5	—	—	—	—
Conductasa[c]	30.7	29.9 ± 0.4	—	—	—	—
Nervobión	—	—	100	103 ± 2	100	114.4 ± 0.3
Neurodavur	—	—	250	237 ± 6	250	241 ± 3
Pazbronquial	1.00	1.00 ± 0.01	0.60	0.6 ± 0.1	—	—
Perduretas de codeína	50.0	46.1 ± 0.4	—	—	—	—
Serfoxide[d]	—	—	300	280 ± 3	—	—

[a] Each value is the mean of 3 replicate determination.
[b] SD = standard deviation.
[c] Expressed as pyridoxine α-cetoglutarate.
[d] Expressed as anhydre pyridoxine phosphoserinate.

Table 7. Recovery[a] of salicylic acid, caffeine, pyridoxine, thiamine, codeine, and paracetamol from fortified commercial pharmaceutical preparations

	Salicylic acid		Caffeine		Pyridoxine		Thiamine		Codeine		Paracetamol	
Pharmaceutical preparation	Added, mg/L	Avg. rec. ± SD, %[b]	Added, mg/L	Avg. rec. ± SD, %	Added, mg/L	Avg. rec. ± SD, %	Added, mg/L	Avg. rec. ± SD, %	Added, mg/L	Avg. rec. ± SD, %	Added, mg/L	Avg. rec. ± SD, %
Analgilasa			50	99 ± 1					100	101 ± 4	50	100 ± 2
			100	105 ± 2					200	98 ± 4	200	98 ± 2
			350	96.2 ± 0.2					300	98 ± 4	400	100 ± 1
Dolmen	20	98 ± 1							100	101 ± 1		
	50	99 ± 2							200	102 ± 1		
	100	99.4 ± 0.6							300	99.3 ± 0.7		
Dovirán	50	101.4 ± 0.3	50	98 ± 1					100	99 ± 2		
	100	100 ± 1	100	101.7 ± 0.6					200	98 ± 2		
	150	99.3 ± 0.4	350	102.4 ± 0.5					300	99 ± 1		
Fiorinal	50	98.4 ± 0.7	50	98 ± 1					100	98.4 ± 0.5		
	100	101.2 ± 0.3	200	99 ± 1					200	100 ± 1		
	150	101 ± 1	300	98.4 ± 0.6					300	102 ± 2		
Nervobión					60	98.8 ± 0.2	60	99 ± 4				
					200	105 ± 2	200	105.5 ± 0.9				
					400	99 ± 2	800	99 ± 1				
Neurovadur					60	101 ± 2	60	102 ± 5				
					200	100.8 ± 0.9	200	104 ± 2				
					400	99 ± 1	800	97 ± 3				
Pazbronquial					100	92.6 ± 0.7			100	95 ± 1		
					200	101.1 ± 0.3			200	96 ± 1		
					300	104.6 ± 0.6			300	97 ± 1		
Rinomicine			150	98 ± 1							400	95.5 ± 0.6
			200	97 ± 2							800	96 ± 2
			300	95 ± 2							1200	95 ± 2

[a] Each value is the mean of 3 replicate determinations.

[b] SD = standard deviation.

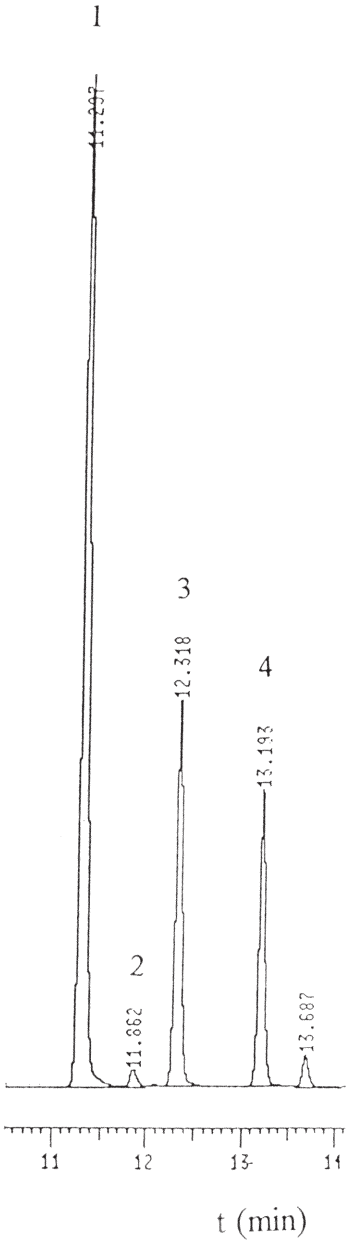

Figure 2. Typical liquid chromatogram obtained by the developed method and showing the separation of the 4 compounds from Fiorinal capsules. Conditions: detector, 285 nm. Peaks: 1 = PCT; 2 = CO; 3 = CF; 4 = SA; and lost peak = salicylamide.

method after dissolution and suitable dilution as indicated in the section on sample treatment. Results and label claims by the manufacturer are summarized in Tables 5 and 6. In addition, as a check on the accuracy of the proposed method, a recovery study was performed in which the respective active principles contained in several pharmaceutical preparations were added at 3 levels. Good recoveries were obtained in all cases (Table 7). Figure 2 shows the separation of the 4 compounds (PCT, CO, CF, and SA) from Fiorinal capsules.

Conclusions

The proposed method allowed successful separation and determination of 6 active principles: 1 organic acid (SA), 2 alkaloids (CF and CO), 2 water-soluble vitamins (PY and TH), and a phenol derivative (PCT). The total time required for the analysis is relatively shorter than that described by other researchers (8, 9, 13) for simpler systems containing some of these analytes (but not all). Its application to pharmaceuticals after validation demonstrated that the proposed method can be used satisfactorily for the determination of these analytes.

We compared the LC procedures proposed by the United States Pharmacopeial Convention (USP; 23) with the developed method described here. The RSDs of the results obtained by the USP methods and this method are similar; however, the mobile phase of this method is simpler, and although the tailing factors, in general, are similar, the resolution obtained with this method is better. In addition, the method proposed in this paper includes more compounds; the USP methods for CO usually include another analgesic (such as PCT or ASA, but not both simultaneously), and in no case do they include water-soluble vitamins.

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