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Simultaneous determination of aspirin, codeine phosphate and propyphenazone in tablets by reversed-phase high-performance liquid chromatography

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Summary

A reversed-phase high-performance liquid chromatographic method has been developed for the simultaneous determination of aspirin, propyphenazone and codeine phosphate in an analgesic tablet formulation. The proposed method is also suitable for the determination of small quantities of salicylic acid. The elution was isocratic using two C-8 columns and methanol-water (45:55) as mobile phase with 1.4% acetic acid and 5 mM tetramethylammonium bromide.

The pharmaceutical combination of aspirin (an analgesic, anti-inflammatory and antipyretic drug), codeine phosphate (an analgesic and antitussive drug) and propyphenazone (an analgesic agent) is used in the treatment of coughs and colds, rheumatic and joint disorders and various mild to moderate pains.

Several procedures for the determination of aspirin-codeine (Yacobs et al., 1966; Galante et al., 1979; Yang et al. 1985), codeine-propyphenazone (Kretschmann and Vogel, 1984; Huettner and Eigendorf, 1986) and aspirin-propyphenazone combinations (Eigendorf et al., 1989, 1990) have been described; furthermore, the TLC detection of codeine, aspirin and propyphenazone (Reimers, 1967; Schmidt, 1968) has been reported. However, no method for the simultaneous assay of the three drugs in a mixture has been developed.

High-performance liquid chromatography (HPLC) with pH control and an organic reagent having the same charge as that of the sample has been employed to improve the analysis of products with amino groups (Goldberg et al., 1984; Gomez-Gomar et al., 1989). Accordingly, in the present work, an acidic pH and a quaternary ammonium salt were necessary to optimize the

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HPLC assay of codeine phosphate. The described procedure was found to be suitable for the simultaneous determination of the opioid, aspirin, propyphenazone and salicylic acid, the principal degradation product of aspirin, in a tablet formulation.

Reagents and chemicals: Aspirin (Sigma reference standard), salicylic acid (Sigma), codeine phosphate (S.A.L.A.R.S.), propyphenazone (Hoechst), paracetamol (Sigma reference standard), acetic acid (Carlo Erba) and tetramethylammonium bromide (Lancaster Synthesis) were used as received. Methanol and water (degassed before use) were HPLC analytical-reagent grade (Carlo Erba). Samples of two batches of tablets, manufactured for the Italian army (labeled to contain: aspirin, 0.3 g; propyphenazone, 0.29 g; codeine phosphate, 0.01 g; microcrystalline cellulose 0.1 g; hydrogenated castor oil, 0.005 g; talc, 0.005 g; magnesium stearate, 0.025 g), were obtained locally from the Stabilimento Chimico Farmaceutico Militare, Florence.

Apparatus: The HPLC system (Perkin-Elmer Series 3B) was connected to a Rheodyne injection valve (Model 7125) fitted with a 20 μl injection loop, a variable-wavelength detector (Perkin-Elmer LC 75 with Autocontrol) set at 284 nm and an LCI-100 (Perkin-Elmer) laboratory computing integrator (0.02–2.56 absorbance units full scale and chart speed 3 mm min^{-1}). Two Technosphere RP C-8 reversed-phase columns, placed in series (5 μm, 10 cm $\times$ 4.6 mm i.d., HPLC Technology), were used at room temperature with a flow rate of the mobile phase of 1.0 ml min^{-1}. Paracetamol (2.5 mg ml^{-1} in methanol) was used as an internal standard.

Calibration curves: Stock solutions (10 mg ml^{-1} in methanol) of each analyte were prepared. Aspirin solutions were prepared immediately before use. These solutions were diluted with absolute methanol to give standard solutions of concentration within the following ranges: aspirin, 0.2–1.0 mg ml^{-1}; codeine phosphate, 0.01–0.03 mg ml^{-1}; propyphenazone, 0.2–1.0 mg ml^{-1}; salicylic acid, 0.001–0.01 mg ml^{-1}. Each solution contained a fixed concentration (5 mg ml^{-1}) of internal standard. Calibration curves for the above concentration range were calculated as a function of the respective area ratio of analyte to the internal standard vs concentration. The regression equations and the symmetry factor (SF) of peaks are reported in Table 1 (average of four determinations).

TABLE 1

Regression equations and symmetry factor (SF) of aspirin, codeine phosphate, propyphenazone and salicylic acid

Drug	r	Intercept	Slope	SF
Aspirin	0.9999	0.2356	22.95	1.00
Codeine phosphate	0.9991	−0.0572	20.47	1.05
Propyphenazone	0.9956	1.8300	117.8	0.93
Salicylic acid	0.9562	0.0001	−0.0013	0.88

Tablet assay: 20 tablets were weighed and finely ground. An amount of powder equivalent to the average weight of one tablet (735 mg) was accurately weighed and transferred into a 100 ml volumetric flask. The flask was half-filled with absolute methanol and the mixture was sonicated for 10 min at room temperature prior to the flask being made up to volume. After centrifugation (3000 rpm for 10 min), a 2.0 ml aliquot of internal standard solution was added and the mixture was then transferred into a 10 ml volumetric flask which was made up to volume with methanol. The resulting solution was filtered through a 0.45 μm membrane filter before injection. A typical chromatogram is shown in Fig. 1.

Table 2 lists the analytical results from two batches of commercial tablets and two authentic admixtures. The accuracy and reproducibility of the proposed method were assessed by preparing two admixtures of aspirin, codeine phosphate and propyphenazone at similar ratios to those labelled on the tablet formulation. Since limit tests for salicylic acid are present in official monographs on aspirin (not more than 0.05–0.3% w/w), synthetic admixtures were spiked with salicylic acid (0.25–0.5% of the claimed amount of aspirin).

The proposed chromatographic conditions allowed the simultaneous determination of aspirin, salicylic acid, codeine phosphate and propyphenazone in tablet dosage form with precision and accuracy. Satisfactory separation between aspirin and salicylic acid peaks was achieved with

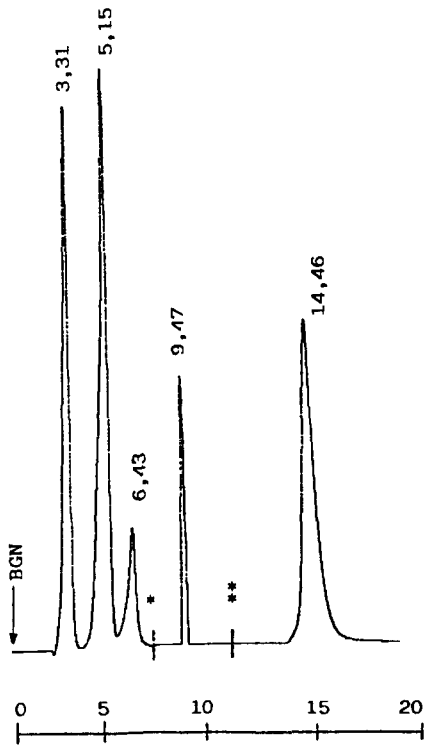

Fig. 1. Typical chromatogram of paracetamol (R_t 3.31 min), aspirint(R_t 5.15 min), salicylic acid (R_t 6.43 min), codeine phosphate (R_t 9.47 min) and propyphenazone (R_t 14.46 min). (*) From 0.64 to 0.02 absorbance units full scale; (**) from 0.02 to 2.56 absorbance units full scale.

two C-8 columns placed in series. The wavelength of 284 nm was chosen in order to optimize the detection of codeine and salicylic acid, however, two attenuation changes were required because of the difference in molar absorptivities and the relative quantities of the four drugs in the tablets. Separation between codeine and propyphenazone was attempted by varying the proportion of water and methanol, the best resolution being achieved at a volume ratio of 55 : 45.

The presence of codeine required particular attention to be paid to the choice of the pH and the eluent modifier in order to improve the selectivity and reduce the retention time. In particular, the functions of acetic acid were to regulate the degree of ionization of codeine, which was in protonated form in the mobile phase, ion suppression for aspirin and salicylic acid and the reduction of the extent of sample interaction with

TABLE 2

Analyses of authentic admixtures [a] and commercial tablets of aspirin, codeine phosphate and propyphenazone

Drug	Taken (mg)	Average recovery (%)	RSD (%, $n = 5$)
Aspirin	308.0 [a]	100.2	1.7
	301.3 [a]	99.1	0.9
	300.0	99.7	0.6
	300.0	98.6	1.2
Codeine phosphate	9.8 [a]	99.6	0.7
	10.3 [a]	99.2	1.9
	10.0	101.1	1.6
	10.0	102.2	1.3
Propyphenazone	285.2 [a]	101.9	1.0
	297.4 [a]	99.7	1.8
	290.0	98.4	1.7
	290.0	99.4	0.9
Salicylic acid	0.75 [a]	0.23 [b]	2.3
	1.5 [a]	0.51 [b]	1.9
		0.38 [b]	2.2
		0.26 [b]	2.0

[b] On the basis of aspirin content.

residual silanol groups on the surface of the stationary phase. However, codeine was eluted in an asymmetric band and with a very high k'. As in our previous chromatographic study with basic

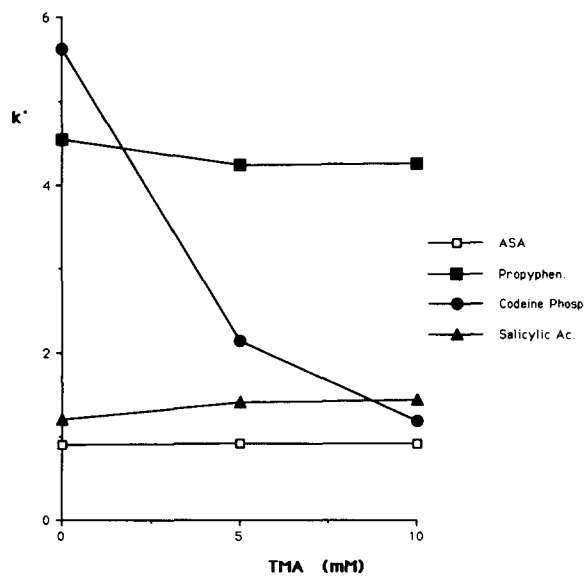

Fig. 2. Capacity factor (k') of aspirin, codeine phosphate, salicylic acid and propyphenazone vs concentration of tetramethylammonium bromide.

compounds (Santoni et al., 1991), only in the presence of 5 mM TMA was the peak symmetry of codeine substantially improved with reduction of the peak tailing and of the retention time to acceptable values (Fig. 2). Fig. 2 also shows that the k' values of aspirin, salicylic acid and propyphenazone are not significantly influenced by the concentration of TMA. Obviously, the chromatographic method could be used to assay tablet preparations which include paracetamol by using another drug as the internal standard.

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