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Simultaneous quantitation of paracetamol, caffeine and propyphenazone by high-pressure liquid chromatography

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Abstract: A reversed-phase high-performance liquid chromatographic method has been developed to determine paracetamol, caffeine and propyphenazone in a typical tablet formulation with high accuracy and precision. The mobile phase is a linear water–methanol gradient.

Keywords: *High-pressure liquid chromatography; quantitation of paracetamol, caffeine and propyphenazone; analysis of dosage forms.*

Introduction

A number of non-prescription pain relievers contain one or more analgesic drugs. Many of them also contain caffeine. The separation and quantitation of these analgesic combinations in dosage forms have been difficult and time-consuming. The determination of analgesics by high-pressure liquid chromatography overcomes many shortcomings of the previously described methods.

HPLC assays for paracetamol in combination with other analgesic and with muscle-relaxant analgesic agents have been published [1–4]. The simultaneous determination of paracetamol (acetaminophen), caffeine and propyphenazone in tablets has not been reported. This report presents an HPLC method for the quantitative determination of the drugs in a tablet formulation. The proposed method eliminates the interferences due to possible impurities, and can be extended to the determination of other drugs which are often combined with propyphenazone in commercial analgesic formulations.

Experimental

Apparatus

The high-pressure liquid chromatograph was a Perkin–Elmer (Series 2 Liquid Chromatograph) equipped with a septumless injector (Reodyne Injector 7105) and connected to a multiple-wavelength detector (Perkin–Elmer LC-55B) and a strip chart

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recorder (Perkin–Elmer R100). The peak area integrations were performed using a chromatographic data system (Perkin–Elmer Sigma 15 Chromatography Data Station). A 25 cm × 4.6 mm i.d. reversed-phase column (Perkin–Elmer, Analytical C_{18}) packed with bonded octadecylsilane on silica (10 μm particles) was used.

Chemical and solvents

Methanol (Carlo Erba, Milano, Italy) was ACS reagent grade. Water and methanol were filtered and degassed prior to use. The following drugs were used: paracetamol, caffeine, phenacetin (Merck-Schuchardt, Darmstadt, FRG), propyphenazone (Hoechst, Riedel-De Haen), phenazone (Sigma Chemical Co., St. Louis, Missouri), salicylamide, ethylparaben (Fluka AG-Chemische Fabrik, Switzerland). All were used without further purification. 4-Aminophenol (Merck-Schuchardt) and 4-chloroacetanilide (Sigma Chemical Co.) were used as received.

Chromatographic conditions

The mobile phase consisted of water and absolute methanol. The analyses were conducted employing the linear gradient elution technique. The following conditions were used. The gradient was linear from a 30:70 methanol–water initial solvent composition to 95:5 final composition. The run time was 13 min. Seven minutes were needed to return to the initial conditions prior to the next analysis. The flow rate was 1.8 ml/min. All determinations were performed at room temperature. The column effluent was monitored at 270 nm and an attenuation of 0.2–0.4 a.u.f.s. was used. The chart speed was 0.5 cm/min.

Stock solutions

Stock solutions of paracetamol (500 mg/100 ml), caffeine (100 mg/100 ml) and propyphenazone (300 mg/100 ml) in absolute methanol were prepared. A stock solution of the internal standard phenacetin (200 mg/100 ml) in absolute methanol was also prepared.

Working standard solution

A working standard solution was prepared in the following manner: 15 ml of each stock solution of the drugs was accurately pipetted into a 100 ml volumetric flask, 15 ml of the internal standard stock solution was added and the resulting solution was brought to volume with methanol.

Calibration curves

Accurately pipetted volumes of 2.5, 5.0, 10, 15 and 20 ml of paracetamol, caffeine and propyphenazone stock solutions were placed in 100 ml volumetric flasks. The internal standard stock solution (15 ml) was added to each flask, followed by the addition of methanol to volume. Samples (10 μl) of each solution were repeatedly injected onto the column, until the ratios of the peak areas of the drugs to that of the internal standard peak were constant. The five concentrations of each drug were subjected to regression analysis and the slope and intercept were calculated (Table 1).

Synthetic mixture

A synthetic mixture was prepared containing the drugs at ratios similar to that claimed to be present in a commercial tablet formulation and five samples were weighed to

Table 1
Calibration data for standard drug solutions

	Concentration* (mg)	$A_D/A_{I.S.}$†	Slope	Intercept	$r^{\pm}s$
Paracetamol	12.5	0.3787 ± 0.0022‡	0.0299	−0.004744	0.9998 (±0.022)
	25.0	0.7525 ± 0.0056			
	50.0	1.4573 ± 0.0083			
	75.0	2.2285 ± 0.0108			
	100.0	2.9973 ± 0.0093			
Caffeine	2.5	0.2173 ± 0.0026‡	0.0855	−0.0007195	0.9999 (±0.0044)
	5.0	0.4241 ± 0.0030			
	10.0	0.8497 ± 0.0052			
	15.0	1.2851 ± 0.0066			
	20.0	1.7099 ± 0.0088			
Propyphenazone	7.5	0.5387 ± 0.0043‡	0.0710	−0.0014854	0.9999 (±0.013)
	15.0	1.0677 ± 0.0056			
	30.0	2.1100 ± 0.0034			
	45.0	3.1947 ± 0.0083			
	60.0	4.2672 ± 0.0047			

* Total mg/100 ml solution.
† Data represent five replicate injections of standard solutions. $A_D/A_{S.I.}$ is the ratio of the integrated area of the drug peak divided by the integrated area of the phenacetin peak at a concentration of 15 mg/100 ml.
‡ Confidence limits at $p = 0.05$ ($n = 5$).

contain various quantities of the components within the range examined for the calibration curves. The samples were dissolved in absolute methanol in five 100 ml volumetric flasks and 15 ml of phenacetin stock solution was added. The mixtures were sonicated for a few minutes and brought to volume with absolute methanol. The supernatant (10 μl) was injected into the column after centrifugation.

Commercial dosage form

Twenty tablets were weighed and the average weight of one tablet was determined. The tablets were finely powdered and quantities calculated on the labelled amount of the drugs were weighed. Each sample contained paracetamol, caffeine and propyphenazone in amounts covered by the calibration curve. The samples were treated in the same way as the synthetic mixture. Samples (10 μl) of the final solutions were injected on to the column.

Assay method

Aliquots (10 μl) of the various solutions obtained from the synthetic mixture or from the commercial dosage form were injected onto the chromatograph using the described conditions. An identical volume of a working standard solution containing an intermediate component concentration was alternatively injected for comparison after the assayed solution was eluted.

Calculations

Peak area ratios were used to quantitate the resulting chromatograms. Equation (1) was used to calculate the amounts of the drug in the assayed aliquots

$$W_D = W_D^S \cdot \frac{A_D/A_{I.S.}}{A_D^S/A_{I.S.}^S}, \quad (1)$$

where W_D and W_D^S are the amounts (mg/100 ml solution) of each drug in the tested solution and in the working standard solution respectively; A_D and A_D^S are the corresponding peak areas; $A_{I.S.}$ and $A_{I.S.}^S$ are the areas of the internal standard in the assayed solution and the working standard solution respectively. The recovery of each compound from the samples was determined by equation (2):

$$\text{Recovery} = \frac{W_D}{W} \cdot 100, \quad (2)$$

where W is the calculated amount (mg/100 ml) of each compound. The weight of each drug found in an assayed aliquot can be easily transformed to the amounts of the drugs contained in an average weight tablet.

Analysis of a single tablet

One tablet (label claim: 250 mg paracetamol, 50 mg caffeine and 150 mg propyphenazone) was placed in a 100 ml volumetric flask. Approximately 80 ml methanol was added and the mixture was sonicated for 10 min and brought to volume with methanol. Twenty millilitres of this solution was pipetted, after centrifugation, into a 100 ml volumetric flask, 15 ml of the internal standard solution was added and the solution was diluted to volume with methanol. Then 10 μl of this solution was injected onto the column. A working standard solution of a similar drug concentration was used as described under assay method. Quantitation was achieved using equation (1), allowing for the dilution factor, 5 in this case.

Results and Discussion

The simultaneous analysis of paracetamol, caffeine and propyphenazone was difficult because of the wide difference in polarity between paracetamol and propyphenazone. Optimal resolution of these drugs on the octadecylsilane column was attempted by varying the proportion of water, methanol and acetonitrile in the eluent mixture. Reduction of the water content produced a partial overlapping of paracetamol and caffeine peaks and paracetamol travelled close to the solvent front. An increase of water content produced an increase of the retention time of propyphenazone and a broadening of the peak. The use of tetrabutylammonium cation as the counter-ion to increase the retention time of paracetamol was unsuccessful because of its high pK_a value. The linear gradient elution technique was more effective for separating the three drugs in the presence of the internal standard phenacetin, and also allowed the separation of other drugs sometimes present in combination with the drugs under study. Under the chromatographic conditions described paracetamol, caffeine and propyphenazone could be separated and quantitated (Fig. 1).

The reproducibility of the method was shown by chromatographing five solutions of the drugs at various concentrations in the presence of the internal standard phenacetin. The area under the curve for each peak on the chromatograms was determined by electronic integration. The ratio of each drug peak area to the area of the internal

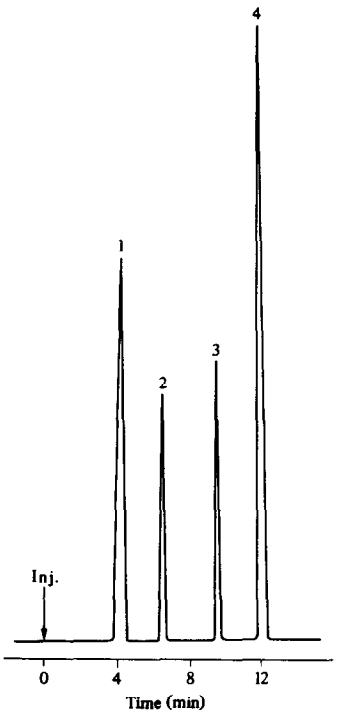

Figure 1
Typical chromatogram of a synthetic mixture containing paracetamol (1), caffeine (2), phenacetin (3) and propyphenazone (4).

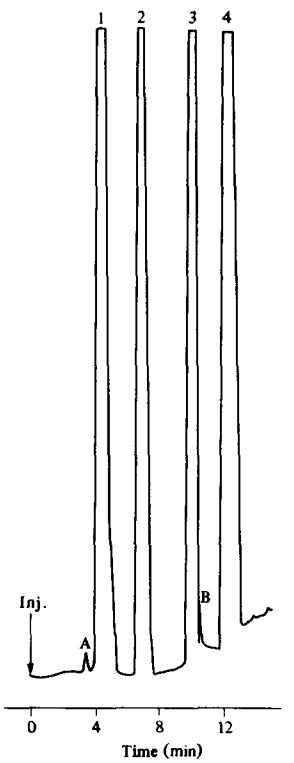

Figure 2
Separation of a synthetic mixture containing paracetamol (1), caffeine (2), phenacetin (3) and propyphenazone (4), spiked with 4-aminophenol (A) and 4-chloroacetanilide (B) at 1% of the paracetamol content.

Table 2
Analysis of acetaminophen, caffeine and propyphenazone in known mixtures

Sample	Paracetamol			Caffeine			Propyphenazone		
	Amount added*	Amount found*	Recovery (%)	Amount added	Amount found	Recovery (%)	Amount added	Amount found	Recovery (%)
1	63.42	63.36 ± 0.24†	99.91	12.68	12.57 ± 0.04	99.13	38.05	38.07 ± 0.15	100.05
2	68.74	68.87 ± 0.18	100.19	13.75	13.65 ± 0.05	99.27	41.24	41.30 ± 0.12	100.15
3	76.10	76.73 ± 0.30	100.08	15.22	15.08 ± 0.03	99.08	45.66	45.64 ± 0.18	99.96
4	87.76	87.99 ± 0.25	100.26	17.57	17.42 ± 0.03	99.15	52.72	52.78 ± 0.20	100.11
5	99.85	100.14 ± 0.16	100.29	19.99	19.89 ± 0.05	99.50	59.98	60.23 ± 0.24	100.42
Mean recovery (%)			100.15			99.22			100.14
RSD (%)			0.15			0.16			0.17

* Total mg/100 ml solution.
† Confidence limits at $p = 0.05$ ($n = 5$).

Table 3
Analysis of acetaminophen, caffeine and propyphenazone in a tablet formulation

Sample	Paracetamol			Caffeine			Propyphenazone		
	Label (mg)*	Found (mg)*	Recovery (%)	Label (mg)	Found (mg)	Recovery (%)	Label (mg)	Found (mg)	Recovery (%)
1	63.42	63.35 ± 0.25†	99.89	12.68	12.57 ± 0.06	99.13	38.05	37.94 ± 0.23	99.72
2	68.42	68.89 ± 0.31	100.22	13.75	13.64 ± 0.05	99.20	41.24	41.10 ± 0.28	99.66
3	76.10	76.23 ± 0.35	100.17	15.22	15.09 ± 0.07	99.14	45.66	45.50 ± 0.34	99.64
4	87.86	88.01 ± 0.33	100.28	17.57	17.45 ± 0.04	99.32	52.72	52.68 ± 0.27	99.92
5	99.85	100.10 ± 0.26	100.25	19.96	19.96 ± 0.05	99.60	59.98	60.05 ± 0.35	100.12
Mean recovery (%)			100.16			99.28			99.81
RSD (%)			0.16			0.20			0.21

* Total mg/100 ml solution.
† Confidence limits at $p = 0.05$ ($n = 5$).

standard was calculated for each chromatogram. Regression analysis of these data of each drug gave the slope, intercept and correlation coefficient for each calibration curve (Table 1). Mixtures containing known quantities of paracetamol, caffeine and propyphenazone in ratios equivalent to those found in a commercial dosage form were studied. The data obtained using equation (1) (Table 2) demonstrate the results obtained. Alternatively the regression data for each drug were used to obtain the concentrations. The values obtained by the two methods were in good agreement. The analytical results from a commercial dosage form (Table 3) indicate that the proposed method can be used for the simultaneous quantitation of these drugs. The method is precise, accurate and free from impurity interference: the chromatogram in Fig. 2 shows that two common contaminants of paracetamol, i.e. 4-aminophenol and 4-chloroacetanilide, were eluted with retention times different from those of the tested components. Other drugs which are often combined with propyphenazone in analgesic tablets have been separated in the same conditions. Figure 3 shows two typical chromatograms showing that paracetamol, caffeine, phenazone, phenacetin, propyphenazone (Fig. 3a) and paracetamol, caffeine, salicylamide, phenacetin, propyphenazone (Fig. 3b) can be effectively separated. In this paper phenacetin has been chosen as the internal standard but it may be replaced by any other cited drugs or, alternatively, by ethylparaben (Fig. 3).

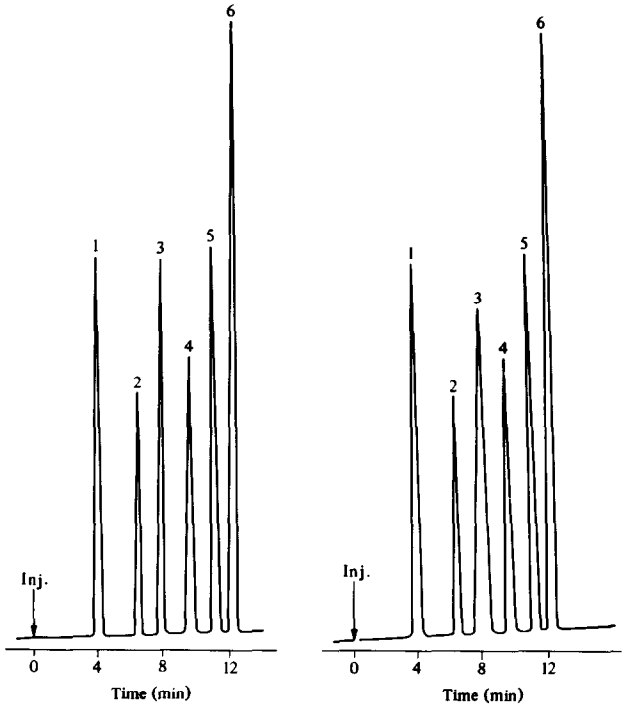

Figure 3
Separation of synthetic mixtures containing (a) paracetamol (1), caffeine (2), phenazone (3), phenacetin (4), propyphenazone (6) and ethylparaben (5), another possible internal standard: (b) paracetamol (1), caffeine (2), salicylamide (3), phenacetin (4), propyphenazone (6) and ethylparaben (5).

References

[1] R. A. Henry and J. A. Schmit, *Chromatographia* **3**, 116–120 (1970).
[2] R. L. Stevenson and C. A. Burtis, *J. Chromatogr.* **61**, 253–261 (1971).
[3] V. D. Gupta, *J. Pharm. Sci.* **69**, 110–113 (1980).
[4] J. T. Stewart, I. L. Honigberg and J. W. Coldren, *J. Pharm. Sci.* **68**, 32–36 (1979).

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