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Short Communication

Simultaneous determination of propyphenazone, paracetamol and caffeine in blood by high-performance liquid chromatography

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Introduction

Propyphenazone (1-phenyl-2,3-dimethyl-4-isopropyl-5-pirazolon), paracetamol (acetaminophenol) and caffeine (1,3,7-trimethylxanthine) are formulated together in medicines such as Pharmazon (Bulgaria) to provide analgesic and antipyretic relief in cases of headache and colds, etc. Analytical methods for the determination of paracetamol [1–6] and caffeine [7–9] in plasma or blood already exist. The simultaneous determination of propyphenazone, paracetamol and caffeine in tablets has been achieved using reversed-phase liquid chromatography and gradient elution [10]. However, gradient elution requires expensive apparatus and often longer analytical times, due to the need to re-equilibrate the column. Normal-phase liquid chromatography can provide better selectivity between compounds with different functional groups that could allow isocratic conditions to be used.

In the present paper a high-performance liquid chromatographic method (HPLC) for the simultaneous determination of propyphenazone, paracetamol and caffeine in blood of rats, using normal-phase HPLC and isocratic elution is reported.

Experimental

Reagents

Propyphenazone, paracetamol, caffeine and phenacetine (analytical-grade purity) were produced by Pharmachim (Bulgaria); acetonitrile (Merck, FRG), n-heptane, isopropanol, methanol and methylenchloride (Fulka, Switzerland) were HPLC-grade.

Blood samples

The blood was collected from rats and placed in tubes containing sodium citrate as anticoagulant.

Standard solutions

Stock solutions of a combined standard propyphenazone, paracetamol and caffeine were prepared in methanol to give a concentration of 50 μg ml^{-1} for each compound. The concentration of phenacetine (internal standard) in methanol was 150 μg ml^{-1}.

Chromatography

HPLC was carried out using a Model Series 4 pump (Perkin–Elmer, USA) with a 20-μl universal loop injector (Rheodyne, USA) and a prepacked LiChrosorb Si-60 (5 μm; 125 × 4 mm i.d.) (Merck, FRG). A pre-column Si-60 (5 μm; 6 × 4.6 mm i.d.) obtained from Waters Assoc. (USA) was connected to the analytical column. The mobile phase was n-heptane–methylenchloride–isopropanol–methanol (85.5:7:4:3.5%, v/v/v/v). The flow rate was 2.3 ml min^{-1}. Detection was by ultraviolet (UV) absorption spectrometry at 266 nm. A model 550 SE spectrometer was connected to an LCI-100 laboratory computing integrator (Perkin–Elmer, USA).

Extraction procedure

Blood (200 μl), internal standard solution (10 μl) and acetonitrile (1.5 ml) were added to a 5-ml tube. The samples were shaken for 15 min, then centrifuged for 3 min at 6000**g**. The organic phase was aspirated and evaporated to dryness at 45°C under a stream of nitrogen. Residues were dissolved in 200 μl of eluent and 20-μl aliquots were injected into the chromatograph. Standard calibration curves of propyphenazone, paracetamol and caffeine over the range 1–10 μg ml^{-1} were prepared using drug-free blood samples. An appropriate volume of the standard solution was transferred into the 10-ml tube prior to the sample preparation procedure described above.

Results and Discussion

Typical chromatograms of a drug-free blood and blood extract, containing propyphenazone, paracetamol, caffeine and the internal standard are shown in Fig. 1. A good

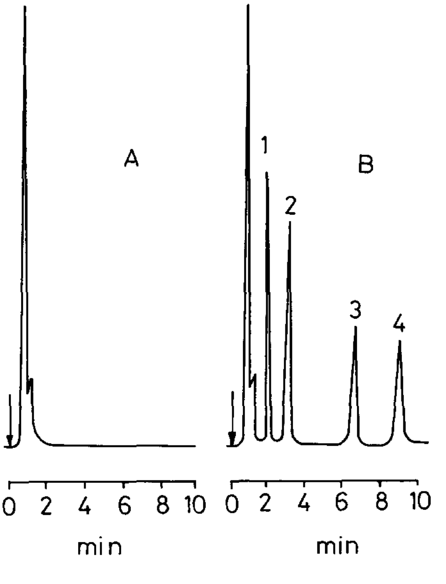

Figure 1
Chromatograms obtained by injecting 20 μl of extract from a drug-free blood (A) and extract from rat's blood (B). Peaks: 1, propyphenazone (4 μg ml^{-1}); 2, phenacetine (internal standard); 3, caffeine (5 μg ml^{-1}); 4, paracetamol (7.5 μg ml^{-1}).

separation was obtained in 8–9 min. Table 1 lists the retention times and capacity factors for the compounds studied and some potential interferents such as theophyline and β-OH-ethyltheophyline. As seen in Table 1 there is no interference of the compounds given.

Quantification of propyphenazone, paracetamol and caffeine was achieved by comparing their respective peak-height ratios to internal standard in rat samples to those of known samples of the standard curve. Recoveries were determined by comparing the peak-heights from the extracted samples without internal standard with those obtained from a direct injection of the same amount of drug in methanol. Table 2 illustrates the recovery and within-day precision data obtained by the analysis of spiked blood samples.

Calibration curves were consistently linear from 1.0 to 10 μg ml^{-1} for all compounds.

The detection limit of sensitivity for propyphenazone, paracetamol and caffeine was 5, 15 and 10 ng, respectively.

Normal-phase liquid chromatography showed high selectivity for the separation of propyphenazone, paracetamol and caffeine. The use of a silica-gel column allows the isocratic elution mode of the compounds studied and gives a good within-day precision of the retention times ($v < 0.5\%$). The column has long-term stability for the assay — after 3 months work the efficiency changes not more than 15%.

In conclusion, a selective and quick normal-phase HPLC method has been developed for monitoring propyphenazone, paracetamol and caffeine using phenacetine as internal standard during pharmacokinetic studies.

Table 1
Elution times for propyphenazone, paracetamol, caffeine, phenacetine (internal standard) and some potential interferents

Compound	Capacity factor, k'	Retention time, t_R (min)
Propyphenazone	2.2	1.9
Paracetamol	13.8	8.9
Caffeine	9.7	6.4
Phenacetine	3.8	2.9
Theophyline	6.2	4.3
β-OH-ethyltheophyline	16.0	10.2

Table 2
Recovery of propyphenazone, paracetamol and caffeine from rat's blood and within-run assay reduction

Compound	Concentration (μg ml^{-1})	Recovery (%)	% RSD
Propyphenazone	1.0		4.5
	5.0	99.0	4.1
	10.0		4.0
Paracetamol	1.0		4.0
	5.0	98.5	3.8
	10.0		3.6
Caffeine	1.0		4.2
	5.0	96.5	4.0
	10.0		3.9

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