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Note

Liquid chromatographic determination of paracetamol and dextropropoxyphene in plasma

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The combination of paracetamol and dextropropoxyphene is used in the treatment of mild-to-moderate pain. The commonly used dosage form contains 325 mg paracetamol and 32.5 mg dextropropoxyphene. Estimation of plasma concentrations of paracetamol has been described using both gas chromatography [1] and high-performance liquid chromatography (HPLC) [2]. Peak plasma concentrations following 325 mg are of the order of 3–6 μg/ml. Concentrations of dextropropoxyphene after administration of a therapeutic dose are low. Administration of 130 mg to six subjects produced a mean maximum concentration of 0.27 μg/ml [3]. The dose used in combination products would be expected to result in peak concentrations well under 0.1 μg/ml. Gas chromatography has been employed for its assay but there are indications of degradation under the conditions used [4]. Mass fragmentography has also been utilised [5] but this technique is not available to the majority of laboratories. Dextropropoxyphene has no useful UV absorbance to enable use of HPLC with detection at the concentrations encountered during therapeutic use.

We describe an assay procedure which requires single one 1-ml specimens of plasma and extracts dextropropoxyphene first, followed by paracetamol. The separate extracts are subjected to HPLC. Paracetamol is detected by UV absorbance and dextropropoxyphene by electrochemical means. The assay is sufficiently sensitive to allow pharmacokinetic evaluation of single doses of paracetamol/dextropropoxyphene combinations.

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EXPERIMENTAL

Reagents

Dichloromethane, diethyl ether and hexane were all HPLC grade from Labscan (Dublin, Ireland). Ethyl acetate, perchloric acid and potassium dihydrogen orthophosphate were all Analar grade from BDH (Poole, U.K.). Methanol was HPLC grade from Rathburn (Walkerburn, U.K.). Sodium hydroxide was laboratory reagent grade from May and Baker (Dagenham, U.K.). Water was reverse osmosis purified (Elga, High Wycombe, U.K.) Paracetamol, dextropropoxyphene, salicylamide and verapamil were purchased from Sigma (Poole, U.K.).

Extraction procedure

Aliquots of plasma (1 ml) were placed in flat-bottomed, glass, screw-capped tubes. Verapamil, 100 ng (internal standard for dextropropoxyphene), and salicylamide, 10 μg (internal standard for paracetamol), were each added. Verapamil was contained in 0.1 ml methanol–water (1:1, v/v) and salicylamide was contained in 0.1 ml water. Specimens were mixed and 0.1 ml of 0.1 *M* sodium hydroxide was added. Diethyl ether–hexane (1:1, v/v; 4 ml) was added and the mixture shaken for 10 min. The layers were separated by centrifuging (1500 *g*, 10 min) and 3 ml of the ether supernatant transferred to conical-bottomed glass tubes. The aqueous portions were retained. The extracts were evaporated to dryness at 50°C under nitrogen. The dried extracts were reconstituted in 0.2 ml dextropropoxyphene mobile phase and 25-μl aliquots injected. The retained aqueous–organic mixtures were now used for paracetamol extraction. A 1-ml volume of a 1/15 *M* KH_2PO4 solution (pH 5) was added to each tube and after mixing, 5 ml ethyl acetate were added. The mixtures were shaked for 8 min and centrifuged (1500 *g* for 5 min). Aliquots (2 ml) of the organic extraction mixtures were transferred to conical-bottomed glass tubes and evaporated to dryness at 50°C under nitrogen. The dried extracts were reconstituted in 0.2 ml of paracetamol mobile phase and 30-μl aliquots injected.

Chromatography of dextropropoxyphene

Mobile phase. To 350 ml of perchloric acid (10 m*M* in methanol) was added slowly and with mixing 0.1 *M* sodium hydroxide in methanol (approximately 30–35 ml) to pH 6.8. To this were added 30 ml dichloromethane. This was mixed well and purged with helium before use.

Chromatography. The column was a Spherisorb 5SW silica column (5 μm particle size, Phase Separations, Queensferry, U.K.), 25 cm × 4.6 mm I.D. The system comprised a Waters Model 710B autosampler, a Shimadzu Model LC5A pump, a BAS Model LC4B electrochemical detector at a potential of 1.2 V and an attenuation of 20 nA f.s.d. and a Shimadzu Model CR3A integrator. The mobile phase flow-rate was 3.0 ml/min at ambient temperature.

Chromatography of paracetamol

Mobile phase. Methanol (190 ml) was mixed with 810 ml of 1/15 *M* KH_2PO4 solution (pH 5). This was filtered and degassed before use.

Chromatography. The column was a Spherisorb 5ODS column (5 μm particle

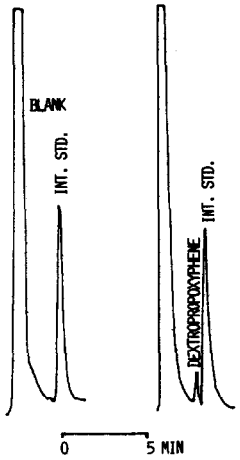

Fig. 1. Typical chromatograms showing extracts from a blank specimen and from a specimen containing 12 ng/ml dextropropoxyphene.

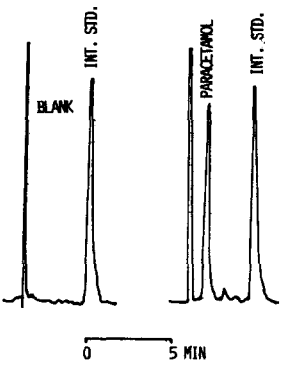

Fig. 2. Typical chromatograms showing extracts from a blank specimen and from a specimen containing 1.1 μg/ml paracetamol.

size, Phase Separations), 25 cm × 4.6 mm I.D. The system comprised a Waters Model 710B autosampler, a Varian Series 5000 HPLC system with a 254-nm detector and a Shimadzu Model CR3A integrator. The mobile phase was delivered at 2.0 ml/min and the column was thermostatted at 30°C. The attenuation settings were 0.002 a.u.f.s. for the detector and 8 for the integrator.

RESULTS AND DISCUSSION

Dextropropoxyphene

Under the conditions described, the integrator baseline was practically noiseless. This is illustrated in Fig. 1, where a typical chromatogram is shown. Typical calibration lines had correlation coefficients of 0.997 or greater. Inter-assay variability was assessed over six days by extracting on each day quality-control specimens spiked with 12.0, 26.5 and 53.5 ng/ml dextropropoxyphene. Coefficients of variation were 9.1, 7.1 and 5.9%, respectively. The limit of determination was taken as 2 ng/ml. This gave a peak in excess of five times any baseline noise. Recovery of dextropropoxyphene was 78%.

Paracetamol

Fig. 2 illustrates typical chromatograms. As for dextropropoxyphene this shows chromatography of an extract obtained from plasma after administration of a paracetamol/dextropropoxyphene combination. Also as for dextropropoxyphene, calibration lines had correlation coefficients of 0.997 or better. Inter-assay variability was measured over six days at levels of 0.75 and 3.02 μg/ml paracetamol. Coefficients of variation were 11.7 and 5.6%, respectively. The limit of deter-

TABLE I

PHARMACOKINETICS OF A PARACETAMOL/DEXTROPROPOXYPHENE COMBINATION IN TEN SUBJECTS

Compound		Peak concentration (μg/ml)	Half-life (h)
Paracetamol	Mean	3.58	1.77
	S.D.	1.16	0.43
	Range	1.95-5.17	1.00-2.37
Dextropropoxyphene	Mean	26	3.74
	S.D.	9.7	2.50
	Range	10-42	1.81-9.37

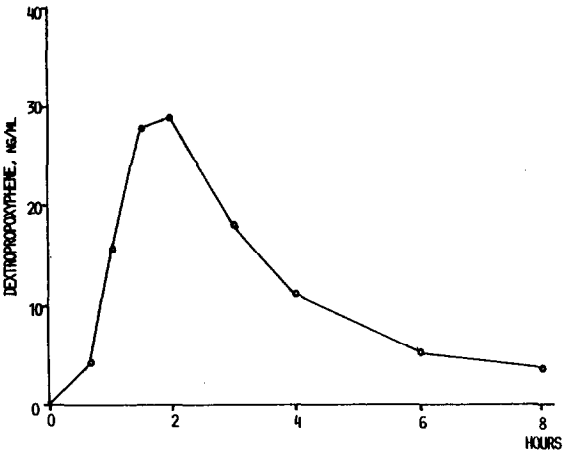

Fig. 3. Plasma concentrations of dextropropoxyphene in a typical subject.

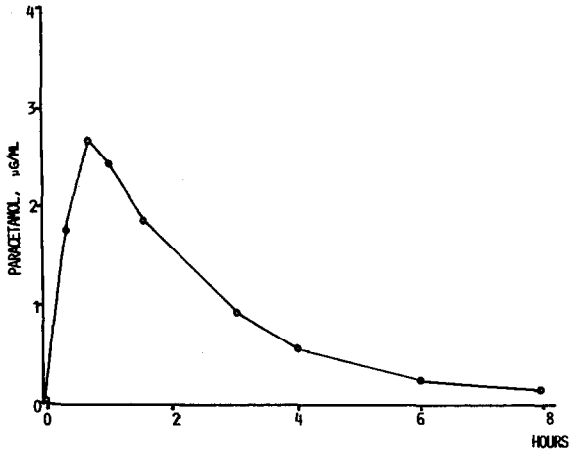

Fig. 4. Plasma concentrations of paracetamol in a typical subject.

mination was taken as 0.1 μg/ml (greater than five times any baseline noise). Recovery of paracetamol after the dextropropoxyphene pre-extraction was 90%.

Pharmacokinetic studies

The commonly used form of this product contains 325 mg paracetamol and 32.5 mg dextropropoxyphene. There is a great disparity in the resulting plasma concentrations of the two components. A single dose containing the quantities above was given to ten volunteer subjects (Table I). The mean peak concentration of paracetamol was 3.58 μg/ml. The mean peak concentration of dextropropoxyphene was 26 ng/ml. This represents a greater than 100-fold difference in the concentrations of two components. Paracetamol had a mean half-life of 1.7 h and dextropropoxyphene a mean half life of 3.9 h (calculated by least-squares regression analysis of the terminal portion of the log concentration–time graphs).

Figs. 3 and 4 show plasma concentrations of the two subtances at various times after administration of the combination product to one subject. The sensitivity of the assays allows adequate definition of the pharmacokinetic properties of the two substances.

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