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Note

High-performance liquid chromatographic method for the analysis of benztropine in human plasma

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Benztropine mesylate is an anticholinergic drug used in the treatment of Parkinsonism. It may be given orally in doses of 1–4 mg once or twice daily but may not exceed 6 mg daily. While there are a number of methods analyzing benztropine mesylate tablets and injectables [1–4], reports on plasma levels of the drug are very rare. Jindal et al. [5] published a paper on gas chromatography–mass spectrometry of the subject but their method achieved a lower limit of quantitation of 5.0 ng/ml and hence lacked the required sensitivity.

This paper reports a more sensitive and simple method, which could be used for a 2.0-mg single-dose pharmacokinetic study of benztropine mesylate.

EXPERIMENTAL

Reagents

Benztropine mesylate was supplied by Sigma (St. Louis, MO, U.S.A.), while desipramine hydrochloride was supplied by Nucro Technics (Rockville, MD, U.S.A.). Phosphoric acid (85%), sodium hydroxide (5 *M*), hexane and triethylamine were of HPLC grade and supplied by Fisher Scientific (Montreal, Canada). HPLC-grade acetonitrile was supplied by Anachemia Canada (Lachine, Canada), reagent-grade ethylene glycol by Caledon (Georgetown, Canada) and Spherisorb C_8 was obtained from Phase Separations (Norwalk, CT, U.S.A.).

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Chromatographic conditions

The chromatographic system consisted of a Waters Model 590 programmable solvent delivery module, a Waters Lambda Max Model 481 UV detector, a Waters WISP 710B autosampler, an analytical column, 150 mm × 3.9 mm, in-house packed with C_8 Spherisorb, 5 μm grain size, and a Spectra Physics 4270 integrator. The data were collected, processed and the regressions run on a Spectra Physics Chrom Station. The detector was set at 199 nm. The flow-rate was 1.5 ml/min with a resulting back-pressure of 75–110 bar at room temperature. Total run time was 9 min.

Mobile phase

Triethylamine (1.5 ml) was diluted with deionized water to a volume of 1 l and adjusted to pH 3.0 by the dropwise addition of 85% phosphoric acid. An 800-ml volume of this buffer was mixed with 1200 ml of acetonitrile, stirred well and filtered through a 0.45-μm Nylon filter.

Standard and quality control preparation

The standard samples in plasma were prepared by adding the appropriate volume of aqueous spiking solution of benztropine mesylate to human plasma containing EDTA as an anti-coagulant. The volume added was always less than or equal to 2% of the total volume of the sample so that the integrity of the plasma was maintained. Quality control samples were prepared in the same way using separately weighed stock solutions. After aliquoting, 2-ml samples were stored at −15°C until required.

Method of extraction

Standard or quality control plasma samples (2 ml) were added to 150 μl of 200 ng/ml desipramine hydrochloride and the sample was basified with 150 μl of 5 *M* sodium hydroxide. After vortex-mixing, 1 ml of ethylene glycol was added, vortex-mixed and then the sample extracted with 10 ml of hexane (30 min on a rotary shaker at 30 rpm). The sample was centrifuged (1000 *g* at 4°C) and the organic layer transferred into a conical tube containing 0.1 *M* hydrochloric acid (300 μl). Benztropine was back-extracted into acid by shaking on an Eberbach shaker for 20 min at high speed. After centrifuging and aspirating the upper organic layer, 200 μl of the lower phase was injected into the chromatographic system.

RESULTS AND DISCUSSION

A set of seven calibration standards, a zero, a blank and three sets of quality controls were analyzed each analytical day. A linear regression describing the calibration curve was calculated using the reciprocal of the drug concentration as weight.

Table I shows precision and accuracy data. Within the range 0.25–5.0 ng/ml, a linear regression line was obtained for the correlation coefficient which ranged from 0.9921 to 0.9985. On two occasions, an extended calibration curve was run, proving that the calibration curve is linear at least up to the concentration 12 ng/ml.

In order to evaluate within-run precision and accuracy and also in-injector stability, a 'Big Batch' of three full standard curves plus quality controls in replicate were extracted and injected. All together, 54 samples were extracted and injected over a period of 10 h.

Coefficients of variation (C.V.) of the between-run precision had ranges of 3.9–12.2% (Table I). Accuracy, expressed as a percentage of nominal, had a range of 90.6–108.8%.

Extracted samples are stable at room temperature for at least 10 h; even after 18 h, neither degradation nor change in peak-height ratios was observed. The efficiency of the extraction is high (ca. 70%) for both the drug and the internal standard.

Fig. 1 shows chromatograms of blank plasma, plasma containing benztropine at 0.25 ng/ml (limit of quantification) and plasma from a patient taking 2 mg of the drug once a day (blood taken 12 h after the last dose, 6.7 ng/ml). The retention times of the drug and internal standard were 7.0 and 5.3 min, respectively.

In order to evaluate the possibility of interference from other drugs used in Parkinsonism therapy with benztropine, several of them were injected into the system. Their capacity factor k' is listed in Table II. None of these drugs interfered with benztropine.

It is worth noting the extracting technique involving ethylene glycol as an intermediate. Benztropine mesylate can be easily extracted from pure basified water with hexane or other organic solvents. However, without the use of an intermediate such an extraction is impossible from plasma. Benztropine, in significant amounts, is retained in the plasma by non-specific binding. The first step of extraction requires freeing the drug from these sites by adding an

TABLE I

BETWEEN-RUN ACCURACY AND PRECISION OF THE METHOD FOR THE DETERMINATION OF BENZTROPINE IN HUMAN PLASMA

Quality control sample concentration (ng/ml)	n	Concentration found (mean ± S.D.) (ng/ml)	C.V (%)	Percentage of nominal concentration
0.60	9	0.621 ± 0.0757	12.2	103.5
2.50	10	2.444 ± 0.2211	9.0	97.8
4.50	9	4.577 ± 0 2597	5.7	101.7

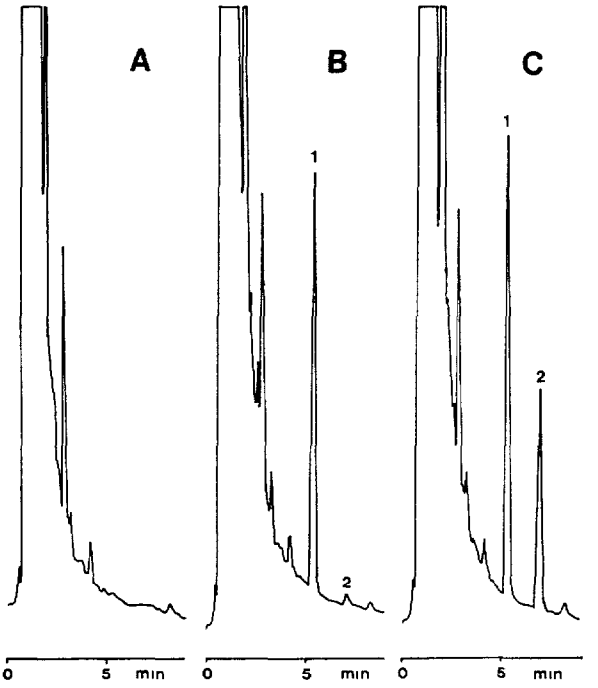

Fig. 1. Chromatograms of (A) blank human plasma, (B) plasma spiked with 0.25 ng/ml benztropine and (C) plasma from a patient (benztropine concentration, 6.7 ng/ml). Peaks: 1 = internal standard (desipramine); 2 = benztropine. a.u.f.s. = 0.005; integrator attenuation = 16.

TABLE II

CAPACITY FACTORS k' OF ANTIPARKINSONISM DRUGS IN CHROMATOGRAPHIC CONDITIONS OF THE METHOD

Compound	Capacity factor
Amantadine	0.00
Carbidopa	0.00
Levodopa	0.00
L-Hyoscyamine	0.36
Orphenadrine	3.90
Bromocriptine	4.17
Biperiden	4.21
Benztropine	5.55

organic solvent miscible with water. In the second step, the drug is extracted with an organic solvent, which must in turn be immiscible in part with the intermediate. Otherwise, an emulsion will form or the extract will be very contaminated. This sets limits on possible combinations of solvents. In this case,

the best ones are extremely non-polar pentane or hexane, while those of intermediate value include various glycols, dimethylformamide or even methanol and acetonitrile. We observed, however, that, as the latter two precipitate proteins, the extracts are much dirtier than in the case of ethylene glycol, which is not. This phenomena has been recorded in our laboratory on several occasions.

Some authors recommend saturating plasma samples with inorganic salts before extraction, in order to break down weak binding between drug and proteins. The binding process described above must be of a different nature, since the addition of inorganic salts did not result in the freeing of benztropine mesylate.

CONCLUSIONS

The method described above is simple, reliable and sensitive enough to be used in a single-low-dose benztropine mesylate pharmacokinetic study. In order to obtain meaningful pharmacokinetic data, plasma levels should be monitored for a period of three to four half-lives. The lower limit of quantitation of 0.25 ng/ml is adequate to achieve this goal. The method may be applied to the study of pharmacokinetics of the drug and therapeutic drug monitoring.

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