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Short Communication

Quantitation of trihexyphenidyl from plasma using a mass-selective detector and electron-impact ionization

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ABSTRACT

A method is described for the measurement of plasma concentrations of trihexyphenidyl, an anti-parkinsonian drug. The drug was extracted from human plasma samples. Then, gas chromatography–mass spectrometry with electron-impact ionization and selected-ion monitoring allowed the specific quantitation of trihexyphenidyl, with bupivacaine used as an internal standard. Linear calibration curves were obtained in the concentration range 5–100 ng/ml. Precision and accuracy were found acceptable for quantitation during pharmacokinetic trials of the drug. This method has been successfully applied to bioavailability studies after Parkinane and Artane administration to humans.

INTRODUCTION

Trihexyphenidyl (THP) has been used for many years in the treatment of Parkinson's disease. It is an anticholinergic drug [1] that displays the same pharmacological properties as atropine. THP is used effectively in the predisposition of neuroleptic extrapyramidal risks, the Parkinson syndrome, the excited and motive crisis due to neuroleptic drugs administered in Parkinson's disease. The drug is said to be rapidly excreted and not to accumulate in tissues [2]. THP undergoes extensive metabolism and *ca.* 60% of the dose is excreted in urine as hydroxylated metabolites.

Analytical methods [2–5] used for THP quantification from plasma and/or serum samples are based on gas chromatography with nitrogen–phosphorus-

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selective detection. They are considered to lack the specificity and sensitivity needed to detect THP in body fluids at low levels.

The aims of this work were to develop a gas chromatographic–mass spectrometric (GC–MS) method for the measurement of THP in plasma and/or serum after a single-dose administration for bioequivalence studies, and to validate the method.

EXPERIMENTAL

Reagents and chemicals

Methanol, HPLC grade (Carlo Erba, Milan, Italy), was used to prepare THP and bupivacaine (BUP) stock and working solutions. Heptane, HPLC grade (Aldrich, Strasbourg, France), was used as the extraction solvent. Ethanol, RPE-ACS (Reagent Puro Erba-American Chemical Society) (Carlo Erba), was used to dissolve the dry extraction residues. Trihexyphenidyl hydrochloride was obtained from Lederle Labs. (Oullins, France). BUP, the internal standard, was obtained from Roger Bellon Labs. (France). Parkinane L. P. and Artane were obtained from Lederle Labs. and Theraplix Labs. (Paris, France), respectively.

Stock solutions

Stock solutions of THP and BUP were prepared in methanol at 100 μg/ml, and stored at 4°C. Working solutions were prepared just before use.

Extraction

The internal standard solution (200 ng/ml) was added to 0.5 ml of serum and mixed by shaking for 10 s. The mixture was extracted after addition of heptane (4 ml), using an automatic shaker for 2 min. After centrifugation (2000 *g*, 5 min), the organic phase was recovered and evaporated to dryness under a stream of nitrogen at room temperature. The dry residue was kept at 4°C until GC–MS analysis.

Chromatographic conditions

The dry residue was dissolved with 30 μl of ethanol, and a 3-μl aliquot was injected into the chromatographic column. The gas chromatograph was a Hewlett Packard Model 5790, equipped with a BP1 methyl silicone capillary column (12.5 m × 0.22 mm I.D., film thickness 0.12 μm, Alltech, Templeuve, France). Samples were injected in the splitless mode (valve time 0.75 min). The injector port was set at 250°C, and the oven temperature was raised from 90°C (45 s) to 270°C (3 min) at 25°C/min. The transfer line between the gas chromatograph and the mass-selective detector was set at 260°C. Helium was used as carrier gas with a head pressure of 0.4 kg/cm^2.

Detection and measurement

A Hewlett Packard mass-selective detector (HP 5970 A) operating between 7

and 9 min after the run start was used for detection. The monitored ions were *m/z* 98 (for quantitation) and *m/z* 218 (as qualifier ion) for THP, and *m/z* 140 for BUP. Fig. 1 shows the structures of the two molecules and of the main ion fragments monitored for their assay. Mass spectra of the two compounds are shown in Fig. 2. Mass spectrometer operations and data processing were under the control of an HP 59970 MS Chemstation.

RESULTS

Chromatographic separation and mass spectra

Fig. 3 shows the total ion current recorded from a blank plasma (a) and from a plasma spiked with THP (100 ng/ml) and BUP (200 ng/ml) (b). Fig. 4 displays the fragmentogram of the monitored ions at *m/z* 98, *m/z* 218 (Fig. 4a and b) and *m/z* 140 from a plasma extract (Fig. 4c). These two chromatographic peaks, with respective absolute retention times of 8.05 and 8.18 min, correspond to THP and BUP, respectively. The total ion current corresponding to the addition of all ion currents between *m/z* 35 and *m/z* 400 and obtained from the same plasma of a subject treated with THP is shown in Fig. 4d.

Linearity

Various serum samples spiked with THP at concentrations of 5, 10, 20, 50 and 100 ng/ml and with BUP (200 ng/ml) were prepared and assayed according to the procedure described above. A linear regression analysis of the values (peak-area ratio *versus* concentration) indicated a good linear fit of the data with slope 1.51, an intercept of 0.00899 and $r^2 = 0.999$.

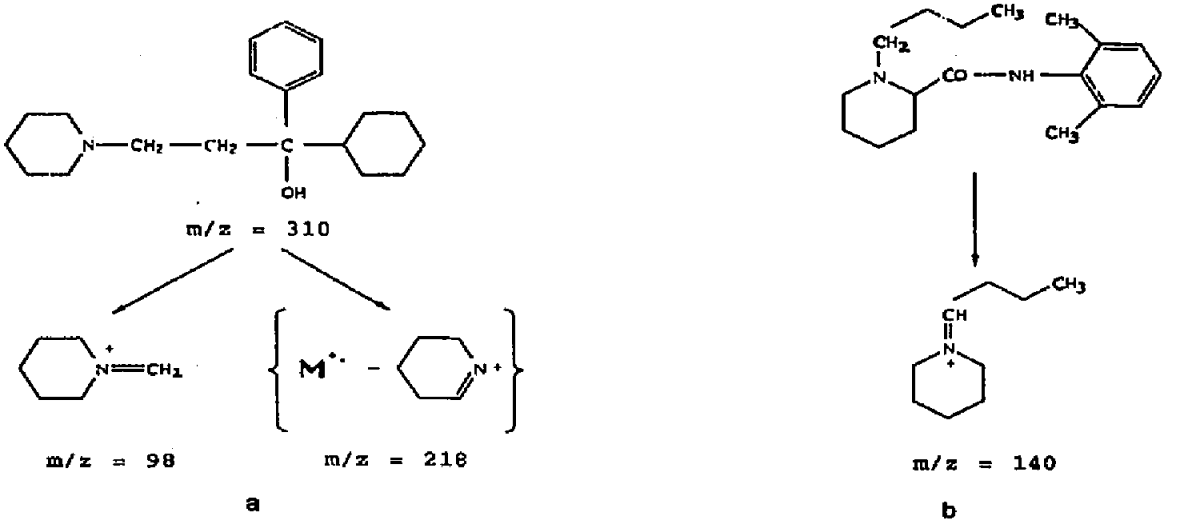

Fig. 1. Structures of trihexiphenidyl (a) and bupivacaine (b) and of the main ion fragments monitored for THP assay.

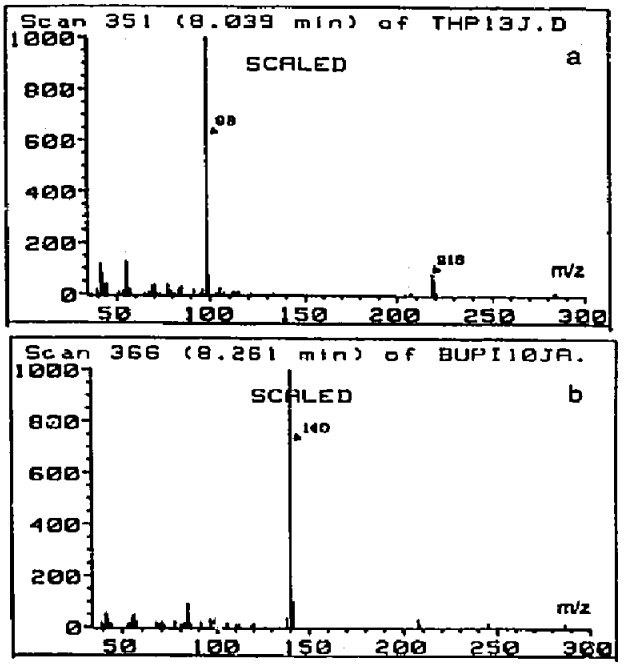

Fig. 2. (a) Normalized (scaled) mass spectrum of trihexiphenidyl obtained by GC–MS and electron-impact ionization from a pure solution. (b) Normalized mass spectrum of the internal standard, bupivacaine, obtained by GC–MS and electron-impact ionization from a pure solution.

Precision

The precision of the method was estimated by the measurement of both the repeatability and the reproducibility within the therapeutic concentration range. The repeatability was studied by measuring THP concentrations for five serum samples spiked with 10, 50 or 100 ng/ml. These measurements were performed on

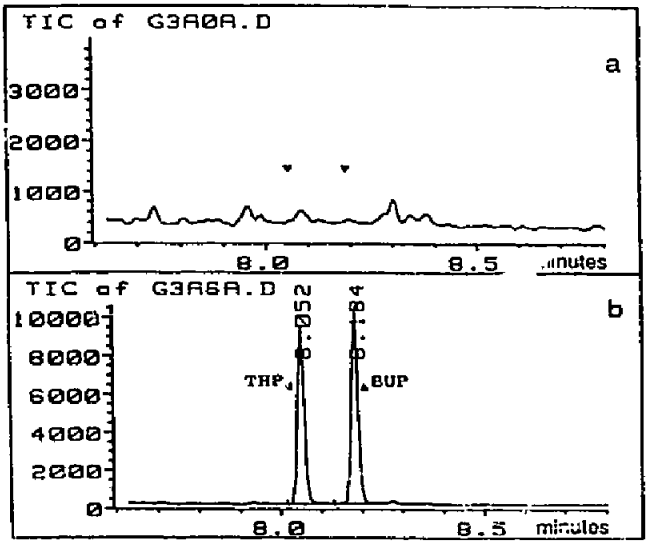

Fig. 3. (a) Total ion current from a blank plasma. (b) Tot ion current from a plasma spiked with THP (100 ng/ml) and BUP (200 ng/ml).

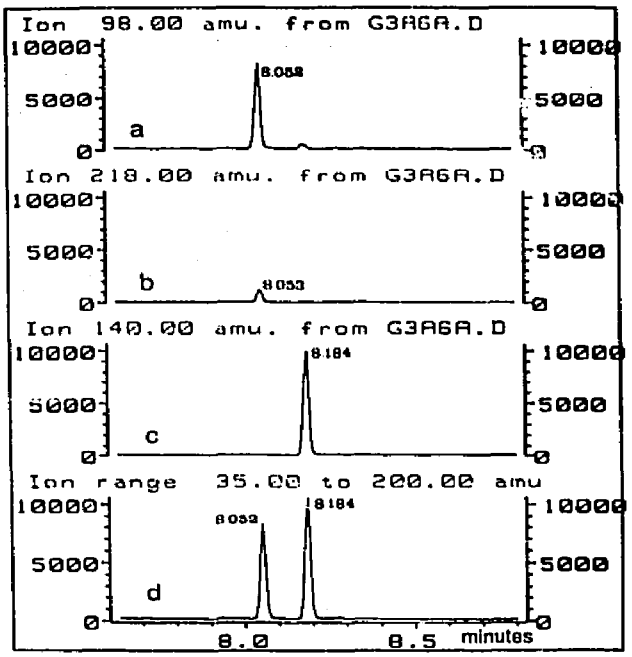

Fig. 4. Fragmentograms of (a and b) ions at *m/z* 98 and 218 for THP and (c) at *m/z* 140 for BUP from the plasma of a patient treated with THP. (d) Total ion current from the same plasma sample.

the same day and under the same experimental conditions. The results are reported in Table I.

The reproducibility was evaluated according to the same procedure as for the repeatability determination, but over a five-week period. The range of concentrations was 5–50 ng/ml. Throughout this period, the mean coefficient of variation (C.V.) for THP measurements was 10.96% for 5 ng/ml and 5.25% for 50 ng/ml.

Accuracy

The comparison (correlation analysis by linear regression) of the measured concentrations against target concentrations in the range 5–100 ng/ml for four different spiked plasma samples gave the following results:

Slope: 1.030 not significantly different from 1 ($p < 0.01$)

Intercept: 0.0702 not significantly different from 0 ($p < 0.01$)

$r^2 = 0.999$

TABLE I

REPEATABILITY OF THP DETERMINATION

Concentration (ng/ml)	Peak-area ratio (*m/z* 98)/(*m/z* 140)					Mean ± S.D.	C.V. (%)
10	0.0875	0.0860	0.0916	0.0968	0.0946	0.0913 ± 0.0041	4.48
50	0.4415	0.4309	0.4222	0.4319	0.4218	0.4297 ± 0.0073	1.69
100	0.7695	0.7904	0.8165	0.8048	0.8178	0.7998 ± 0.0181	2.26

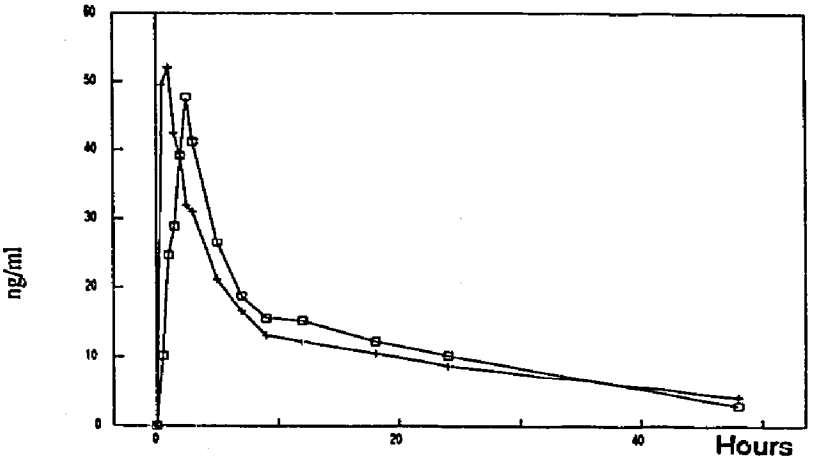

Fig. 5. Plasma concentration of THP *versus* time curve after oral administration of Parkinane L.P. (□) or Artane (+) to a human subject.

Limit of detection

The limit of detection was taken as the lowest concentration yielding an integrated area corresponding to ten times the area measured in the same integration window after injection of an extract from a blank serum. For THP this limit was *ca.* 1 ng/ml.

Example of human kinetics

This method has been used for the quantitative determination of THP in human serum after oral administrastion of 15 mg of either Parkinane L.P. or Artane, in order to study and compare the pharmacokinetics of these pharmaceutical preparations. Fig. 5 shows the THP mean serum levels *versus* time curve obtained from eleven subjects after administration of one Parkinane L.P. 15-mg capsule or one Artane 15-mg capsule.

CONCLUSION

The GC–MS method developed was found to be suitable for the measurement of the concentrations of THP from plasma samples collected during pharmacokinetic and biopharmaceutical trials. The analytical parameters were sufficient for the accurate determination of bioavailability and bioequivalence between pharmaceutical preparations containing THP. Moreover this technique, using GC separation and mass-selective detection, is sensitive enough for such trials and does not need chemical ionization. The use of the ion at m/z 98 for THP quantitation, associated with the ion at m/z 218 as qualifier ion, makes the method selective for accurate THP determination.

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