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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF PLASMA OXYBUTYNIN USING A DEUTERATED INTERNAL STANDARD

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SUMMARY

A gas chromatographic–mass spectrometric method is described for the quantitative analysis of plasma oxybutynin. Deuterated oxybutynin served as the internal standard and its synthesis is described. Chromatographic separation on a methylsilicone capillary column avoided the thermal decomposition observed using a packed column. Electron-impact ionization and selected-ion monitoring of the α-cleavage fragments of drug and internal standard permitted quantitation of oxybutynin down to 0.25 ng/ml of plasma. At the 2 ng/ml level the accuracy and precision are 4 and 10%, respectively, and improved at higher drug concentrations. Application of the method to the pharmacokinetics of oral oxybutynin in man demonstrated rapid absorption and elimination of the drug.

INTRODUCTION

Oxybutynin is a synthetic anticholinergic drug with underlying structural elements in common with atropine. It is efficacious in the treatment of various bladder control problems and appears to provide a slightly longer duration of action than the prototypic agent propantheline. This may permit less frequent dosing with oxybutynin [1]. The anticholinergic side-effects have limited the use of such agents in many individuals [2]. It has not been established whether the frequent intolerance to oxybutynin is due to individual differences in drug bioavailability or pharmacodynamics.

After a dose of oxybutynin, circulating concentrations are so low that thera-

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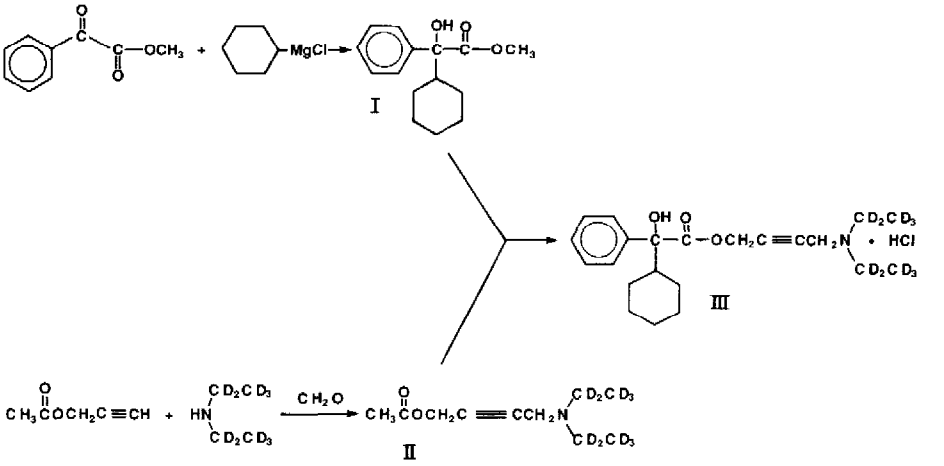

Fig. 1. Synthetic route for the preparation of the internal standard [$^2H_{10}$]oxybutynin hydrochloride, compound III.

peutic drug monitoring studies have previously not been possible [3]. A procedure for the quantitation of oxybutynin extracted from dog serum has been published [4]. The method involves packed column gas chromatography (GC) which risks thermal decomposition of this labile drug (see Results and discussion). Further, the assay gives inadequate separation from a purported metabolite, the retention time of the internal standard limits sample throughput, and the method was not validated at therapeutic serum concentrations.

The present study describes a sensitive capillary gas chromatographic–mass spectrometric (GC–MS) method for the analysis of therapeutic plasma concentrations of oxybutynin. A deuterated internal standard (Fig. 1) was incorporated to enhance accuracy and precision. Application of the method to the evaluation of the pharmacokinetics of oxybutynin after administration of a single oral dose in man is reported.

EXPERIMENTAL

Chemicals and reagents

Diethyl ether, heptane and hexane were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Methanol and toluene were from Mallinckrodt (St. Louis, MO, U.S.A.). Isoamyl alcohol and acetonitrile were from Fisher Scientific (Fairlawn, NJ, U.S.A.). Oxybutynin hydrochloride was provided by Sidmak Labs. (East Hanover, NJ, U.S.A.). Oxybutynin hydrochloride 5-mg tablets (Ditropan®) were from Marion Labs. (Kansas City, MO, U.S.A.). Dimethyldichlorosilane, cyclohexylmagnesium chloride, methyl benzoylformate, paraformaldehyde and sodium methoxide were obtained from Aldrich (Milwaukee, WI, U.S.A.). The [$^2H_{10}$]diethylamine was purchased from MSD Isotopes (Montreal, Canada). Propargyl acetate was from Wiley Organics (Columbus, OH, U.S.A.).

Synthesis of [$^2H_{10}$]oxybutynin

Methyl cyclohexylphenylglycolate (I). Compound I was synthesized using the Grignard conditions of Ryan and Ainsworth [5] with the exception that commercially available cyclohexylmagnesium chloride was substituted for the corresponding bromide.

4-[$^2H_{10}$]Diethylamino-2-butynyl acetate (II). The Mannich condensation procedure of Lindeke et al. [4] was used with an equivalent of [$^2H_{10}$]diethylamine substituted for piperidine.

[$^2H_{10}$]Oxybutynin hydrochloride (III). Using the method of Majewski et al. [6], compounds I and II were condensed by an ester–ester exchange. Uncorrected m.p. 120–122 °C. Mass spectrum, see Fig. 2.

Plasma sample preparation

Screw-top centrifuge tubes (15 ml) were treated with a 10% solution of dimethyldichlorosilane in toluene, then rinsed with methanol and oven-dried. The caps were fitted with PTFE liners. A 1-ml aliquot of plasma was added to each tube, and the pH was adjusted to 1.5–2.0 by adding 1 ml of 0.5 *M* hydrochloric acid containing 12.6 ng of deuterated oxybutynin (calculated as the free base). The tubes were extracted with 3 ml of diethyl ether–hexane (3:1) by vortexing for 10 s, then shaking horizontally for 10 min. After centrifugation (500 *g*) for 15 min, the organic phase and interfacing film were aspirated to waste, then the aqueous phase was alkalinized to pH 9.0–9.5 using 1 ml of 0.75 *M* sodium carbonate buffer. Heptane–isoamyl alcohol (98:2), 3 ml, was added to each tube and the samples were extracted by vortex-mixing (3×5 s), then shaking horizontally for 15 min. The organic phases were transferred to disposable 4-ml screw-cap vials fitted with PTFE cap liners. The organic phases were evaporated under streams of nitrogen using gentle heating from a hot air gun, then the vials were capped and refrigerated (5 °C) until analysis.

Instrumentation and analysis

All GC–MS analyses utilized a Finnigan Model 9610 gas chromatograph–4000 mass spectrometer interfaced to an IBM-AT computer using a Teknivent Vector/One data system and software (St. Louis, MO, U.S.A.). Fragmentation was accomplished by electron impact at 35 eV ionizing voltage and 250 μA ionizing current (an ionizing voltage of 70 eV at 350 μA provided similar results). The electron multiplier was operated at 1850 V. The instrument was calibrated daily using perfluorotributylamine. The data system acquired two channels of selected-ion current: that at *m/z* 342 representing the demethylated oxybutynin fragment and that at *m/z* 349 (de-[$^2H_3$]methylation) for [$^2H_{10}$]oxybutynin (Fig. 2). The scan rate was every 0.2 s with a sweep width of 0.1 a.m.u. integrating each acquisition sample for 4 ms.

Samples were placed on ice, then individually reconstituted with 20 μl of hexane immediately prior to injection. This volume was then reduced to 2–4 μl under a stream of nitrogen and 0.1 μl was injected into the chromatograph using a Hamilton 0.5-μl syringe. The injector port was adapted to capillary bore using a 17.8-cm conversion sleeve and reducing union (Supelco, Bellefonte, PA, U.S.A.).

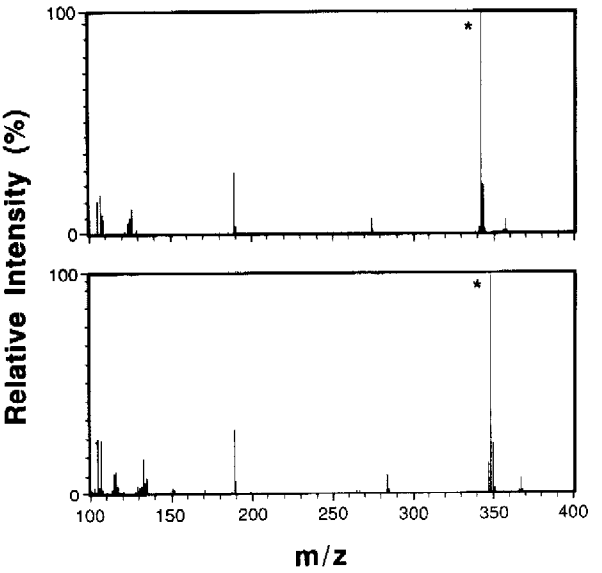

Fig. 2. Electron-impact mass spectra of oxybutynin (upper) and [$^2H_{10}$]oxybutynin (lower). The indicated ions (*) *m/z* 342 and 349 correspond to the fragments of oxybutynin and [$^2H_{10}$]oxybutynin which were selected for monitoring.

Chromatographic separation was accomplished using a 30 m×0.32 mm DB-1 (J and W Scientific, Folsom, CA, U.S.A.) methylsilicone fused-silica column operated at 250°C. The injector port and interface oven were operated at 265 and 250°C, respectively. The helium carrier gas linear velocity was 50 cm/s. Under these conditions, the retention times for oxybutynin and [$^2H_{10}$]oxybutynin were 3.00 and 2.95 min, respectively.

Calculations

Calibration plots (seven or eight points) were prepared by analyzing a series of 1-ml aliquots of drug-free plasma to which 0.5–20 ng of oxybutynin (calculated as the free base) were added using a 1 ng/μl solution of oxybutynin used as the hydrochloride in acetonitrile.

Quality control plasma samples were prepared from a separate weighing and dilution of oxybutynin and stored frozen with the subject samples. These samples were prepared at an oxybutynin concentration of 2, 10 and 20 ng/ml and analyzed in duplicate with each set of unknown samples and calibration standards.

The ion current ratios generated by monitoring the ions characteristic for oxybutynin/[$^2H_{10}$]oxybutynin (*m/z* 342/349) were determined from their peak areas at the appropriate GC retention times. The concentrations of oxybutynin in unknowns and quality control samples were then calculated from their ratios using the slope of the standard curve.

Human samples

Oxybutynin (two 5-mg tablets) was administered to healthy male volunteers with 180 ml of water, at 7:00 am, after an overnight fast. No food or beverage

other than water was permitted until a standard lunch was served 5 h after dosing. Blood samples (10 ml) were obtained before dosing and then 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 h after dosing. Samples were collected in heparinized tubes by venipuncture or through an indwelling venous catheter, using a Becton Dickinson 10-ml plastic syringe. If the sample was obtained from a catheter, the line was cleared of residual heparin before obtaining the sample. The blood was centrifuged, then the plasma was transferred to glass vials for storage at $-20°C$ until analysis.

RESULTS AND DISCUSSION

Extraction and recovery

The oxybutynin plasma samples required washing with diethyl ether–hexane prior to the extraction of the oxybutynin to eliminate neutral components, notably cholesterol which otherwise dominates with the *m/z* 342 ion profile (*m/z* 342). Because of the low basicity of oxybutynin [4], the pH was adjusted down to 1.5–2.0 to retain the analyte in the aqueous phase during the wash. The inclusion of 2% isoamyl alcohol in the heptane used in the subsequent extraction of oxybutynin prevented intractable emulsion formation.

The extraction recovery of oxybutynin from plasma spiked at 2 and 10 ng/ml ($n=4$) was 40.5% (coefficient of variation, C.V., 21.0%) and 47.1% (C.V. 3.6%), respectively. The low values are in part the consequence of using two extraction steps. After the ether wash and centrifugation, the plasma samples present a thin gelatinous interface which must be completely aspirated to waste along with the ether. This sacrifices some aqueous phase and recovery and dictates the use of an internal standard with partitioning characteristics parallel to that of the analyte.

Choice of internal standard

Many commercial and synthesized compounds structurally related to oxybutynin were evaluated as potential internal standards before selecting deuterated oxybutynin. Of those standards not selected, two are of potential value in the analysis of oxybutynin. The N-methyl-N-isopropyl isomer of oxybutynin was synthesized as described for [$^2H_{10}$]oxybutynin except methylisopropylamine was substituted for [$^2H_{10}$]diethylamine. The base peak ion of oxybutynin and this isomer are of the same mass which permitted selected-ion monitoring of only one ion, with resolution by chromatography rather than by mass. While monitoring one rather than two ions may improve the signal-to-noise ratio, the deuterated standard was chosen because of the well documented advantages of isotopic standards for analytical control [7].

The N-methyl-N-*n*-propyl isomer of oxybutynin was similarly synthesized and permitted a rigorous validation of the oxybutynin assay. In this instance, α-cleavage base peak ions *m/z* 342 and 328 were monitored for drug and internal standard, respectively. However, standard plots were not as linear as those generated using [$^2H_{10}$]oxybutynin ($r \geqslant 0.99$ versus > 0.999). Either of these alternative isomeric standards are available in the event that extracted matrix components were to interfere with the deuterated ion profile, *m/z* 349.

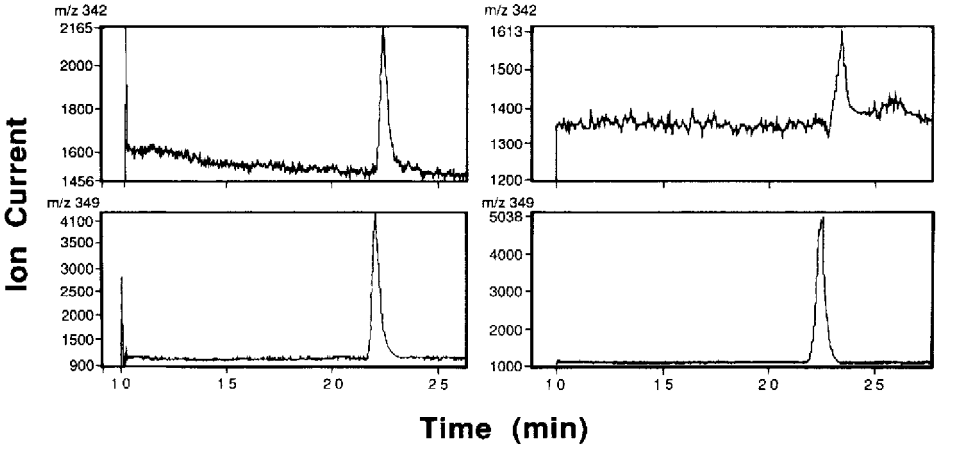

Fig. 3. Selected-ion chromatograms for oxybutynin (upper profile, *m/z* 342) and the internal standard, [$^2H_{10}$]oxybutynin (lower profile, *m/z* 349), extracted from 1-ml plasma samples containing 2 ng/ml (left) and 0.5 ng/ml (right) oxybutynin.

Chromatography and detection

Capillary GC using a bonded methylsilicone stationary phase was used in the present study to chromatograph oxybutynin and the internal standard, [$^2H_{10}$]oxybutynin, from plasma extracts. Detection was by electron-impact mass fragmentography. Selected-ion profiles were acquired for the α-cleavage base peak fragments of the drug and internal standard, *m/z* 342 and 349, respectively. Typical chromatograms are shown in Fig. 3. Note the slightly shorter retention time for the internal standard, characteristic of a deuterated species relative to the protium form.

Initial efforts using conventional packed column chromatography with a base-deactivated stationary phase (SP-2100-DB, Supelco) were unsatisfactory due to the thermal decomposition of oxybutynin. A substantial decomposition product was detected as an early eluting chromatographic peak when the relatively high abundance *m/z* 189 cyclohexylphenyl $C=O^+H$ ion (*m/z* 189) of oxybutynin was monitored.

Sensitivity, accuracy and precision

The detector response (oxybutynin/[$^2H_{10}$]oxybutynin) was linear throughout the 0.5–20 ng/ml oxybutynin plasma concentration range examined in this study. The reliable limit of detection of plasma oxybutynin was 0.25 ng/ml, based on the signal-to-noise ratio observed for the 0.5 ng/ml sample (Fig. 3).

The correlation coefficients for the standard curves were consistently greater than 0.999. The accuracy of the assay, as determined by the analysis of the three concentrations of control samples, extracted in duplicate with each of five sets of unknowns, is indicated in Table I. The relative differences between observed and true values were 4, 1 and 2% at the 2, 10 and 20 ng/ml concentrations, respectively.

TABLE I

ASSAY OF OXYBUTYNIN PLASMA CONTROL SAMPLES ($n=10$)

Oxybutynin added (ng/ml)	Mean concentration detected (ng/ml)	C.V. (%)	Accuracy (%)
2	2.07	10	104
10	10.09	6	101
20	20.43	7	102

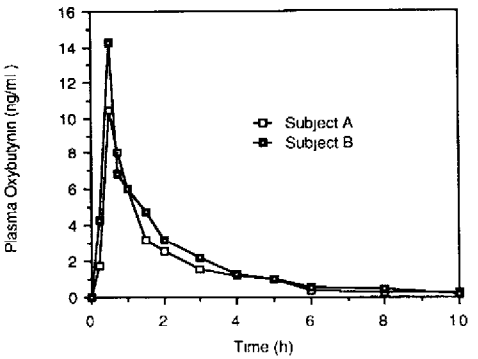

Fig. 4. Plasma concentration–time profiles of oxybutynin from two normal male volunteers after each received two 5-mg tablets.

Human data

The described method provided sufficient sensitivity to permit quantitation of oxybutynin in clinical samples for at least 8 h after a single 10-mg dose. The oxybutynin plasma concentration–time profile for two human subjects administered oxybutynin is illustrated in Fig. 4. Rapid absorption and elimination of the drug is apparent. These results indicate that the short time to peak plasma oxybutynin concentration occurs in advance of reported peak effects and that the effects may last long after most of the absorbed oxybutynin has been cleared from the bloodstream [1,8]. Application of this method to clinical studies may be of value in the improvement (individualization) of the pharmacotherapy of neurogenic bladder.

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