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Note

Capillary gas chromatographic–mass spectrometric analysis of plasma methylphenidate

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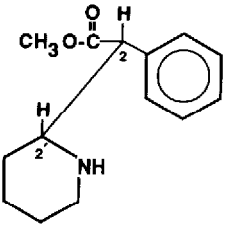
Administration of the central stimulant *dl-threo*-methylphenidate (MPH, Fig. 1) frequently improves the classroom behavior of children diagnosed with



(2*R*, 2'*R*) - MPH

Fig. 1. Saw-horse representation of the *d*-isomer of the racemic drug MPH.

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attention deficit disorder (hyperactivity). Therapeutic blood values of MPH rarely exceed 10 ng/ml and the circulating concentrations decay rapidly due to the facile metabolic deesterification of the drug [1]. Accordingly, pharmacokinetic profiles of MPH require quantitation of blood MPH as low as 1 ng/ml. Though no liquid chromatographic method has achieved this sensitivity, packed-column gas chromatography (GC) utilizing nitrogen–phosphorus [2,3] or chemical [4–6] and electron-impact [7,8] ionized mass spectrometric (MS) detection permit such trace analysis.

The present paper describes a sensitive and specific capillary GC–MS procedure for plasma MPH analysis appropriate for large-scale pharmacokinetic studies. Isotopically labeled MPH was incorporated for analytical control. The method evolved from a published packed-column procedure [7] and provides cleaner plasma extractions while benefiting from the capillary column as an MS inlet.

EXPERIMENTAL

Chemicals

The MPH·HCl was obtained as the U.S.P. reference standard NDC No. 00216-1303-05. The 10-mg MPH·HCl immediate-release tablets were obtained from Ciba-Geigy (Summit, NJ, U.S.A.). Deuterated MPH·HCl was synthesized according to the method of Patrick et al. [9] and contains [$^2H_2$-$^2H_8$]MPH. Diethyl ether, toluene, hexane and heptane were from Burdick and Jackson (Muskegon, MI, U.S.A.). Isoamyl alcohol and ethyl acetate were from Fisher Scientific (Fairlawn, NJ, U.S.A.). Pentafluoropropionic anhydride was from Pierce (Rockville, IL, U.S.A.) and dichlorodimethylsilane was from Aldrich (Milwaukee, WI, U.S.A.).

Human subject

A healthy male volunteer (88 kg) fasted overnight, then was dosed with two 10-mg tablets of MPH·HCl. Immediately after dosing, the subject was administered a breakfast of eggs, bacon, hash brown potatoes, toast and milk. A lunch consisting of a cheeseburger, french fries and milk was served 5 h later.

Blood samples (10 ml) were obtained through a heparinized indwelling venous catheter before dosing and then 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 h after dosing, using 10-ml plastic syringes. The line was cleared of residual heparin before sampling. The blood was transferred to 10-ml heparinized Vacutainers®, then centrifuged and the plasma transferred to glass vials and stored at −70°C until analysis. Under these storage conditions the samples are reported to be stable [10].

Plasma sample preparation

Screw-top centrifuge tubes (15 ml) were treated with a 10% solution of di-

methyldichlorosilane 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 the addition of 1 ml of 0.5 *M* hydrochloric acid containing 24 ng of [^2H_n]MPH·HCl. The samples were extracted with 3 ml of diethyl ether–hexane (3:1) by vortex-mixing for 10 s, then shaking horizontally for 10 min. After centrifugation (1800 *g*) at 4°C for 15 min, the organic phases and interfacing films were aspirated to waste, then the aqueous phases were alkalinized to pH 9.0–9.5 using 0.75 ml of 0.75 *M* 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. After centrifugation (1800 *g*) at 4°C for 15 min, the organic phases were transferred to disposable 4-ml screw-cap vials fitted with PTFE cap liners. The organic phases were then evaporated to dryness under streams of nitrogen using gentle heating from a hot air gun. Ethyl acetate (20 μl) and pentafluoropropionic anhydride (50 μl) were added to each vial and then the capped vials were heated for 40 min at 60°C. Upon cooling to room temperature, the vials were evaporated to dryness under nitrogen, then the vials were capped and refrigerated (5°C) until analysis.

Instrumental 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.). The mass spectrometer was calibrated using perfluorotributylamine (FC-43). The injector port was adapted to capillary bore using a 17.8-cm conversion sleeve and a reducing union (Supelco, Bellefonte, PA, U.S.A.). Detection was by mass fragmentography accomplished with electron-impact ionization at 32 eV (adjusted for maximum peak height using *m/z* 219 of FC-43) and 280–300 μA ionizing current. The electron multiplier was operated at 1700–1750 V. The data system acquired two channels of selected-ion current with a mass defect of 0.4 μ: that at *m/z* 229 representing the pentafluoropropionylpiperidyl fragment and that at *m/z* 234 for the corresponding $^2H_5$ isotopic variant (high-mass resolution will shift these ions to 230/235, respectively). The scan rate was every 0.2 s with a sweep width of 0.1 μ, integrating each acquisition sample for 4 ms.

Extracted samples were individually reconstituted with 15 μl of hexane immediately prior to injection and 0.1 μl was injected into the GC system using a Hamilton 0.5-μl syringe. The remaining volume was available for multiple injections if the need arose. Chromatographic separation was accomplished using a 30 m×0.32 mm, 0.25 μm film thickness (DB-1 J. & W. Scientific, Folsom, CA, U.S.A.) dimethylsilicone fused-silica column operated isothermally at 205°C. The injector port and MS interface oven were operated at 220 and 215°C, respectively. The helium carrier gas linear velocity was 50 cm/s. Under these conditions, the retention time for MPH was 1.85 min from injection.

Analytical calculations

Standard plots were prepared by analyzing duplicate 1-ml aliquots of blank plasma, fortified to contain 1, 2, 3.9, 7, 8, 11.7 and 14.6 ng/ml MPH using methanolic spiking solutions derived from serial dilutions of separate weighings for each concentration. These standards were extracted with the subject samples. The concentrations of MPH in unknown samples were calculated from the slope and intercept of the standard curve, plotted as peak-area ratio (MPH/[$^2H_5$]MPH) versus known MPH plasma concentrations. All MPH values are reported as the free base.

RESULTS AND DISCUSSION

Extraction and chromatography

The clean extraction of MPH from plasma was critically important for consistent capillary chromatographic performance during the analysis of large numbers of samples. To this end, neutral components were removed by an organic wash from the acidified samples, then the plasma was alkalinized and the drug extracted into heptane–2% isoamyl alcohol rather than into diethyl ether, benzene and/or hexanes [2–14]. The heptane functions to minimize the coextraction of non-volatiles which otherwise accumulate at the injector end of the column and ultimately contribute to chromatographic peak tailing. The isoamyl alcohol was included to prevent the formation of intractable emulsions during the extraction. To also reduce injector port contamination, only approximately 1% of the reconstituted plasma extract was injected into the splitless GC inlet.

This GC–MS selected-ion monitoring method for plasma MPH analysis provided chromatograms with very few interfering peaks (Fig. 2), a consequence of the mass specificity of the detector. Perhaps owing to the high cholesterol content of the described food regimen, the m/z 229 ion profile sometimes revealed a large cholesterol chromatographic peak eluting approximately 5 min after MPH. The occurrence of this cholesterol peak in some sample sets may limit the injection rate. This inconvenience is avoidable through the complete aspiration to waste of the organic phase and the interfacing film after the wash step of the sample work-up.

A prior packed-column GC–MS method for MPH analysis similarly incorporated deuterated MPH as an internal standard [7]. However, use of a capillary column eliminated the requirement for a solvent diverter and sample–carrier gas separator, both systems subject to blockage, breakage and leaks. Accordingly, to facilitate the analysis of the large number of MPH samples commonly involved in pharmacokinetic studies, the GC–MS system in the present study was fitted with a dimethylsilicone capillary column threaded directly into the ion source to provide a maintenance-free MS inlet.

The separation efficiency of the non-polar capillary column, unlike that of

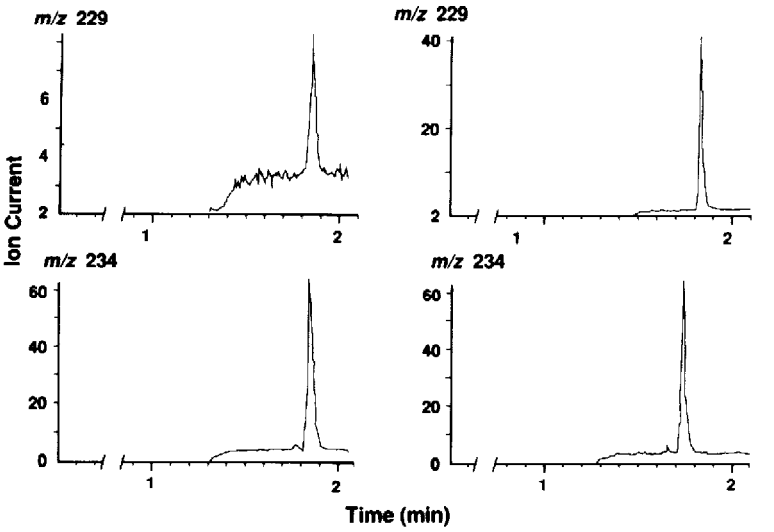

Fig. 2. Selected-ion chromatograms of MPH (upper) and [$^2H_5$]MPH (lower) detecting 1 ng/ml (left) and 10 ng/ml (right) MPH extracted from 1-ml aliquots of plasma The electron impact-generated pentafluoropropionylpiperidyl ion fragment (m/z 229) and the corresponding penta-deuterated species (m/z 234) were the ion currents monitored. The injection was at 0 min and the filament was powdered approximately 1.3 min later

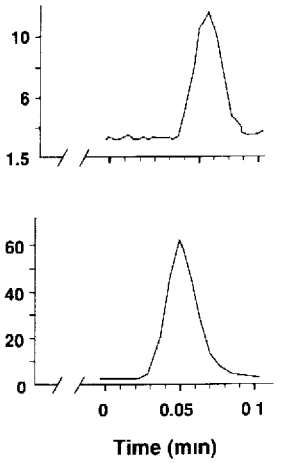

Fig. 3. Deuterium isotope effect facilitating the elution of [$^2H_5$]MPH (lower) relative to the protium form (upper), illustrated on an exploded time scale.

the packed columns used in earlier pharmacokinetic studies [11,12], revealed an MPH isotope effect whereby the unlabeled form was retained approximately 1 s longer than the deuterium form (Fig. 3). This required that the baseline values used for the drug and internal standard peak-area integrations

be separately assigned to maximize the accuracy and precision of the determinations.

Accuracy and precision

This capillary GC–MS method was recently applied to over 1000 clinical plasma samples and provided reliable and expedient analysis of 50–60 plasma unknowns per regular working day. The quality of the chemical measurements was evaluated by back-calculating each individual calibration standard data point against the associated calibration plot (Table I). The means of the plasma MPH concentrations determined from the 1–14.6 ng/ml fortified samples were all within 3% of theory and the precision improved from a coefficient of variation (C.V.) of 16–8% in progressing from the lowest to the highest concentrations analyzed.

TABLE I

ACCURACY AND PRECISION

Concentration of MPH (ng/ml)		*n*	C.V. (%)
Added	Detected[a] (mean)		
1	1.01	79	16
2	2.00	73	12
3.9	3.87	81	12
7.8	7.60	78	9
11.7	11.69	84	8
14.6	14.54	81	8

[a]Calculated from slope and intercept of the respective calibration plots based on the equation (MPH/[$^2H_5$]MPH) = (slope) [MPH] added + intercept (all r values exceeded 0.99).

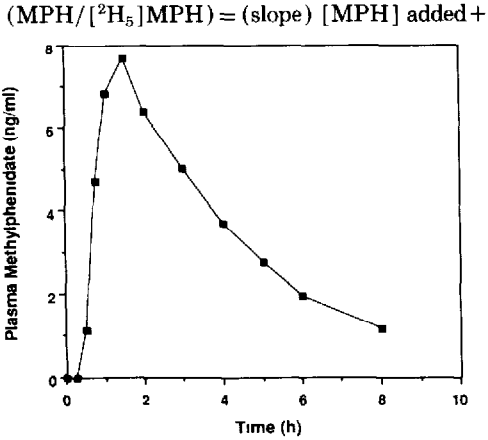

Fig. 4 Plasma MPH–time profile after an 88-kg male volunteer received two 10-mg immediate-release tablets with breakfast. The MPH concentration at 10 h was below the lowest calibration standard.

Human data

Application of this analytical method to the pharmacokinetics of MPH in a male volunteer demonstrated the rapid absorption and elimination of the drug (Fig. 4). The dose was administered with breakfast, a practice which appears to increase the rate of absorption relative to the fasted state [13]. The peak plasma concentration in this individual reached approximately twice the mean peak value associated with MPH at half the dose [12], consistent with the documented dose proportionality of the drug [14]. The rapid hydrolysis of MPH [11], producing a pharmacologically inactive amino acid metabolite [15], accounts for the observed steep decay of MPH from plasma.

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