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Short Communication

Gas chromatographic–mass spectrometric determination of plasma selegiline using a deuterated internal standard

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ABSTRACT

A gas chromatographic–mass spectrometric method is described for the determination of plasma selegiline. Tetradeuteroselegiline was synthesized and served as the internal standard. Human plasma samples (1 ml) containing 1–6 ng of selegiline were acidified, washed with diethyl ether–hexane, then alkalinized and extracted with heptane–isoamyl alcohol. Analytical separations were performed on a dimethylsilicone capillary column. Detection was by selected ion monitoring of the electron impact generated m/z 96 and 100 α-cleavage fragments of drug and internal standard, respectively.

INTRODUCTION

Selegiline [(−)-*R**-deprenyl, Fig. 1], used alone [1] or in combination with levodopa [2], alleviates symptoms of Parkinson's disease. This lipophilic amine [3] regionally localizes within the brain [4] where it appears to indirectly facilitate dopaminergic transmission by a selective "suicide" inhibition of monoamine oxidase (MAO)-type B [5].

Significant presystemic metabolism [6], rapid tissue distribution [3,4,7] and irreversible protein binding [4,8,9] of selegiline ostensibly relate to the failure to detect unlabelled selegiline in biological samples [10–13] except in human serum immediately following intravenous administration [7] or in rat urine after a suprapharmacological dose [9]. Accordingly, pharmacokinetic studies of selegiline have largely been limited to the use of the radiolabelled drug [3,4,7].

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Only a single published method for selegiline analysis specifies a detection limit below 10 ng/ml. Juvancz *et al.* [14] used gas chromatography (GC)–nitrogen–phosphorus detection (GC–NPD) to assay 3–300 ng/ml selegiline serum concentrations. No pharmacokinetic applications were reported and the procedure required a technically difficult serum steam distillation step which practically precluded the multiple sample analyses involved in drug concentration–time profiling. The present study describes a gas chromatographic–mass spectrometric (GC–MS) method for plasma selegiline analysis which utilizes a conventional plasma extraction, provides a limit of quantitation of 1 ng/ml and incorporates a deuterated internal standard for analytical control.

EXPERIMENTAL

Synthesis of [$^2H_4$]selegiline

The deuterated internal standard, [$^2H_4$]selegiline (Fig. 1), was synthesized by reductive amination of phenylacetone (Sigma, St. Louis, MO, USA) using methyl-[$^2H_3$]amine hydrochloride and sodium cyanoborodeuteride (Aldrich, Milwaukee, WI, USA) in methanol according to the method of Borch *et al.* [15]. This yielded racemic [$^2H_4$]methamphetamine which was then reacted with propargyl bromide (Aldrich) in toluene [16] to provide racemic [$^2H_4$]selegiline.

Plasma extraction

Screw-top centrifuge tubes (10 ml) were silanized with 10% dimethyldichlorosilane (Aldrich) in toluene, then rinsed with methanol and oven-dried. The caps were fitted with PTFE liners. An aliquot (1 ml) of blank human plasma was added to each tube. These aliquots were then fortified with selegiline hydrochloride to contain 0, 1, 2, 4 and 6 (or 5) ng/ml as the free base using a 1 ng/μl methanolic spiking solution. The pH was adjusted to 1.0–2.0 by the addition of 1 ml of 0.5 *M* hydrochloric acid containing 10 ng of [$^2H_4$]selegiline. The samples were washed with 3 ml of diethyl ether–hexane (3:1) by vortex-mixing for 5 s, then shaking horizontally for 30 min. After centrifugation (3000 *g*) for 15 min, the organic phases and interfacing films were aspirated to waste, then the aqueous phases were alkalinized to pH 9.0–10.0 using 1 ml of 0.75 *M* sodium carbonate. Heptane–isoamyl alcohol (98:2), 3 ml,

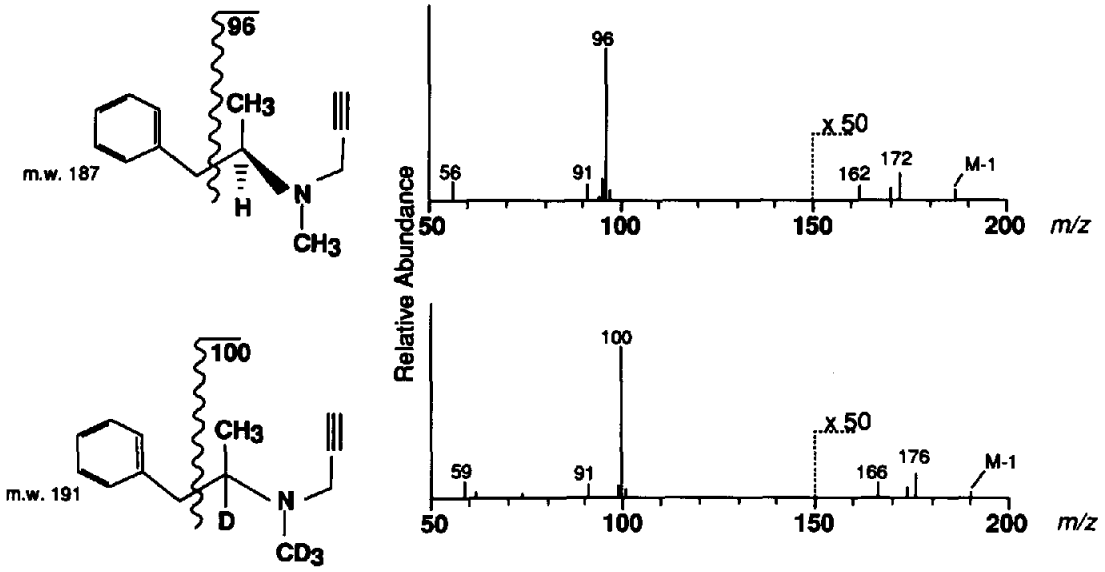

Fig. 1. Structures and electron impact mass spectra of selegiline (upper) and the internal standard, [$^2H_4$]selegiline (lower), illustrating the base peak ions *m/z* 96 and 100, respectively. These fragments result from α-cleavage and were chosen for selected ion monitoring of plasma extracts.

was added to each tube, and the samples were extracted by vortex-mixing for 5 s, then shaking horizontally for 30 min. After centrifugation (3000 *g*) for 15 min, the organic phases were transferred with silanized pipets to silanized disposable 4-ml screw-cap vials fitted with PTFE cap liners. The organic phases were evaporated to dryness under streams of nitrogen at 25°C, then the vials were capped and stored at −20°C until analysis.

Instrumental analysis

All analyses utilized a Finnigan Model 9610 gas chromatograph–Model 4000 mass spectrometer interfaced to an IBM-AT computer using a Teknivent Vector/One data system and software (St. Louis, MO, USA). The MS system was calibrated using perfluorotributylamine (FC-43). The injector was adapted to a capillary bore using a 17.8-cm conversion sleeve and a reducing union (Supelco, Bellefonte, PA, USA) and was operated in the splitless mode. Detection was by selected ion monitoring accomplished by electron impact ionization at 60–70 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 1825–1925 V. The data system acquired two channels of ion current: that of the selegiline base peak ion at *m/z* 96 and that of the corresponding tetradeuterated fragment at *m/z* 100 (Fig. 1). 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.

Each extracted sample was reconstituted with 20 μl of acetonitrile immediately prior to injection. This was concentrated to approximately 2 μl under a stream of nitrogen, and 0.1 μl was injected (Hamilton 0.5 μl syringe) onto a 30 m × 0.32 mm I.D., 0.25 μm film thickness dimethylsilicone fused-silica column (DB-1, J.&W. Scientific, Folsom, CA, USA) operated at 155°C. The injector port and interface oven were operated at 220°C and the helium carrier gas linear velocity was 50 cm/s. Under these conditions, selegiline and [$^2H_4$]selegiline eluted 2.05 and 2.03 min after injection, respectively.

Four separate sets of calibration standards were analyzed on four separate days. Back-calculated concentrations of selegiline were then determined for each individual standard data point using the slope and intercept of the associated standard curve, plotted as peak-area ratio (selegiline/[$^2H_4$]selegiline) *versus* known selegiline plasma concentrations (Table I). All selegiline values are reported as the free base.

The percentage recovery of selegiline was determined by comparing selegiline/[$^2H_4$]selegiline peak-area ratio values for 4 ng/ml calibration points relative to values from samples where 4 ng of selegiline were added to the final heptane–isoamyl alcohol extract of drug-free, but internally standardized, plasma samples.

RESULTS AND DISCUSSION

The low nanogram selegiline plasma concentration range analyzed in the present study was examined because methods with a limit of detection at approximately 10 ng/ml selegiline have failed to detect therapeutic blood levels of the drug.

The quality of the selegiline analysis is presented in Table I and represents the results of four sets of calibration standards analyzed on four separate days. One of these four sets used a 5 ng/ml sample in place of the 6 ng/ml sample. Pre-

TABLE I

ACCURACY AND PRECISION

Concentration of selegiline (ng/ml of human plasma)		*n*	Coefficient of variation (%)
Added	Detected (mean)[a]		
1	0.94	4	12.9
2	2.25	4	8.4
4	3.88	4	4.8
5	4.95	1	
6	5.92	3	0.4

[a] Evaluated by back-calculating each individual calibration standard data point against the associated calibration plot. All *r* values exceeded 0.99 (mean 0.9964).

cision improved from 12.9 to 0.4% (coefficient of variation) in progressing from the 1 to 6 ng/ml plasma selegiline samples. All calibration plot correlation coefficients exceeded 0.99 (mean 0.9964). Chromatograms from 0, 1 and 6 ng/ml plasma selegiline samples are shown in Fig. 2.

Without silanization of the glassware used in the plasma extraction, the drug was not recovered; after silanization the selegiline recovery was 83–88%. The stability of selegiline in plasma was not evaluated in this study. However, any potential instability of selegiline during sample preparation will be controlled for by the deuterated internal standard, barring an appreciable kinetic isotope effect.

The structurally related MAO inhibitors pargyline and clorgyline were initially examined as internal standards for this assay. Pargyline was found to elute too close to the solvent front and clorgyline exhibited an unacceptably long retention time. A homologue of selegiline, 3-(N-methyl-N-2-propynyl)amino-1-phenylbutane, was synthesized as described for [$^2H_4$]selegiline, only substituting benzylacetone for phenylacetone and using non-deuterated reagents. This provided a potential internal standard which generated an electron impact base peak ion in common with the analyte (*m/z* 96) and was readily separable from selegiline by GC. While use of this selegiline homologue showed promise as an internal standard, the tetradeuterated derivative provided better analytical control. Use of trideuterated selegiline as an internal standard was not ideal. This isotopic variant was obtained as described for the $^2H_4$-labelled species, only using sodium cyanoborohydride as the reducing agent. Unlike [$^2H_4$]selegiline, the [$^2H_3$]selegiline gave rise to an appreciable (5% relative abundance) *m/z* 96 ion which contributed to the apparent analyte ion current.

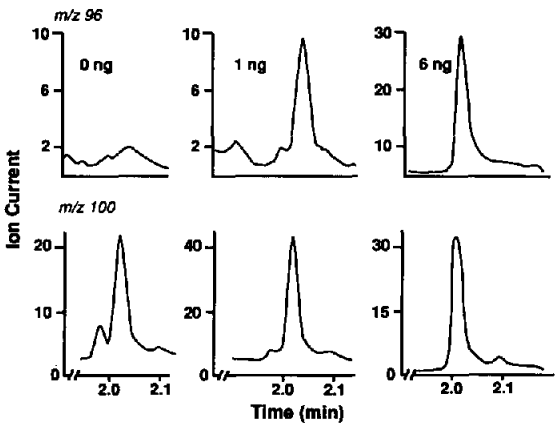

Fig. 2. Selected ion chromatograms shown for 0 ng (left), 1 ng (center) and 6 ng (right) of selegiline (upper) extracted from 1-ml aliquots of plasma. The internal standard, [$^2H_4$]selegiline (lower), was added at a concentration of 10 ng/ml for each. Selegiline (*m/z* 96) and [$^2H_4$]selegiline (*m/z* 100) were detected 2.05 and 2.03 min after injection, respectively.

On a standard regimen of selegiline (5–10 mg per day orally), urinary elimination of the metabolites (−)-*R*-methamphetamine and (−)-*R*-amphetamine has been reported to account for most of the dose [10,11]. These metabolites attain 5–15 ng/ml concentrations in serum and cerebrospinal fluid [12], and accumulate in human brain tissue [17]. Whereas the *R*-enantiomers of methamphetamine and amphetamine are generally less active in inducing catecholaminergic effects than the corresponding *S*-enantiomers [18], these metabolites may [19,20] or may not [21] contribute to the clinical pharmacology of selegiline. A therapeutically significant MAO inhibitory role for desmethylselegiline, possibly the major selegiline metabolite [22], has also been considered [6].

Under the chromatographic conditions used in the present study, these three metabolites do not interfere with the selegiline determination. Selegiline elutes 2.05 min after injection while amphetamine, desmethylselegiline and methamphetamine elute 1.3, 1.55 and 1.6 min after injection, respectively. Further, desmethylselegiline (10 ng/ml) was not detectable from plasma samples prepared for analysis by the present method and using selected ion monitoring of the *m/z* 83 base peak ion (which provides a strong response for a 50 pg direct GC injection of desmethylselegiline). Amphetamine and methamphetamine (2–10 ng/ml) were readily detectable from these plasma extracts after derivatization with trifluoroacetic anhydride and using selected ion monitoring of the α-cleavage fragments *m/z* 140 and 154, respectively. The corresponding elution times were 1.52 and 1.91 min after injection.

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