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Simultaneous determination of selegiline and desmethylselegiline in human body fluids by headspace solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

A method for the simultaneous determination of selegiline and its metabolite, desmethylselegiline, in human whole blood and urine is presented. The method, which combines a fiber-based headspace solid-phase microextraction (SPME) technique with gas chromatography–mass spectrometry (GC–MS), required optimization of various parameters (e.g., salt additives, extraction temperatures, extraction times and the extraction properties of the SPME fiber coatings). Pargyline was used as the internal standard. Extraction efficiencies for both selegiline and desmethylselegiline were 2.0–3.4% for whole blood, and 8.0–13.2% for urine. The regression equations for selegiline and desmethylselegiline extracted from whole blood were linear ($r^2 = 0.996$ and 0.995) within the concentration ranges 0.1–10 and 0.2–20 ng/ml, respectively. For urine, the regression equations for selegiline and desmethylselegiline were linear ($r^2 = 0.999$ and 0.998) within the concentration ranges 0.05–5.0 and 0.1–10 ng/ml, respectively. The limit of detection for selegiline and desmethylselegiline was 0.01–0.05 ng/ml for both samples. The lower and upper limits of quantification for each compound were 0.05–0.2 and 5–20 ng/ml, respectively. Intra- and inter-day coefficients of variation for selegiline and desmethylselegiline in both samples were not greater than 8.7 and 11.7%, respectively. The determination of selegiline and desmethylselegiline concentrations in Parkinson's disease patients undergoing continuous selegiline treatment is presented and is shown to validate the present methodology.

Keywords: Selegiline; Desmethylselegiline; Solid-phase microextraction (SPME)

1. Introduction

Selegiline is a potent, irreversible and selective inhibitor of monoamine oxidase type B [1] used widely in the treatment of Parkinson's disease due to its ability to inhibit the breakdown of biogenic amines, most importantly dopamine, in the human brain [2,3]. Selegiline is metabolized rapidly in the gastrointestinal tract and in the liver into desmethylselegiline, a specific metabolite of selegiline, and *l*-methamphetamine [4]. Selegiline and its metabolites are identified in blood and urine after therapeutic administration of selegiline [5–8]. In Japan, the daily recommended dose of selegiline is between 2.5 and 7.5 mg, and the maximum recommended daily dose is 10 mg. A recent pharmacokinetic study suggests that selegiline elimination is considerably retarded in Parkinson's disease patients with liver disorders [9]; serum concentrations of selegiline 0.5–1 h after administration of the drug were approximately 22 ng/ml in Parkinson's disease patients with impaired liver function, although the therapeutic serum concentration of selegiline is reported to be 3.1 ng/ml. High selegiline concentrations in patients may cause toxicity, and symptoms, such as hallucination, dyskinesias, dizziness and hypotension. Therefore, methods for determining selegiline and desmethylselegiline concentrations in human samples are required in order to allow selegiline dosage adjustments, and to predict the outcome of any toxic effects of the drug and potential treatments.

Many methods have been reported for determining selegiline and desmethylselegiline concentrations in human plasma,

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serum, urine and hair using gas chromatography (GC)–mass spectrometry (MS) [5,6,10–12], GC with electron capture detection (ECD) [13], GC with nitrogen phosphorus detection [9,14], high-performance liquid chromatography (HPLC) with fluorescence detection [15,16], and HPLC–MS [17–19]. Most of these methodologies employ extraction methods, such as liquid–liquid extraction (LLE) [6,11,13,15–17,19] or solid-phase extraction (SPE) [7,10,12,18] in order to remove impurities contained in various matrices. Although LLE and SPE methods may successfully extract selegiline and desmethylselegiline from biological samples, the large amounts of organic solvent used in the extraction procedure can cause health and environmental problems. Moreover, the off-line LLE and SPE methods are laborious and time-consuming and often require preconcentration of the extract prior to instrumental anlaysis.

Solid-phase microextraction (SPME), introduced by Pawliszyn's group in 1990, is an excellent alternative to the above methods since it is a solvent-free extraction technique that incorporates sample extraction, concentration and sample introduction into a single procedure [20]. In SPME, the analyte is partitioned between the stationary phase on a fused-silica fiber and the aqueous phase (or the headspace phase). After equilibration, the absorbed analytes are thermally desorbed by exposing the fiber in the injection port of a gas chromatograph. Although the usefulness of SPME for analyzing drug compounds in biological samples has been explored [21–36], to the best of our knowledge the SPME method has not been used to determine concentrations of selegiline and its metabolites in biological samples. This paper presents a detailed procedure for headspace SPME–GC–MS analysis of selegiline and desmethylselegiline in human whole blood and urine.

2. Materials and methods

2.1. Materials

Selegiline hydrochloride and desmethylselegiline hydrochloride were obtained from Fujimoto Pharmaceutical Corporation (Osaka, Japan). Pargyline hydrochloride for use as an internal standard (IS) was obtained from Sigma Chemicals (St. Louis, MO, USA). Methanol was reagent grade and was purchased from Wako Pure Chemical Industries (Osaka, Japan). A manual fiber holder for SPME was purchased from Supelco (Bellefonte, PA, USA). Five types of fiber, 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate, 65 μm PDMS/divinylbenzene (DVB), 65 μm Carbowax®/(CW)/DVB and 50/30 μm StableFlex DVB/carboxen (CAR)/PDMS, were obtained from the same manufacturer. Other common chemicals used were of the highest purity commercially available. Drug-free whole blood and urine samples were obtained from healthy volunteers.

2.2. Preparation of standard solutions and quality control (QC) samples

Stock standard solutions of selegiline, desmethylselegiline and the IS were prepared separately by dissolving an appropriate amount of each compound in methanol to achieve a concentration of 1 mg/ml; all stock solutions were stored at 4 °C. Working standard solutions of the compounds were prepared by serial dilution of the stock standard solutions with methanol. A series of 0.1 ml standard solutions were evaporated to dryness under a gentle stream of nitrogen. The residues were reconstituted in 1 ml of the drug-free whole blood and the drug-free urine to prepare the calibration standards containing 0.05–10 ng/ml for selegiline, 0.1–20 ng/ml for desmethylselegiline and 20 ng/ml for the IS. QC samples were prepared by the same procedure as used for the calibration standards, and concentrations were 0.2–10 ng/ml for selegiline, 0.4–20 ng/ml for desmethylselegiline or 20 ng/ml for the IS.

2.3. Conditioning of SPME fibers

New PDMS fibers were conditioned in the injection port of a gas chromatograph (Shimadzu GC-14B, Shimadzu Corp., Kyoto, Japan) at 250 °C for 60 min in order to remove fiber contaminants. Similarly, polyacrylate was conditioned at 300 °C for 120 min, PDMS/DVB at 260 °C for 30 min, CW/DVB at 250 °C for 30 min, and DVB/CAR/PDMS at 270 °C for 240 min. Used fibers were cleaned by heating at the appropriate conditioning temperature for 15 min prior to extraction.

2.4. GC–MS conditions

All analyses were performed using a Shimadzu GC-2010 gas chromatograph interfaced with a Shimadzu QP-2010 quadrupole mass spectrometer (Shimadzu Corp.). The GC–MS was operated with an interface temperature of 280 °C and an ionization source temperature of 220 °C. The mass spectrometer was tuned daily using perfluorotributylamine. A solvent delay of 3.5 min was set to protect the filament from oxidation. Chromatographic separation was achieved using a DB-17 fused-silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm, J & W Scientific, Folsom, CA, USA). Helium with a minimum purity of 99.99995% was used as the carrier gas at a flow rate of 2 ml/min. The gas chromatograph was equipped with a split/splitless injection port operated at 220 °C. The samples were injected in the splitless mode at a column temperature of 60 °C, then the splitter was opened after 1 min. The gas chromatograph oven temperature was programmed as follows: initial temperature 60 °C for 1 min, from 60 to 200 °C at a rate of 20 °C/min, and finally from 200 to 280 °C at a rate of 40 °C/min. The final temperature was held for 5 min.

The mass spectrometer was operated in the positive-ion electron impact (EI) mode using an ionizing energy of 70 eV and an emission current of 60 μA. Full-scan data were obtained with a mass range of *m/z* 50–500. A 0.1 μl aliquot (2 ng each on column) of the standard samples dissolved in methanol was used for direct injection.

2.5. Headspace SPME procedure

To a 7.5-ml glass vial, 1 ml of whole blood spiked with selegiline, desmethylselegiline and the IS, 1 ml of distilled water, 0.2 ml of 1 M NaOH solution and a magnetic stirring bar were

added. The vial was sealed with a silicone septum cap and heated on an aluminum block heater (Reacti-Therm™ Heating-Stirring Model, Pierce, Rockford, IL, USA) at 90 °C with stirring. After 5 min, the septum-piercing needle of the SPME device pierced the septum of the vial, and the conditioned fiber was exposed to the headspace in the vial, allowing extraction of the compounds at 90 °C for 25 min. The fiber was then withdrawn into the needle, removed from the vial and immediately inserted into the injection port of the gas chromatograph at 220 °C for 2 min to ensure desorption of the analytes. Urine samples (1 ml) spiked with selegiline, desmethylselegiline and the IS were mixed with 0.2 ml of 1 M NaOH solution and 0.5 g of NaCl and extracted as described above.

2.6. Evaluation of extraction efficiency, quantification and linearity

Extraction efficiencies were calculated by comparing the chromatographic peak areas obtained from extracts of QC samples with those obtained by direct GC injection of the standard compounds dissolved in methanol. The efficiencies were determined at two different concentrations of selegiline and desmethylselegiline. Regression equations for selegiline and desmethylselegiline extracted from human whole blood and urine were obtained by fitting the ratio of the peak area of the analyte to that of the IS (20 ng) versus concentration of the analyte. The equations were then used to calculate the concentrations of QC samples or practical clinical samples. Intra-day coefficient of variation (CV) and accuracy were determined by replicate analysis of QC samples spiked with two different concentrations of selegiline and desmethylselegiline. The same procedure was repeated for 5 days in order to determine the inter-day CV and accuracy. The limit of detection (LOD) was defined as the lowest concentration of analyte spiked in whole blood and urine that could be detected with a signal-to-noise ratio of at least 3. The lower limit of quantification (LLOQ) was determined as the lowest concentration on the calibration curve that could be detected with a signal-to-noise ratio of at least 10; the upper limit of quantification (ULOQ) for both compounds was set at <30 ng/ml, to protect the MS detector from excessive ions.

2.7. Patient sample collection

Whole blood and urine samples for selegiline and desmethylselegiline concentration determinations were obtained from two Parkinson's disease patients taking selegiline hydrochloride for more than 1 year. Whole blood was draw into 7-ml heparinized tubes 0.5–1 h after morning administration of the medication (2.5 mg). Urine samples were collected approximately 17 h after drug administration. All samples were stored at −80 °C until analysis. The patients received both verbal and written information on the study, and written informed consent was obtained. This study was approved by the Ethics Committee of Showa University School of Medicine.

3. Results and discussion

3.1. Mass spectra

In order to select the monitoring ion for quantitative analysis of selegiline, desmethylselegiline and the IS, positive-ion EI mass spectra in full-scan mode were initially obtained by injecting working solutions of the analyte standards into the

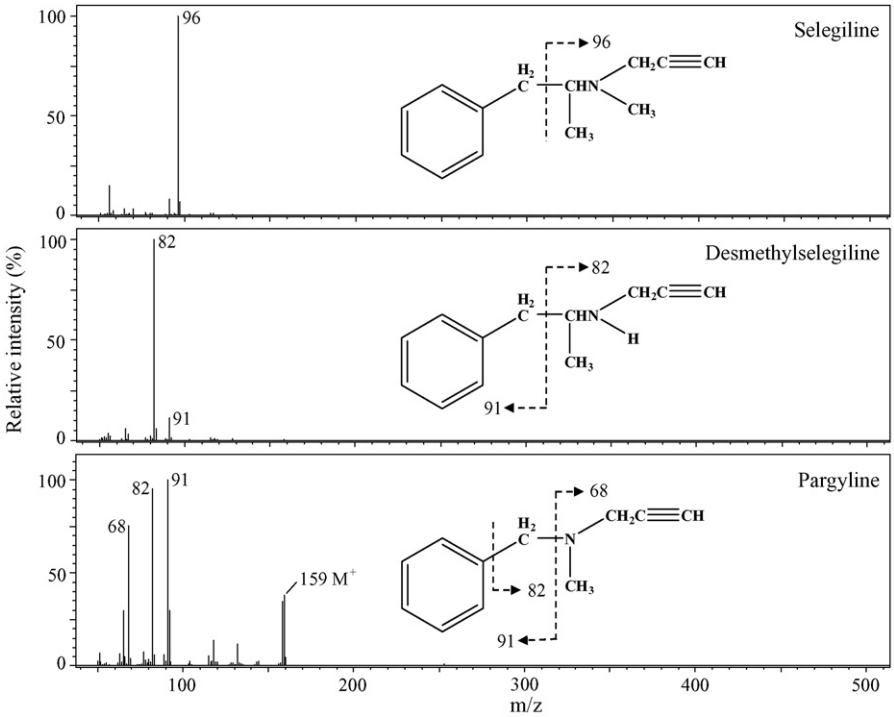

Fig. 1. MS spectra of selegiline, desmethylselegiline and pargyline.

Table 1
Comparison of different fiber coatings on the extraction efficiency for headspace SPME of selegiline, desmethylselegiline and pargyline (IS) from human whole blood and urine

Sample	Compound	Concentration added (ng/ml)	PDMS	Polyacrylate	PDMS/DVB	CW/DVB	DVB/CAR/PDMS
Whole blood	Selegiline	20	15	22	37	113	100[a]
	Desmethylselegiline	40	15	40	34	149	100
	Pargyline (IS)	40	4	5	72	39	100
Urine	Selegiline	20	18	27	93	57	100
	Desmethylselegiline	40	14	31	80	54	100
	Pargyline (IS)	40	9	10	86	22	100

Each fiber was exposed in the headspace of the vial for 25 min at 90 °C after pre-heating for 5 min at the same temperature.
[a] The amount of each compound extracted by the DVB/CAR/PDMS fiber was set at 100%. Each value represents the mean of duplicate determinations.

GC–MS system. Mass spectra and probable fragmentation pathways for the analyte compounds are shown in Fig. 1. Selegiline and desmethylselegiline gave a base peak ion at *m/z* 96 and 82, respectively, corresponding to cleavage of the C–C bond in the *N*-propargyl group-containing side chain. IS fragment ions at *m/z* 91 (base peak) and 68 correspond to cleavage of the C–N bond in the *N*-propargyl group-containing side chain; the ion at *m/z* 82 was due to loss of the phenyl group from the molecule. Quantification was performed by selected ion monitoring (SIM) using each base peak ion at *m/z* 96 for selegiline, at *m/z* 82 for desmethylselegiline and at *m/z* 91 for the IS.

3.2. Optimization of conditions for headspace SPME

In order to select optimal conditions for the simultaneous analysis of selegiline, desmethylselegiline and the IS, SPME fiber coatings, salt additives, extraction temperatures and extraction times were systematically examined using 1 ml of whole blood or urine to which known concentrations of the three compounds had been added.

The first step in the development of any SPME methodology is the selection of a fiber coating for extracting the analyte of interest in the biological sample. Five types of SPME fiber coatings (PDMS, polyacrylate, PDMS/DVB, CW/DVB and DVB/CAR/PDMS) were evaluated in this study for their efficiency in extracting selegiline, desmethylselegiline and the IS (Table 1). CW/DVB fiber was more efficient than DVB/CAR/PDMS fiber for extracting selegiline and desmethylselegiline from whole blood samples. However, the CW/DVB fiber was not suitable for headspace SPME because the fiber coating was stripped after nine or ten extraction procedures. In the case of urine samples, DVB/CAR/PDMS fiber showed the highest extraction efficiency for all three compounds; this fiber was therefore chosen for further methods development.

Decreasing the water solubility of organic compounds by adding salt is known as the "salting out" effect [37,38]. The addition of a salt to an aqueous sample increases the ionic strength of the sample, thereby increasing the partitioning of organic compounds into the SPME fiber coating. In the present study, Na_2SO_4, NH_4Cl, $(NH_4)_2SO_4$, $CaCl_2{\cdot}2H_2O$ and NaCl, in the presence of 0.2 ml of 1 M NaOH solution and/or 1 ml of distilled water, were tested to determine their effect on the extraction efficiency of selegiline and desmethylselegiline from 1 ml of whole blood or urine (Table 2). Salt addition to whole blood samples did not enhance extraction, and alkalinization using only NaOH gave the best extraction efficiencies for all compounds. In contrast, the addition of 0.5 g of NaCl to the urine samples resulted in the highest extraction efficiencies for all compounds.

Headspace SPME is dependent on the equilibrium between the aqueous phase of the sample, the sample headspace and the solid-phase fiber coating, and thus extraction temperature and time critically impact the headspace SPME process [39]. The effect of extraction temperature (50, 70 and 90 °C) and time (5–30 min) on the extraction of selegiline, desmethylselegiline and the IS are shown in Figs. 2 and 3. Temperatures above 90 °C were not tested in order to avoid excessive vapor pressure in the glass vials. For whole blood, the peak areas of all compounds

Table 2
Effects of different salts on the extraction efficiency for headspace SPME of selegiline, desmethylselegiline and pargyline (IS) from human whole blood and urine using a DVB/CAR/PDMS fiber

Sample	Compound	Concentration added (ng/ml)	Na_2SO_4	NH_4Cl	$(NH_4)_2SO_4$	$CaCl_2{\cdot}2H_2O$	NaCl	no salt
Whole blood	Selegiline	20	28	36	40	75	40	100[a]
	Desmethylselegiline	40	45	34	49	104	79	100
	Pargyline (IS)	40	37	51	56	79	46	100
Urine	Selegiline	20	92	73	81	82	100[a]	61
	Desmethylselegiline	40	93	46	79	71	100	44
	Pargyline (IS)	40	106	98	94	42	100	72

The DVB/CAR/PDMS fiber was exposed in the headspace of the vial for 25 min at 90 °C after pre-heating for 5 min at the same temperature.
[a] The amount of each compound extracted from whole blood and urine without salt and with NaCl, respectively, was set at 100%. Each value represents the mean of duplicate determinations.

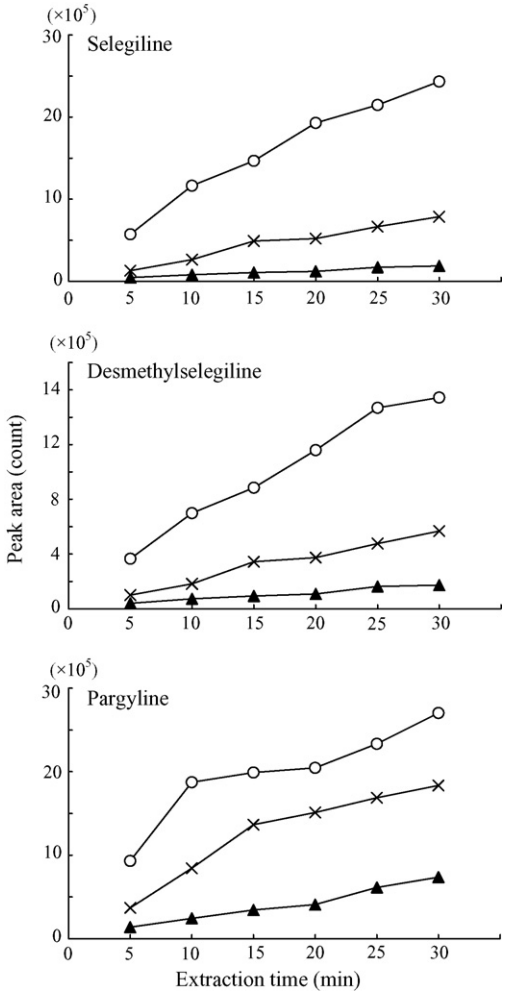

Fig. 2. Effects of different extraction temperatures and times on the extraction efficiency of selegiline, desmethylselegiline and pargyline (IS) from human whole blood by headspace SPME using DVB/CAR/PDMS fibers. The amounts of selegiline, desmethylselegiline and IS added to 1 ml of whole blood in the presence of 1 ml distilled water plus 0.2 ml 1 M NaOH were 20, 40 and 40 ng, respectively. Each point represents the mean of duplicate determinations. Key: (▲) 50 °C, (×) 70 °C and (◯) 90 °C.

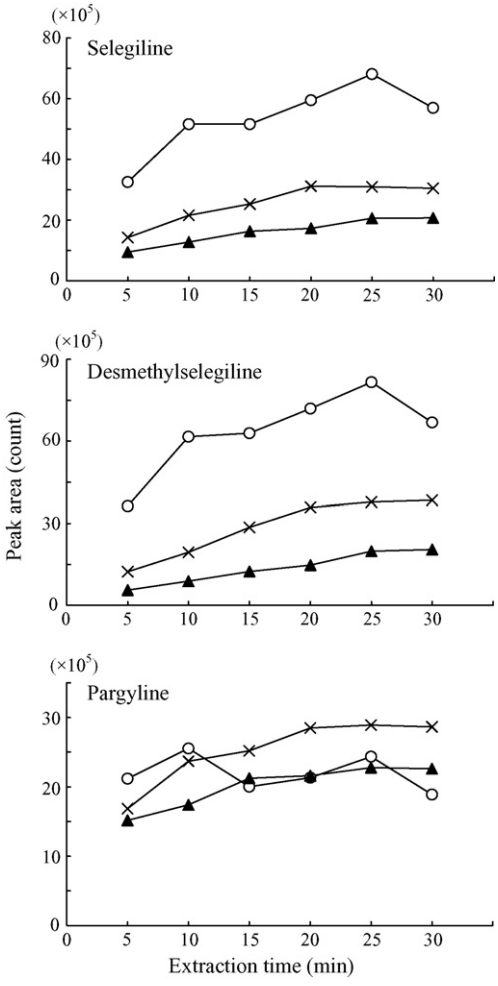

Fig. 3. Effect of different extraction temperatures and times on the extraction efficiency of selegiline, desmethylselegiline and pargyline (IS) from human urine by headspace SPME using DVB/CAR/PDMS fibers. The amounts of selegiline, desmethylselegiline and IS added to 1 ml of urine in the presence of 0.2 ml 1 M NaOH plus 0.5 g NaCl were 20, 40 and 40 ng, respectively. Each point represents the mean of duplicate determinations. Key: (▲) 50 °C, (×) 70 °C and (◯) 90 °C.

increased with increased temperature and time (Fig. 2). However, equilibria for all compounds were not attained even after 30 min. Although SPME has maximum sensitivity at the equilibrium point, full equilibration is not necessary for accurate and precise analysis due to the linear relationship between the amount of analyte adsorbed by the SPME fiber and its initial concentration in the sample matrix at non-equilibrium conditions [40]. It was therefore necessary to establish the best compromise between extraction efficiency, extraction temperature and extraction time.

An increase in temperature during extraction negatively affects extraction efficiency because the distribution constant between the sample and the fiber coating decreases [39]; this was observed for the urine samples (Fig. 3). The peak areas of all compounds at 50 and 70 °C increased with increased time, and reached equilibrium after 20–25 min. At 90 °C, the peak areas of selegiline and desmethylselegiline reached a maximum after 25 min, and then decreased. In contrast, the peak area of the IS became maximum after 10 min, and then decreased. However, the sensitivity of the IS was sufficient for quantification even after 15 min.

Using the GC conditions described above, the total analysis cycle (including separating the analytes, cooling the gas chromatograph and equilibration) was 25 min per sample. Therefore, in order to best compromise acceptable extraction, rapid analysis and reliability, the DVB/CAR/PDMS fibers were exposed to the headspace at 90 °C for 25 min.

3.3. Reliability of the method

The present paper demonstrates the quantitative analysis of selegiline and desmethylselegiline, using pargyline as the IS. The best IS is a stable isotope-labeled version of the analyte. However, isotope-labeled selegiline and desmethylselegiline are not commercially available. This necessitated the choice of another compound to use as an IS having chromatographic retention, ionization and structural properties similar to

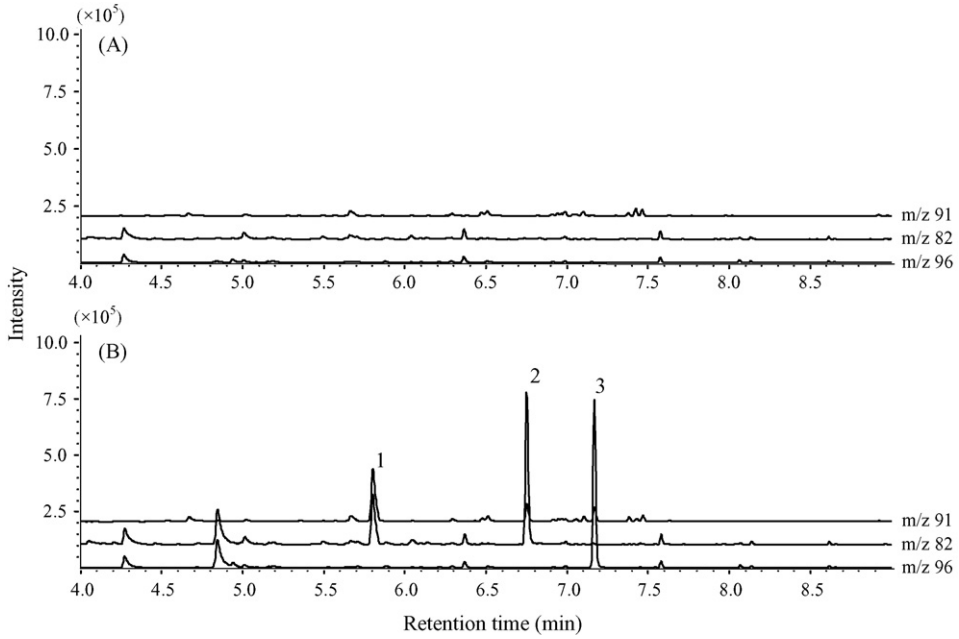

Fig. 4. SIM chromatograms for selegiline, desmethylselegiline and IS extracted from human whole blood by headspace SPME using DVB/CAR/PDMS fibers. (A) Whole blood blank, (B) spiked whole blood. The amounts of selegiline, desmethylselegiline and IS added to 1.0 ml of whole blood in the presence of 1 ml distilled water plus 0.2 ml 1 M NaOH were 10, 20 and 20 ng, respectively. The fiber was exposed to the headspace of the vial for 25 min at 90 °C after pre-heating for 5 min at the same temperature. Peaks: 1, IS; 2, desmethylselegiline; 3, selegiline.

those of selegiline and desmethylselegiline. Pargyline, an irreversible MAO inhibitor, is similar in structure to selegiline and desmethylselegiline (Fig. 1). In preliminary trials, pargyline was assessed for its suitability as an IS, was found to sufficiently fulfill the above criteria, and was thus chosen as the IS for the quantitative analysis of selegiline and desmethylselegiline in human body fluids.

Figs. 4 and 5 show chromatograms of SIM for headspace SPME from 1 ml of human whole blood and urine samples in the presence or absence of selegiline, desmethylselegiline and the IS. Distinct peaks are apparent for all compounds, and the retention times for the IS, desmethylselegiline and selegiline were 5.8, 6.8 and 7.2 min, respectively (Figs. 4 and 5, lower panel). While some small impurity peaks were observed in the

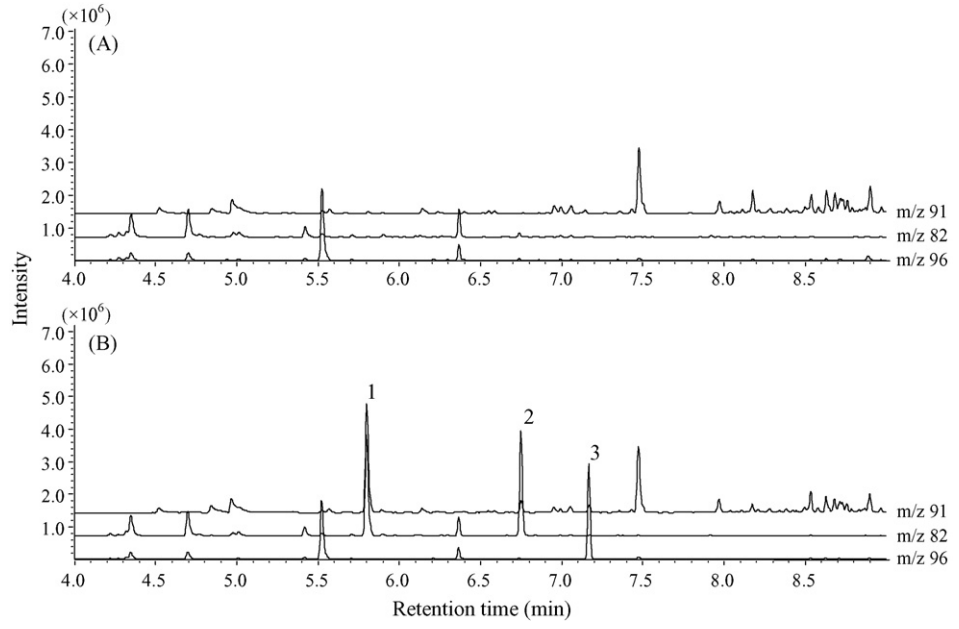

Fig. 5. SIM chromatograms for selegiline, desmethylselegiline and IS extracted from human urine by headspace SPME using DVB/CAR/PDMS fibers. (A) Urine blank, (B) spiked urine. The amounts of selegiline, desmethylselegiline and IS added to 1 ml of urine in the presence of 0.5 g NaCl plus 0.2 ml 1 M NaOH were 2, 4 and 20 ng, respectively. The fiber was exposed to the headspace of the vial for 25 min at 90 °C after pre-heating for 5 min at the same temperature. The key numbers are the same as those specified in Fig. 4.

Table 3
Extraction efficiencies for selegiline and desmethylselegiline from QC samples by the present method

Sample	Compound	Concentration added (ng/ml)	Concentration measured (ng/ml)	Extraction efficiency (%)
Whole blood	Selegiline	1.0	0.026 ± 0.0006[a]	2.6
		10	0.335 ± 0.0315	3.4
	Desmethylselegiline	2.0	0.039 ± 0.0019	2.0
		20	0.454 ± 0.0401	2.3
Urine	Selegiline	0.2	0.016 ± 0.0005	8.0
		2.0	0.183 ± 0.0106	9.2
	Desmethylselegiline	0.4	0.064 ± 0.0067	13.2
		4.0	0.342 ± 0.0105	8.4

[a] The values are mean ± S.D. of three experiments.

Table 4
Regression equations and detection limits for selegiline and desmethylselegiline in human whole blood and urine measured by the present method

Sample	Compound	Regression equation[a]	Correlation coefficient (r^2)	Concentration range (ng/ml)	LOD (ng/ml)	LLOQ (ng/ml)	ULOQ (ng/ml)
Whole blood	Selegiline	$y = 0.2520x - 0.0705$	0.996	0.1–10	0.03	0.1	10
	Desmethylselegiline	$y = 0.1224x - 0.0844$	0.995	0.2–20	0.05	0.2	20
Urine	Selegiline	$y = 0.3917x - 0.0228$	0.999	0.05–5.0	0.01	0.05	5.0
	Desmethylselegiline	$y = 0.2984x - 0.0496$	0.998	0.1–10	0.03	0.1	10

[a] Results from 7 to 8 different concentrations (each point represents the mean of duplicate determinations) for each compound were used to obtain the equations.

whole blood and urine blanks (Figs. 4 and 5, upper panel), no interfering peaks were found near the peaks of the test compounds.

Extraction efficiencies of selegiline and desmethylselegiline from QC samples are presented in Table 3. For whole blood, the efficiencies for selegiline and desmethylselegiline were 2.6–3.4% and 2.0–2.3%, respectively, whereas for urine they were 8.0–9.2% and 8.4–13.2%, respectively. The low extraction efficiencies observed for QC samples are to be expected owing to the equilibrium nature of the extraction process; this is not problematic because small variation and satisfactory quantification can be achieved by headspace SPME, as shown in Tables 4 and 5.

Table 4 shows the regression equations, LOD, LLOQ and ULOQ obtained by the present method. The equations for these compounds exhibit good linearity in the ranges shown in the table, with correleation coefficients of at least 0.995. In whole blood, the LOD of selegiline and desmethylselegiline under optimal conditions were 0.03 and 0.05 ng/ml, respectively, and 0.01 and 0.03 ng/ml, respectively, in urine. The LLOQ and ULOQ, which correspond to the lowest and highest concentration levels of the concentration range, were 0.05–0.2 and 5–20 ng/ml, respectively.

The intra- and inter-day CVs and accuracy were evaluated by assaying QC samples prepared in human whole blood and urine and are summarized in Table 5. The intra-day CVs at all concentrations examined were less than 7.4 and 8.7% for whole blood and urine, respectively, whereas the inter-day CVs at all concentrations examined were less than 9.9 and 11.7% for whole blood and urine, respectively. Accuracy was in the range 84.0–117.5% for all concentrations.

Table 5
Intra-day and inter-day coefficients of variation (CV) and accuracy for selegiline and desmethylselegiline concentrations in QC samples measured by the present method[a]

Sample	Compound	Concentration added (ng/ml)	Intra-day[b]			Inter-day[c]		
			Concentration measured (ng/ml)	Accuracy (%)	CV (%)	Concentration measured (ng/ml)	Accuracy (%)	CV (%)
Whole blood	Selegiline	1.0	0.86 ± 0.06[d]	86.0	7.4	0.84 ± 0.05	84.0	6.0
		10.0	8.81 ± 0.37	88.1	4.2	9.57 ± 0.93	95.7	9.7
	Desmethylselegiline	2.0	1.68 ± 0.07	84.0	4.3	1.68 ± 0.09	84.0	5.6
		20.0	17.1 ± 0.71	85.4	4.1	19.2 ± 1.90	95.8	9.9
Urine	Selegiline	0.2	0.21 ± 0.01	105.0	5.5	0.21 ± 0.01	105.0	4.2
		2.0	2.15 ± 0.19	107.5	8.7	2.29 ± 0.27	114.5	11.7
	Desmethylselegiline	0.4	0.47 ± 0.01	117.5	2.5	0.45 ± 0.01	112.5	2.4
		4.0	4.17 ± 0.28	104.3	6.6	3.82 ± 0.38	95.5	9.9

[a] All data were obtained using IS calibration.
[b] Intra-day CV were calculated from measurements of three spiked samples on the same day.
[c] Spiked samples were analyzed on 5 separate days, with one sample analyzed each day.
[d] The values are mean ± S.D.

Table 6
Stability of selegiline and desmethylselegiline in QC samples at −80 °C

Sample	Compound	Concentration added (ng/ml)	Concentration measured (ng/ml)						
			<1 day	1 week	3 weeks	7 weeks	Mean	S.D.	CV (%)
Whole blood	Selegiline	0.2	0.004[a]	0.005	0.005	0.004	0.004	0.0004	9.9
		2.0	0.048	0.055	0.048	0.048	0.050	0.0029	5.9
	Desmethylselegiline	0.4	0.011	0.010	0.012	0.013	0.011	0.0011	10.0
		4.0	0.062	0.070	0.065	0.073	0.068	0.0054	7.9
Urine	Selegiline	0.2	0.013	0.015	0.015	0.015	0.014	0.0008	5.4
		2.0	0.134	0.161	0.150	0.156	0.150	0.0117	7.8
	Desmethylselegiline	0.4	0.040	0.040	0.039	0.037	0.039	0.0014	3.6
		4.0	0.250	0.309	0.282	0.281	0.280	0.0238	8.5

[a] The values are the means of triplicate determinations.

3.4. Stability

Methanol stock standard solutions containing 1 mg/ml of selegiline, desmethylselegiline and the IS, and the working standard solutions, were all stable for at least 3 months when stored at 4 °C. The DVB/CAR/PDMS fiber showed excellent stability: one fiber could be used for at least 50 extractions with good reproducibility.

To assess the stability of selegiline and desmethylselegiline in whole blood and urine stored at −80 °C, we evaluated QC samples containing two concentrations of these compounds (0.2 and 2.0 ng/ml for selegiline, 0.4 and 4.0 ng/ml for desmethylselegiline) following storage at −80 °C for up to 7 weeks. Selegiline and desmethylselegiline concentrations were determined in samples retrieved from storage after 1 day, 1 week, 3 weeks and after 7 weeks ($n = 3$ at each concentration). In the stability experiments, the extraction efficiencies of selegiline and desmethylselegiline from all samples were 1.6–3.3% for whole blood and 6.3–10% for urine. As shown in Table 6, the CVs of the measured concentrations were less than 10% at all concentrations, showing that selegiline and desmethylselegiline are stable in whole blood and urine at −80 °C for at least 7 weeks.

3.5. Application of the method to dosed samples from Parkinson's disease patients

To demonstrate the clinical applicability of the present method, the concentrations of selegiline and desmethylselegiline in whole blood and urine were determined in two female Parkinson's disease patients receiving selegiline medication. The amount of pargyline added as an IS was 20 ng to 1 ml of whole blood or urine. However, due to the high concentrations of selegiline and desmethylselegiline present in the urine samples, 0.1 ml of the patient urine was diluted with 0.9 ml of blank urine obtained from healthy subjects. Typical SIM chromatograms of selegiline and desmethylselegiline in whole blood and urine samples from the patients are shown in Fig. 6. The levels of these compounds in the samples are summarized in

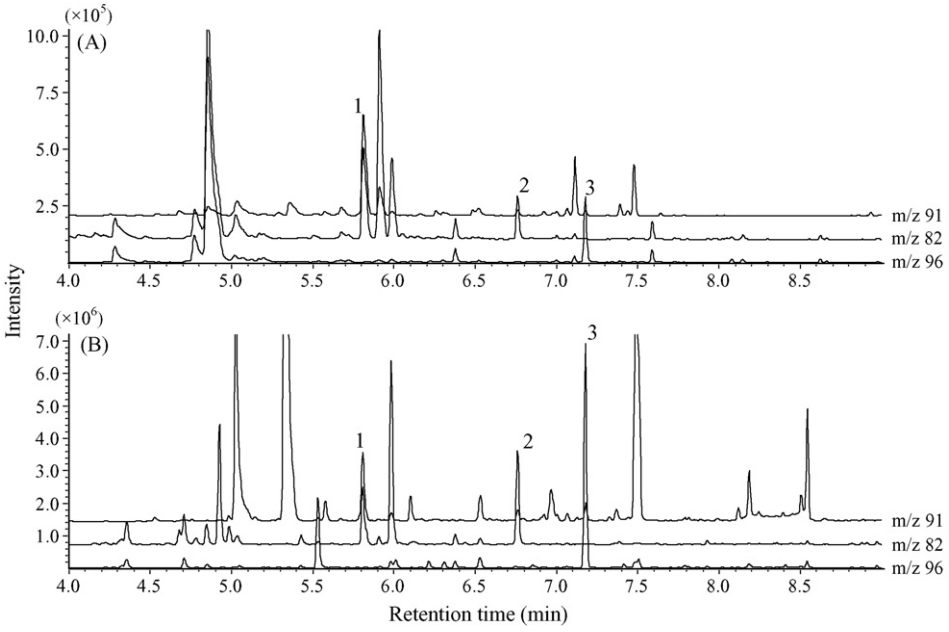

Fig. 6. Typical SIM chromatograms obtained from extracts of whole blood (A) and urine (B) from Parkinson's disease patients receiving selegiline medication. In each sample, 20 ng of pargyline was spiked as the IS. The key numbers are the same as those specified in Fig. 4.

Table 7
Concentrations of selegiline and desmethylselegiline in whole blood and urine samples obtained from Parkinson's disease patients

Patient	Gender	Age (years)	Body weight (kg)	Selegiline medication		Concentration of the compound (ng/ml)[a]			
				Oral dose (mg)	Duration (months)	Whole blood		Urine[b]	
						Selegiline	Desmethylselegiline	Selegiline	Desmethylselegiline
1	Female	71	48	2.5–5	21	0.39	1.26	60.7	39.6
2	Female	70	45	2.5–5	15	1.85	2.93	32.5	56.5

[a] The values are the means of duplicate determinations.
[b] Data obtained by sample dilution with drug-free urine.

Table 7. The observed concentrations were within therapeutic levels [7–10,18].

4. Conclusions

The extraction and detection of selegiline and desmethylselegiline in human whole blood and urine samples have been achieved using headspace SPME with a DVB/CAR/PDMS fiber and SIM of GC–MS. Under optimal conditions, good sensitivity, linearity and reproducibility were obtained. The present method was successfully applied to quantify selegiline and desmethylselegiline simultaneously in blood and urine of Parkinson's disease patients receiving selegiline medication.

References

[1] J. Knoll, K. Magyar, Adv. Biochem. Psychopharmacol. 5 (1972) 393.
[2] G.P. Reynolds, P. Riederer, W.D. Rausch, J. Neural Transm. 16 (Suppl.) (1980) 173.
[3] C.W. Olanow, J. Neural Transm. 48 (Suppl.) (1996) 75.
[4] G.P. Reynolds, J.D. Elsworth, K. Blau, M. Sandler, A.J. Lees, G.M. Stern, Br. J. Clin. Pharmacol. 6 (1978) 542.
[5] E.H. Heinonen, V. Myllylä, K. Sotaniemi, R. Lamintausta, J.S. Salonen, M. Anttila, M. Savijärvi, M. Kotila, U.K. Rinne, Acta Neurol. Scand. 126 (1989) 93.
[6] R. Kronstrand, M.C. Andersson, J. Ahlner, G. Larson, J. Anal. Toxicol. 25 (2001) 594.
[7] M. Katagi, M. Tatsuno, H. Tsutsumi, A. Miki, T. Kamata, H. Nishioka, K. Nakajima, M. Nishikawa, H. Tsuchihashi, Xenobiotica 32 (2002) 823.
[8] A. Clarke, F. Brewer, E.S. Johnson, N. Mallard, F. Hartig, S. Taylor, T.H. Corn, J. Neural Transm. 110 (2003) 1241.
[9] M. Anttila, E.A. Sotaniemi, O. Pelkonen, A. Rautio, Clin. Pharmacol. Ther. 77 (2005) 54.
[10] R. Kikura, Y. Nakahara, Biol. Pharm. Bull. 18 (1995) 267.
[11] H.-S. Shin, Drug Metab. Dispos. 25 (1997) 657.
[12] M. Hasegawa, K. Matsubara, S. Fukushima, C. Maseda, T. Uezono, K. Kimura, Forensic Sci. Int. 101 (1999) 95.
[13] J.S. Salonen, J. Chromatogr. 527 (1990) 163.
[14] K. Laine, M. Anttila, L. Nyman, A. Wahlberg, L. Bertilsson, Eur. J. Clin. Pharmacol. 57 (2001) 137.
[15] R.L. Croix, E. Pianezzola, M.S. Benedetti, J. Chromatogr. B 656 (1994) 251.
[16] H.J. Mascher, C. Kikuta, A. Millendorfer, H. Schiel, G. Ludwig, Int. J. Clin. Pharmacol. Ther. 35 (1997) 9.
[17] J. Lengyel, K. Magyar, I. Hollósi, T. Bartók, M. Báthori, H. Kalász, S. Fürst, J. Chromatogr. A 762 (1997) 321.
[18] M. Katagi, M. Tatsuno, A. Miki, M. Nishikawa, K. Nakajima, H. Tsuchihashi, J. Chromatogr. B 759 (2001) 125.
[19] M.H. Slawson, J.L. Taccogno, R.L. Foltz, D.E. Moody, J. Anal. Toxicol. 26 (2002) 430.
[20] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[21] T. Kumazawa, K. Sato, H. Seno, A. Ishii, O. Suzuki, Chromatographia 43 (1996) 59.
[22] M. Nishikawa, H. Seno, A. Ishii, O. Suzuki, T. Kumazawa, K. Watanabe, H. Hatttori, J. Chromator. Sci. 35 (1997) 275.
[23] X.-P. Lee, T. Kumazawa, K. Sato, O. Suzuki, J. Chromatogr. Sci. 35 (1997) 302.
[24] H. Seno, T. Kumazawa, A. Ishii, K. Watanabe, H. Hattori, O. Suzuki, Jpn. J. Forensic Toxicol. 15 (1997) 16.
[25] T. Kumazawa, H. Seno, X.-P. Lee, A. Ishii, K. Watanabe-Suzuki, K. Sato, O. Suzuki, Anal. Chim. Acta. 387 (1999) 53.
[26] S.-W. Myung, S. Kim, J.-H. Park, M. Kim, J.-C. Lee, T.-J. Kim, Analyst 124 (1999) 1283.
[27] S. Ulrich, S. Kruggel, H. Weigmann, C. Hiemake, J. Chromatogr. B 731 (1999) 231.
[28] C.M. Lock, L. Chen, D.A. Volmer, Rapid Commun. Mass Spectrom. 13 (1999) 1744.
[29] T. Kumazawa, H. Seno, K. Watanabe-Suzuki, H. Hattori, A. Ishii, K. Sato, O. Suzuki, J. Mass Spectrom. 35 (2000) 1091.
[30] G. Frison, L. Tedeschi, S. Maietti, S.D. Ferrara, Rapid Commun. Mass Spectrom. 15 (2001) 2497.
[31] C. Paradis, C. Dufresne, M. Bolon, R. Boulieu, Ther. Drug Monit. 24 (2002) 768.
[32] M. Iwai, H. Hattori, T. Arinobu, A. Ishii, T. Kumazawa, H. Noguchi, O. Suzuki, H. Seno, J. Chromatogr. B 806 (2004) 65.
[33] Y.F. Sha, S. Shen, G.L. Duan, J. Pharmacol. Biomed. Anal. 37 (2005) 143.
[34] H. Bagheri, A. Es-haghi, M.R. Rouini, J. Chromatogr. B 818 (2005) 147.
[35] A.R. de Oliveira, E.J. Cesarino, P.S. Bonato, J. Chroamtogr. B 818 (2005) 285.
[36] A. Aresta, F. Palmisano, C.G. Zambonin, J. Pharm. Biomed. Anal. 39 (2005) 643.
[37] T. Kumazawa, H. Seno, X.-P. Lee, A. Ishii, O. Suzuki, K. Sato, Chromatographia 43 (1996) 393.
[38] B.J. Hall, J.S. Brodbelt, J. Chromatogr. A 777 (1997) 275.
[39] H.L. Lord, J. Pawliszyn, Anal. Chem. 69 (1997) 3899.
[40] J. Ai, Anal. Chem. 69 (1997) 3260.