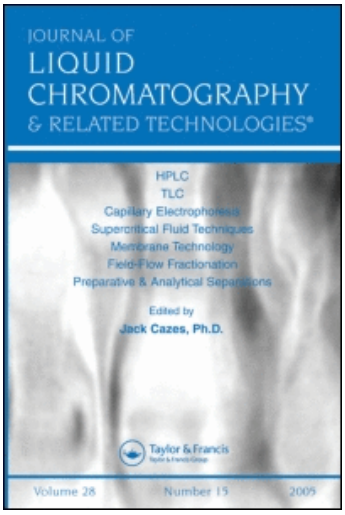

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Determination of Desmethyl- Selegiline, Methamphetamine and Amphetamine - The Main Metabolites of Selegiline in Plasma by HPLC After Derivatization

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DETERMINATION OF DESMETHYL-SELEGILINE, METHAMPHETAMINE AND AMPHETAMINE - THE MAIN METABOLITES OF SELEGILINE IN PLASMA BY HPLC AFTER DERIVATIZATION

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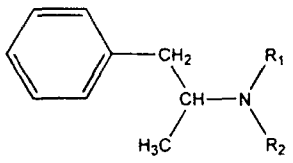
ABSTRACT

Determination of desmethylselegiline, methamphetamine and amphetamine in plasma requires a very sensitive and selective method. These substances are metabolites of the MAO-inhibitor selegiline. This is the first published HPLC method which is able to determine these three substances down to a limit of quantification of 0.2 ng/mL human plasma. After extraction as free bases into an organic solvent and reextraction as salts into an inorganic acid all three substances and an internal standard (phentermine) are derivatized for fluorescence detection after reversed phase chromatography. The recovery is better than 90 % for all substances. The linearity in the range tested (0.2 - 15 ng for each substance/mL plasma) is very good indeed. The precision and accuracy is usually smaller than 5 %. Pharmacokinetic results are presented.



	R_1	R_2
Selegiline	CH_3	CH2CCH
Desmethylselegiline	H	CH2CCH
Methamphetamine	CH_3	H
Amphetamine	H	H

Figure 1. Structures of selegiline and ist amine metabolites.

INTRODUCTION

Selegiline (Deprenyl) is a selective and irreversible inhibitor of monoamino oxidase B. Therefore it is used in treatment of Parkinson's disease. Selegiline is readily absorbed from the gastrointestinal tract. As a lipophilic substance selegiline is showing a high volume of distribution and also a high rate of biotransformation. Therefore the plasma concentrations of selegiline are very low and the half-life of eliminination is very short with about 9 minutes. Selegiline is converted via metabolism to desmethylselegiline (DMS), methamphetamine (MA) and amphetamine (A). These metabolites have much longer elimination half-lifes than selegiline (see Figure 1).

Amphetamines have been of considerable interest in forensic science and toxicology and some methods for their analysis exist. Few of them, however, allow for determination at low ng/mL or pg/mL plasma.

Recently published methods for determining selegiline with GC-MS[1] or its metabolites DMS, MA and A in plasma were using GC-ECD after derivatization[2] or GC-negative ion CI-MS.[3] No more published methods can be found in the last 5 years.

This study describes the determination of DMS, MA and A in plasma down to determination values of 0.25 ng/mL for DMS and 0.20 ng/mL for MA and A. HPLC with fluorescence detection was used after extraction into an organic solvent and reextraction into an anorganic acid with following derivatization of the secondary amino group (DMS and MA) and the primary amino group (A) with the same derivatization agent.

EXPERIMENTAL

Amphetamine and methamphetamine was from the university institute of organic chemistry (Vienna/Austria). Desmethylselegiline was synthesized. The internal standard phentermine was a gift from Gerot (Vienna/Austria). Reagents of GR quality were supplied by E. Merck (Darmstadt, FRG) and by Rathburn (Scotland). The derivatization agent (AQC) was first published for amino acids by the Millipore Corporation (Milford, USA)[4,5] and was synthesized in our laboratory.

AQC: 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate

Apparatus and Chromatographic Equipment

The chromatographic system consisted of a HP-1090 (Hewlett Packard, USA) with a fluorescence detector FP-920 (Jasco, Japan) connected to a PE-Nelson 900 software for integration.

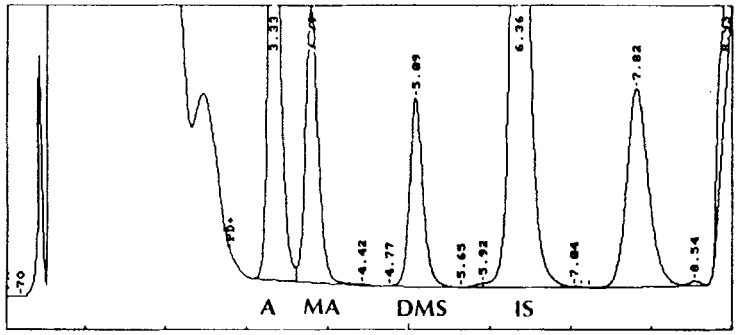
The analytical column 125 x 4 mm i. d. (SRD, Vienna, Austria) was filled with Supersphere RP 18 e μm. The mobile phase was a step gradient mixture of :

A. 19 % acetonitrile/27 % methanol/54 % buffer (v/v)
B. 75 % acetonitrile, 25 % buffer (v/v)

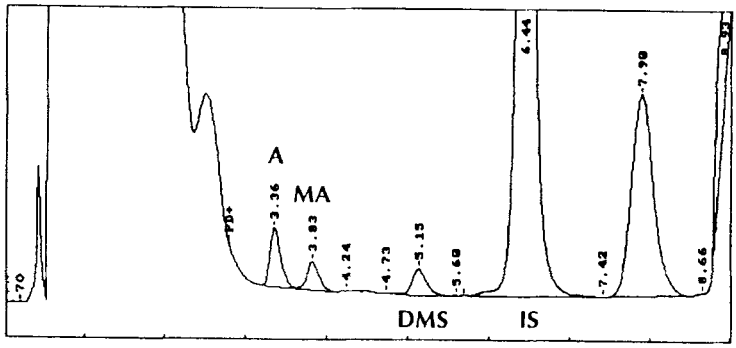
The step to mixture B was after 8 minutes for 2 minutes in order to clean the column. The flow rate was 2.0 mL/min at 60°C column temperature.

Preparation of Plasma Samples

1.0 mL plasma was mixed with internal standard solution (20 μL) and 50 μL 2 M sodium hydroxide. After adding 4 mL of extraction solvent (mixture of ethylacetate/hexane [1:1]) the samples were shaken for 1 minute and then centrifuged. 3 mL of the organic phase was reextracted by 0.3 mL of diluted hydrochloric acid. 0.2 mL of the acid phase was mixed with diluted sodium hydroxide solution and buffer. After adding 100 μ of derivatization agent (3 mg AQC/mL acetonitrile) the samples were mixed. After 10 minutes at room temperature, the samples could be injected.



A



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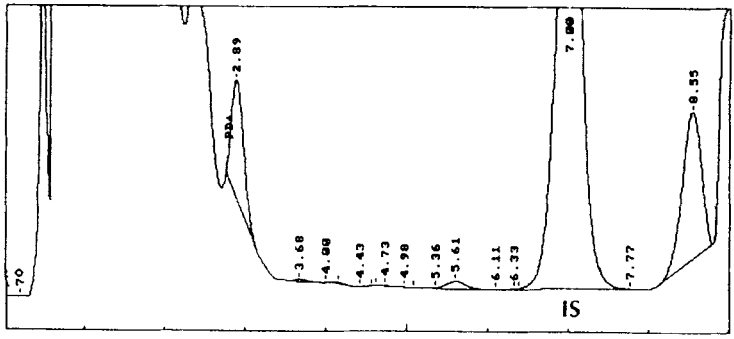

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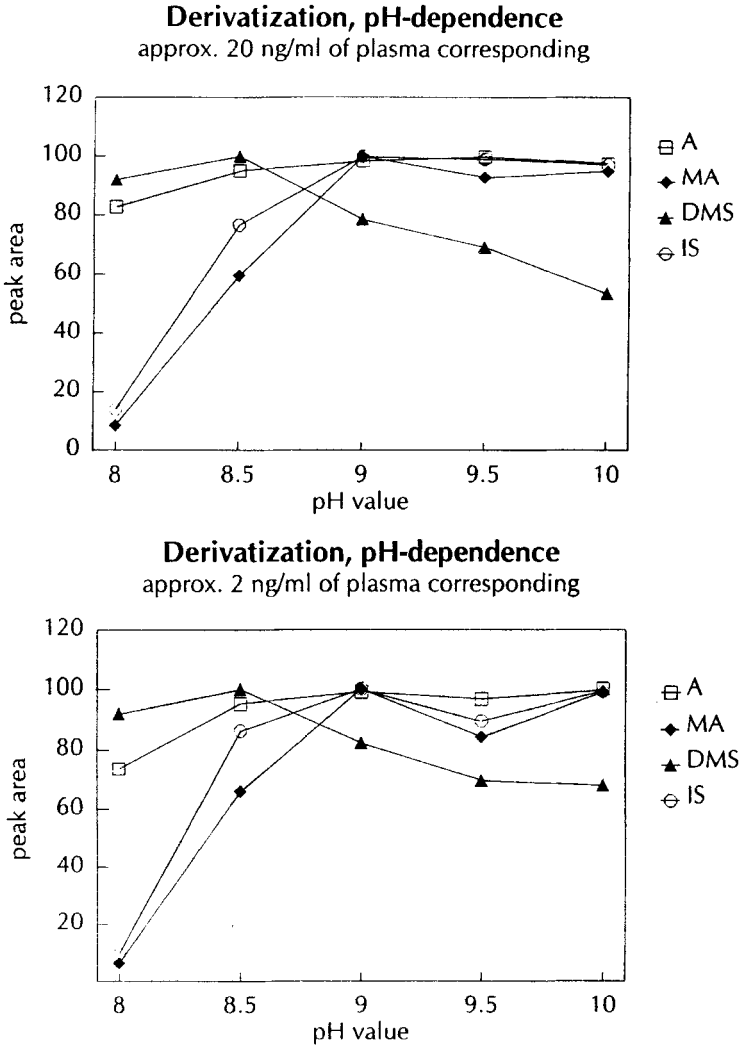

Figure 3. pH-dependence of the derivatization at 2 different concentration levels for A, MA, DMS and the Internal Standard (phentermine).

Figure 2. (left) Plasma calibration samples:
A: 5.22 ng A, 5.12 ng MA and 5.45 ng DMS / ml of plasma.
B: 0.42 ng A, 0.41 ng MA and 0.45 ng D MS / ml of plasma.
C: Blank pool-plasma.

Validation

The method was validated by adding various different quantities of DMS, MA and A to pooled human plasma. The resulting concentrations were between 0.2 and 15 ng/mL pool-plasma. These calibration series were subjected to the entire analytical procedure, so as to test the linearity, precision and accuracy of the method.

RESULTS AND DISCUSSION

Extraction and Reextraction

The absolute recovery from plasma at 4 - 9 ng/mL were 94.3 % for DMS (± 1.90 %, n = 4), 103.3 % for MA (± 0.85 %, n = 4) and 92.2 % for A (± 0.79 %, n = 4).

Chromatographic Separation

Our investigations showed some problems with endogenous plasma substances. Therefore we tried different reversed phase columns from different suppliers.

Also we changed the composition of the mobile phase with different percentages of acetonitrile and methanol. Figure 2 shows plasma calibration samples.

Derivatization Conditions

Different compositions of the reaction mixture were tried for derivatization. Fig. 3 shows the pH-dependence at 2 ng/mL and 20 ng/mL of the 3 substances and the internal standard.

Linearity, Precision, and Accuracy

In the calibration series, the linear regression between the spiked plasma concentrations and the peak area was determined after analysis of the calibration samples (Table 1).

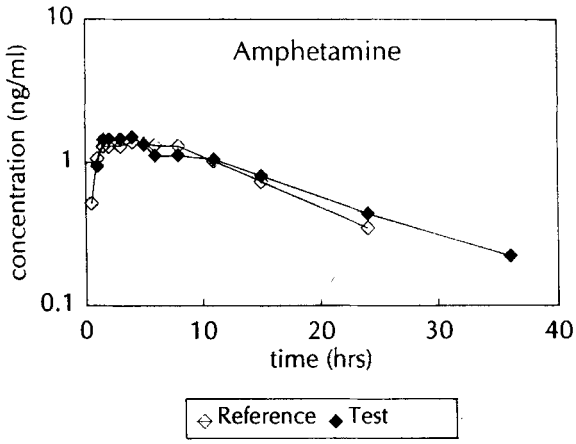

Figure 4. Plasma levels of A of a selected volunteer (vol. 7) after oral administration of 5 mg of selegiline.

Table 1

Linear Regression of A, MA, and DMS

	Slope	Intercept	Corr. Coeff	Range	Number
A	0.9545	0.0252	0.9989	0.21 - 15.29	18
MA	0.5397	0.0000	0.9981	0.20 - 14.97	18
DMS	0.3522	0.0989	0.9987	0.22 - 15.95	18

Table 2

Limit of Quantification for A, MA, and DMS

	Conc. -spiked/ mL Plasma	Precision (CV%)	Accuracy
amphetamine	0.17 ng	± 8.50 %	- 2.25 %
methamphetamine	0.18 ng	± 5.30 %	+ 12.90 %
desmethylselegiline	0.25 ng	± 12.17 %	- 8.30 %

Table 3

Amphethamine: Linearity, Precision, and Accuracy in Plasma

Sample	Conc-Obs ng/mL	Mean ng/mL	±CV%	Conc-Calc ng/mL	Accuracy %
St 0	0.013 -0.012 -0.025				
St 1	0.217 0.226 0.222	0.222	2.14	0.211	4.87
St 2	0.427 0.416 0.408	0.417	2.24	0.423	-1.32
St 3	0.848 0.843 0.807	0.832	2.68	0.844	-1.41
St 4	2.091 2.156 2.144	2.130	1.63	2.105	1.22
St 5	4.948 4.918 5.032	4.966	1.19	5.223	-4.92
St 6	15.390 15.402	15.529 15.795	1.49	15.291	1.56

Table 4

Methamphethamine: Linearity, Precision, and Accuracy in Plasma

Sample	Conc-Obs ng/mL	Mean ng/mL	±CV%	Conc-Calc ng/mL	Accuracy %
St 0	0.068 0.018 0.018				
St 1	0.232 0.223 0.218	0.224	3.25	3.207	8.38
St 2	0.425 0.403 0.383	0.404	5.14	0.414	-2.40
St 3	0.816 0.853 0.774	0.814	4.91	0.827	-1.55
St 4	2.051 2.085 2.000	2.045	2.09	2.061	-0.77
St 5	4.903 4.629 4.924	4.819	3.42	5.115	-5.79
St 6	15.513 15296 15.067	15.292	1.46	14.975	2.12

Table 5

Desmethylselegiline: Linearity, Precision, and Accuracy in Plasma

Sample	Conc-Obs ng/mL	Mean ng/mL	±CV%	Conc-Calc ng/mL	Accuracy %
St 0	-0.006				
	-0.274				
	0.020				
St 1	0.241	0.230	15.42	0.221	4.28
	0.190				
	0.259				
St 2	0.448	0.442	1.39	0.441	0.33
	0.436				
	0.444				
St 3	0.849	0.862	3.40	0.881	-2.19
	0.895				
	0.841				
St 4	2.221	2.205	1.01	2.196	0.39
	2.179				
	2.2`4				
St 5	5.170	5.217	2.19	5.449	-4.26
	5.347				
	5.133				
St 6	16.500	16.185	2.15	15.953	1.45
	16.243				
	15.812				

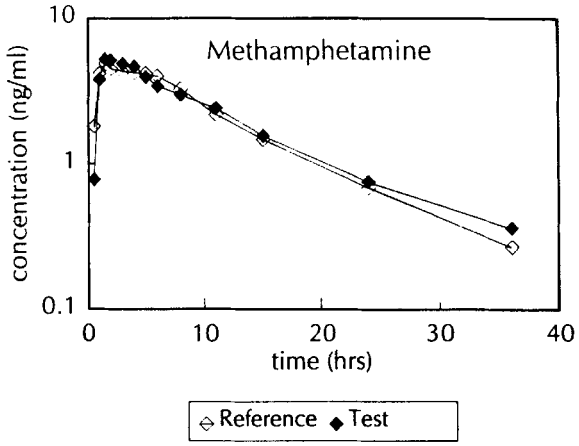

Figure 5. Plasma levels of MA of a selected volunteer (vol. 7) after oral administration of 5 mg of selegiline.

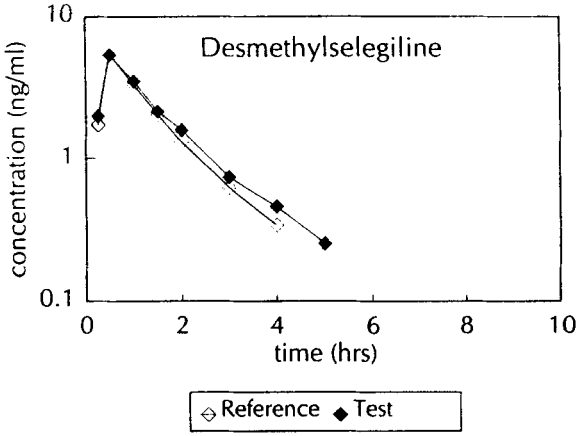

Figure 6. Plasma levels of DMS of a selected volunteer (vol. 7) after oral administration of 5 mg of selegiline.

The values for precision and accuracy[6] are listed in Table 3 for amphetamine, A, Table 4 for methamphetamine, MA, and Table 5 for desmethylselegiline, DMS.

Limit of Quantification

6 spiked plasma samples at low levels were analyzed. The following results were obtained (Table 2).

Stability

The stability was tested in spiked plasma and injection solution. A, MA and DMS were stable in plasma at room temperature over 4.5, 8 and 24 hours. The same results were obtained after two freeze and thaw cycles. The derivatized A, MA and DMS was stable in the injection solution over 27 hours at room temperature and was also stable after freezing and thawing.

Pharmacokinetics

After oral application of a single dose of selegiline the following results (see Figures 4, 5 and 6) were obtained. More detailed pharmacokinetic results from bioequivalence studies are published elsewhere.[7]

CONCLUSION

To sum up, it can be said that the analytical method described herein is the first HPLC method for measuring A, MA and DMS together to a very low level of about 200 pg/mL plasma. Only one published method is able to measure all 3 substances to such a low level and this method used GC-negative-ion CI-MS.[3]

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