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HPLC Analysis and Detection of L-Deprenyl

V. CSOMOR[1], R. LAUFER[1,2], Z. PÖSTÉNYI[2], H. KALÁSZ[2,*], E. ADEGHATE[3], S. M. NURULAIN[4], K. TEKES[1], AND A. ADEM[4]

[1]Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary
[2]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
[3]Department of Anatomy, United Arab Emirates University, Al Ain, UAE
[4]Department of Pharmacology and Therapeutics, United Arab Emirates University, Al Ain, UAE
*E-mail: huba.kalasz@gmail.com

Summary. L-Deprenyl and its major metabolites were subjected to reversed-phase high-performance liquid chromatography (HPLC). The separation was monitored using both ultraviolet (UV) and electrochemical detectors. Ultraviolet absorbance detected L-deprenyl, L-nordeprenyl, L-methamphetamine, and L-amphetamine. Amperometric detection was specific and sensitive to the parent compound (L-deprenyl, a tertiary amine) only. Peaks of the major L-deprenyl metabolites did not give any comparable signal using amperometric monitoring.

Key Words: L-deprenyl, HPLC, amperometric detection, UV detection, metabolites

Introduction

L-Deprenyl (also called as Selegiline, Movergan, Anipryl, Eldepryl, Emsam, Zelapar, etc.) has widely been used in the treatment of Parkinson's disease to delay its progress, as well as to postpone starting L-3,4-dihydroxyphenylalanine (L-DOPA) administration [1, 2]. Further, recent uses include treatment of depression and senile dementia. Published potential indications refer to an increase in both longevity and male sexual performance [3].

L-Deprenyl and its major metabolites (L-nordeprenyl, L-methamphetamine, and L-amphetamine) can be determined in serum and urine using various separation methods, such as gas chromatography (GC) [4, 5], gas chromatography–mass spectrometry (GC–MS) [6, 7], high-performance liquid chromatography (HPLC) [8, 9], HPLC–MS [10–12], and capillary electrophoresis [13, 14]. Specificity for the parent compound is given by the use of selected ion monitoring of either GC–MS or HPLC–MS.

Capillary electrophoresis studies made evident that mutarotation (change in optical activity) does not take place in L-deprenyl metabolism [13, 14].

Regarding the pharmacological activity, major metabolites of L-deprenyl have only minor pharmacological effect (L-nordeprenyl) or no

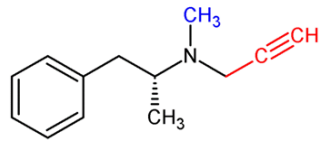
effect (L-methamphetamine and L-amphetamine) in comparison to that of the parent drug.

This paper gives an account on how a parent drug (L-deprenyl, here), and not the metabolites, can selectively be monitored by amperometric mode of detection.

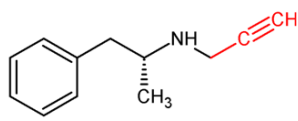
Experimental

Materials

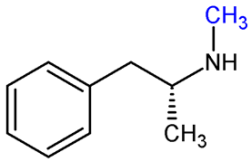
Chemicals were purchased from Merck (Darmstadt, Germany) in the possible best available quality of purity. L-Deprenyl, L-nordeprenyl, L-methamphetamine, and L-amphetamine were kind gifts from Chinoin Pharmaceutical Company (present name: sanofi-aventis/Chinoin), Budapest, Hungary. The chemical structures of L-deprenyl, L-nordeprenyl, L-methamphetamine, and L-amphetamine are given in *Fig. 1*.



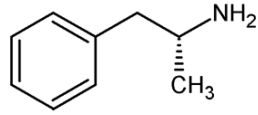
L-deprenyl



L-nordeprenyl



L-methamphetamine



L-amphetamine

Fig. 1. The chemical structures of L-deprenyl, L-nordeprenyl, L-methamphetamine, and L-amphetamine

Rat brain homogenate originated from a control sample of our previous experiment (permission no. 1810/003/2004, ANTSz, Budapest, Hungary) [15].

Methods

HPLC was performed with a JASCO (Tokyo, Japan) system consisting of DG-2080-54 Degasser, PU-1580 HPLC Pump, AS-2057 Automatic Injector, Jasco MD-1510 Multiwavelength Detector (operated at 265 nm), Antec Leyden Decade Digital Amperometric Detector (Zoeterwoude, The Netherlands), and JMBS Hercule 2000 Chromatography Interface (Le Fontanil, France) (E_{ox} = 0.9 V). Chromatograms were electronically stored and evaluated using Borwin 1.50 Chromatography Software (JMBS, Le Fontanil, France). The separation was done using a 4.6 mm × 250 mm, 5 μm) Zorbax Rx-C18 octadecyl silica column (Agilent Technologies, supplied by Kromat Kft., Budapest, Hungary). The mobile phase was sodium phosphate–citrate buffer (10.03 g $Na_2HPO_4 \cdot 2H_2O$ + 10.08 g citric acid + 10 mg Na_2EDTA + 100 mg ion-pairing agent (1-octane sulfonic acid sodium salt) dissolved in water, pH adjusted to 3.7 with phosphoric acid and completed to 1000 mL) containing 20%, 25%, and 30% acetonitrile as organic modifier, in the various experiments. Mobile phase flow rate was 1 mL min^{-1}.

Results

Figure 2 shows separation of L-deprenyl and its major metabolites (L-nordeprenyl, L-methamphetamine, and L-amphetamine) on Zorbax Rx-C18 octadecyl silica column monitored at 265 nm. Resolution of these four components depends on the extent of organic modifier in the mobile phase. Twenty percent acetonitrile in the sodium phosphate–citrate buffer at pH 3.7 gave proper resolutions between each pair of compounds; however, higher acetonitrile content shortened elution time definitely together with a certain decrease of resolution.

The parent L-deprenyl can solely be detected if amperometric monitoring is employed *(Fig. 3)*. A calibration curve for L-deprenyl (amperometric detection) is given in *Fig. 4*.

There was not any interfering peak from the rat brain homogenate coeluted with L-deprenyl (not shown here) when the mobile phase was sodium phosphate–citrate buffer containing 20% acetonitrile.

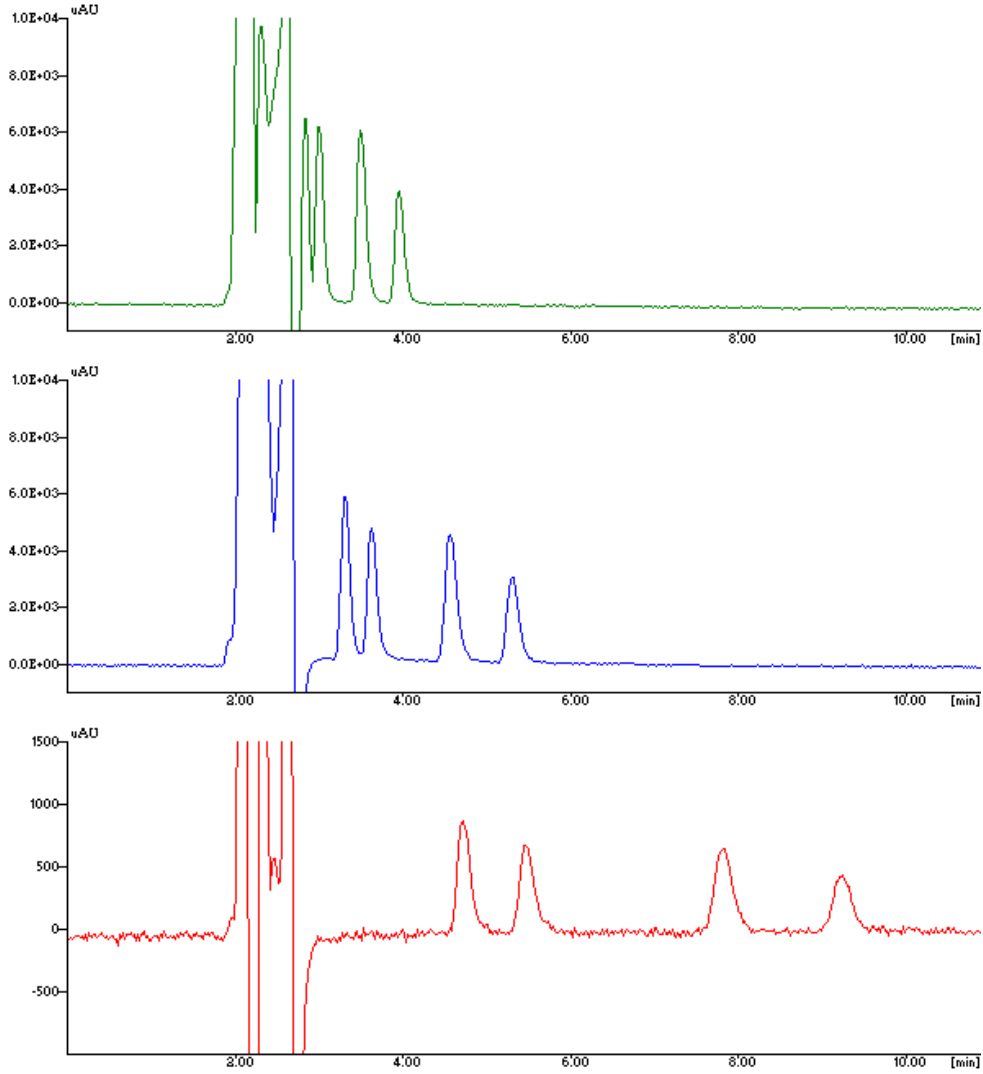


Fig. 2. Elution sequence of HPLC separation of L-amphetamine, L-methamphetamine, L-nordeprenyl, and L-deprenyl using UV detection at 265 nm. The mobile phase contained 30% acetonitrile (top chromatogram), 25% acetonitril (center chromatogram), and 20% acetonitrile (bottom chromatogram). Injected amounts of L-amphetamine, L-methamphetamine, L-nordeprenyl, and L-deprenyl were 80 ng each (top and center chromatograms) and 20 ng each (bottom chromatogram)

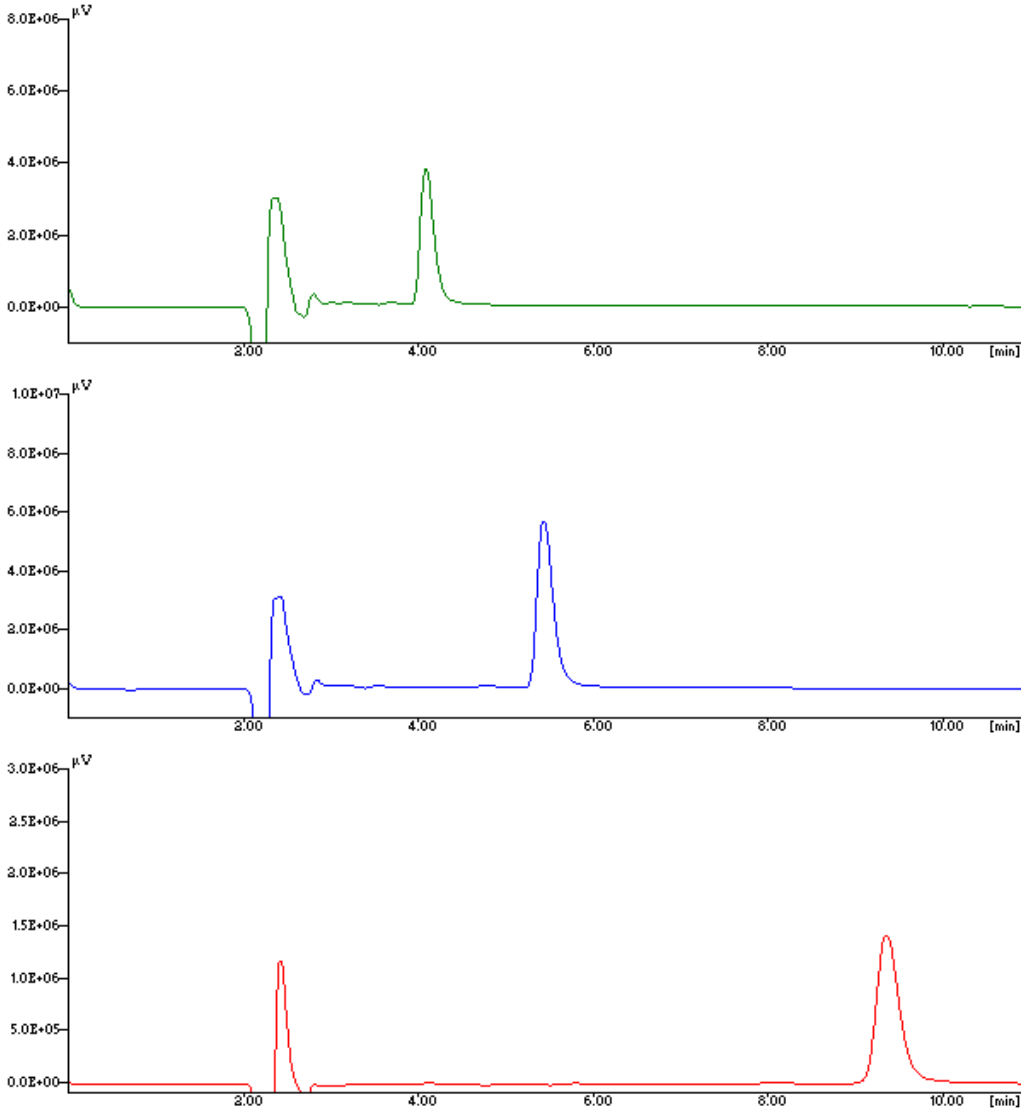


Fig. 3. Changes in the elution time of L-deprenyl using amperometric detection (E_{ox} = 0.9 V). The mobile phase contained 30% acetonitrile (top chromatogram), 25% acetonitril (center chromatogram), and 20% acetonitrile (bottom chromatogram). Injected amounts of L-deprenyl were 80 ng (top and center chromatograms) and 20 ng (bottom chromatogram)

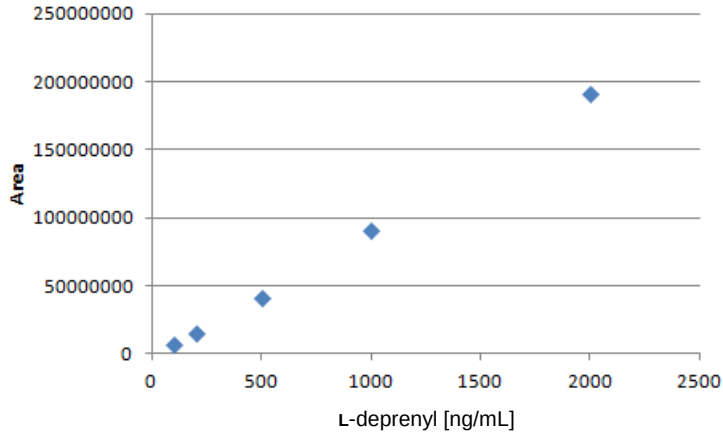


Fig. 4. Calibration curve for L-deprenyl when amperometric detection (E_{ox} = 0.9 V) was used. The mobile phase contained 20% acetonitrile. R^2 = 0.99921

Discussion

HPLC has generally been used to determine drug concentration in various body fluids and tissues. Ultraviolet absorbance and mass spectrometry are the two most generally used detection methods. Amperometric detection of HPLC separation became widely used for determination of biogenic amines in various animal (and human) specimens as a consequence of the overall simplicity of the instrumentation required.

Determination of the level of a drug and that of its metabolites is an integral part of pharmacokinetics when both the drug and some of its metabolites have pharmacological activity at a comparable level. It is the case of prodrugs and drugs with active metabolites. At the same time, the overwhelming majority of drugs do not have active metabolite(s). To determine concentration of drug metabolites is a time- and money-consuming procedure that should be avoided. Amperometric detection makes possible to determine L-deprenyl without any overlapping peak originated from either its own metabolites or from the background peaks of tissue (here, rat brain) homogenate. Sensitivity of amperometric detection is higher than that of the ultraviolet (UV) detection. Specificity of amperometric detection offers optimization based on the presence/absence of background peaks originated from the tissue homogenate, while separation of parent drug from its metabolites is avoided.

Parallel experiments (not shown here) indicate that various analogues of L-deprenyl (p-fluoro-deprenyl, p-bromo-deprenyl, p-methyl-deprenyl, etc.) and their metabolites behave like L-deprenyl. The parent drug is well detectable using amperometric monitoring, and neither one of its desalkylated metabolites gives peak at the chromatogram.

Amperometric detection could be used to determine L-deprenyl level in the rat brain having absence of interfering HPLC peaks with its metabolites.

Conclusion

Employment of amperometric detection is going to open new ways in the study of pharmacokinetics of tertiary amino compounds. Optimization of chromatographic separation can easily be done by changing the acetonitril concentration.

Acknowledgments

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Conflict of interest statement

The authors have declared no conflict of interest.

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