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DETERMINATION OF LOW PLASMA CONCENTRATIONS OF 3-METHOXY-4-HYDROXYPHENYLETHYLENE GLYCOL USING GAS CHROMATOGRAPHY—NEGATIVE-ION CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

Gas chromatography—negative-ion chemical ionization mass spectrometry (GC—NICI-MS) allowed the detection of extremely low plasma concentrations of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). Glucuronide and sulphate conjugates of MHPG were determined after enzymatic hydrolysis of plasma with β-glucuronidase—arylsulphatase. A 1-ml plasma sample was extracted at the pH of the hydrolysis (pH 4.8) with ethyl acetate, and the dry extract was derivatized with pentafluoropropionic anhydride in ethyl acetate. After evaporation of the solvent, the residue was dissolved in benzene and an aliquot was analysed by GC—NICI-MS. A trideuterated analogue of MHPG was used as an internal standard. Negative-ion chemical ionization of the pentafluoropropionyl derivatives was carried out using ammonia. The ion—molecule adducts at *m/e* 766 and 785 (MHPG) and *m/e* 769 and 788 (internal standard) were formed from the pentafluoropropionyl derivatives with the ions of *m/e* 163 ($CF_3CF_2COO^-$) and *m/e* 144 (loss of fluorine from *m/e* 163). The concentrations of the ions of *m/e* 163 and 144 play a major role in the sensitivity and precision of this technique, which allows the detection of free MHPG plasma concentrations as low as 100 pg/ml in routine analysis.

INTRODUCTION

3-Methoxy-4-hydroxyphenylethylene glycol (MHPG) is a major metabolite of noradrenaline and results from the O-methylation (by catechol O-methyltransferase) and deamination (by monoamine oxidase, MAO) of the neurotrans-

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mitter. MHPG is present in plasma in the free form (20–30%) or as a conjugate with glucuronic and sulphuric acids [1–4]. The degree and the kinetics of the MAO inhibition can be characterized by the determination of MHPG in body fluids following the administration of MAO inhibitors [1, 2]. The extremely low concentrations of MHPG, particularly after the depletion induced by an MAO inhibitor, requires a very sensitive and specific technique of analysis, such as gas chromatography–mass spectrometry (GC–MS).

Almost all of the previously published GC–MS methods are based on electron-impact (EI) mass fragmentography after acylation [3–7] or silylation [8] of MHPG, using deuterated internal standards. The determination of the pentafluoropropionyl (PFP) derivative by chemical ionization (CI) with methane and isobutane reagent gas has also been reported [9].

In this study, we used the PFP derivative of MHPG, because it is more stable than the corresponding trifluoroacetyl derivative [10]. The assay reported demonstrates the advantage of negative-ion chemical ionization (NICI) for the routine quantification of the extremely low plasma MHPG concentrations, which may occur during treatment with monoamine oxidase inhibitors.

EXPERIMENTAL

Instrumentation

GC–MS analyses were performed with a Girdel (Model 32) gas chromatograph coupled with a Ribermag R10-10B mass spectrometer. A silanized glass column (2 m × 2 mm I.D.) was packed with 3% OV-1 on 80–100 mesh Supelcoport (Supelco) and carefully conditioned. Helium was used as the carrier gas and ammonia as the auxiliary gas for CI.

The operating temperatures were column oven 165°C, injection port 230°C and interface 250°C. The ion source pressure was optimized between $6 \cdot 10^{-2}$ and $8 \cdot 10^{-2}$ Torr, with an as high as possible ammonia/helium ratio. *m/e* 766 (MHPG) and *m/e* 769 ([$^2H_3$]MHPG) were chosen for fragmentography.

The ion source and electron energy were tuned daily. The electron multiplier was set at 2.2 kV. The time of integration was 100 msec per ion at an electronic gain of 10^6 V/A.

Chemicals and reagents

Stock solutions in redistilled water of authentic MHPG hemipiperazine salt (Sigma) and of the glycol side-chain trideuterated [$^2H_3$]MHPG hemipiperazine salt (Merck Sharp & Dohme) were stable at 4°C for at least two weeks. The deuterium enrichment of [$^2H_3$]MHPG was more than 97.6%, with a ratio between MHPG and [$^2H_3$]MHPG of less than 0.1% as determined by the relative intensities of the corresponding ions, using the NICI method discussed in this paper.

Pentafluoropropionic anhydride (PFPA) was obtained from Pierce, and ethyl acetate and benzene of RPE-ACS grade from Carlo Erba.

Enzyme hydrolyses were carried out with β-glucuronidase and aryl sulphatase (H_1, Sigma). Enzyme solutions of 1000 U/ml of plasma were prepared in 0.2 *M* acetate buffer (pH 4.8).

Sample preparation

Samples and standards were prepared in silanized conical tapered glass tubes.

Free MHPG. Plasma (1 ml) was mixed with 1 ml of 0.2 *M* acetate buffer (pH 4.8) and with 5 or 10 ng of [$^2H_3$]MHPG interal standard (10 μl of aqueous solution). Samples were extracted with two 7-ml volumes of ethyl acetate on a mechanical shaker (20 min). After centrifugation (10 min at 1000 *g*), the organic phase was transferred into a second tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The remainder was lyophilized (3 h) to eliminate the residual water retained by gel formation with lipids [11].

The dry extracts were derivatized at 60°C (5 h) with a mixture of 100 μl of PFPA and 40 μl of dried ethyl acetate (over anhydrous sodium sulphate). After evaporation of the excess of the reagent mixture at 25°C under a stream of nitrogen, the reaction product was dissolved in 20 μl of benzene, and an aliquot (2 μl) of the solution was injected into the gas chromatograph. The derivatives were stable for two weeks in the PFPA—ethyl acetate mixture at 4°C and for several days in benzene solution at the same temperature.

Total MHPG. Plasma (1 ml) was mixed with 1 ml of 0.2 *M* acetate buffer (pH 4.8), containing the enzyme, and then 10 ng of [$^2H_3$]MHPG internal standard were added. Samples were incubated at 37°C for 16 h and extracted as described for free MHPG.

Calibration procedure

As MHPG is an endogenous compound in plasma, it is not possible to

TABLE I

PRECISION AND ACCURACY OF THE NEGATIVE-ION CHEMICAL IONIZATION GC–MS METHOD

Direct calibration graph from dog plasma; internal standard, [$^2H_3$]MHPG (10 ng/ml). $x_{(y=0)}$ = −1.118 ng/ml. Correlation = 0.9998.

Spiked MHPG concentration (ng/ml) (x)	y = area 766 / area 769	Mean y	Relative S.D. (%) of y	Found MHPG concentration (ng/ml) (x')	Mean found concentration ($\bar{x}'$)	Relative S.D. (%) of x'	Relative error (%) $\left(\frac{\bar{x}' - x}{x}\right)$
0	0.1090	0.1150	5.7	−0.0724	−0.0148	—	—
	0.1140			−0.0244			
	0.1220			0.0523			
1	0.2201	0.2186	4.1	0.9933	0.9789	8.8	−2.1
	0.2090			0.8868			
	0.2267			1.0566			
2	0.3094	0.3138	3.5	1.8499	1.8917	5.6	−5.4
	0.3264			2.0129			
	0.3055			1.8124			
5	0.6509	0.6501	0.8	5.1256	5.1176	0.9	+2.4
	0.6447			5.0661			
	0.6546			5.1611			
10	1.1545	1.1683	1.7	9.9562	10.0882	1.8	+0.9
	1.1598			10.0070			
	1.1905			10.3015			
20	2.2061			20.0432	19.9383	0.7	−0.3
	2.2016			20.0000			
	2.1778			19.7718			

TABLE II

PRECISION AND ACCURACY OF THE NEGATIVE-ION CHEMICAL IONIZATION GC–MS METHOD

Reversed calibration graph from dog plasma; internal standard, endogenous MHPG. $x_{(y=0)}$ = −4.0 pg/ml. Correlation = 0.9996. Concentration of free MHPG calculated from slope: 1106 pg/ml dog plasma [see $x_{(y=0)}$ in Table I].

Spiked concentration of [$^2H_3$]MHPG (pg/ml) (x)	y = area 769 / area 766	Mean y	Relative S.D. (%) of y	Found concentration (pg/ml) (x')	Mean found concentration ($\bar{x}'$)	Relative S.D. (%) of x'	Relative error (%) $\left(\frac{\bar{x}' - x}{x}\right)$
200	0.1728	0.1875	9.6	187.1	203.3	9.7	+1.65
	0.2075			225.4			
	0.1821			197.4			
1000	0.8283	0.8964	6.7	911.8	987.1	6.7	−1.29
	0.9405			1035.8			
	0.9204			1013.6			
2000	1.8262	1.8235	1.5	2015.0	2012.0	1.5	+0.6
	1.8493			2040.5			
	1.7950			1980.5			
5000	4.4862	4.5241	2.0	4055.8	4997.7	2.0	−0.05
	4.6258			5110.1			
	4.4603			4927.1			

prepare the calibration graph in plasma directly when the concentrations of MHPG are below the basal values. In fact, the endogenous MHPG (given by the intercept value on the calibration graph) makes the analysis of concentrations below this level uncertain (i.e., a decrease of MHPG following inhibition of MAO). Average values in the literature are 5.8 ± 0.6 ng/ml [4] and 3.3 ± 1.3 ng/ml [7] for free MHPG, and 10.2 ± 1.0 ng/ml [3] and 16.5 ± 4.4 ng/ml [6] for total MHPG. For this reason, two parallel calibration graphs were prepared. A direct calibration graph (Table I) was obtained with 1 ml of plasma by addition of 1, 2, 5, 10 and 20 ng of MHPG standard (in 10–50 μl of aqueous solution) and 10 ng [$^2H_3$]MHPG internal standard (in 10 μl of aqueous solution), followed by the previously described sample preparation. To evaluate the detection limit and the sensitivity of the method, we used a "reversed calibration graph", inverting the role of the internal standard [$^2H_3$]MHPG and MHPG (Table II). A series of samples containing 200, 1000, 2000 and 5000 pg/ml [$^2H_3$]MHPG were prepared in dog plasma and the endogenous free MHPG (1.1 ng/ml) was used as an internal standard. Fig. 1 shows the ion chromatogram obtained from a sample containing 200 pg/ml [$^2H_3$]MHPG in plasma (20 pg of [$^2H_3$]MHPG injected). Both calibration graphs showed reproducible results: the free MHPG level in dog plasma was 1.12 ng/ml when determined from the intercept on the abscissa of the direct calibration graph and 1.11 ng/ml calculated from the slope of the reversed calibration graph.

RESULTS AND DISCUSSION

The aim of this study was to establish a routine method for the determination of decreased concentrations of MHPG in plasma during treatment with IMAO A-type antidepressants. For this reason we chose a very simple sample preparation protocol.

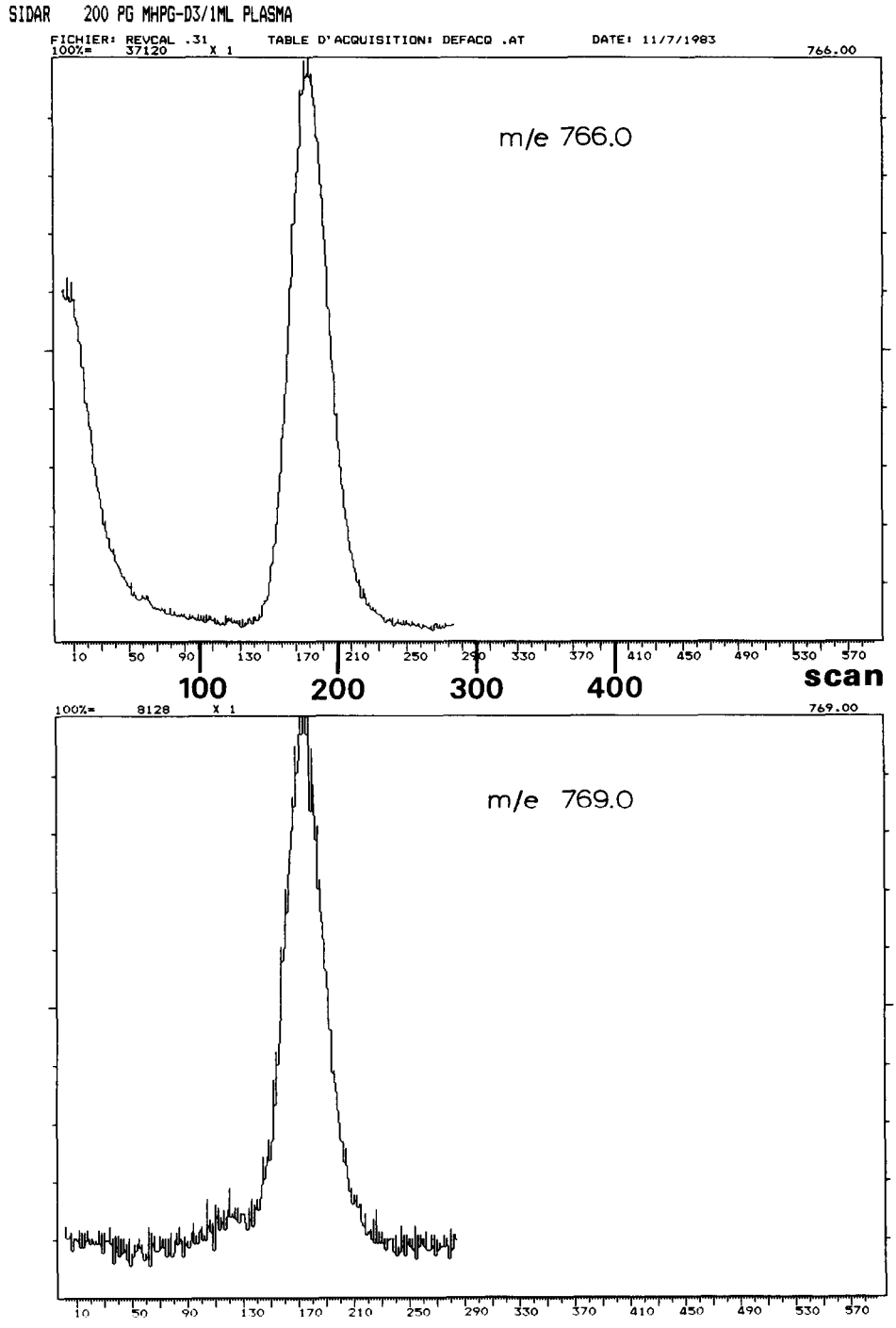

Fig. 1. Reversed calibration graph: 200 pg/ml [$^2H_3$]MHPG in plasma (*m/e* 769). Internal standard, endogenous MHPG (*m/e* 766) at a concentration of 1.1 ng/ml.

The maximum efficiency for hydrolysis with β-glucuronidase and aryl sulphatase was found between pH 4.2 and 5.2. As MHPG is a weak acid it was possible to carry out the extraction without changing the pH after hydrolysis. To mininize the need for manual intervention, a direct extraction method was adopted [3] without pre-extraction or precipitation of proteins.

It is well known that NICI-MS is a very sensitive technique for the analysis of those compounds which contain groups of high electron affinity [13—15]. The excellent sensitivity obtained with an electron-capture detector in the determination of perfluoroacyl-MHPG derivatives [11] could even be increased using NICI-MS [16]. Nevertheless, the optimal sensitivity in the NICI mode can be achieved only with very accurate tuning of the MS source [16]. In particular, control of the pressure in the ion source is a critical parameter [14].

CI-MS by "capture" of negative ions on the neutral molecules was established by Von Ardenne et al. [17]. They systematically examined the occurrence of ion—molecule reactions, using an "auxiliary gas" to slow down the electrons by collision. It was shown that halogen ions readily attach to neutral molecules, even at low concentrations, because of their high electron affinity [17, 18]. The formation of negative molecule—ion adducts with trifluoroacetyl derivatives [17] by the so-called "auto-ionization" was described.

Our results demonstrated the advantage of NICI-MS for determination of the MHPG(PFP)$_3$ derivative. Using ammonia as the auxiliary gas and helium as the carrier gas for chromatography, we observed the formation of characteristic anion—molecule adducts at *m/e* 785 and 766 (see Fig. 2), resulting in a considerably higher sensitivity compared with other MS methods (EI and positive CI with methane and ammonia).

The first assays showed a weak and irreproducible sensitivity for the PFP derivatives of the MHPG and [$^2H_3$]MHPG standard solutions, whereas good sensitivity and accuracy were obtained with samples prepared from plasma extracts. These apparently conflicting results were explained as follows: when a standard MHPG(PFP)$_3$ derivative is analysed, the reactive ions are supplied by the derivative itself (auto-ionization), the excess of PFPA reagent being

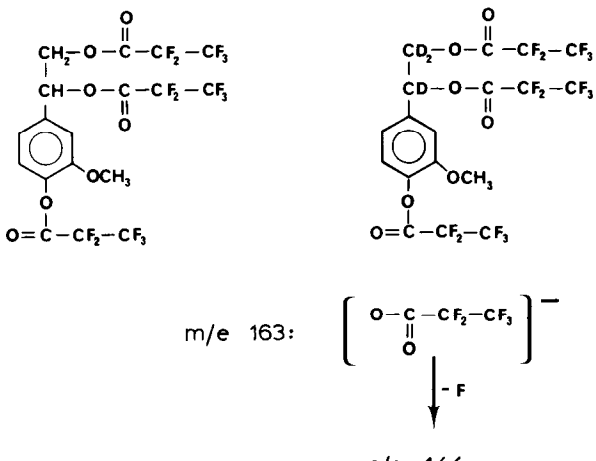

Fig. 2. Structures of the MHPG derivatives.

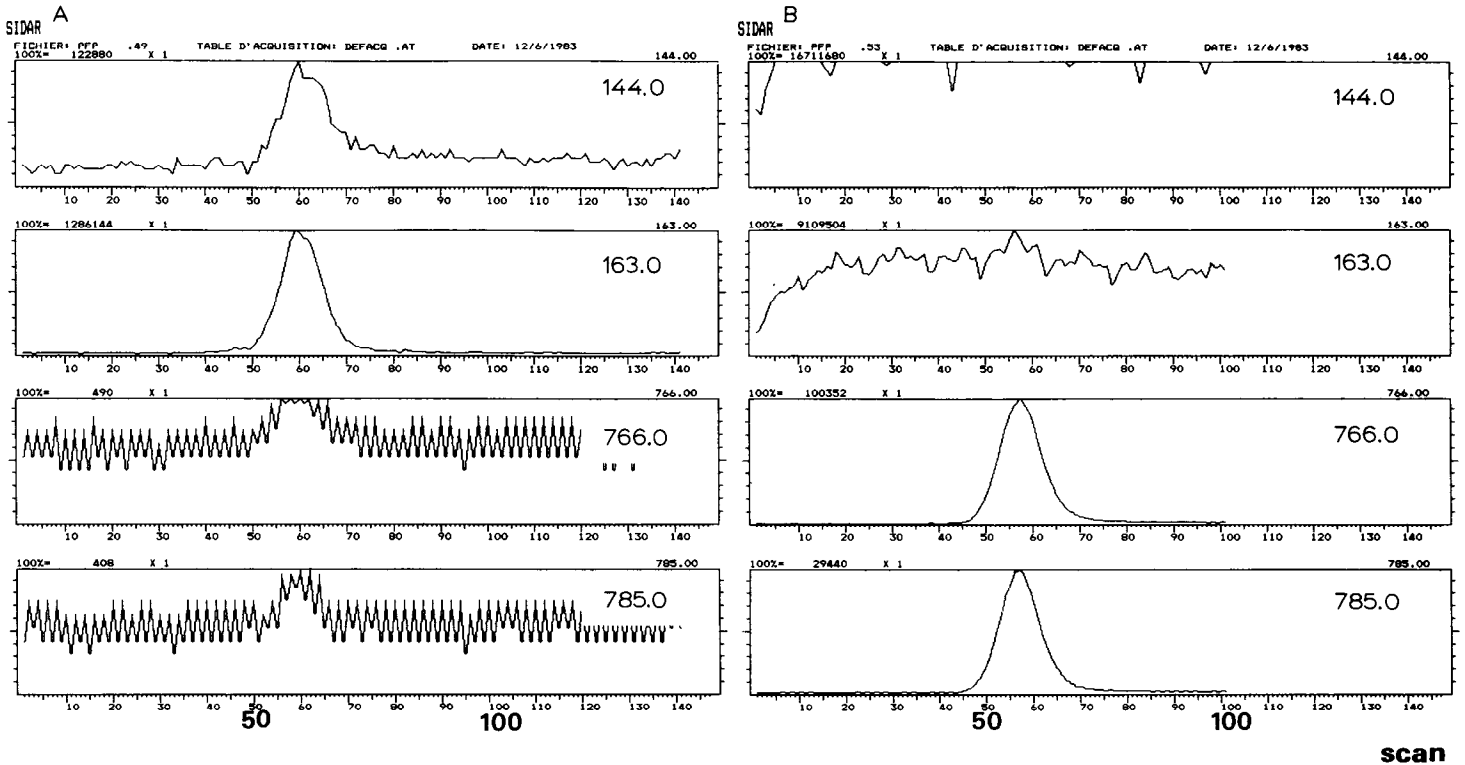

Fig. 3. Selected-ion chromatograms after injection of 1 ng of MHPG(PFP)$_3$. Effect of PFPA concentration in the ion source on the sensitivity: complete elimination of PFPA (A); presence of trace amounts of PFPA (B). *m/e* 144 and 163, integration time 1 msec; *m/e* 766 and 785, integration time 100 msec.

eliminated almost completely. With a plasma extract, the concentration of the reactive ions is increased by the large amount of PFP derivatives formed in the extract. This hypothesis was confirmed by the following experiment: a standard sample of MHPG$(PFP)_3$ and a plasma extract were analysed after complete removal of the reactive mixture (wash out used for the perfluoroacylation; see ref. 12) and compared with the corresponding reactive mixture containing samples. No formation of ion–molecule adducts was seen with the PFPA-free standard samples (Fig. 3A), whereas even 10 min after injection of 0.5 μl of PFPA—ethyl acetate reaction mixture the same sample gave these adduct ions (Fig. 3B). No difference was observed for the plasma extracts.

The formation of the adduct ions seems to be regulated by the concentration of the reactive ions in the ion source and the pressure and temperature of the ion source. For example, at 110°C, the intensities of the ions of *m/e* 766 and 785 are three and five times higher, respectively, than at 200°C, but the pollution of the source, with a consequent reduction in sensitivity, rapidly became unacceptable.

Our experience showed that when [$^2H_3$]MHPG is used as the internal standard, with a correctly tuned mass spectrometer, it is not necessary to repeat the entire calibration graph daily (e.g., 2 × 3 points). Using ammonia as the reagent gas, the pollution of the ion source can be eliminated by baking at 300°C, and permits operation for one to two weeks without a significant decrease in sensitivity. Below 100 pg/ml MHPG in plasma (10 pg of MHPG injected) further precautions were necessary. In particular, adsorption of the sample on the GC septum must be minimized [13]. This effect was checked by injections of solvent blanks.

Application

This technique has been used extensively to measure MHPG in plasma samples taken from depressed patients during clinical trials with cimoxatone*. Free and conjugated MHPG plasma concentrations were monitored during treatment for one month in an open clinical study with cimoxatone [1, 2]. After the first dose of cimoxatone, a marked decrease of the MHPG concentration in plasma was observed. The decrease in free MHPG was about 85% of the control from the first dose of cimoxatone and remained nearly constant during the whole treatment. When the treatment was stopped, five to six days were necessary for the MHPG levels to return to the control values [1, 2].

These studies demonstrated the sensitivity and the precision of NICI-MS, as concentrations of free MHPG as low as 100—800 pg/ml were detected.

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*Cimoxatone, 3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone (synthesized at the Delalande Research Centre), is a selective and reversible inhibitor of type A MAO and possesses antidepressant activity [1, 2].

REFERENCES

1 M.F. Poirier, J.R. Susini, P. Deniker, V. Rovei, M. Strolin Benedetti and J.M. Languillat, in K.F. Tipton, P. Dostert and M. Strolin Benedetti (Editors), Monoamine Oxidase and Disease, Academic Press, London, 1984, p. 341.
2 H. Loo, M.F. Poirier, P. Deniker, M. Strolin Benedetti, N. Sontag and A. Lesage, in K.F. Tipton, P. Dostert and M. Strolin Benedetti (Editors), Monoamine Oxidase and Disease, Academic Press, London, 1984, p. 353.
3 B. Sjöquist, B. Lindström and E. Anggard, J. Chromatogr., 105 (1975) 309.
4 F. Karoum, J. Moyer-Schwing, S.G. Potkin and R.J. Wyatt, Brain Res., 125 (1977) 333.
5 F. Karoum, J.C. Gillin, R.J. Wyatt and E. Costa, Biomed. Mass Spectrom., 2 (1975) 183.
6 S. Takahashi, D.G. Godse, J.J. Warsh and H.C. Stancer, Clin. Chim. Acta, 81 (1977) 183.
7 F.A.J. Muskiet, G.T. Nagel and B.G. Wolthers, Anal. Biochem., 109 (1980) 130.
8 D.C. Jimerson, S.P. Markey, J.A. Oliver and I.J. Kopin, Biomed. Mass Spectrom., 8 (1981) 256.
9 D.J. Edwards, M. Rizk and J. Neil, J. Chromatogr., 164 (1979) 407.
10 E. Gelpi, E. Peralta and J. Segura, J. Chromatogr. Sci., 12 (1974) 701.
11 H. Dekirmenjian and J. Maas, Clin. Chim. Acta, 52 (1974) 203.
12 Pierce Handbook and General Catalog, Pierce, Rockford, IL, 1982.
13 D.F. Hunt and F.W. Crow, Anal. Chem., 50 (1978) 1781.
14 R.C. Dougherty, Anal. Chem., 53 (1981) 625A.
15 H. Budzikiewicz, Angew. Chem., Int. Ed. Engl., 20 (1981) 624.
16 P.J. Arpino, Actual. Chim., April (1982) 19.
17 M. von Ardenne, K. Steinfelder and R. Tümmler, Elektronenanlagerungs Massenspektrographie Organischer Substanzen, Springer, Berlin, 1971.
18 H.P. Tannenbaum, J.D. Roberts and K.C. Dougherty, Anal. Chem., 47 (1975) 49.