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CHROMATOGRAPHIC DETERMINATION OF NANOGRAM LEVELS OF 2-(METHYLPROPARGYLAMINO)-1-PHENYLPROPANE (JUMEX, DEPRENYL) IN PLASMA

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SUMMARY

A quantitative capillary gas chromatographic method for the determination of 2-(methylpropargylamino)-1-phenylpropane (Jumex, Deprenyl) in plasma has been developed. The main problems in the development of the method were (1) very low therapeutic doses and consequently the necessity of very high sensitivity, and (2) the volatility of the free base form of Deprenyl. Various methods for the extraction of the compound and its pre-purification were tried, such as direct extraction from alkaline solutions, ion-pair extraction from acid solutions, extraction using Extrelut and Celite columns, and preconcentration and extraction on reversed-phase columns. As these methods turned out to be unsatisfactory, steam distillation of the free base was used for preseparation, and direct extraction of the distillate using a small amount of chloroform was used as a concentration step. The apparatus of Schulek, Constructed originally for the determination of CO_2, was used for the steam distillation step.

The final determination was carried out on a glass capillary column coated with Carbowax 20M impregnated with potassium hydroxide. A home-made all-glass direct injector and a Perkin-Elmer nitrogen–phosphorus detector were used. The quantitation limit of the method is 3 ng Deprenyl per ml plasma.

INTRODUCTION

2-(Methylpropargylamino)-1-phenylpropane (Jumex, Deprenyl) is an anti-Parkinson drug produced by Chinoin Pharmaceutical Works, and for its determination in plasma a very sensitive method had to be developed, because the human daily dose of the drug is 5 mg. We therefore developed a pre-purification and a gas chromatographic method for the determination of plasma levels of Deprenyl in the ng/ml

range. Various extraction methods were tried, and finally pre-purification was achieved by steam distillation.

It is well established that amines can be successfully chromatographed on packed columns if the support is treated with potassium hydroxide[1] and Carbowax or Apiezon liquid phases are used. High sensitivity was achieved by using a capillary column impregnated with potassium hydroxide and coated with Carbowax 20M, and nitrogen–phosphorus selective detection (NPD) was applied.

EXPERIMENTAL

Materials and apparatus

Deprenyl and its possible metabolites were prepared by Dr. Z. Ecseri (Chinoin).

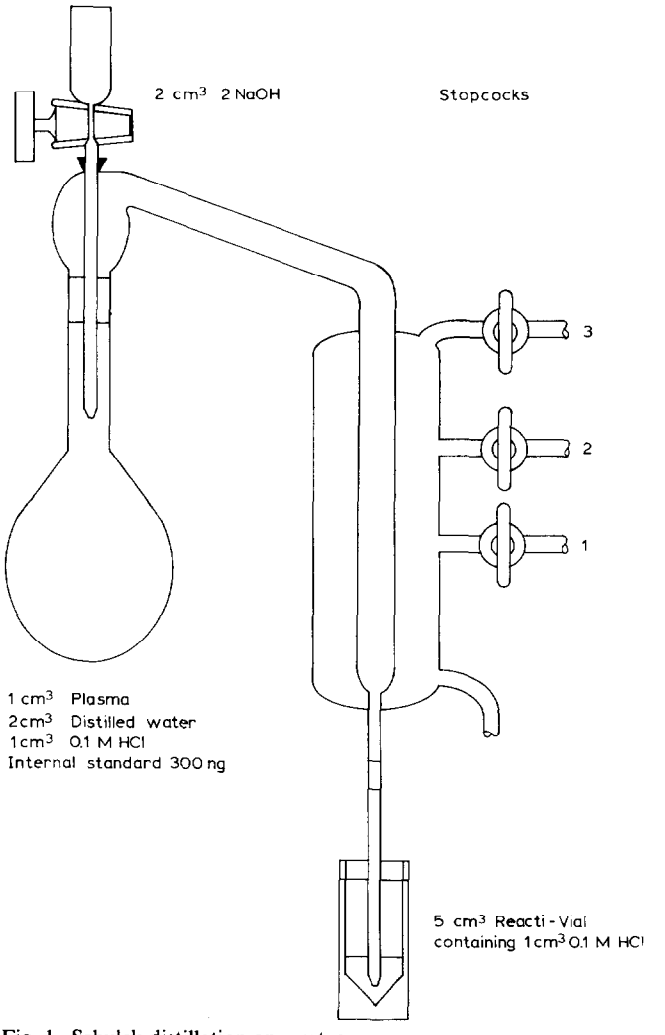

Fig. 1. Schulek distillation apparatus.

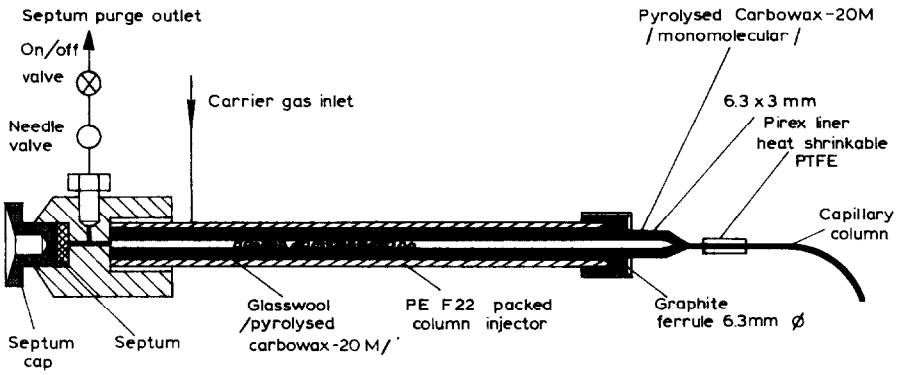

Fig. 2. Laboratory-made direct capillary injector.

Solvents were of analytical-reagent grade (Reanal); chloroform was freshly purified before use.

Extrelut and LiChroprep were purchased from Merck and Celite from Johns-Manville.

A Schulek apparatus[2] was constructed in our glass-blowing workshop. It is basically a Kjehldahl-type distillation apparatus, but it has a wide-bore condenser that is equipped with three exit tubes for cooling water, which are fitted with glass stopcocks (Fig. 1).

Using the stopcocks, the cooling of the condenser can be controlled. If conditions can be found that preclude codistillation in the first heating period, the condensation tube can be filled with steam by boiling the solution, expelling the air from the tube. If stronger cooling is now switched on, the steam is condensed and the partial vacuum produced sucks up liquid from the collection flask. If codistillation is started (in this instance by adding potassium hydroxide solution to the distillation flask) the vapour does not pass through the solution, forming bubbles, but is dissolved in the solution in the receiver or condenses with water vapour.

A Perkin-Elmer F22 gas chromatograph was used, equipped with a capillary inlet system (No. 069711) and a direct injector, which was made as follows (Fig. 2).

A 6.3 mm O.D., 3 mm I.D. Pyrex glass tube was inserted into the packed on-column injector of the instrument. One end of the glass tube was drawn to 0.6 mm O.D. in the flame. The capillary column was joined to the drawn-out end of the tube with heat-shrinkable PTFE tubing. A septum flush was also made in our workshop. The insert was passivated by pyrolysing Carbowax 20M on to it. We applied Cronin's method[3], modified for our circumstances: 5 ml of a 5% solution of Carbowax 20M in chloroform was percolated through the tube, which was then dried in a stream of nitrogen, heated at 270°C for 1 h, extracted by percolating 20 ml of pure chloroform through it and dried in a stream of nitrogen. A 16 m × 0.25 mm I.D. AR glass capillary column was pre-treated with potassium hydroxide solution and coated with Carbowax 20M (courtesy of Dr. G. Alexander).

A Perkin-Elmer nitrogen–phosphorus detector (No. 073897) was used, and evaluation was carried out with a PE Sigma 10 Data System.

TABLE I

TESTS OF EXTRACTION METHODS

Method	*Results*	*Reasons for losses*	*Conclusions drawn*	*Ref.*
Direct extraction from alkaline solution	Good recovery from standard solution (80%). Poor recovery from plasma (30%)	Bad separation of phases, protein-stabilized emulsions at phase boundary	Protein should be removed	4
Extraction after ultrafiltration (Amicon CF 25 membranes)	Poor recovery, even after washing the precipitate with ethanol. Protein incompletely removed. Method very time consuming	Emulsion still formed on phase boundary, adsorption on precipitate causes further losses	Method unsatisfactory for our purposes	5
Extraction on Extrelut column. Application of sample solution in acidic solution; alkalinization, extraction	Recovery poor, amount of coextracted impurities large. Pre-purification of Extrelut did not help	Deprenyl seems to be bound to proteins in alkaline solutions at room temperature	If extraction is carried out at room temperature it should be done at acidic pH values	6
Extraction on Celite or Extrelut in acid solution using ion-pairing agent	Recovery poor, amount of coextracted impurities still large, (ion-pairing agents used: iodide, hexanesulphonate)	Distribution coefficients unfavourable with solvents used. (ethyl acetate, chloroform, isopropanol)	Other column materials should be used	7
Extraction on C_{18} reversed-phase column (LiChroprep C_{18}, 30 × 4 mm) with ion pairing (various solvents)	Recovery better (50%) but variable. Amount of solvent used fairly large. Solvent impurities and coextracted materials give impurity peaks	Irreproductibility probably due to evaporation of material with solvent and adsorption on walls of vessels	As the amount of solvent cannot be reduced, a fundamentally different method should be tried	8

Methods

The following extraction methods were first tried: (1) direct extraction from alkaline solution[4]; (2) extraction after ultrafiltration (Amicon CF 25 membranes)[5]; (3) extraction on an Extrelut column; application of sample solution in acidic solution; alkalinization, extraction[6]; (4) extraction on Celite or Extrelut in acidic solution using an ion-pairing agent[7]; and (5) extraction on a C_{18} reversed-phase column (LiChroprep C_{18}, 30 × 4 mm I.D.) with ion-pairing (various solvents)[8].

As these extraction methods were unsuccesful we finally used the following steam distillation and concentration procedure.

Steam-distillation and concentration. A 1-ml volume of 0.1 *M* hydrochloric acid is placed in a 5-ml Reacti-Vial. A 1-ml volume of plasma (in calibration runs, containing the appropriate amount of Deprenyl hydrochloride, added in 0.01 *M* hydrochloric acid is acidified with 3 ml of 0.03 *M* hydrochloric acid in the distillation flask, internal standard (N-ethyl-*p*-toluidine, 300 ng in 0.01 *M* hydrochloric acid) and a few boiling chips are added, the flask is connected to the apparatus, the end of the distilling tube is dipped under the surface of the solution in the receiver and distillation is started by gently warming the flask. Cooling water is shut off, with the bottom stopcock open (*i.e.*, the level of the water is low). After distillation has started, and no more air bubbles pass through the receiver, cooling is switched on, the bottom stopcock is closed, the middle stopcock is opened and 2 ml of 2 *M* sodium hydroxide solution are added to the distillation flask through the dropping funnel. Addition of the sodium hydroxide solution must take place slowly and care should be taken that boiling does not stop during the addition. If the level of the receiving solution in the distilling tube falls too low, cooling can be enchanced by closing the middle and opening the top stopcock.

After collecting 2 ml of distillate, the condensing tube is raised and 2–3 drops more of condensate are collected. The tube is rinsed externally with a few drops of 0.01 *M* hydrochloric acid. The receiver is removed and distillation is stopped. Chloroform (200 μl) and 10 *M* sodium hydroxide solution (100 μl) are added to the receiver, which is closed immediately and mixed on a Vortex mixer for 2 min. After mixing, the sample is centrifuged (3000–4000 rpm, 2000 *g*). After centrifugation the chloroform layer is transferred (using a PTFE hypodermic needle and an all-glass syringe) into a 0.3 ml Reacti-Vial containing 10 μl of concentrated hydrochloric acid. The syringe is rinsed with 100 μl of chloroform. After mixing, the solution is evaporated at room temperature in a stream of nitrogen. Chloroform (20 μl) and 1 *M* sodium hydroxide solution (100 μl) are added to the residue, the vial is closed immediately and the resulting mixture is shaken on a Vortex mixer and centrifuged (3000–4000 rpm, 2000 *g*). The sample is cooled in an ice-bath and a 2-μl aliquot from the chloroform layer is injected.

Gas chromatographic conditions

Nitrogen was used as the carrier gas (2.5 ml/min) and make-up gas (25 ml/min), with a septum purge at 5 ml/min (septum purge stream closed for 2 min after injection). The injector temperature was 200°C and the detector temperature 280°C. A two-step temperature programme was used: from 70°C (2 min) to 105°C at 20°C/min, followed by 4°C/min to 170°C (10 min). The detector was optimized according to the age of the bead.

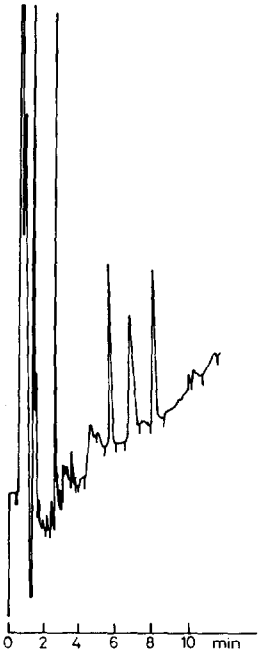

Fig. 3. Chromatogram of 1 ml of blank plasma. For gas chromatographic conditions, see text.

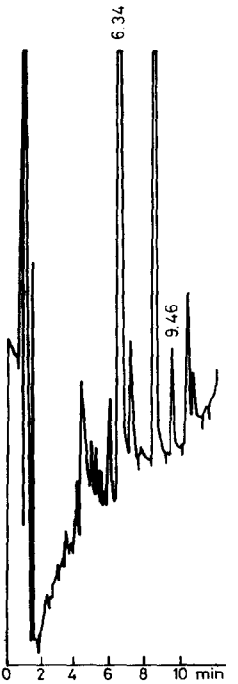

Fig. 4. Chromatogram of internal standard N-ethyl-*p*-toludine (300 ng/ml plasma) (6.42 min) and Deprenyl (7 ng/ml plasma) (9.57 min). For gas chromatographic conditions, see text.

RESULTS AND DISCUSSION

The results obtained with the various extraction methods and the conclusions drawn from them are collected in Table I. These results showed that for a satisfactory pre-purification the following had to be taken into account:

(1) The Deprenyl base is very volatile with water vapour and it coevaporates with solvents. Solutions of Deprenyl can therefore be concentrated by evaporation only if Deprenyl is in the salt form.

TABLE II

CALIBRATION DATA FOR DEPRENYL*

Deprenyl in plasma (ng/ml)	*Relative peak-area ratio:* $\left(\frac{A_{\text{Deprenyl}}}{A_{\text{internal standard}}}\right) \cdot 10$					*Mean value* ($\bar{x}$)	*Standard deviation* (s)
3	0.059	0.078	0.063	0.050	0.071	0.064	0.011
7	0.142	0.113	0.134	0.146	0.129	0.132	0.013
30	0.577	0.498	0.634	0.584	0.552	0.569	0.050
70	1.32	1.32	1.25	1.21	1.36	1.29	0.060
300	5.57	5.72	5.28	5.65	5.35	5.51	0.191

* Constants of the calibration line based on the above data: $r^2 = 0.9984$; $a = 0.0099$; $b = 0.0183$.

(2) The small amounts to be determined make it necessary to restrict the amount of solvent used for the extraction, to reduce the solvent impurity peaks by avoiding the concentration of the solvent impurities on evaporation.

(3) most interfering plasma constituents are not volatile with steam, and protein binding of Deprenyl is negligible at high temperatures.

Therefore, the steam distillation method described here was developed. The procedure gave fairly clean samples. Impurity peaks could be traced to the solvents used, even if the amounts of carefully purified solvents were kept to the minimum. All evaporation steps were carried out with Deprenyl in the salt form, to avoid evaporation losses.

Contact of the sample with the metal bottom of the original PE F22 capillary injector caused large losses in the Deprenyl peak area and distortion of the peak shape at nanogram levels. Therefore, a direct injector had to be constructed. The inner volume of the direct injector is large enough to contain the sample vapour without contact with metal parts or sample losses. The passivation of Carbowax 20M makes it sufficiently inert.

The liner was changed every day without any difficulties. The solvent (chloroform) did not give a signal in the interesting part of the chromatogram, although the chloroform caused faster ageing of the alkali source. No impurity peaks interfere with the internal standard and Deprenyl peaks. This is evident by comparing the chromatogram of blank plasma (Fig. 3) with that of a plasma sample containing 7 ng/ml of Deprenyl (Fig. 4).

The calibration graph is linear between 3 and 300 ng/ml (see Table II). The correlation coefficient (r^2) is 0.9984. The lower limit of quantitation is 3 ng/ml. The limit of detection is 400 pg/ml, but quantitation is not reliable below 3 ng.

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