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A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF AMANTADINE IN HUMAN PLASMA

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SUMMARY

A simple, sensitive and specific method for amantadine determination in human plasma is described. After extraction with toluene, the compound is converted to its N-trichloroacetyl derivative and determined by GLC (electron capture detector) on an OV-17 column. Endogenous materials were found not to interfere. The linearity of the method ranges from 25 to 1000 ng per ml of plasma. Plasma values obtained in patients under chronic treatment are reported.

INTRODUCTION

The importance of knowing drug plasma levels during chronic treatment has been emphasized[1–3]. It is known that for some therapeutic agents the assessment of plasma levels may lead to valuable information, such as therapeutic and toxic thresholds[4–6].

The increasing use of amantadine, used alone or in combination with L-Dopa, for Parkinsonism patients, the individual variability in clinical responses[7–10] and the lack of sensitivity of the methods available[11], has prompted us to develop a sensitive, simple and specific method for routine amantadine determination in human biological fluids.

MATERIALS AND METHODS

Standards and reagents

Amantadine hydrochloride was supplied by Ist. De Angeli S.p.A., Milan, and amphetamine sulphate by Recordati S.p.A., Milan.

The following reagents were used: toluene, scintillation grade (Merck); trichloroacetyl chloride, purum (Fluka); sodium hydroxide (Carlo Erba); 37% hydrochloric acid (Carlo Erba); trimethylchlorosilane (Applied Science Labs.).

Apparatus

For gas chromatography a Carlo Erba Fractovap G 1 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used. The chromatographic column was glass tubing, 2 m long and 4 mm I.D., packed with 100–120 mesh Chro-

mosorb Q, coated with 3% OV-17 (Applied Science Labs.), conditioned for 1 h at 250° (40 ml/min nitrogen flow), 4 h at 340° (no nitrogen flow) and 24 h at 275° (40 ml/min nitrogen flow)[12].

The operating conditions were: injection port temperature, 270°; column temperature, 220°; detector temperature, 260°; carrier gas and scavenger gas (nitrogen) flow, 60 ml/min.

The ECD was used with a pulse current at an excitation voltage of 50 V and a pulse interval of 100 μsec.

For mass spectrometry an LKB 9000 combined with a gas chromotograph was used. Mass spectrometric conditions were: ionization beam, 70 eV; ion source temperature, 290°; accelerating voltage, 3.5 kV; and trap current, 60 μA. A 2-m glass column of 3% OV-17 on Gas-Chrom Q (100–120 mesh) operated at 220° with a helium flow of 30 ml/min was used.

Extraction procedure

All the glassware used was previously silanized with a 1% aqueous solution of trimethylchlorosilane and then washed with methanol. To 1 ml of plasma were added 100 ng of amphetamine-free base (1 ml of an aqueous solution of amphetamine sulfate) as internal standard, 1 ml of 1 *N* NaOH and 5 ml of toluene. The test tubes were subjected to gentle mechanical shaking for 20 min. After centrifugation at 4° for 15 min, 4–5 ml of the organic phase were transferred to a second test tube, and the compound

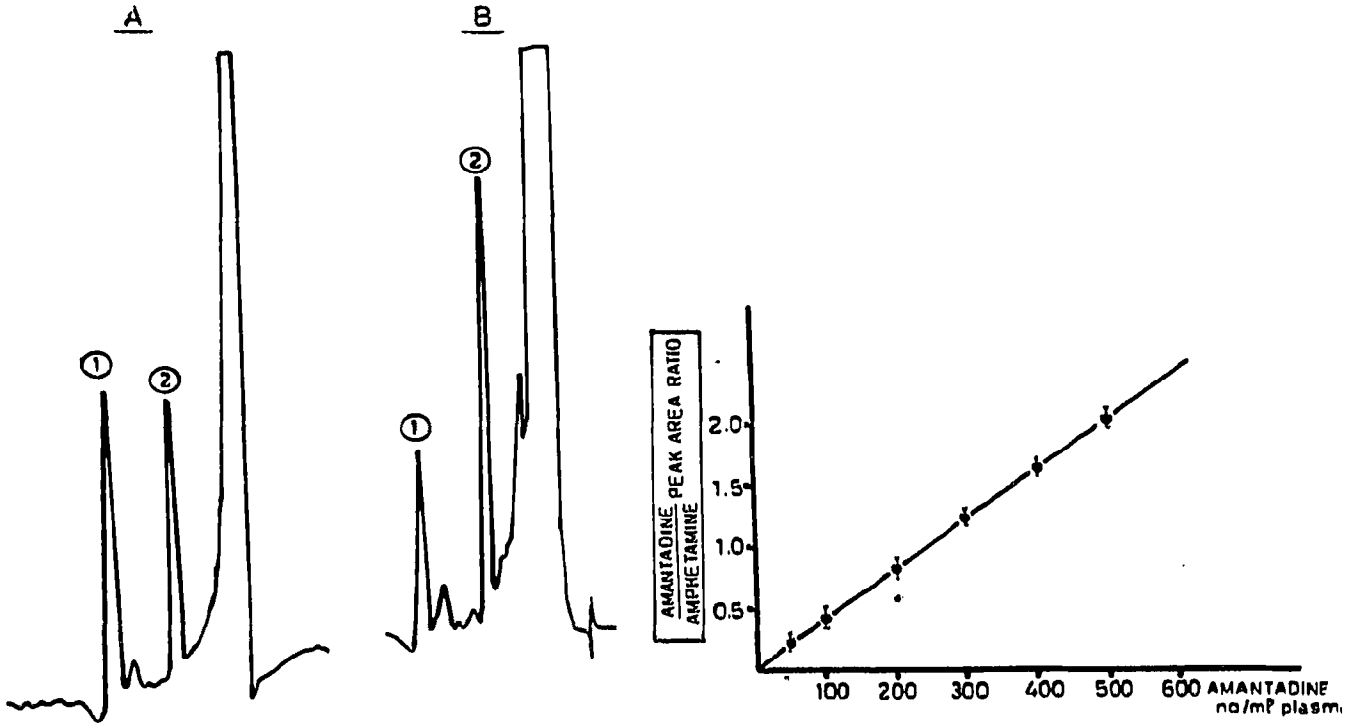

Fig. 1. Gas chromatograms of the N-trichloroacetyl derivative of amantadine extracted from water (A) and from plasma (B): 1 = amantadine; 2 = amphetamine (internal standard). Conditions as described in the text.

Fig. 2. Standard calibration curve of the N-trichloroacetyl derivative of amantadine: ordinate, the peak area ratio of amantadine to the internal standard; abscissa, the drug concentrations in plasma.

was back extracted with 2 ml of 1 *N* HCl. The organic phase was discarded and the aqueous phase, after being made alkaline with 0.5 ml of 6 *N* NaOH, was extracted with 2 ml of toluene. Next, 1.5 ml of the organic phase were transferred to a third test tube, 20 μl of 2% trichloroacetyl chloride solution in toluene (prepared immediately before use) was added and the stoppered tubes heated at 70° for 30 min. After cooling to room temperature 1–3 μl of the solution was injected for gas chromatographic (GC) analysis.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms of extracts from water and plasma. No interfering peaks from endogenous substrates were noted in a series of samples obtained from different patients and volunteers.

The calibration curves for the N-trichloroacetyl derivative of amantadine, obtained by plotting peak area ratios of the drug to the internal standard against known amounts of the drug added to the biological specimen, are illustrated in Fig. 2. The linearity of the method ranges from 25 to 1000 ng per ml of plasma.

The completeness of the reaction was established by checking the consistency of the GC response while changing the time of contact with the trichloroacetyl chloride. The use of amphetamine as an internal marker for quantitation by the relative area technique has therefore been proved satisfactory.

The identity of the GC peak obtained after reaction of amantadine with trichloroacetyl chloride was checked by means of gas chromatography–mass spectrometry (GC–MS). The mass spectra of amantadine and its N-trichloroacetyl derivative

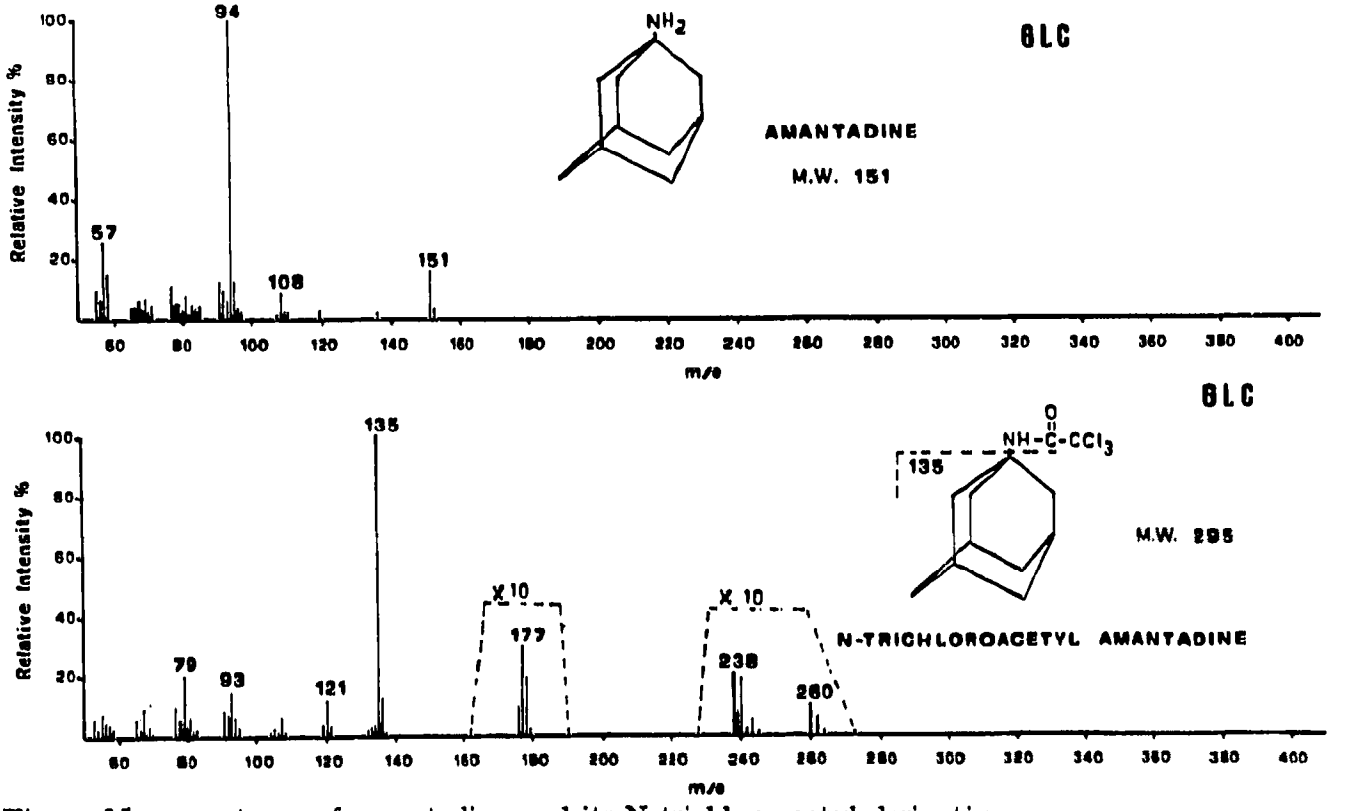

Fig. 3. Mass spectrum of amantadine and its N-trichloroacetyl derivative.

are shown in Fig. 3. The fragmentation pattern is supported by previous data[13]. The recovery from plasma or water was constant at 98.6 ± 0.65%.

The method has been applied to the determination of amantadine in plasma of patients receiving the drug chronically, as reported in Table I.

TABLE I

PLASMA LEVELS (μg/ml) OF AMANTADINE IN PATIENTS RECEIVING THE DRUG CHRONICALLY (200–300 mg/day)

Amantadine was administered at 8 a.m. (200 mg) for the first week and at 8 a.m. (200 mg) and 8 p.m. (100 mg) for the following weeks. Blood sampling was performed at 2 p.m.

Case	*Age*	*Body weight (kg)*	*Sex*	*Days of treatment*		
				7	*14*	*21*
A G	77	64	M	0.58	0.95	1.01
B L	68	62	M	0.27	n.p.[a]	0.79
L L	70	78	M	0.77	1.06	0.99
C A	79	81	M	0.48	0.72	0.68

[a] n.p. = not performed.

CONCLUSION

The described analytical procedure, because of its simplicity, sensitivity and specificity, as well as for the small amount of blood required, may well satisfy requirements for routine clinical application.

The relationships between drug plasma levels and the therapeutic efficacy are now under investigation.

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