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QUANTITATIVE ANALYSIS OF AMINEPTINE (S-1694) IN BIOLOGICAL SAMPLES BY GAS CHROMATOGRAPHY—MASS FRAGMENTOGRAPHY

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SUMMARY

A sensitive, specific method for the quantitative analysis of amineptine in rat blood and brain is described. After extraction and purification amineptine is detected by mass fragmentography, monitoring the fragment ion at *m/e* 192. The method allows the quantitative analysis of as little as 50 ng of amineptine per ml blood and 200 ng per g for brain.

INTRODUCTION

Amineptine (S-1694), [dihydro-10,11-dibenzo(*a*,*d*)cycloheptenyl-5-amino]-7-heptanoic acid, is a new stimulant of the central nervous system whose biochemical and pharmacological effects are different from amphetamine: in rats amineptine causes increases in locomotor activity, stereotyped movements and hyperthermia, like amphetamine, whereas it produces little or no anorectic effect in this animal species [1,2]. Moreover, while amphetamine affects both dopaminergic and noradrenergic systems [3—5], amineptine appears to act preferentially on the dopaminergic system [2].

As a basis for future work in exploring the possibility of a relationship between the pharmacological effect of amineptine and its concentration in different brain areas, a specific, sensitive method was developed for measuring amineptine levels in biological materials.

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MATERIALS AND METHODS

Standard and reagents

Amineptine was obtained from Servier Labs. (Paris, France). The following reagents were used: methanol, ethyl acetate, *n*-hexane (R.P.E.; Carlo Erba, Milan, Italy). Trimethylanilinium hydroxide (TMHA) was synthesized in our laboratories [6].

Apparatus

An LKB Model 9000 gas chromatograph—mass spectrometer equipped with an accelerating voltage alternator was used. The gas chromatographic (GC) conditions were as follows: the chromatographic column was a glass tube (2 m long and 2 mm I.D.) packed with 1% OV-17 on Gas-Chrom Q, 60—80 mesh (Applied Science Labs., State College, Pa., U.S.A.); the column and injector temperature was 290° and the carrier gas (helium) flow-rate was 25 ml/min.

The mass spectrometer was operated under the following conditions: molecular separator temperature, 290°; trap current, 60 μA; electron energy, 70 eV; accelerating voltage, 3.5 kV; filters, 20 Hz.

The mass spectrometer was focused on the ion at *m/e* 192. characteristic of the tricyclic nucleus, allowing continuous monitoring of amineptine and its internal standard, opipramol, in the effluent of the GC column. Fig. 1 shows a typical mass fragmentogram of amineptine and its internal standard.

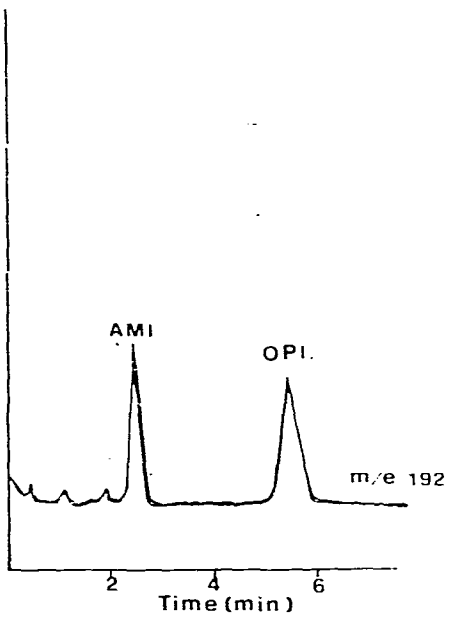

Fig. 1. Mass fragmentogram of amineptine (AMI) and its internal standard opipramol (OPI) obtained focusing at *m/e* 192.

Construction of the calibration graphs and quantitative analysis of amineptine

For calibration and quantitative analysis, the internal standard technique was used. Amineptine was dissolved in methanol in concentrations from 250 to 2000 ng/ml; 1 ml of each solution was evaporated and the residue was dissolved in 0.1 ml of methanol containing 100 ng/ml of opipramol. An aliquot of these solutions (1—3 μl) was injected into the GC column together with 1 μl of 0.2 *M* TMAH in methanol. Amineptine can be quantitated when the relative peak height is used as an index of concentration, as a linear relationship exists between relative peak heights of amineptine in the range 2.5—20 ng.

Extraction from blood

Two ml of heparin-treated rat blood were made alkaline by adding 5 ml of phosphate buffer (pH 8.5) and extracted twice with 10 ml of ethyl acetate by shaking for 10 min on an automatic shaker. After centrifugation the combined organic phases were evaporated to dryness. The residue was dissolved in 5 ml of 0.1 *M* HCl and washed twice with 5 ml of *n*-hexane. After washing, the acid phase was adjusted to pH 8.5 by adding 5 ml of 0.2 *M* phosphate buffer (pH 8.5) and extracted twice with 10 ml of ethyl acetate. The combined organic phases were evaporated to dryness in a rotating evaporator. The residue was dissolved in 0.1 ml of methanol containing a fixed amount of the internal standard (opiramol), and submitted to mass fragmentographic analysis.

Extraction from brain

Rat brain was homogenized in a glass Potter apparatus with absolute ethanol (1:10, w/v). After centrifugation the alcoholic phase was separated from the precipitate and evaporated to dryness. The residue was dissolved in 5 ml of 0.1 *M* HCl and the acid phase was submitted to the clean-up procedure described above for blood.

Recovery studies

The addition of amineptine, in amounts ranging from 100 to 2000 ng, to drug-free blood and brain samples resulted in over-all recoveries of 91.7 ± 5.2% for blood and 83.1 ± 4.8% for brain. The minimum detectable amount of amineptine under these experimental conditions was 50 ng/ml for blood and 200 ng/g for brain.

Animal studies

To check the sensitivity and specificity of the method for pharmacokinetic studies in vivo, adult male CD-COBS rats (Charles River, Calco, Italy) weighing 230—250 g were used. The animals were maintained on a standard diet and in standardized environmental conditions, fasted overnight before experiments and treated intraperironeally with amineptine at a dose of 20 mg/kg. At various times after drug administration, groups of 4 animals were killed and their blood and brains were collected and analysed as described above.

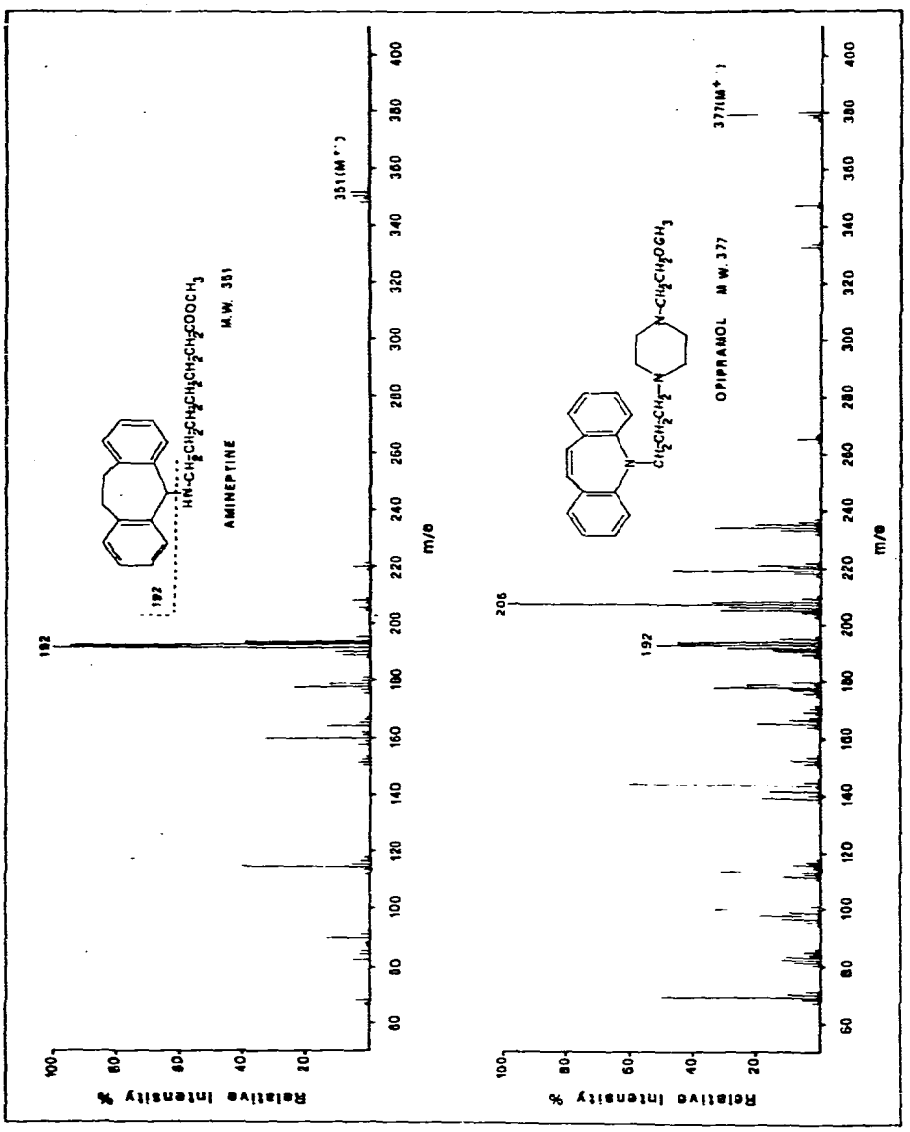

Fig. 2. Mass spectra of amineptine and opipramol after reaction with TMAH.

RESULTS AND DISCUSSION

The mass spectra of amineptine and its internal standard opipramol, after methylation with TMAH, are shown in Fig. 2. Since no stable isotope-labelled amineptine was available, the internal standard was added at the end of the extraction procedure in order to correct possible errors due to variability of injected volumes or changes in instrumental conditions. The variability of the recovery efficiency was taken into account by analysing, together with each set of samples, control samples enriched with a known amount of amineptine. Opipramol was chosen as an internal standard because of its suitable retention time and because, like amineptine, it gives an intense ion at *m/e* 192, characteristic of the tricyclic nucleus, allowing the detection of both amineptine and opipramol by single-ion monitoring.

Typical mass fragmentograms obtained from a blood sample of a control rat treated with 20 mg/kg (i.p.) of amineptine (B) are shown in Fig. 3.

A peak (C) with a retention time shorter than that of amineptine was found in all the blood and brain samples from treated rats but not in samples from control animals, suggesting the formation of a metabolic product of amineptine. Like amineptine, the peak corresponding to the unknown compound was not eluted from the column unless the sample was injected by the on-column methylation technique using TMAH; this suggests there may be a polar group in the molecule.

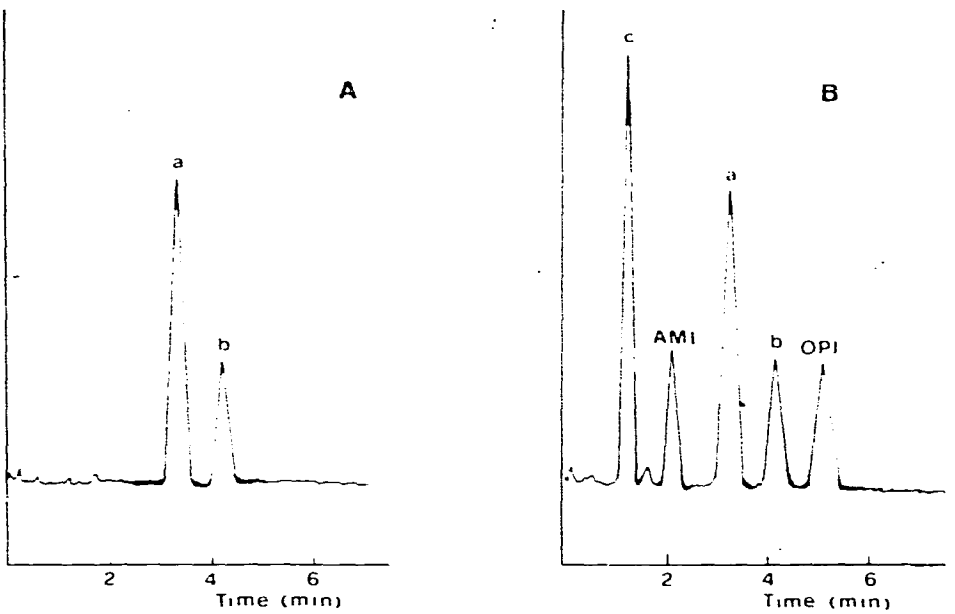

Fig. 3. Mass fragmentograms obtained from rat blood. A, control animal; B, 15 min after treatment with amineptine (AMI) (20 mg/kg, i.p.) with the addition of the internal standard opipramol (OPI). a and b, endogenous substances; c, amineptine metabolite.

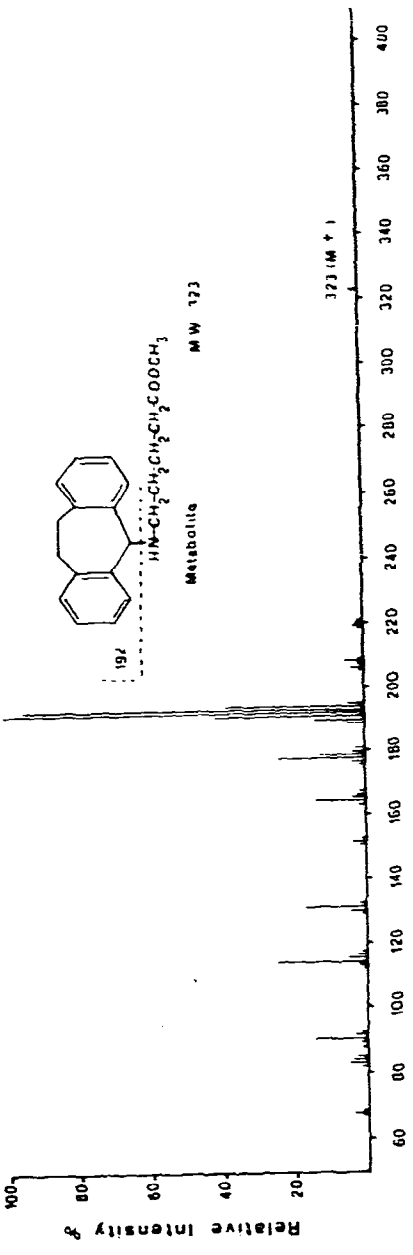

Fig. 4. Mass spectrum of amineptine metabolite after methylation using TMAH.

The mass spectrum corresponding to the unknown compound is given in Fig. 4. There is a base peak at *m/e* 192, suggesting that the tricyclic nucleus is not modified, and an apparent molecular ion at *m/e* 323, suggesting that the aliphatic chain in the amineptine molecule might be biotransformed with the loss of 2 CH_2 units. The structure of the metabolite was also confirmed by reaction of the biological extract with diazomethane and BSTFA in pyridine. The reaction with diazomethane gave a peak with the same retention time and the same mass spectrum of the TMAH product, while the reaction with BSTFA gave a peak with a longer retention time and with a mass spectrum retaining the base peak at *m/e* 192 and an apparent molecular ion at *m/e* 381.

Blood and brain levels at various times after amineptine treatment are shown in Fig. 5. Amineptine brain levels decrease rapidly and are not detectable 120 min after treatment, while in blood amineptine is still measurable. 240 min after treatment.

The results indicate that this method is suitable for measuring amineptine in blood and brain of animals and may possibly be useful for the determination of amineptine biotransformation products.

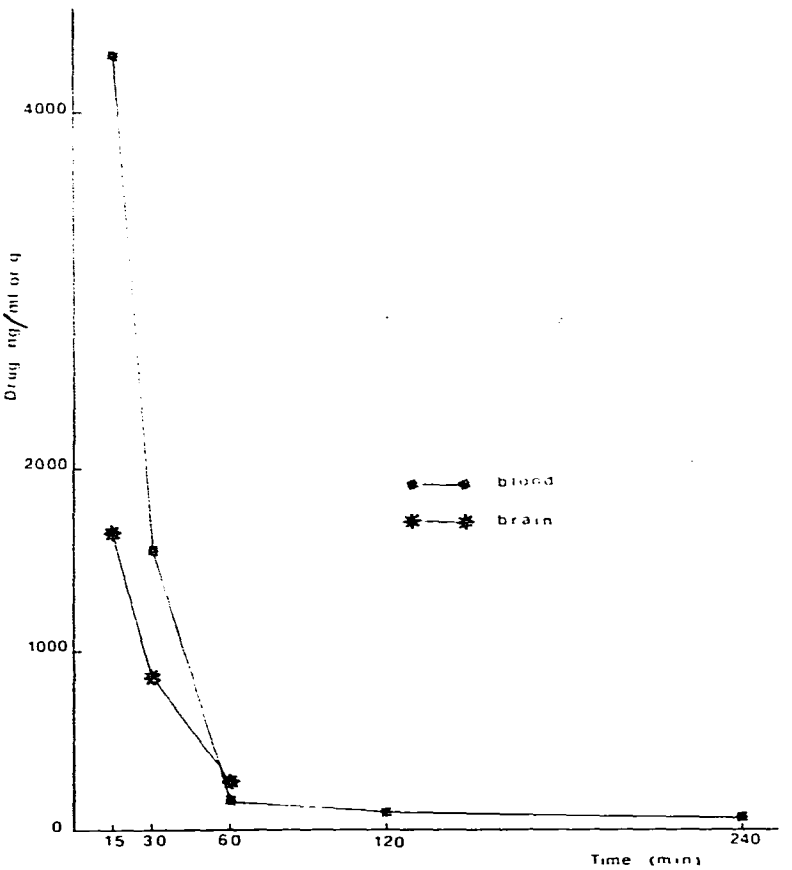

Fig. 5. Blood and brain levels of amineptine in rats treated at a dose of 20 mg/kg (i.p.).

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