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Note

Highly sensitive method for determination of isopropyl-*p*-iodoamphetamine by gas chromatography with electron-capture detection

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Isopropyl-*p*-iodoamphetamine (IIMP) is a new brain tracer, currently used with a single photon emission computerized tomograph. Because its accumulation in the brain is proportional to cerebral blood flow, it is useful in detecting neurological abnormalities, including ischemia, epilepsy and low-pressure hydrocephalus [1–5]. IIMP has been shown to readily cross the blood–brain barrier and to have a relatively long retention time [6,7]. Clearance of IIMP in humans has been described [8]. However, relatively little else is known about the pharmacokinetic properties of this compound. It appears that work in this area is slow to progress because the analytical methodologies previously reported [9], including high-performance liquid chromatography (HPLC), lack sufficient sensitivity.

In this paper, we describe a sensitive, rapid and specific gas chromatography (GC) method using electron-capture detection (ECD) and its use in analysing plasma samples from patients receiving IIMP (intravenous injections).

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EXPERIMENTAL

Chemicals

p-Iodoamphetamine (IAMP), IIMP and *p*-iodophenyl ketone (IPK) were purchased from Oris (Saclay, France) and *p*-iodoaniline (IA), which was used as internal standard, from Aldrich (Milwaukee, WI, U.S.A.) (Fig. 1). IAMP, IIMP, IPK and IA were dissolved in methanol to concentrations ranging from 0.2 ng/ml to 0.2 μg/ml. All reagents used were of analytical grade: methanol from Carlo-Erba (Milan, Italy), trifluoracetic anhydride (TFA) from Aldrich, ethyl acetate from Merck (Darmstadt, F.R.G.) and sodium hydroxide (1 *M*) from Prolabo (Paris, France).

Apparatus

GC–ECD analysis was carried out on a Girdel 330 gas chromatograph (Delsi, Paris, France) equipped with a ^{63}Ni electron-capture detector. A glass column (2 m × 2 mm I.D.) packed with 3% OV-225 on Gas Chrom Q, 80–100 mesh (Delsi), was used.

The injection port temperature was 290°C and the detector temperature 290°C. The oven temperature was set at 160°C for 2 min and then increased to 175°C (5°C/min) after injection. The carrier gas was nitrogen at a flow-rate of 60 ml/min. All chromatograms were recorded on a servo-trace recorder (Sefram, Paris, France) at a chart speed of 5 mm/min.

Extraction procedure

A 1-ml volume of plasma was mixed with 30 μl of internal standard (IA, 1 ng/ml), 100 μl of sodium hydroxide (1 *M*) and 8 ml of ethyl acetate. The solution was vortexed for 1 min and then centrifuged for 15 min at 1000 *g* at 4°C. The organic phase was transferred to a clean tube and then evaporated to dryness in a water-bath (40°C) under a gentle stream of nitrogen gas. The residue was dissolved in 500 μl of ethyl acetate and 25 μl of TFA and had to be incubated at 60°C for 30 min because IIMP is not derivatized at room temperature. The mixture was cooled and washed twice, first with 3 ml of sodium hydroxide (1 *M*),

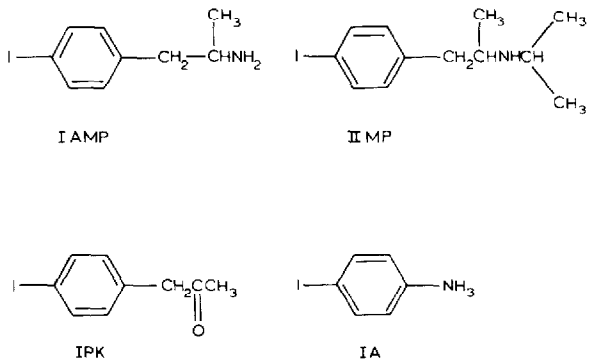

Fig. 1. Chemical structures of IAMP, IIMP, IPK and IA (internal standard).

then with 2 ml of distilled water to eliminate the free TFA. An aliquot (1–3 μl) was injected into the GC–ECD system.

RESULTS AND DISCUSSION

Because the metabolism of IIMP is unclear [9], and IIMP could therefore be metabolized through dealkylation to IAMP and through deamination to IPK, we determined the retention times of IA, IIMP, IAMP and IPK. A preliminary trial using OV-17 3% on Gas Chrom Q, which is currently used for the determination of the amphetamines [10–13], had been set up. Under these conditions IAMP and IIMP chromatographic peaks were not resolved. However, OV-225 3% on Gas Chrom Q gave high resolution of IAMP and IA without interference from endogenous compounds, IAMP or IPK. Fig. 2 illustrates typical chromatograms obtained after extraction of blank (A) and spiked (B) human plasma samples. The retention times for IPK, IA, IIMP and IAMP were 2.0, 4.0, 5.4 and 6.5 min, respectively. Since no chromatographic interference with IAMP and IPK was observed, the assay of IIMP using IA as an internal standard could be performed. Fig. 2C shows a chromatogram obtained after injection of a standard solution of 30 ng/ml IA, and 25 ng/ml IPK, IIMP and IAMP. The calibration curve was linear over the range 0–100 ng/ml (correlation coefficient 0.998). The linear equation of the curve for IIMP was $y = 0.0274x + 0.0539$, where y was the ratio of

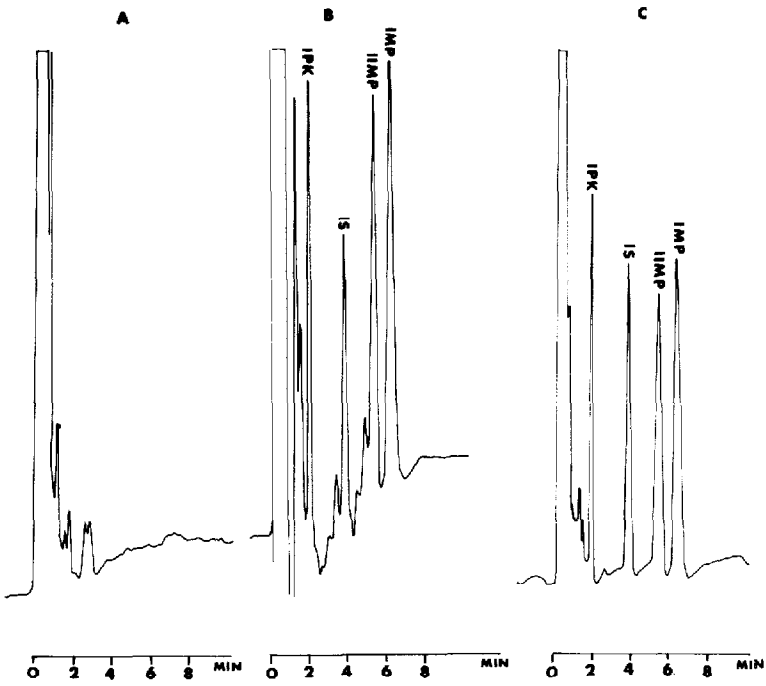

Fig. 2. Typical chromatograms obtained from (A) a blank plasma control sample, (B) a plasma sample containing 40 ng/ml IPK, IAMP and IIMP and 30 ng/ml IA as internal standard (IS) and (C) 25, 30, 25 and 25 ng/ml of the trifluoroacetyl derivatives of IPK, IA, IIMP and IAMP, respectively.

TABLE I

DAY-TO-DAY AND WITHIN-DAY REPRODUCIBILITY OF IIMP

Concentration (ng/ml)	Coefficient of variation (%)	
	Day-to-day	Within-day
5	7.0	8.2
10	9.6	9.0
25	8.5	7.7
50	5.1	4.5

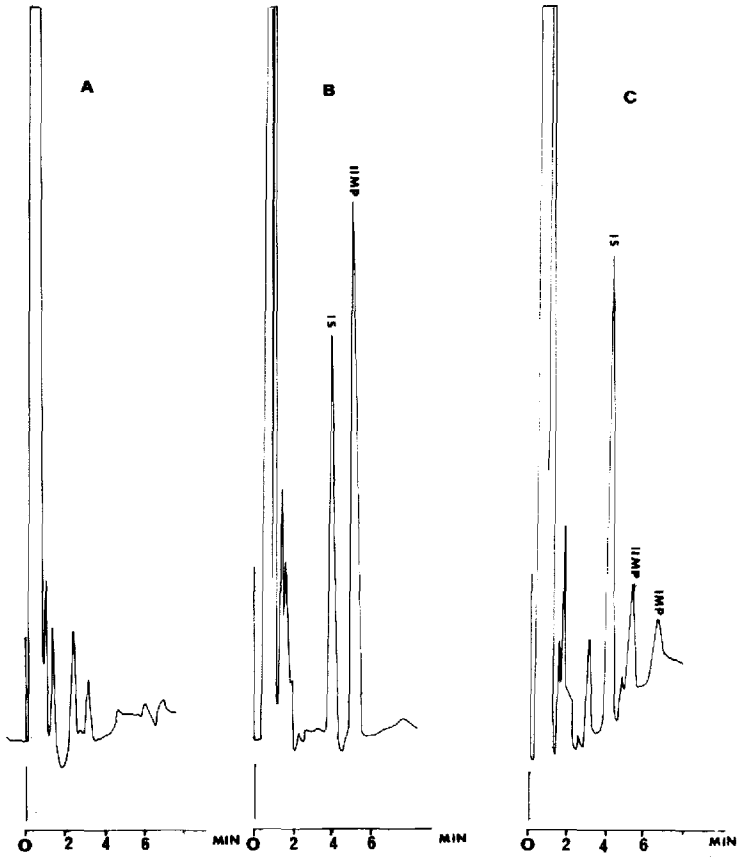

Fig. 3. Chromatograms of human plasma before and after a bolus dose of 10 mg of IIMP. (A) Blank plasma control; (B) diluted plasma (1:1) 20 min after intravenous injection; (C) diluted plasma (1:1) 5 h after intravenous injection.

the peak height of IIMP to the peak height of the internal standard and x was the concentration of IIMP in ng/ml.

The within-day reproducibility of the method was determined at plasma concentrations of 5, 10, 25 and 50 ng/ml IIMP. Ten determinations of each were made. The day-to-day reproducibility was assessed over the same concentration range over a period of five days. The coefficients of variation are shown in Table

I. The recoveries of IIMP and IA were estimated by comparing the peak heights of a pure solution of IIMP and IA with those of extracted plasma containing the same amounts of IA and IIMP. The recoveries of the extraction procedure were $76 \pm 4\%$ and $79 \pm 3\%$, respectively ($n = 10$). The detection limit for IIMP was 3 pg and the minimum detectable concentration was 1.5 ng/ml in a 1-ml plasma sample.

CONCLUSION

The iodine atom in the *para* position of the ring of IIMP makes the compound particularly well suited for detection by electron capture. As an application of the aforementioned method, plasma samples from patients receiving IIMP (intravenously) were analysed. Fig. 3 shows chromatograms of plasma extracts from a patient before and after administration of IIMP (10 mg, intravenously). The data of IIMP obtained in humans demonstrate that the method has a sufficiently high sensitivity for measuring IIMP concentrations in biological fluid and that the combination of sensitivity and simplicity makes it well adapted for use in pharmacokinetic studies.

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