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Note

High-performance liquid chromatographic determination of atrazine in human plasma

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The S-triazines are among the most widely used pre-emergent herbicides in the world. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a selective herbicide commonly used on many crops, including maize, sorghum, sugar cane, pineapple and asparagus for the control of annual grasses and broad-leaf weeds. Residues cause problems in soil; subsequent rotational crops may be affected and atrazine residues can contaminate the water-table [1]. This latter fact may pose problems for the health of animals or even humans. The acute ingestion of atrazine by humans may cause neurological problems, cytolytic hepatitis damage and acute tubulopathy. Experimental studies have shown morphological and functional damage of the liver [2] and kidney [3] in animals.

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To measure atrazine in biological samples, a sensitive, rapid method is required. Atrazine can be measured by a gas chromatographic method in tissues [4] or by high-performance chromatography (HPLC) in water [5].

This paper describes an HPLC method for the measurement atrazine in human plasma and results obtained in a case of an acute intoxication by a preparation containing atrazine.

EXPERIMENTAL

Chemicals and reagents

Atrazine and propazine were purchased from Riedel de Haën Seelze (Hannover, F.R.G.). Methanol and dichloromethane were HPLC grade. Distilled water was purified by passing it through a reverse-osmosis four-filter system (Millipore, Bedford, MA, U.S.A.).

Chromatographic conditions and instrumentation

A Beckman (Gagny, France) Model Gold 126 liquid chromatograph equipped with a UV variable-wavelength detector (Model 167 Beckman) and connected to a microcomputer (NEC PC 8300) and an integrator (Model 427) was used. Chromatography was performed on a 125 mm × 4.6 mm I.D. stainless-steel C_{18} Hypersil ODS 5 μm particle size column (Chromasciences, France) with a precolumn (20 mm × 4 mm I.D.) that contained the same stationary phase. The mobile phase was water–methanol (40:60, v/v) at a flow-rate of 1.0 ml/min and a pressure of 14 MPa. The separation was performed at 20–22°C. Atrazine and propazine were detected at a wavelength of 254 nm.

Standard solutions

Standard stock solutions of atrazine and propazine as the internal standard were prepared in methanol at a concentration of 5.0 mg/l. These were refrigerated at 4°C and found to be stable for several weeks in the dark.

A 200-μl volume of the stock solution of propazine was used for internal standardization (1000 ng).

Plasma standards (calibration standards) were prepared at concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 ng/ml. The 400 ng/ml standard was prepared by adding 50 ml of drug-free human plasma to the dry residue of 4 ml of methanolic solution atrazine (5 mg/l). The other standards were then prepared by stepwise dilution with drug-free plasma. These calibration standards were stored deep-frozen (−20°C) in small portions until needed for analysis.

Plasma extraction procedure

Calibration curve. To 2.0 ml of each solution of plasma standard in a 50-ml centrifuge tube containing 1000 ng of internal standard (propazine) were added 6 ml of dichloromethane. The tube was mechanically shaken for 5 min and

then centrifuged at 4000 *g* for 5 min. The aqueous phase was re-extracted in a similar manner. The two organic phases were mixed and evaporated to dryness under nitrogen at 20°C. The residue was redissolved in 50 μl of water–methanol (40:60, v/v), and 20 μl were injected into the chromatograph.

Samples. Plasma samples from patients were stored at −20°C until analysis. A suitable volume of plasma (up to 2.0 ml) was combined with 1000 ng of internal standard, and 6 ml of dichloromethane were added. This mixture was further treated as described for the calibration curve.

Quantitation

Calibration standards covering the anticipated concentration range (6.25–400 ng/ml) in methanol and plasma were processed. Peak-area ratios of atrazine to the internal standard were measured, and the calibration was obtained from linear regression of the peak-area ratio against concentrations. This line was then used to calculate the concentration of atrazine in unknown samples.

Recovery

Extracts from plasma, prepared as described above, were compared with a direct assay of standards in methanolic solution. The relative recoveries were determined for two different concentrations.

Interferences

Interferences from endogenous material and from other pesticides were investigated (Table I). Pesticides were tested at a concentration of 200 ng/ml.

Human case study

The procedure was used to analyse the in vivo disposition of atrazine in a poisoned patient, found after absorption of 500 ml of a commercial product containing 200 g/l atrazine. Blood was collected frequently into heparinized tubes over a period of 4 h after the admission. The plasma was separated, frozen and stored in the dark at −20°C until analysis.

TABLE I

PESTICIDES TESTED FOR POSSIBLE INTERFERENCE IN THE HPLC ASSAY OF ATRAZINE

Triazines	Carbamates
Aminotriazole	Propham
Simazin	Chlorpropham
Desmetryne	
Ametryne	
Prometryne	

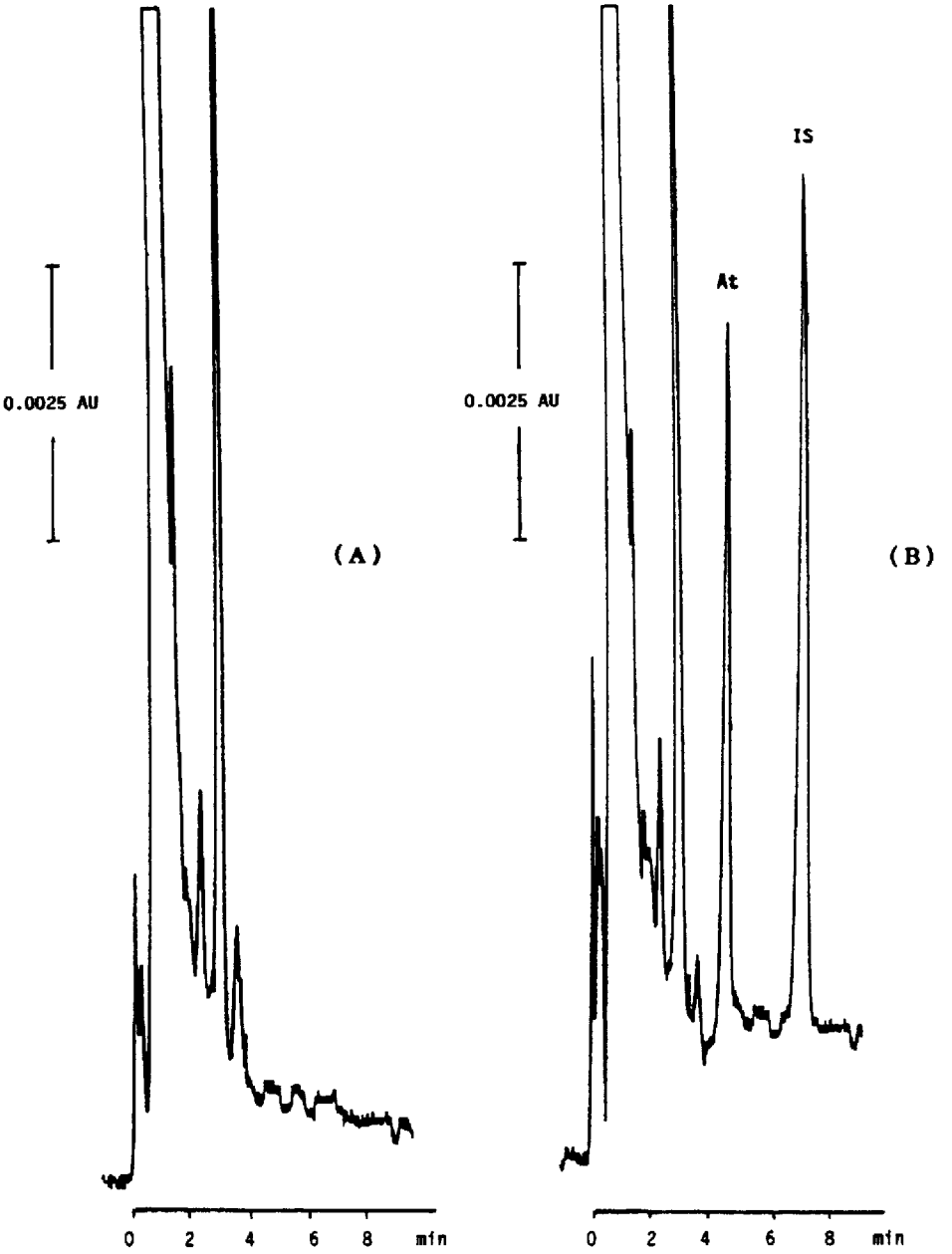

Fig. 1. Representative chromatograms of (A) extracted drug-free plasma and (B) human plasma (2 ml) containing 200 ng/ml atrazine and 500 ng/ml internal standard. Peaks: At = atrazine, IS = internal standard.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of atrazine in human plasma using propazine as internal standard. In this chromatogram, which was obtained after extraction of 2.0 ml of drug-free plasma, no additional peaks that could interfere with the determination of atrazine and internal standard were present.

The calibration curves were obtained using a methanolic solution of standards or human plasma spiked with 6.25–400 ng/ml atrazine and 500 ng/ml internal standard. There was a good correlation between the amount of atrazine added to plasma and the amount detected in the samples of 2.0 ml of plasma. The linear regression equations were $y=0.00290x+0.017$ ($r=0.998$) for methanolic solution and $y=0.00255x-0.013$ ($r=0.996$) for standards in plasma (y=peak-area ratio of atrazine to internal standard; x=atrazine concentration). Calibration curves in plasma showed good linearity between peak-area ratios and concentrations from 6.25 to 400 ng/ml. The limit of quantitation in plasma was defined as the lowest concentration of atrazine resulting in a signal-to-noise ratio of 2. The present method was able to detect 2 ng/ml atrazine in methanolic solution, corresponding to 6 ng/ml atrazine in plasma. The intra- and inter-assay precision data for atrazine are summarized in Table II. There was little variation in atrazine determination with coefficients of variation below 10%.

The analytical relative recoveries of atrazine in plasma determined at 6.25 and 100 ng/ml were 72 and 88%, respectively, and the analytical relative recoveries of propazine in plasma determined at 6.25 and 100 ng/ml were 82 and 98%, respectively, using atrazine as internal standard.

The assay was shown to be selective, without interferences from endogenous material or from other pesticides (triazines or carbamates).

This HPLC procedure has been used for the assay of human plasma samples from one poisoned patient. After ingestion of 500 ml of a commercial product containing atrazine at a concentration of 200 g/l, the atrazine plasma level was 2.1 μg/ml at 5 h after absorption and 1 μg/ml after 3 h of hemodialysis.

In summary, this HPLC assay shows good reproducibility, sensitivity and

TABLE II

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION FOR ATRAZINE IN PLASMA

Concentration (ng/ml)	Coefficient of variation (%)	
	Intra-assay ($n=10$)	Inter-assay ($n=10$)
6 25	8.25	9.01
100	4.82	6.88
400	4.59	6.81

selectivity. It has the advantage of being a relatively convenient, rapid and simple method. It was developed in response to a clinical problem and can provide an indication of the atrazine concentration in as little as 8 min and an accurate answer within 1 h, once the standards have been processed. This assay could be also used for the determination of propazine with atrazine as internal standard; for propazine, the sensitivity is 4.3 ng/ml. This method was applied to the analysis of samples from one poisoned patient and could be readily applied to determine the toxicokinetic parameters of atrazine in humans.

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