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Note

Determination of amoxapine and metabolites in plasma by liquid chromatography with electrochemical detection

RAYMOND F. SUCKOW*

Analytical Psychopharmacology Laboratory, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962 (U.S.A.)

and

THOMAS B. COOPER

Analytical Psychopharmacology Laboratory, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962 (U.S.A.) and New York State Psychiatric Institute, 722 West 168th Street, New York, NY 10032 (U.S.A.)

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Amoxapine is the N-demethylated metabolite of the neuroleptic dibenzoxazepine loxapine. It has been shown to be a useful antidepressant and a review of its pharmacology and efficacy has been presented [1]. Since there are conflicting reports as to the clinical efficacy versus plasma concentration relationships routine plasma level monitoring of amoxapine and its metabolites appears to be unwarrented at this time [2, 3]. However, concentrations of amoxapine and its metabolites in biofluids may be necessary in various clinical situations as well as in determining their pharmacokinetic profiles.

To date, a number of analytical methods have been reported for amoxapine determinations. Cooper and Kelly [4] reported a gas chromatographic method for loxapine, amoxapine and their respective 7- and 8-hydroxymetabolites in urine and serum. A number of liquid chromatographic procedures have been reported for amoxapine and their major metabolites [5—9]. However, some of these latter procedures do not adequately resolve all the compounds of interest, while others do not have sufficient sensitivity for pharmacokinetic studies or require different chromatographic conditions for the analysis of

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amoxapine and its metabolites. All procedures used an ultraviolet detector at 254 nm or 214 nm to monitor the peaks.

We have developed an alternative approach to the detection of amoxapine and its metabolites using liquid chromatography coupled with an electrochemical detector. The method affords adequate sensitivity for single-dose pharmacokinetic studies as well as simultaneously resolving all compounds of interest in a single chromatogram.

EXPERIMENTAL

Reagents and standards

Phosphoric acid, potassium phosphate monobasic, *n*-butylamine, 1-heptanesulfonic acid, sodium salt, were all reagent or HPLC grade (Fisher Scientific, Fairlawn, NJ, U.S.A.). Acetonitrile, UV grade, and methyl *tert.*-butyl ether were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.).

Stock solutions (1 mg/ml) of amoxapine, 8-hydroxyamoxapine, 7-hydroxyamoxapine and 8-hydroxyloxapine were prepared in 0.01 *M* hydrochloric acid. Working solutions were prepared by dilution of the above to 1 μg/ml. All standards were supplied by American Cyanamid, Lederle Labs. (Pearl River, NY, U.S.A.).

Apparatus

Chromatography was performed using a Model 6000A solvent delivery pump, and a WISP 710B injector (Waters Assoc., Milford, MA, U.S.A.). Separations were achieved with a 25 cm $\times$ 4.6 mm I.D. column packed with trimethylsilyl bonded silica (LC-1, particle size 5 μm; Supelco, Bellafonte, PA, U.S.A.) and detected by a Model 5100A coulometric analyzer having a Model 5011 dual porous graphite electrode cell (ESA, Bedford, MA, U.S.A.). Chromatograms were recorded on a Model B5217-5 omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.).

Extraction

To 1 ml of plasma standard or unknown sample, 50 μl (50 ng) of internal standard 8-hydroxyloxapine and 1.0 ml of 0.6 *M* carbonate buffer (pH 9.8) were added in a 15-ml round-bottom screw cap culture tube. Methyl *tert.*-butyl ether (7 ml) was added and the mixture shaken for 15 min and centrifuged at 1500 *g* for 15 min. The organic layer was then transferred to a 15-ml tapered centrifuged tube containing 250 μl of 0.1 *M* acidic phosphate buffer (pH 2.0). After mixing for 10 min and centrifuging at 1500 *g* for 10 min, the organic layer was aspirated and discarded and the aqueous contents transferred to small glass vials suitable for automatic injection by the WISP 710B.

Chromatographic conditions

The mobile phase consisted of 0.05 *M* potassium dihydrogen phosphate—acetonitrile (75:25) with 1.2 ml/l *n*-butylamine, 1.0 ml/l orthophosphoric acid (85%) and 0.005 *M* heptanesulfonic acid added. The flow-rate was 1.8 ml/min and the temperature ambient. The detector potential was set at +0.75 V versus

the proprietary reference electrode. A Model 5020 guard cell (placed between the pump and injector) was also set at a potential of +0.75 V versus the proprietary reference electrode.

Quantitation

All determinations of plasma samples were calculated based upon peak height ratios using the internal standard method.

RESULTS AND DISCUSSION

Analyses of amoxapine, 7-hydroxyamoxapine and 8-hydroxyamoxapine in plasma were performed by liquid chromatography with electrochemical detection. This method is able to separate and quantitate the 7- and 8-hydroxy metabolites as well as amoxapine in a single chromatogram. A sample chromatogram appears in Fig. 1A. The use of a trimethylsilyl column with the addition

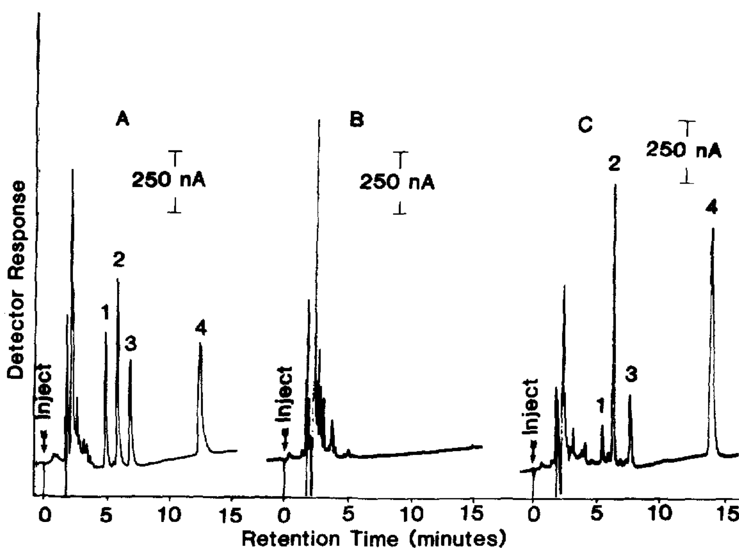

Fig. 1. Sample chromatogram of: (A) a spiked 1-ml plasma sample containing 75 ng of 7-hydroxyamoxapine (1), 8-hydroxyamoxapine (2) and amoxapine (4), with 50 ng of internal standard 8-hydroxyloxapine (3); (B) a drug-free plasma blank; and (C) a 1-ml plasma sample from a patient receiving amoxapine: 30 ng of 7-hydroxyamoxapine (1), 137 ng of 8-hydroxyamoxapine (2) and 194 ng of amoxapine (4).

TABLE I

RECOVERY OF AMOXAPINE AND METABOLITES (50 ng/ml) FROM 1 ml OF PLASMA (n = 8)

Compound	Recovery (%)	C.V. (%)
Amoxapine	88	5.0
8-Hydroxyamoxapine	85	4.4
7-Hydroxyamoxapine	81	4.6

of n-butylamine in the mobile phase enhanced peak symmetry and affords resolution between the peaks. No interfering endogenous peaks were observed (Fig. 1B).

The absolute recovery of amoxapine and metabolites from plasma was determined by spiking 1-ml blank plasma samples with 50 ng of amoxapine and metabolites, and processing as described, except for the addition of the internal standard which was added to the final extract just before injection on column. The peak height ratios of these compounds to the internal standard were compared to those recorded when the same concentration of unextracted amoxapine and metabolites and internal standard were injected. The results appear in Table I.

The precision of the procedures was determined by spiking 1-ml aliquots of drug-free plasma with various concentrations of amoxapine and its metabolites together with the internal standard. The samples were processed as described. The results appear in Table II. Day-to-day reproduciblity was assessed by analysis of the slopes of the calibration curve for each day (Table III).

The lower limit of detection of this assay is about 5 ng/ml for all compounds. This assay is therefore suitable for single-dose pharmacokinetic studies if 2—3 ml of sample are used.

The use of an electrochemical detector for this method yielded an increase in sensitivity over an ultraviolet absorbance detector (254 nm). Furthermore, in this application the coulometric type of electrochemical detector was observed to be more sensitive than the thin-layer amperometric transducer. This

TABLE II

WITHIN-RUN PRECISION OF ASSAY BASED UPON PEAK HEIGHT RATIOS AT VARIOUS CONCENTRATIONS (n = 8)

Compound	Concentration (ng/ml)					
	10		30		100	
	Peak height ratio	C.V. (%)	Peak height ratio	C.V. (%)	Peak height ratio	C.V. (%)
Amoxapine	0.12	4.5	0.35	3.4	1.21	1.2
8-Hydroxyamoxapine	0.21	7.4	0.67	1.3	2.25	0.8
7-Hydroxyamoxapine	0.11	13.4	0.38	5.3	1.51	2.0

TABLE III

DAY-TO-DAY PRECISION OF THE ASSAY BASED UPON LINEAR REGRESSION SLOPES (n = 5)

Compound	Slope (ng/ml)	C.V. (%)
Amoxapine	69.3	8.8
8-Hydroxyamoxapine	43.4	3.5
7-Hydroxyamoxapine	58.0	5.0

TABLE IV

STEADY-STATE PLASMA LEVELS OF AMOXAPINE AND METABOLITES IN NINE PATIENTS RECEIVING AMOXAPINE

Subject	Dose (mg per day)	Amoxapine (ng/ml)	8-Hydroxyamoxapine (ng/ml)	7-Hydroxyamoxapine (ng/ml)
1	200	71	157	8
2	250	150	226	5
3	150	74	133	T*
4	250	88	170	8
5	150	104	239	5
6	150	195	137	30
7	150	26	70	T
8	350	222	582	28
9	300	92	204	11

*T, Level below 5 ng/ml.

may be due in part to the "pre-conditioning" (pre-oxidation) of the mobile phase by the guard cell prior to the actual sample detection, thus eliminating the potential interference from any impurities in the mobile phase and increasing signal-to-noise ratio.

Table IV illustrates the plasma levels of amoxapine and metabolites of several depressed patients receiving amoxapine. The concentration of the 8-hydroxy metabolite was usually found to be equal to or greater than the parent compound. The 7-hydroxy metabolite was found in concentrations near the limit of detection for most patients. These data agree with those previously reported [2—6, 9].

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