Journal of Chromatography, 431 (1988) 438–443
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4306

Note

High-performance liquid chromatographic procedure with fluorescence detection for the *m*-O-dealkylated lactam metabolite of flecainide acetate in human plasma

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(First received March 4th, 1988; revised manuscript received May 11th, 1988)

Flecainide acetate (Tambocor®, R-818) is a new antiarrhythmic that has been shown to be effective for the suppression of ventricular arrhythmias [1–4]. Flecainide acetate has undergone extensive clinical evaluation world-wide and is marketed in the United States and most European countries. Two major metabolites of flecainide have been isolated from human urine and identified [5]. Both major metabolites have shown measurable effects on cardiac electrophysiologic

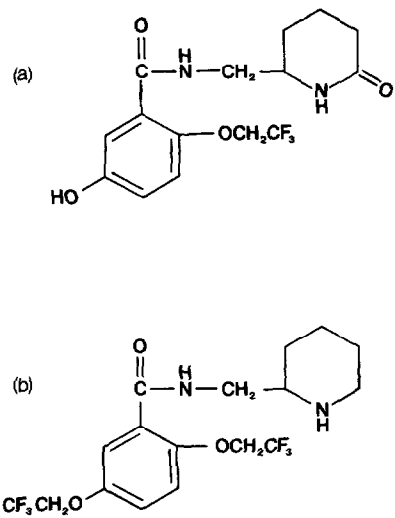

Fig. 1. Chemical structures of (a) MODLF and (b) flecainide acetate.

0378-4347/88/$03.50

parameters in a laboratory animal model [6]; however, the observed effects were much less than those reported for the parent drug. A high-performance liquid chromatographic (HPLC) procedure to quantitate one of the major metabolites (*m*-O-dealkylated flecainide, MODF) in human biological fluids has been reported previously [7].

This paper describes a sensitive and selective procedure to quantitate the second major metabolite of flecainide (*m*-O-dealkylated lactam of flecainide, MODLF;5-hydroxy-N-[2-(6-oxopiperidylmethyl)]-2-(2,2,2-trifluoroethoxy)-benzamide, Fig. 1) in human plasma. It is a reversed-phase HPLC method with sample preparation accomplished through the use of solid-phase extraction columns.

EXPERIMENTAL

Chemicals and reagents

Methanol and acetonitrile were Omnisolv® grade (EM Science, Cherry Hill, NJ, U.S.A.). Water was deionized and all other chemicals were analytical reagent grade.

Apparatus

HPLC analyses were performed using a Waters M6000A pump, a Waters 710B automatic sample delivery system (Milford, MA, U.S.A.), and a Shimadzu RF-530 fluorescence spectromonitor (Kyoto, Japan). The excitation and emission wavelengths were 310 and 380 nm, respectively. Peak heights were measured by a Hewlett-Packard 3390A integrator (Avondale, PA, U.S.A.). Separation was achieved on a Waters μBondapak® phenyl column (10 μm, 30 cm × 3.9 mm I.D.). The mobile phase consisted of acetonitrile–0.1 *M* sodium acetate (23:77, v/v) which was filtered through a 0.45-μm Nylon 66 membrane prior to use [8]. The flow-rate was 2 ml/min.

The vacuum manifold and the solid-phase extraction columns (octyl, 200 mg) were from Analytichem International (Harbor City, CA, U.S.A.).

Preparation of standard solutions

All standard solutions of MODLF were prepared in acetonitrile and were made by diluting a 1 mg/ml stock solution. Concentrations used were 100, 200, 300, 400, 600, and 1000 ng/ml.

Sample preparation

Each solid-phase extraction column was initially activated by wetting with two column volumes of methanol followed by two column volumes of water. After activation, the following was added to the top of the column: 1 ml of plasma, 1 ml of 0.2 *M* acetate buffer (pH 5.0), and 50 μl of acetonitrile (replaced with standard solution of MODLF for the calibration curve). Each sample was drawn through a column and was then rinsed with two column volumes of water. The aspirator on the vacuum manifold was left on for 5 min to dry the columns. The MODLF was eluted with two 500-μl aliquots of methanol and the combined eluent was

evaporated to dryness under nitrogen at 45 °C. The residue was reconstituted in 100 μl of mobile phase and 50 μl were injected into the HPLC system.

Calibration and quantitation

A least-squares linear regression line of the peak height for MODLF versus concentration was calculated. The slope and intercept obtained from this line were used for the calculation of MODLF concentrations in unknown samples. A calibration curve was run with each set of unknowns.

RESULTS AND DISCUSSION

Chromatography

The retention time of MODLF in this HPLC system was 6.5 min and no interfering peaks were detected for blank human plasma (Fig. 2). Although flecainide acetate and the other major metabolite, MODF, were totally resolved in this elution system (retention times were 83 and 10 min, respectively), they were not analyzed concurrently because of the need for different sample preparation procedures and mobile phases [7,9]. The possibility of combining the analyses will be examined in the future.

Linearity and sensitivity

Several compounds were tested as possible internal standard candidates; however, none were found to be acceptable due to either poor response in the chromatographic system or non-reproducible recovery in the sample preparation procedure. Due to the consistent recovery of MODLF, an external standard method afforded acceptable linearity over the range tested. The linear range for this method was 5–50 ng/ml MODLF with a 1-ml plasma sample. The lowest quantifiable concentration was the lowest calibration standard and linearity was not tested beyond the highest calibration standard. The linear regression results were good with a correlation coefficient (r) value greater than 0.997 in all cases.

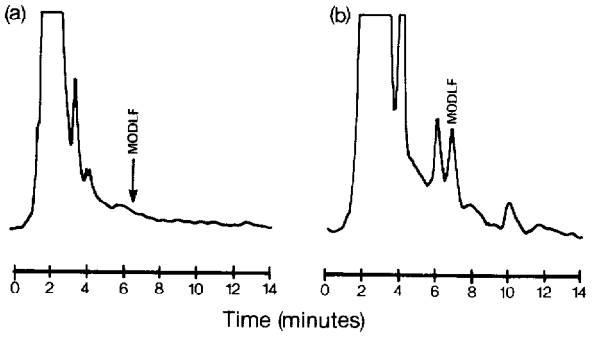

Fig. 2. Typical chromatograms of human plasma. (a) Blank plasma; (b) plasma from a subject dosed with flecainide acetate, concentration of MODLF is 19.6 ng/ml.

Precision and accuracy

Intra-day precision and accuracy were determined by analyzing five replicate samples at each of six concentrations (Table I). The precision, expressed as the coefficient of variation, ranged from 1.3 to 9.0%. The accuracy, expressed as the relative error, ranged from −9.6 to −2.1%. Inter-day precision was determined over a fifteen-day period (Table II) and the range was from 1.0 to 5.0%.

Extraction recovery

The extraction recovery of MODLF using this method was examined by analyzing five replicate samples spiked with MODLF standard solutions at each of six concentrations. The peak heights of these samples were compared with peak heights obtained by direct injection of a series of MODLF standard solutions. The mean recovery was 118, 113, 110, 111, 109, and 110% for the MODLF concentrations 5, 10, 15, 20, 30, and 50 ng/ml, respectively. Although the recovery was consistent over the entire calibration curve range, it appeared to be high. We do not feel that the high recovery is caused by an interference in the plasma

TABLE I

INTRA-DAY PRECISION AND ACCURACY

Values represent five replicate samples.

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Relative error (%)
5	4.5 ± 0.1	3.3	−9.6
10	9.5 ± 0.9	9.0	−4.6
15	14.1 ± 0.3	2.1	−6.1
20	18.9 ± 0.7	3.6	−5.5
30	28.9 ± 0.6	2.0	−3.5
50	49.0 ± 0.6	1.3	−2.1

TABLE II

INTER-DAY PRECISION

Values represent eight replicate samples over a fifteen-day period.

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
5	5.1 ± 0.3	5.0
10	9.7 ± 0.2	1.8
15	15.0 ± 0.4	2.4
20	20.2 ± 0.8	4.1
30	30.6 ± 0.6	1.9
50	49.6 ± 0.5	1.0

TABLE III

COMPOUNDS TESTED FOR INTERFERENCE

Acetylsalicylic acid	Lidocaine
Captopril	Mexilitine hydrochloride
Chlorothiazide	Nifedipine
Diazepam	Procainamide hydrochloride
Digoxin	Propranolol hydrochloride
Diltiazem	Quinidine sulfate
Disopyramide phosphate	Tocainide hydrochloride
Encainide	Verapamil hydrochloride
Furosemide	

because repeated analyses of blank human plasma from different subjects did not show a peak at the retention time of MODLF.

Selectivity

A series of drugs which might be given concurrently with flecainide in the clinical environment (Table III) were tested for possible interference with quantitation of MODLF by direct injection into the HPLC system. With one exception, the drugs tested did not interfere, either because of different retention times or very little fluorescence intensity at the wavelengths used. Procainamide hydrochloride had a retention time just prior to MODLF and was not baseline-resolved. When blank plasma was spiked with procainamide hydrochloride (concentration 4.9 μg/ml) and carried through the sample preparation procedure, the interference was still present. This procedure should not be used to quantitate MODLF in patients receiving procainamide.

Application

Plasma samples collected from three healthy elderly human subjects after eleven days of a 100-mg twice-daily dosing regimen with flecainide acetate were successfully analyzed for MODLF levels with the described method. Samples collected just prior to the last dose (trough) and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h post-dose were analyzed. Trough levels of MODLF were low and ranged from 8 to 10 ng/ml for the three subjects (Table IV). Since steady-state levels in plasma for the parent drug, flecainide, are normally achieved in patients by three to five days after beginning a twice-daily dosage regimen [10], the very low trough levels of MODLF found in these subjects indicate no consequential accumulation of MODLF in plasma with chronic twice-daily oral dosing of flecainide acetate. The absence of any consequental accumulation in plasma of the other major metabolite (MODF) of flecainide after multiple oral dosing of flecainide acetate has been previously reported [7].

After the last dose of flecainide acetate, the highest plasma levels of MODLF were very low (range 14–20 ng/ml) and occurred between 2 and 6 h post-dose in the three subjects (Table IV). By 24 h post-dose, plasma levels of MODLF had declined to about one third to one half of the highest levels. Both MODF and

TABLE IV

PLASMA LEVELS OF MODLF IN HEALTHY ELDERLY SUBJECTS AFTER ELEVEN DAYS OF 100-mg TWICE-DAILY ORAL DOSING OF FLECAINIDE ACETATE

Collection time (h)	Plasma concentration (ng/ml)		
	Subject No. 1	Subject No. 2	Subject No. 3
Trough*	8	10	9
0.5	9	—**	10
1	14	12	16
2	13	13	20
3	15	14	16
4	12	13	16
6	18	13	15
8	13	—**	14
12	13	10	9
24	5	7	6

*Sample collected just prior to last dose of flecainide acetate.
**Insufficient volume for analysis.

MODLF have been reported to be extensively excreted in urine primarily as conjugates [5]. Thus, it is likely that the low levels of MODLF in human plasma are due to rapid and extensive conjugation with excretion of the conjugate in urine.

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