Journal of Chromatography, 534 (1990) 223–227
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO 5494

Note

Analysis of encainide and its three major metabolites in plasma by column liquid chromatography

JEAN-MARIE POIRIER*, MARTINE LEBOT and GEORGES CHEYMOL

Department of Pharmacology, Saint-Antoine Hospital, 184 Rue du Faubourg Saint-Antoine, 75012 Paris (France)

(First received May 9th, 1990, revised manuscript received July 17th, 1990)

Encainide (E), 4-methoxy-2′-(2-(1-methyl-2-piperidyl)ethyl)benzanilide, is a new antiarrhythmic agent used in the treatment of ventricular arrhythmias [1,2]. In extensive metabolizers (>90% of the population) O-desmethylencainide (ODE) and 3-methoxy-O-desmethylencainide (MODE) are present at higher concentrations than the parent drug. In contrast, in poor metabolizers 10–20 fold higher plasma concentrations of E were found with little or no formation of ODE and MODE; instead, N-desmethylencainide (NDE) was present [3,4]. Accurate determination of E and its metabolites is of clinical importance because the metabolites possess cardiovascular activity equal or greater than that of parent drug [5,6].

The first column liquid chromatographic (LC) assay allowed a simultaneous determination of E, ODE and NDE using an external standard quantification method [7]. Other LC assays [8,9] were based on that in the original publication [7] for the quantitative determination of E and its three major metabolites, but either the assay was conducted without an internal standard [8] or an incomplete resolution of NDE from the solvent front was encountered [9], leading in both cases to loss of accuracy. Another LC method for the quantification of E, ODE and MODE has been published [10], but without information about NDE determination.

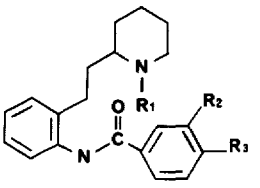
The purpose of this paper is to present a simple and sensitive LC assay for the co-determination of encainide and its three major metabolites in plasma, without the disadvantages of the previously published methods.

EXERIMENTAL

Chemicals and reagents

Pure samples of E and its three metabolites ODE, MODE and NDE (Fig. 1) were kindly supplied by Bristol Myers Labs. (Evansville, IN, U.S.A.). 4-Methyl-

0378-4347/90/$03 50



	R_1	R_2	R_3
ENC	CH_3	H	OCH_3
ODE	CH_3	H	OH
MODE	CH_3	OCH_3	OH
NDE	H	H	OCH_3

Fig 1 Structures of encainide (ENC) and its three major metabolites O-desmethylencainide (ODE), 3-methoxy-O-desmethylencainide (MODE) and N-desmethylencainide (NDE)

propranolol was used as internal standard (ICI Labs., Macclesfield, U.K.). Methanol, ethanol, diethyl ether, dichloromethane (analytical grade) and triethylamine (HPLC grade) were purchased from Prolabo (Paris, France). Methanesulphonic acid was obtained from Aldrich Chimie (Strasbourg, France). The carbonate–bicarbonate buffer (pH 10.5) was prepared from 0.2 *M* stock solutions of anhydrous sodium carbonate and sodium bicarbonate (Normapur grade, Prolabo).

Calibration standards

Stock solutions of E and its metabolites (320 μg/ml) and 4-methylpropranolol (500 μg/ml) were prepared by dissolving appropriate amounts of pure samples in ethanol and methanol, respectively. They were stable for at least one month without observable degradation when stored at −20°C.

A working solution of E and its metabolites at 3.2 μg/ml was prepared by a 1:100 dilution of the stock solution with distilled water.

Plasma standards were made by appropriate dilutions of the working solution with drug-free human plasma in order to give concentrations of E and its metabolites ranging from 5 to 320 ng/ml.

The working solution of 4-methylpropranolol was prepared by a 1:100 dilution of the stock solution with distilled water to provide a concentration of 5 μg/ml.

Chromatography

The LC unit consisted of an 501 solvent-delivery system, an automatic WISP 710 B injector and a 484 variable-wavelength UV detector set at 270 nm (Millipore-Waters, Saint Quentin en Yvelines, France).

Separations were done at ambient temperature on a 5-μm Resolve silica column (15 cm × 4.6 mm I.D.), equipped with a 1-cm precolumn packed with the same material (Millipore-Waters). The mobile phase was methanol–water–methanesulphonic acid–triethylamine (400:4:0.02:0.02, v/v), pumped at a flow-rate of 1 ml/min. The chromatograms were recorded on an SP 4290 integrator (Spectra-Physics, Les Ulis, France) and areas were reported.

Sample preparation

To 1 ml of plasma in a 10-ml glass tube, were added 0.1 ml of internal standard solution (500 ng), 0.5 ml of pH 10.5 carbonate–bicarbonate buffer and 5 ml of extraction solvent consisting of dichloromethane–diethyl ether (70:30, v/v). The tube was shaken vigorously for 5 min and centrifuged for 5 min at 2000 *g*. The upper phase was discarded by vacuum aspiration, and the organic layer was removed into a conical glass tube and evaporated to dryness at 45°C under a stream of nitrogen. The residue was dissolved in 200 μl of mobile phase, and an aliquot (100 μl) was introduced into the LC unit.

RESULTS AND DISCUSSION

Typical chromatograms of plasma extracts are shown in Fig. 2. Assays performed on drug-free plasma samples show the absence of any endogenous interfering peaks (Fig. 2A). The retention times of the internal standard (4-methylpropranolol), NDE, ODE, MODE and E were 3.6, 4.5, 5.6, 6.3 and 7.0 min, respectively.

A representative chromatogram of an extract of a blank plasma sample spiked with 20 ng/ml E and its metabolites and 500 ng/ml internal standard is presented in Fig. 2B. The chromatogram of an extract of a plasma sample obtained from a patient 2.5 h after a 25-mg oral dose of encainide, containing 0, 80, 39 and 23 ng/ml NDE, ODE, MODE and E, respectively, is shown in Fig. 2C.

A least-squares linear regression was used to calculate the equation relating peak-area ratio between each drug and the internal standard, and the concentration of the compounds. Calibration curves were linear ($r^2 \geqslant 0.999$) in the range 5–320 ng/ml for E and its metabolites in plasma. The daily fluctuation of the plasma calibration graphs ($n = 5$) was slight, with coefficients of variation (C.V.) of 2.5, 1.2, 3.2 and 2.6% and intercepts of -0.1 ± 0.1, 0.1 ± 0.2, -0.2 ± 0.5, -0.3 ± 0.5 ng/ml for NDE, ODE, MODE and E, respectively.

Within-day and day-to-day precision and accuracy data for plasma analysis of E and its metabolites were evaluated on the basis of mean ± S.D. of five determinations over the concentration range 5–80 ng/ml.

At the level corresponding to the determination limit, estimated at 5 ng/ml for the four compounds, C.V were less than 10% for intra-assay studies and ranged from 7.8 to 12.7% for inter-assay studies. For NDE, ODE, MODE and E the overall accuracy was 100.5 ± 1.3, 99.0 ± 3.5, 101.5 ± 4.7 and 101.6 ± 2.3%,

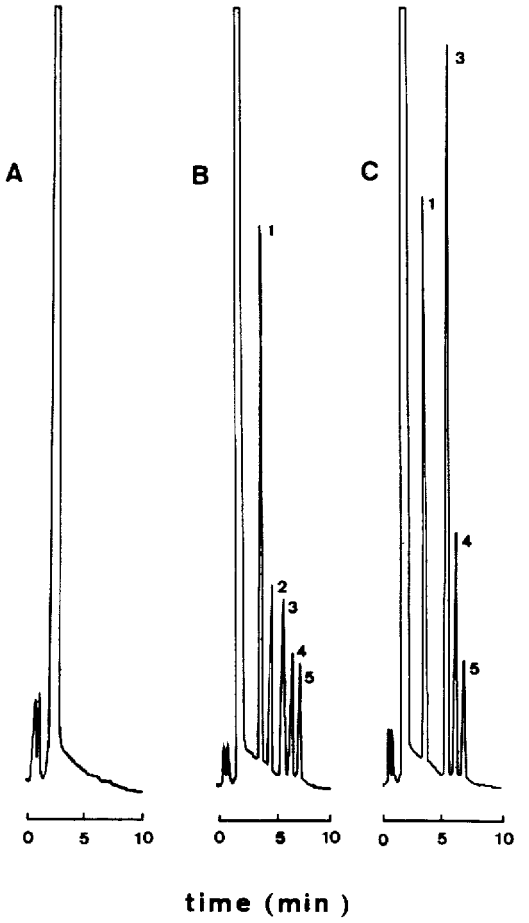


Fig. 2. Chromatograms of 1-ml plasma extracts (A) Drug-free plasma, (B) drug-free plasma spiked with 20 ng/ml each of NDE, ODE, MODE and E, and 500 ng/ml internal standard, (C) sample obtained from a patient 2.5 h after a 25-mg oral dose of encainide containing 0, 80, 39 and 23 ng/ml NDE, ODE, MODE and E, respectively Peaks 1 = internal standard; 2 = NDE, 3 = ODE, 4 = MODE; 5 = E.

respectively, for within-day analysis, and 103.1 ± 4.6, 97.8 ± 3.8, 100.4 ± 3.6 and 100.5 ± 3.0%, respectively, for day-to-day analysis.

The efficiency of the plasma extraction procedure was determined at a level of 80 ng/ml for E and its metabolites ($n = 3$) by comparing the peak areas for spiked plasma samples with those of the corresponding amounts of pure standards in mobile phase injected directly. The results were 86.4 ± 2.7, 86.0 ± 2.4, 86.0 ± 3.4 and 85.8 ± 2.2% for NDE, ODE, MODE and E, respectively, and indicate that the percentage extraction was the same for the four compounds.

Pure samples of some commonly administered drugs used for the treatment of cardiovascular diseases (acebutolol, amiodarone, disopyramide, hydroquinidine,

nadoxolol, propranolol, quinidine and sotalol) and their main metabolites (diacetolol, N-desethylamiodarone, N-dealkyldisopyramide, 3-hydroxyhydroquinidine, 4-hydroxypropranolol and 3-hydroxyquinidine) were assayed under the described chromatographic conditions. More xanthine derivatives, such as caffeine, theobromine and theophylline, were also tested under these LC conditions. Of the assayed drugs N-desethylamiodarone, propranolol and sotalol co-eluted with the internal standard, and amiodarone with ODE In the liquid–liquid extraction procedure described, amiodarone, its main metabolite and sotalol were not recovered. Therefore only propranolol interfered with the internal standard.

In conclusion, an LC method was developed for the co-determination of encainide and its three major metabolites in plasma. The method has some advantages, such as the use of an internal standard and an improvement of accuracy over other reported assays. The reproducibility and the sensitivity of the method allow pharmacokinetic and polymorphic studies of encainide.

REFERENCES

1 M. Sami, J. W. Mason, F Peters and D C Harrison, *Am J Cardiol*, 44 (1979) 526
2 R A Winkle, F. Peters, R. E Kates, C Tucker and D C Harrison, *Circulation*, 64 (1981) 290
3 T Wang, D M. Roden, H. T Wolfenden, R L Woosley, A. J. J Wood and G R Wilkinson, *J Pharmacol Exp Ther*, 228 (1984) 605.
4 R. L. Woosley, D. M. Roden, G Dai, T Wang, D Altenbern, J A Oates and G R Wilkinson, *Clin Pharmacol Ther.*, 39 (1986) 282
5 A W. Gomoll, J E Byrne and R F Mayol, *Pharmacologist*, 23 (1981) 209
6 D M. Roden, H. J Duff, D Altenbern and R L Woosley, *J Pharmacol. Exp Ther.*, 221 (1982) 552
7 R F Mayol and R E Gammans, *Ther Drug Monit*, 1 (1979) 507
8 M J Bartek, R K Mayol, M P. Boarman, R. E Gammans and D G. Gallo, *Ther Drug Monit*, 10 (1988) 446.
9 J Turgeon, C Funck-Brentano, H. T. Gray and D. M Roden, *J Chromatogr*, 490 (1989) 165
10 D J. Kazierad, T. J Hoon and M B Bottorff, *Ther Drug Monit*, 11 (1989) 327