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## Note

# Encainide and metabolites analysis in serum or plasma using a reversed-phase high-performance liquid chromatographic technique

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Encainide is a recently approved class I antiarrhythmic agent structurally distinct from other fast sodium channel blocking drugs [1]. Encainide was reported to be effective in suppressing life-threatening and non-life-threatening ventricular arrhythmias and would appear to be the drug of choice for potentially malignant ventricular arrhythmias [2,3]. Recently as a result of CAST (Cardiac Arrhythmia Suppression Trial), the FDA (Food and Drug Administration) has requested restriction of encainide use to only life-threatening arrhythmias [4].

Two distinct phenotypes for encainide metabolism are extensive and non-extensive metabolizers, including 90 and 10% of the patient population, respectively. Extensive metabolizers have an encainide half-life of $1.7 \pm 0.3$ h producing the two active metabolites O-demethylencainide (ODE) and 3-methoxy-O-demethylencainide (MODE), while non-extensive metabolizers have an encainide half-life of $9.8 \pm 4$ h producing the inactive metabolite N-demethylencainide (NDE) [5,6]. Non-extensive metabolizer plasma encainide concentrations correlate with antiarrhythmic activity; however, active metabolites produced by extensive metabolizers need to be assayed for effective clinical evaluation [7,8].

Plasma encainide concentrations are currently being determined by either radioimmunoassay (RIA) or high-performance liquid chromatography (HPLC) [9], whereas HPLC would be the preferred technique due its ability

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to measure parent compound as well as active and inactive metabolites. A previously described, normal-phase HPLC encainide analysis used a silica gel column and a mobile phase consisting of methanol and water [9]. However, methanol and water dissolve silica and shorten the effective life-time of the column. A new, fast and effective reversed-phase HPLC protocol to determine encainide and its metabolites in serum or plasma is described in this paper. Furthermore, column-dependent mobile phase adjustments which improve resolution and efficiency are also described.

## EXPERIMENTAL

### *Reagents*

Acetonitrile (UV grade), methanol and *n*-butyl chloride (HPLC grade) were purchased from American Burdick and Jackson (Muskegon, MI, U.S.A.). Phosphoric acid (85%) and nanograde isopropyl alcohol were purchased from Mallinckrodt (St. Louis, MO, U.S.A.). Reagent-grade boric acid, anhydrous sodium carbonate and potassium chloride were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Octylamine and nonylamine were purchased from Aldrich (Milwaukee, WI, U.S.A.). Encainide hydrochloride, ODE, MODE and NDE were obtained from Bristol Myers (Evansville, IN, U.S.A.). Trimethobenzamide hydrochloride was obtained from Beecham Labs. (Bristol, TN, U.S.A.).

Working borate buffer solution was made by mixing 63 parts of 1 mol/l boric acid–1 mol/l potassium chloride solution with 37 parts of 1 mol/l sodium carbonate. The pH of the borate buffer was adjusted to 9.0.

Working internal standard (I.S.) was made by diluting trimethobenzamide hydrochloride to a concentration of 10 $\mu$g/ml in methanol. High and low encainide controls were prepared by spiking human plasma obtained from Puget Sound Blood Center (Seattle, WA, U.S.A.), with pure drug and metabolites. High control contained 400 ng/ml each of encainide, ODE, MODE and NDE, while the low control contained 20 ng/ml each. The standard was prepared in a similar way containing 200 ng/ml of encainide and each metabolite.

### *Extraction of encainide and its metabolites*

A 2-ml volume of serum or plasma was required and may be stored at $-20^\circ$C for at least one week without affecting the analysis. I.S., 100 $\mu$l, was added, followed by 1.0 ml of working borate buffer. Extraction solvent, 10 ml of *n*-butyl chloride–isopropyl alcohol (95:5, v/v) was added, then shaken for 10 min on an Eberbach shaker (Baltimore Biological Lab., Baltimore, MD, U.S.A.). The organic layer was separated by centrifugation at 500 *g* for 5 min, then transferred to a 15-ml centrifuge tube and evaporated to dryness under air at 40°C. Chloroform and 0.025 mol/l hydrochloric acid, 200 $\mu$l each, were

added to the dried extract, vortex-mixed for 10 s and centrifuged at 500 *g* for 3–5 min. The upper aqueous layer (50–60 μl) was injected into the HPLC system.

*HPLC separation*

The HPLC apparatus consisted of Waters (Milford, MA, U.S.A.) Model 590 pump and Model 440 single-channel UV detector with a Rheodyne (Cotati, CA, U.S.A.) Model 7125 manual injector. A Supelco (Bellefonte, PA, U.S.A.) 25 cm×4.6 mm I.D., 5 μm particle size, cyanopropyldimethylsilyl (PCN) column was used. The mobile phase consisted of 0.06% phosphoric acid–acetonitrile (85:15, v/v) with 0.01% octylamine. Elution was monitored at 254 nm, flow-rate was 2 ml/min and chart-speed was 1 cm/min. After analysis the system was washed with acetonitrile–water (20:80, v/v).

## RESULTS AND DISCUSSION

Resolution of encainide and metabolites along with the I.S. was achieved with the order of elution being ODE, MODE, I.S., NDE and encainide (Fig. 1). Elution of all analytes was achieved within 15 min of injection. Mobile phase for separation shown in Fig. 1 included 0.02% nonylamine. Resolution and speed of analysis can be adjusted by changing the concentration of nonylamine in the mobile phase or using octylamine. Increased amine concentrations will decrease analysis time at the expense of resolution. Octylamine will increase resolution and slow analysis time. However, over time columns using nonylamine will lose resolution and a switch to octylamine will improve resolution and extend the life-time of the column. We have found that resolution is mostly dependent on the column and mobile phase adjustment will adequately provide for analyte resolution (Fig. 2).

No endogenous compound was found either in serum or plasma which interfered with the analysis. Back extraction of the analytes into 0.025 mol/l hydrochloric acid provided a cleaner preparation than a simple liquid–liquid extraction without significant loss of recovery. Extraction efficiencies were 67% ODE, 89% MODE, 92% NDE, 96% encainide and 93% for the internal standard. Recovery of ODE was independent of concentration over the linear range and although recovery was incomplete it did not cause any complication in the assay technique.

Linearity of encainide and metabolites was from 10 to 1000 ng/ml with the minimum detection limit being 10 ng/ml. Similar assay ranges were also reported using the normal-phase technique [9]. Effective plasma levels of ODE are 100–300 ng/ml for suppression of arrhythmia while the plasma levels of MODE are 60–280 ng/ml during long-term therapy in extensive metabolizers [10]. Non-extensive metabolizers require concentrations of encainide greater than 265 ng/ml for therapeutic effects [11,12]. Plasma levels of encainide are 250–1000 ng/ml in non-extensive metabolizers, twenty times higher than extensive metabolizers due to greater oral bioavailability and longer half-life of

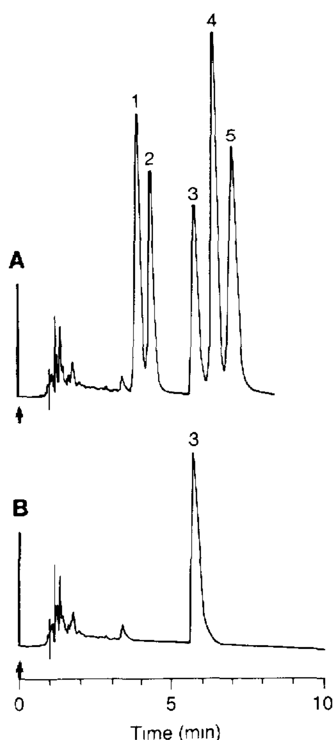


Fig. 1. (A) HPLC profile of encainide and metabolites at a concentration of 400 ng/ml. The peaks in order of elution are: (1) ODE; (2) MODE; (3) I.S.; (4) NDE; (5) encainide. (B) Blank plasma extracted with I.S. PCN column with a mobile phase of 0.06% phosphoric acid–acetonitrile (85:15 v/v) with 0.02% nonylamine.

elimination [12,13]. Toxicity has been observed with ODE concentrations greater than 300 ng/ml [12]. Following oral administration, plasma concentrations can vary sixteen-fold, indicating a wide range of bioavailability. Protein binding is 70.5 and 78% for extensive and non-extensive metabolizers, respectively [13].

Between-run precision for the assay was evaluated for both low and high controls (Table I). Interference was also evaluated among commonly used drugs. Other antiarrhythmic drugs as lidocaine, quinidine, procainamide, tocainide, propranolol, mexiletine, flecainide and amiodarone do not interfere with the assay. The assay is also free from interference by the anticonvulsants phenobarbital, phenytoin, ethosuximide and primidone as well as chloral hydrate. The tricyclic antidepressants and methadone do not interfere with the assay but are retained by the column with longer elution profiles and may elute with subsequent injections. Caffeine and nicotine have short retention times

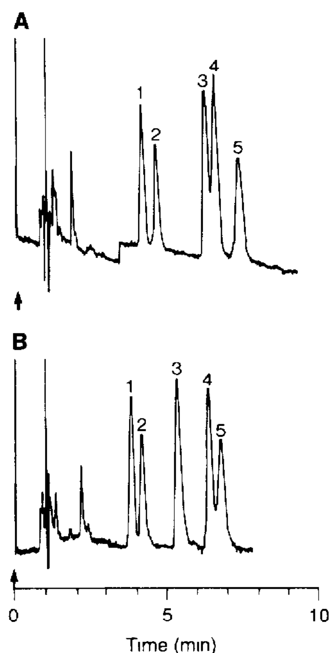


Fig. 2. Two columns from same source, but different lot numbers using identical mobile phase. Encainide and metabolites at a concentration of 200 ng/ml were extracted from plasma. Resolution between the I.S. and NDE is different for column A versus column B. Mobile phase adjustment will optimize resolution. Order of elution is the same as in Fig. 1. PCN columns with a mobile phase of 0.06% phosphoric acid–acetonitrile (85 15, v/v) and 0.01% octylamine

TABLE I

PRECISION ANALYSIS OF LOW AND HIGH CONTROLS

| Analyte | High control | | | Low control | | |
|---|---|---|---|---|---|---|
| | *n* | Mean ± S.D. (ng/ml) | C.V. (%) | *n* | Mean ± S.D. (ng/ml) | C.V. (%) |
| ODE | 50 | 437 ± 27.0 | 6.2 | 51 | 21 ± 3.9 | 18.2 |
| MODE | 50 | 412 ± 20.4 | 5.0 | 52 | 21 ± 3 0 | 14 2 |
| NDE | 48 | 402 ± 14.9 | 3.7 | 51 | 22 ± 3.6 | 16 5 |
| Encainide | 50 | 403 ± 15.6 | 3.9 | 52 | 23 ± 2.5 | 10.7 |

and do not interfere. Anxiolytics, diazepam and chlordiazepoxide do not interfere, however, oxazepam produced an eluent peak between the I.S. and NDE. The extra peak produced by oxazepam did not interfere with the analysis, but could be mistaken for the wrong substance if elution times are not checked carefully. Dipyridamole was found to coelute with MODE and would thus interfere with the metabolite analysis, but not the parent drug.

A reversed-phase HPLC assay for encainide and metabolites can effectively be used for both therapeutic monitoring and drug overdose. Moreover, both extensive and non-extensive metabolizers can easily be identified by this analysis. Extensive metabolizers do not have NDE, whereas non-extensive metabolizers do not have MODE. As expected 90% of the samples analyzed in this laboratory are from extensive metabolizers. Encainide analysis has been found to be useful in monitoring antiarrhythmic therapy, especially when used with other cardiac agents [10]. We conclude that our reversed-phase HPLC assay for encainide and metabolites has great utility in the clinical laboratory.

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