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Determination of encainide and its metabolites by high-performance liquid chromatography

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Abstract Encainide (ENC) and its metabolites *O*-demethylencainide (ODE), 3-methoxy-*O*-demethylencainide (MODE), *N*-demethylencainide (NDE) and bis-*N*,*O*-demethylencainide (NODE) have been measured by two HPLC procedures The method of Mayol using a μPorasil column with ethanol–water–methanesulphonic acid as mobile phase was not able to separately measure NODE and NDE in plasma A new method is described using a μBondapak Phenyl column with acetonitrile–phosphate buffer (0 05 M, pH 7 5) that yields satisfactory separation of ENC and its metabolites NODE was not identified as a metabolite in 23 patients analysed

Keywords *Encainide, Encainide metabolites, HPLC, metabolic demethylation*

Introduction

Encainide (ENC) is a newly introduced antiarrhythmic drug which is effective in the suppression of ventricular arrhythmias [1, 2] It undergoes extensive metabolism to *O*-demethylencainide (ODE), 3-methoxy-*O*-demethylencainide (MODE), *N*-demethylencainide (NDE) and bis-*N*-*O*-demethylencainide (NODE) (Fig 1) Encainide, taken orally, undergoes a polymorphic pattern of "first pass" metabolism in the liver [1] Two distinctive groups of patients are apparent, namely "extensive" (fast) metabolizers and "poor" (slow) metabolizers The fast metabolizers demethylate the parent drug very rapidly in the liver so that plasma concentrations of ODE and MODE are high (200–500 ng ml^{-1}), whilst that of ENC is usually low (50–100 ng ml^{-1}) The slow metabolizers (about 10% of the population) have very high concentrations of ENC in peripheral plasma (ca 500–1000 ng ml^{-1}), whilst the concentrations of ODE and MODE are very low or undetectable The third metabolite, NDE, is usually present in measurable quantities, but so far NODE has not been found in any of the authors' studies In earlier studies, encainide was measured in plasma by radioimmunoassay [3] However, high-performance liquid chromatography (HPLC) with UV detection can be used to measure the various metabolites as well as the parent compound [3, 4], and at present, the method of Mayol and coworkers [4] is frequently used However, the

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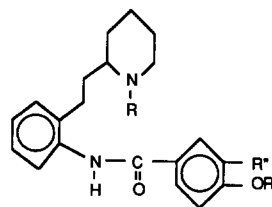

	R	R'	R''
ENCAINIDE	CH_3	CH_3	H
NDE	H	CH_3	H
ODE	CH_3	H	H
NODE	H	H	H
3-MODE	CH_3	H	OCH_3

Figure 1
Structure of ENC and its metabolites

authors have encountered problems in measuring the two metabolites NDE and NODE because they eluted in a portion of the chromatogram where other unknown peaks not derived from ENC were present Kates *et al* [2] modified Mayol's method by using an internal standard (acebutolol), but details of the procedure were not reported The aim of the present study was to develop a new HPLC method which used internal standard quantitation and solved the problems mentioned above

Experimental

Apparatus

The liquid chromatographic system was made by Waters Associates (Massachusetts) and consisted of a solvent delivery system M-6000A, a U6K injector, a 10-μm μBondapak Phenyl column (300 × 3 9 mm, i d), UV detector model 441 and data module M-730

Reagents

Encainide and its metabolites used as standards were supplied by the Mead Johnson Corp (Pharmaceutical Division, Evansville, Indiana) All other chemicals were of HPLC grade and were used without further purification

Analytical procedure of Mayol [4]

To 1 0 ml of plasma (patient or standard), 0 5 ml of 0 5 M Tris buffer (pH 8 5) and 5 0 ml of butyl chloride–isopropanol (95 5) were added and shaken at high speed for 10 min After centrifugation at 2000 rpm for 5 min, 3 5 ml of the upper organic phase was transferred into a conical tube and evaporated under a stream of dry nitrogen at 35°C The residue was dissolved in 100 μl of the mobile phase ethanol–water–methanesulphonic acid (500 30 0 1, v/v/v) At a flowrate of 1 5 ml min^{-1}, 70 μl of the dissolved residue was injected into a 10-μm μPorasil C18 Bondapak column (300 × 3 9 mm, i d) Quantitation was by measurement of peak height at 254 nm using an external standardization method

Proposed analytical procedure

To 1 0 ml of standard or patients' plasma, 0 2 ml of 6 M ammonia buffer (consisting of ammonium hydroxide and ammonium chloride) and 200 μl of an aqueous solution of propranolol hydrochloride (25 μg ml^{-1}) were added and then mixed for 10 min on a rotary mixer (60 rpm) with 5 ml iso-propyl ether–isopropanol (9 1) Water (200 μl) was added to the blanks After shaking and centrifugation (10 min, 2000 rpm), the upper organic phase was transferred to a conical tube and evaporated to dryness at 40°C under a stream of dry nitrogen The residue was dissolved in 100 μl of the mobile phase acetonitrile–0 05 M phosphate buffer (pH 7 5) (30 70, v/v), centrifuged for 5 min at 2000 rpm and 60 μl injected onto a 10-μm μBondapak phenyl column (300 × 3 9 mm, i d)

Results

Typical chromatograms using the proposed method are shown in Fig 2 No large or overlapping peaks in the relevant portion of the chromatogram after 3 5 min were observed

Calibration curves were constructed by measuring peak heights (as integrated by the data module) of the respective substances related to the peak height of the internal standard Linear regression of these calibrations was determined over the concentration range 20–500 ng ml^{-1} Spiked plasma containing ENC and its metabolites was used to compare the precision of the proposed method and that of Mayol [4] For the proposed method at analyte concentrations of 105 ng ml^{-1} (ENC), 290 ng ml^{-1} (ODE), and

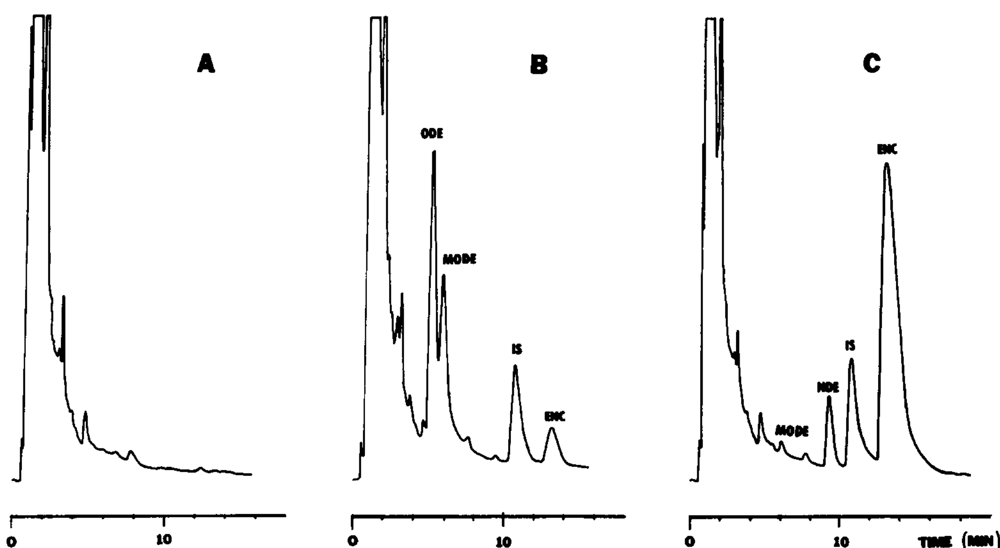

Figure 2

Chromatograms of plasma samples obtained by the proposed procedure (A) Patient not receiving ENC (B) Plasma sample obtained from a patient showing fast metabolism of ENC (ODE, 420 ng ml^{-1}, MODE, 500 ng ml^{-1}, ENC, 120 ng ml^{-1}) (C) Plasma sample obtained from a patient showing slow metabolism of ENC (MODE, 45 ng ml^{-1}, NDE, 130 ng ml^{-1}, ENC, 960 ng ml^{-1}) HPLC conditions, as in Experimental at a flowrate of 2 5 ml min^{-1} and detection at 254 nm and 0 01 AUFS Retention times ODE, 5 3 min, MODE, 6 0 min, NDE, 9 1 min, Internal standard, 10 7 min, ENC, 13 2 min Under these conditions NODE, if present, would have a retention time of 4 0 min

185 ng ml^{-1} (3-MODE), the observed mean concentrations (n = 6) were 104 2, 275 8 and 172 5 ng ml^{-1}, respectively, with relative standard deviations (RSD) of ± 7 1, 4 2 and 7 9%, respectively This compared favourably with the results obtained using Mayol's method with the same spiked plasma sample that gave observed mean concentrations (n = 8) of 108 8, 323 8 and 180 6 ng ml^{-1}, respectively, with RSD values of ± 5 4, 6 9 and 7 0%, respectively In addition the proposed method was able to measure NODE (135 ng ml^{-1}) and NDE (150 ng ml^{-1}), respectively The day-to-day precision (RSD, n = 9) for measuring ENC, ODE, 3-MODE, NODE and NDE in the same spiked plasma sample was ± 7 8, 3 8, 7 2, 6 8 and 7 2%, respectively

The percentage recovery of encainide and its metabolites from spiked plasma was also measured by comparison of the peak height ratios with those obtained by direct injection of the pure compounds The percentage analytical recoveries of NODE, ODE, MODE, NDE and ENC were 55, 85, 75, 100 and 100%, respectively, adjusted to account for injection volume Examples of the analysis of plasma by this technique are shown in Fig 2 The first chromatogram (A) is a normal control plasma, (B) shows the usual pattern of metabolites from a fast metabolizer, and 2C shows the metabolites excreted in plasma by the less common slow metabolizer Figure 3 shows chromatograms obtained using the Mayol procedure [4], as described above, and illustrates the poor resolution between ENC and MODE

The efficiency of the μPorasil column decreased rapidly after 50 injections and this was partially responsible for the inability to detect the fourth metabolite, NODE, in patients' plasma However, when plasma from many patients treated with ENC was examined using the new technique in which NODE did elute separately, the authors were also unable to find any evidence of NODE as a metabolite in human plasma

The choice of propranolol as an internal standard could be criticized because patients may be prescribed propranolol However, the concentration of propranolol used as an

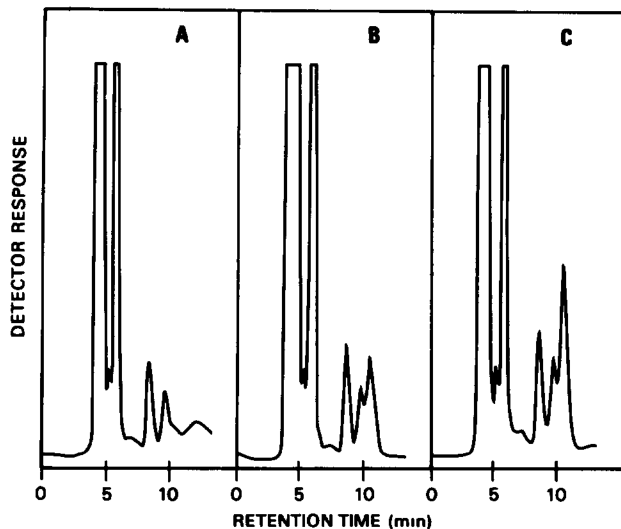

Figure 3
Typical chromatograms obtained by the Mayol procedure (A) No ENC added (B) ENC (58 ng) added (C) ENC (116 ng) added HPLC conditions as in Experimental, at a flowrate of 1 0 ml min^{-1}, and detection at 254 nm, 0 02 AUFS Retention times ODE, 8 3 min, MODE, 9 5 min, ENC, 10 2 min

internal standard was 5000 ng ml^{-1}, which is large compared with the anticipated clinical therapeutic level of propranolol For instance, after a dose of 80 mg the plasma concentration of propranolol 6 h later is about 100 ng ml^{-1} [5], so that in the worst case an error of 2% could be introduced

Discussion

The essential differences between the two methods described here are that the Mayol procedure used a μPorasil column, which is a normal phase column packed with irregular silica, whereas the proposed method uses a μBondapak phenyl column which contains phenyl groups bound to silica For the mobile phase, Mayol used ethanol–water–methane sulphonic acid whereas the proposed method uses acetonitrile with a phosphate buffer and no ion-pairing reagent This resulted in a more consistent separation of NDE from NODE In the authors' experience the Mayol procedure could achieve this separation on a new column but this capacity was lost after about 50 injections The exact reason for this was not elucidated but this phenomenon was not seen with the proposed method However, despite the fact that it is possible to continuously determine these metabolites separately, the authors were unable to find NODE in the serum of 23 patients in this study There appears to be no significant difference in precision between the proposed method and that of Mayol *et al* The latter, however, have reported a slightly lower limit of detection (about 10 ng ml^{-1}) Both methods allow the determination of ENC and metabolites with precision (RSD) of 4–8%

The advantage of the proposed method is considered to be that it permits the identification and determination of the metabolite, NDE, which occurs in the group of slow metabolizers, who make up to 10% of the population This alternative method for the determination of ENC and its metabolites also fulfills the need for a column and mobile phase system that does not rapidly deteriorate with time

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