

METHOD FOR THE QUANTITATIVE DETERMINATION OF ENCAINIDE

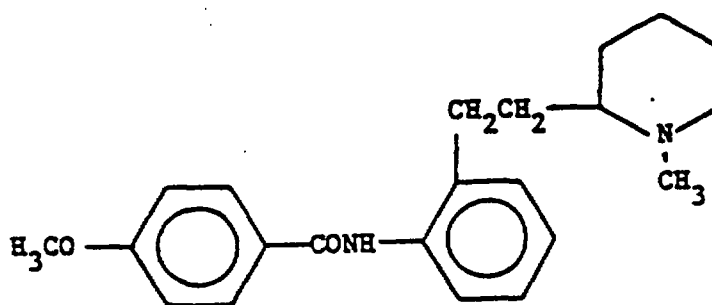
This invention relates to the method for the quantitative determination of encainide by non-ionic bonding.

5 According to the invention there is provided a process for the quantitative determination of encainide and the O-demethyl and 3-methoxy-O-demethyl metabolites thereof in a biological fluid involving extraction thereof from said fluid and separation thereof by high pressure liquid chromatography
10 on a silicic acid column using an organic solvent as mobile phase, wherein said mobile phase is the mixture ethanol:water: methanesulfonic acid 500:30:0.1.

Encainide hydrochloride is an antiarrhythmic compound which
15 is also referred to in the literature as MJ 9067 (USAN And The USP Dictionary of Drug Names 1980, page 122, United States Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, Library of Congress Catalog Card No. 72-88571). Encainide has the following structural formula.

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The following publications describe the chemical synthesis of encainide, a number of analogs thereof, and the antiarrhythmic properties of these compounds in animals.

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Dykstra, et al., J. Med. Chem., 16 1015-1020 (1973).

Dykstra and Minielli, U.S. Patent No. 3,931,195 patented January 6, 1976.

Byrne, et al., J. Pharmacology and Experimental Therapeutics,
5 200, 147-154 (1977).

Assay methods for encainide and its metabolites are disclosed in the following references.

Mayol and Gammans, Therap. Drug Monitoring, 1, 507-524
10 (1979).

Mayol, U.S. Patent Application Serial No. 155,338 filed June 2, 1980.

In patent application No. 8127079 (Specification 2084570)
15 there is described and claimed 4-Hydroxy-3-methoxy-N-[2-[2-(1-methyl-2-piperidinyl)-ethyl]phenyl]benzamide.

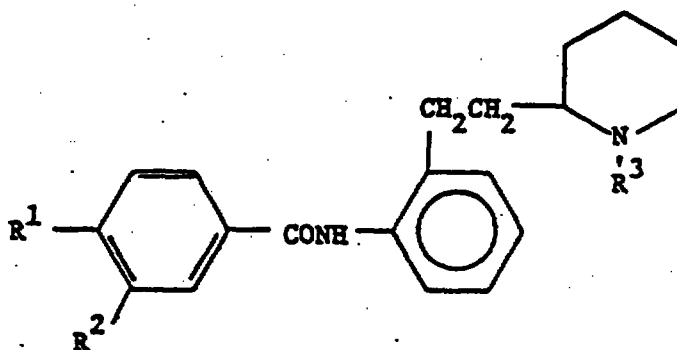
Detailed Description of the Invention

20 The structures of encainide and a number of analogs thereof which are described in the foregoing Dykstra, et al. publication and patent are shown in the following table as well as the compound of specification 2084570. The compound numbers used in the table are the same as the example numbers employed in the Dykstra,
25 et al. patent. Compound No. A is the compound of specification 2084570.

Structures

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<u>Compound No.</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>
89 (O-demethylenecainide, or ODE)	HO-	H-	CH ₃ -
107 (encainide, or E)	CH ₃ O-	H-	CH ₃ -
5 108	H-	CH ₃ O-	CH ₃ -
109	CH ₃ O-	CH ₃ O-	CH ₃ -
139 (N-demethylenecainide, or NDE)	CH ₃ O-	H-	H-
A (3-methoxy-O-demethyl-encainide, or 3-methoxy ODE)	HO-	CH ₃ O-	CH ₃ -

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Compound Nos. 89 and 139 have been identified by Mayol and Gammans (op. cit.) as metabolites of encainide (Compound No. 107) in which publication the former is referred to as ODE and the latter of NDE. The former (ODE) has been identified as a principal metabolite which is sometimes present in plasma in amounts several times the concentration of encainide following treatment with the latter. As a result of an improvement of the high pressure liquid chromatography assay method described in the Mayol and Gammans publication, which improvement constitutes the present invention, it has been found that compound A is also a principal metabolite of encainide in man following oral administration of the latter, and that it is present in concentrations in the blood plasma substantially greater than that of Compound No. 89 two or more hours following the administration of a 50 mg dose of encainide hydrochloride orally. The HPLC assay method described in the Mayol and Gammans publication has now been found to have been incapable of distinguishing between Compound Nos. 89 and Compound A. It is thought that Compound A is produced in the body from Compound No. 89 by a biological transformation process.

For comparative purposes the biological activity of the compounds whose structures are shown in the above table are presented in the following table in which comparative antiarrhythmic activity is given as determined by the laboratory screening test referred to in the Dykstra, et al. paper above.

This is referred to in the table as Test I. Test II is an approximation of the oral toxicity of these compounds in mice, and Tests III and IV refer to the human metabolism results observed following oral administration of an antiarrhythmic oral dose of 75 mg total dose of encainide hydrochloride to patients.

Biological Activity

- I Antiarrhythmic activity for ventricular arrhythmia in mice produced by chloroform inhalation; ED₅₀ expressed as mg/kg body weight, intraperitoneal administration, J.W. Lawson J. Pharmacol. Expt. Ther. 160, 22 (1968).
- II ALD₅₀/ATD₅₀ mouse treated orally; ALD₅₀ is the approximate lethal dose of half the animals; ATD₅₀ is the approximate lowest dose where signs of physiologic or neurologic deficit appear in half the animals, expressed as mg/kg body weight.
- III Time following oral dosing of humans with 75 mg. of encainide hydrochloride for clearance (plasma concentration <20 ng./ml.) from blood plasma; hours.
- IV Approximate half life in human blood plasma following oral dosing with encainide hydrochloride; hours.

<u>Compound No.</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>
89	1.7-2.8*	25-50/5-10	>16	4-6
107 (encainide)	5.3-15*	50-100/5-10	6	2.5-3
108	10	100/10-25	**	**
109	10	100/25	**	**
139	28	147/15.7	+	+
A	9.5	250/31.3-62.5	>16	4.6

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- * Range of various determinations.
- ** Compounds have not been administered to man nor observed as metabolites of encainide in man or animals.
- + Occurrence in plasma following oral administration of encainide to man has been rare, and value has not been determinable.

The foregoing results reflect approximately equivalent antiarrhythmic activity for each of these substances except for Compound No. 89 which is somewhat more potent but also somewhat more toxic in mice. Compound A differs from and enjoys the advantages over each of Compound Nos. 89 and 107 of reduced toxicity and of a more prolonged presence in the blood.

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EXAMPLE
Assay Procedure

A. Extraction of Biological Fluid.- To 1 ml of plasma or urine in a screw-capped tube there is added 0.2 ml of 0.5 M. Tris-HCl buffer (2-amino-2-hydroxymethyl-1,3-propanediol), pH 8.5, and 10 ml of n-butyl chloride containing 5% by volume of isopropanol. The sample is then shaken on an oscillating mixer followed by centrifugation to separate the phases. A 9 ml aliquot of the organic layer is removed and evaporated to dryness under a stream of nitrogen and the residue is redissolved in 100 ml of ethanol. A 50 ml aliquot of this solution is injected onto the column for high pressure liquid chromatography.

B. High Pressure Liquid Chromatography (HPLC) Conditions.- A normal phase silicic acid column having dimensions 3.9 mm by 30 cm in length equipped with a variable wavelength UV detector set at 254 nm is used. The mobile phase employed consists of 500 ml of ethanol, 30 ml of water, and 0.1 ml of methanesulfonic acid at a flow rate of 1 ml per minute. A series of standards containing 0, 25, 100 and 500 ng/ml of each compound to be assayed was prepared using pooled human plasma. These standards were treated as described above. Quantification of the detector

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response was achieved by digital integration or measurement of peak heights. The standard curve for each component was constructed by linear regression of the detector response versus concentration from the plasma standards. The concentration
5 of compound in the sample was then interpolated from these curves

C. Results. - The relative order of elution of encainide and its O-demethyl and 3-methoxy O-demethyl metabolites from the column and their retention times is as follows: Compound
10 No. 89, 8.3 minutes; Compound A, 9.7 minutes; Compound No. 107, 11.4 minutes. The last endogenous plasma component eluted from the column has a retention time of 3.6 minutes and the baseline is very stable in the region where the metabolites elute. Thus,
15 no interference from endogenous plasma components occurs. Due to differences in extraction efficiencies, specific molar absorptivities, and retention times for the individual components, the sensitivity of the assay for each of these components is as follows: Compound No. 89, 10 ng/ml; Compound A, 20 ng/ml; Compound No. 107, 15 ng/ml.

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CLAIMS:

1. A process for the quantitative determination of encainide and the O-demethyl and 3-methoxy-O-demethyl metabolites thereof in a biological fluid involving extraction thereof from said fluid and separation thereof by high pressure liquid chromatography on a silicic acid column using an organic solvent as mobile phase, wherein said mobile phase is the mixture ethanol:water: methanesulfonic acid 500:30:0.1 by volume.
2. A process according to claim 1, substantially as herein-
before described.

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