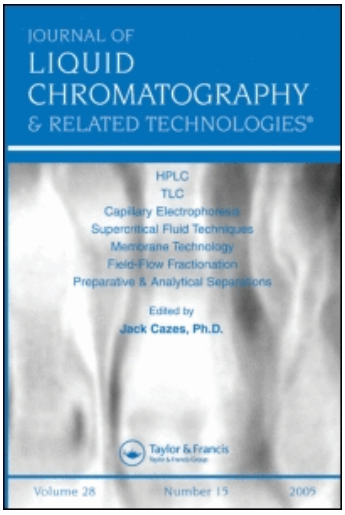

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Boutagy, J. , Rumble, F. M. and Shenfield, G. M.(1984) 'A Rapid Liquid Chromatographic Method for Determination of Flecainide in Human Blood Plasma Using Ultraviolet Detection', Journal of Liquid Chromatography & Related Technologies, 7: 13, 2579 — 2590
To link to this Article: DOI: 10.1080/01483918408067026
URL: http://dx.doi.org/10.1080/01483918408067026

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(13), 2579-2590 (1984)

A RAPID LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF FLECAINIDE IN HUMAN BLOOD PLASMA USING ULTRAVIOLET DETECTION

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ABSTRACT

A liquid chromatographic method for the assay of the antiarrhythmic drug flecainide in plasma has been developed. The method is rapid, simple and with sufficient detection sensitivity to render it suitable for therapeutic drug monitoring. Flecainide and added internal standard, a non-fluorinated analogue, were extracted by a single ether extraction from alkalinized plasma followed by a back-extraction of the ether with dilute phosphoric acid. A portion of the acid extract was then applied directly to a 30 cm ODS column eluting isocratically with 30% acetonitrile in water containing 0.01M dibutylamine phosphate. Monitoring was by ultraviolet detection at 214 nm and the total run time was 8 min. This method is specific and can quantitate plasma levels to less than 30 ng/ml (free base) from 0.5 ml of plasma without interference from antiarrhythmic drugs commonly used in therapy.

INTRODUCTION

Flecainide acetate (R-818) is a relatively new antiarrhythmic drug recently introduced into clinical medicine for evaluation (1-3). It is an orally active drug effective in suppressing ventricular arrhythmias and has been designated a Class 1 antiarrhythmic agent (4-7). The pharmacological properties of

 0148-3919/84/0713-2579$3.50/0

flecainide derived from clinical and animal studies have been well documented in a recent symposium devoted to this drug (8).

To date, there is no established therapeutic range in man for flecainide plasma levels although there is mounting evidence that there is an effective plasma concentration range within which the drug is clinically active. This range is between 200 and 900 ng/ml (10,11) but as more data becomes available these values may be modified. It is therefore likely that as for other Class 1 antiarrhythmic drugs the routine monitoring of plasma flecainide levels will be a useful adjunct to therapy.

There are methods described for the assay of flecainide in blood or plasma. These include a spectrophotometric method (9) which suffers the disadvantage of interference from quinidine and propranolol, gas-liquid chromatography methods requiring derivatization (10-11) and several high-performance liquid chromatographic (HPLC) methods (12-16). The methods by HPLC all use fluorescence detection for selectivity and sensitivity and most require one millilitre of plasma. Of the two more simple of these procedures one method (14) uses a phenyl column to achieve separation together with a fluorinated isomer of flecainide as the internal standard but without good baseline resolution of standard and drug. Also with that chromatographic procedure diazepam co-elutes with flecainide becoming a potential source of interference should ultraviolet detection be used (15). The second method (16) uses normal-phase chromatography requiring small plasma volumes. In our own drug assay laboratory where an HPLC instrument used for

routine drug assay is dedicated to reverse-phase chromatography the constant switching to normal-phase would become inconvenient. We have therefore developed a simple and rapid assay for the analysis of flecainide in plasma using reverse-phase chromatography and ultraviolet detection at 214 nm giving sufficient sensitivity and selectivity to enable use in routine drug monitoring. The procedure is essentially a modification of the method used in our laboratory for the analysis of most commonly used antiarrhythmic drugs (unpublished data). The method consists of a single extraction of alkalinized plasma with diethyl ether followed by a back extraction into dilute aqueous acid. The chromatography is performed on a standard C18 reverse-phase column eluting with acetonitrile-water mobile phase containing di-butylamine phosphate as a modifier and monitoring using a fixed wavelength detector with a zinc lamp and filter for 214 nm output. The internal standard used for the assay is the non-fluorinated analog of flecainide.

MATERIALS

Flecainide acetate, 2,5-bis-(2,2,2-trifluoroethoxy)-N-(2-piperidylmethyl) benzamide monoacetate and the internal standard 2,5-diethoxy-N-(2-piperidylmethyl) benzamide hydrochloride (S-15177) were supplied by Riker Laboratories (Thornleigh, N.S.W. Aust). Acetonitrile was L.C. grade (Ashland Reagent, Ashland Chemical Co., Columbus, OH. U.S.) Water was double distilled through an all glass still and filtered through a 0.45 μm filter (Millipore). Dibutylamine phosphate was purchased as D-4 reagent

(Millipore-Waters Associates, Lane Cove, N.S.W., Aust.) All other reagents were A.R. grade. Drug free plasma was obtained from pooled blood supplied by the Blood Bank at this hospital.

METHODS

Chromatography

Analysis was performed on a Varian Model 5000 liquid chromatograph coupled to a Vista 401 controller. Detection was with a Waters Model 441 U/V detector operating with zinc lamp at 214 nm (0.02 auf). Sample injection was by a Rheodyne injector or by an automated injector (Wisp Model 710B, Waters Associates). The chromatography was performed isocratically on a 30 cm, 10 μm μ-Bondapak C18, reverse-phase column (Waters Associates) in line with a Bondapak-C18/Corasil pre-column. The mobile phase was acetonitrile-water (30:70), the water containing 0.01 mole/litre dibutylamine phosphate. The pH of the mobile phase was 3.2 and was not adjusted. The mobile phase was pumped at a flow rate of 1.5 ml/min and the chromatography was performed at ambient temperature.

Standard Solutions for Assay Calibration

A stock solution of flecainide acetate in water containing 10 mg/100 ml (8.73 mg/100 ml of flecainide base) was prepared and stored at 4 degrees C. Similarly a stock solution of the internal standard (8 mg/100 ml) was prepared in water and stored at 4 degrees. To prepare standard solutions in plasma, 0.25 ml of flecainide stock solution was made up to 20 ml with drug free plasma giving a final concentration of 1092 ng/ml of flecainide as

free base. This was further serially diluted with drug free plasma to give a series of concentrations to 27.3 ng/ml of flecainide base. The standard curve was constructed from drug free plasma containing added flecainide in the range between these two concentrations.

Plasma Extraction and Assay Calibration

To either standard control or patient plasma samples (0.5 ml) in 15 x 115 mm glass tubes was added internal standard solution (0.2 ml) made by diluting the stock internal standard 1:50 with water. Aqueous sodium hydroxide (2M, 50 µl) was then added and the samples were extracted once with diethyl ether (3 ml) by vortex for 30 sec. After centrifugation at 3000 rpm for 5 min the ether extract was transferred to a second tube containing 100 µl 0.1M phosphoric acid. The mixture was again vortexed for 30 sec and after centrifugation the ether layer was removed as completely as possible and discarded. A gentle stream of nitrogen was blown for approximately one minute over the remaining aqueous acid extract to remove traces of volatile material and an aliquot 20 or 50 µl) was injected into the liquid chromatograph. The assay was calibrated from serial plasma samples containing added flecainide at concentrations of 27.3 to 1092 ng/ml (free base) by measuring peak area ratio of flecainide to that of internal standard.

RESULTS AND DISCUSSION

The presence of dibutylamine phosphate as a modifer in the mobile phase considerably enhanced the chromatography, and the

monitoring at 214 nm gave excellent response. Monitoring at this wavelength was made because of the enhanced intensity of absorption by flecainide at shorter wavelength compared to the intensity at the absorption maxima at 295 nm. The chromatograms obtained following extraction of control and patient plasma are shown in Figure 1. These are largely free from endogenous interference and the flecainide response from the lowest calibrator 27 ng/nl plasma is well quantitated above background. The total run time for the assay was 8 minutes. The non-fluorinated analog of flecainide was a suitable standard being well separated from the parent. The fluorinated isomer (2,3-bis analog of flecainide) has been used as an internal standard (14,15) but under the conditions of this assay it co-eluted with flecainide and was therefore not suitable.

Calibration

The calibration of the assay from plasma containing added flecainide produced a linear calibration curve from 27.3 to 1092 ng/ml (free base) (Figure 2). These values are well within the apparent therapeutic range and from the regression data generated for each calibration the concentration of flecainide in unknown samples was determined.

Recovery

To determine extraction efficiency known amounts of flecainide in 0.5 ml of plasma were extracted with ether as previously described followed by back-extraction in 0.1 M

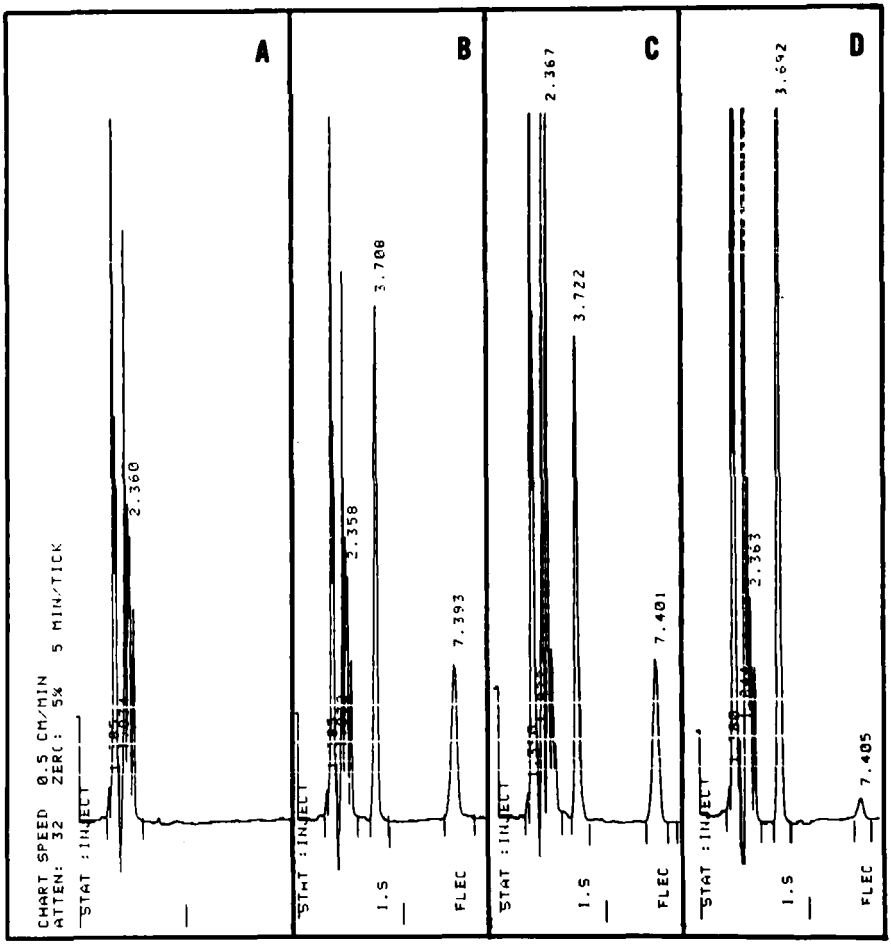

FIGURE 1. Chromatograms obtained following plasma extraction and injection of 20 ul aliquots. A = drug free human plasma with no added internal standard (I.S). B = Control plasma with flecainide (FLEC) concentration of 365 ng/ml. C = Patient plasma sample found to contain 394 ng/ml flecainide. D = Lowest calibrator plasma 27.3 ng/ml (50 µl aliquot injection).

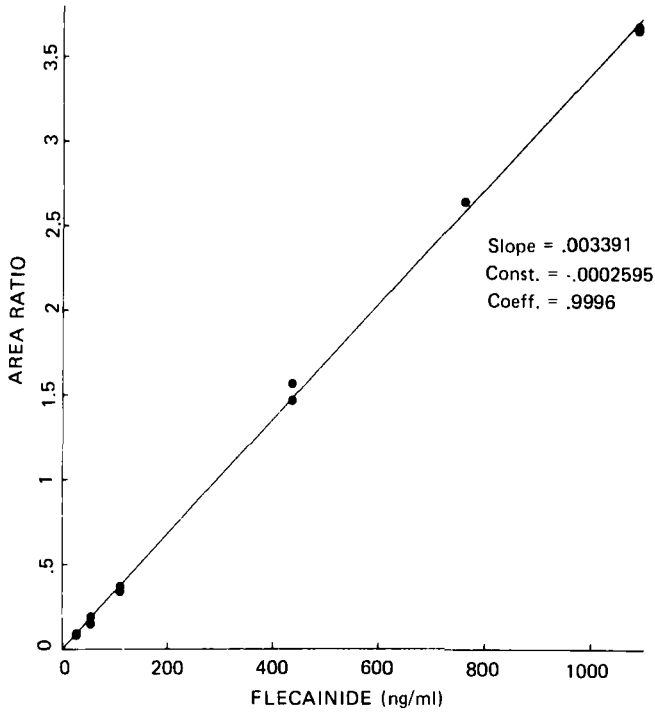

FIGURE 2. Calibration curve from human plasma with duplicate measurements of area ratio of flecainide to internal standard (arbitary units) versus flecainide concentration (ng/ml).

phosphoric acid. The acid extract was then adjusted to a known volume and aliquot injected onto the chromatograph. The response was then compared to that obtained from equivalent amounts of flecainide dissolved in the same volume of phopshoric acid. The results obtained are shown in Table 1. As can be seen the recovery of flecainide is consistently good at different concentration values indicating good extraction efficiency with ether at elevated pH followed by back-extraction into aqueous acid. There is also good recovery of the internal standard.

TABLE 1

Recovery of Flecainide and Internal Standard from Plasma. Extraction Efficiency for Flecainide was Calculated at Four Different Concentrations. For the Internal Standard, Recovery was at the Concentration Added to the Plasma (n = 5 for Each Concentration).

Compound	Concentration (ng/ml)	Recovery (Mean % ± S.D.)
Flecainide	109	88.7 ± 4.1
	218	90.5 ± 3.2
	436	93.1 ± 3.0
	873	94.8 ± 3.0
Internal Std.	640	86.0 ± 2.9

Precision

The reproducibility and precision of the assay following repeated determinations at different concentration values are shown in Table 2. Within-run precision was determined by analysing five samples from the same specimen. The day-to-day precision was determined by analysing samples from three control specimens eight times at random periods over two months.

Selectivity

Potential interference with the assay would arise from other commonly used antiarrthymic drugs which may be concomitantly administered and would extract from plasma to varying degrees under conditions of the assay. The relative retention times of these compounds and related substances are shown in Table 3. It

TABLE 2

Assay Reproducibility. Within-Run Precision (n = 6 for each concentration) and the Day-to-Day Precision (n = 8) Calculated for Flecainide.

	Mean Conc. Found (ng/ml ± S.D.)	C.V. %
Within-Run	26 ± 1.5	5.7
	96 ± 2.0	2.1
	194 ± 5.7	2.9
	582 ± 6.7	1.2
	962 ± 19.6	2.0
Day-to-Day	45 ± 3.0	6.7
	140 ± 8.0	5.7
	461 ± 19.7	4.3

TABLE 3

Relative Retention Times of Commonly used Antiarrhythmic Drugs and Related Compounds.

Compound	Relative Retention Time (Flecainide = 1.0)
Procainamide	0.23
N-Acetylprocainamide	0.26
Tocainide	0.30
Lidocaine	0.31
Mexiletine	0.37
Quinidine	0.38
Quinine	0.38
Dihydroquinidine	0.41
Internal Standard	0.50
Propranolol	0.61
Flecainide	1.0

can be seen that each is resolved from both flecainide and internal standard and do not interfere with the assay. Also tested were the basic drugs cimetidine and ranitidine as well as theophylline, diazepam and amiodarone. None of these had retention times close to flecainide or the internal standard and would not interfere.

Conclusion

The procedure described in this paper is a practical method from the standpoint of simplicity, reproducibility and relative speed. The chromatograms produced are clear with sufficient sensitivity using ultraviolet detection to carry out routine analysis of this drug. The method has now been incorporated in our laboratory as a standard procedure for a drug with a rapidly increasing clinical use.

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