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Comparison of gas–liquid chromatography and fluorescence polarization immunoassay for therapeutic drug monitoring of flecainide acetate*

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Abstract: A gas chromatographic (GC) method for quantitation of flecainide acetate in human plasma is described and compared with a fluorescence polarization immunoassay (FPIA) for therapeutic drug monitoring. The GC method includes a solid-phase extraction procedure and electron capture detection (ECD) without the need of derivatization. Within-day and between-day coefficients of variation were <7% for GC and FPIA. Recovery was between 89–101% for the GC method. Plasma from 36 patients were analysed by both GC and FPIA and the results showed a good correlation (slope = 0.96; intercept = 0.009 μg ml^{-1}; r = 0.987).

Keywords: *Gas chromatography assay; fluorescence polarization immunoassay (FPIA); flecainide; therapeutic drug monitoring.*

Introduction

Flecainide acetate (Fig. 1) is a class I antiarrythmic drug used in the treatment of chronic ventricular arrythmias. It is a first choice drug because of its efficacy, ease of administration, pharmacokinetic properties (half-life, 12–27 h) [1] and favourable tolerance. Therapeutic plasma concentrations range from 0.2 to 1 μg ml^{-1}. However, major adverse cardiac effects have usually been associated with concentrations higher than 1 μg ml^{-1}. Owing to this potential proarrythmic effect, monitoring of plasma concentrations is recommended [2–4]. Currently, measurement of flecainide plasma levels is accomplished by high-performance liquid chromatography (HPLC) with fluorescence detection [5] and by fluorescence polarization immunoassay (FPIA) [6]. A gas chromatographic (GC) method which includes derivatization and electron capture detection has also been developed [7]. In this work, a new GC method which does not require derivatization and the FPIA have been compared for therapeutic drug monitoring.

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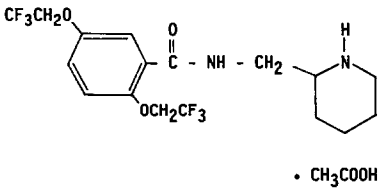
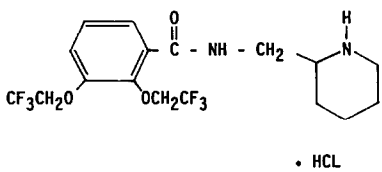


FLECAINIDE ACETATE



INTERNAL STANDARD

Figure 1
Structure of flecainide acetate and the internal standard.

Experimental

Reagents

All reagents and solvents for the GC method were of analytical grade.

Flecainide acetate and the internal standard [*N*-(2-piperidylmethyl)2,3-bis (2,2,2-trifluoroethoxy) benzamide hydrochloride], a positional isomer (Fig. 1), were obtained from Riker Laboratories (Loughborough, UK). Reagents, calibrators and controls for the FPIA method were obtained from Abbott Diagnostics Division.

Standard solutions

For the GC method, a 1 μg ml^{-1} primary standard water solution was diluted with human plasma to give standard solutions containing 0.500, 0.250, 0.125 and 0.062 μg ml^{-1}. A stock solution of internal standard from Riker Laboratories was diluted with water to give a concentration of 1 μg ml^{-1}. Plasma controls were prepared according to the same method.

For the FPIA method, calibrators and controls were obtained from Abbott Diagnostics Division. The concentrations used were 0, 0.100, 0.250, 0.500, 1 and 1.5 μg ml^{-1}.

Extraction

The extraction procedure for the GC method was a solid-phase extraction. Bond-Elut C8-columns (Analytichem) were activated by washing with 3 ml methanol and 3 ml water; 0.5 ml of patient plasma, standard or control, 0.1 ml of internal standard and 0.1 ml of 0.2 M sodium carbonate solution were applied to the extraction columns. The samples were eluted under vacuum and the columns washed with 3 ml water and 3 ml acetonitrile. Vacuum was held on for 2 min and the compounds were eluted with 1 ml methanol.

The eluate was evaporated under a stream of nitrogen at 50°C. The residue was reconstituted with 100 μl methanol and 2.5 μl were injected into the gas chromatograph. The FPIA method did not require any sample extraction or preparation.

Instrumentation

GC analysis was performed on a gas chromatograph from Perkin–Elmer, model 8500, equipped with an electron capture detector (ECD, 15 mCi of Nickel-63). A special syringe for solid injection (type B, SGE, Australia) was used.

The column was a polyphenylmethylsiloxane (OV 17) capillary column (25 m × 0.32 mm) from Alltech. The operating conditions were as follows: injection port temperature, 275°C; and detector, 300°C. Oven temperature was programmed from 230°C with a 1-min hold, to 245°C with a range rate of 2°C min^{-1} and from 245 to 280°C with a range rate of 15°C min^{-1}. Carrier gas was helium.

The FPIA analysis was performed on a TDx assay system (Abbott Diagnostics Division) according to Abbott instructions without modification.

Precision and recovery

For the GC method, a standard curve was calculated every day. Within-day precision was assessed by assaying eight replicates of low (0.125 μg ml^{-1}) and high (0.500 μg ml^{-1}) plasma controls in the same run. Between-day precision was assessed by 10 single determinations of each of the two controls over 10 days. The extraction recovery from human plasma was assessed by eight determinations of each of the two controls. In this assay, internal standard was added after the extraction procedure and the recovery was calculated by comparison with direct injection of methanolic solutions.

For the FPIA method, precision and recovery were obtained from Abbott Diagnostics Division [6]. The methods used by Abbott were as follows: within- and between-day precision was determined on 10 different days by assaying five replicates each of flecainide acetate in human serum at 0.30, 0.60 and 1.20 μg ml^{-1} (only results for 0.30 and 0.60 μg ml^{-1} are presented in this paper). The concentration of each replicate was determined from a single calibration curve run on the first day of the study. Recovery was assessed by three determinations of blank human plasma spiked with 0.10, 0.25, 0.50, 1.0 and 1.5 μg ml^{-1} of flecainide acetate (only results for 0.10, 0.25 and 0.50 μg ml^{-1} are presented in this paper).

Correlation between GC and FPIA

Plasma specimens were obtained from 36 patients receiving flecainide acetate for the treatment of ventricular arrythmias. The samples were analysed by FPIA and stored at −20°C until analysis by GC was performed. For the GC assay, samples over 0.500 μg ml^{-1} by FPIA were diluted with water before the extraction procedure.

Least-squares regression was used to determine the linear relationship between the two methods.

Results and Discussion

Representative chromatograms from the GC method are shown in Fig. 2. Although the drug and the internal standard differ only in the position of the trifluoroethoxy group in the aromatic ring, sufficient separation was achieved by using a capillary OV 17 column. Under the chromatographic conditions used, there was no interference with the

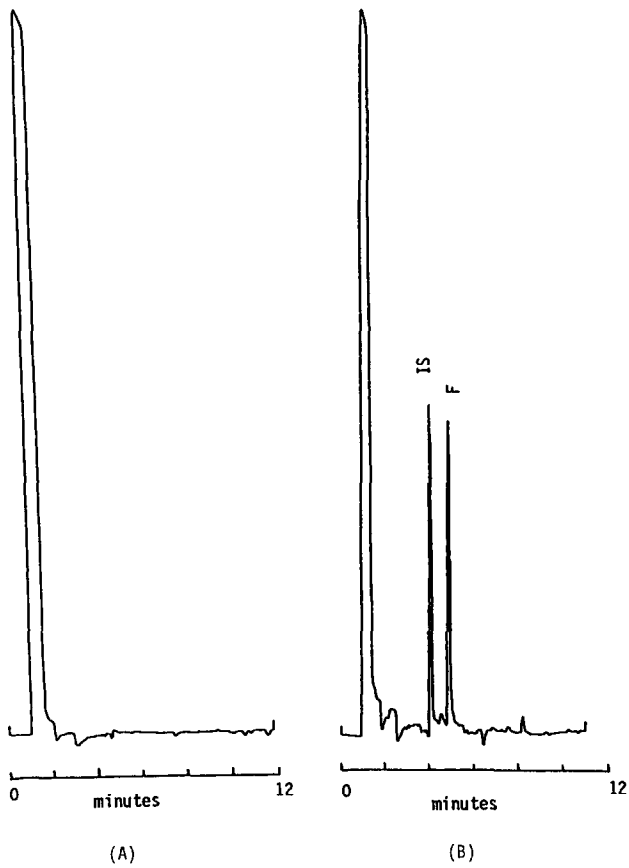


Figure 2
Chromatograms of (A) human blank plasma and (B) plasma spiked with 0.5 μg ml^{-1} of flecainide acetate (F).

drug or the internal standard by any extractable endogenous material present in plasma. The retention times of internal standard and flecainide acetate were 4.6 and 5.7 min, respectively.

Precision for GC and FPIA methods is summarized in Tables 1 and 2. Within- and between-day relative standard deviation (RSD) was <7% for both methods. However, larger RSDs were observed for the low plasma controls (0.125 μg ml^{-1} for GC and 0.300 μg ml^{-1} for FPIA).

Recovery data are summarized in Tables 3 and 4. For the GC procedure, recovery was slightly lower for 0.500 μg ml^{-1} (89%) than for 0.125 μg ml^{-1} (101%). It was higher for FPIA, varying between 96–110%.

Three patient plasma samples were not included in the statistical analysis. One was below the limit of sensitivity, a second was much higher than the therapeutic range and the third was very dirty and presented a large interference peak. Least-squares regression of 33 plasma specimens demonstrates a very good correlation between the two methods (Fig. 3). The slope (0.96) and intercept (0.009 μg ml^{-1}) indicate that the two methods measure similar concentrations.

Table 1
Precision of the GC method

Target value ($\mu g\ ml^{-1}$)	0.125	0.50
Within-day ($n = 8$)		
Mean ($\mu g\ ml^{-1}$)	0.13	0.54
SD ($\mu g\ ml^{-1}$)	0.01	0.02
RSD (%)	6.3	4.1
Between-day ($n = 10$)		
Mean ($\mu g\ ml^{-1}$)	0.12	0.49
SD ($\mu g\ ml^{-1}$)	0.01	0.03
RSD (%)	7	6.1

Table 2
Precision of the FPIA method* (10 determinations of five replicates)

Target value ($\mu g\ ml^{-1}$)	0.30	0.60
Mean observed ($\mu g\ ml^{-1}$)	0.29	0.61
Within-day		
SD ($\mu g\ ml^{-1}$)	0.01	0.01
RSD (%)	4.5	2.2
Between-day		
SD ($\mu g\ ml^{-1}$)	0.02	0.02
RSD (%)	6	3.4

* Data from Abbott Diagnostics Division [6].

Table 3
Recovery of the GC procedure ($n = 8$)

Expected concentration ($\mu g\ ml^{-1}$)	Recovered from plasma ($\mu g\ ml^{-1}$)	Recovery (%)
0.12	0.13	101
0.51	0.45	89

Table 4
Recovery of the FPIA procedure* ($n = 3$)

Expected concentration ($\mu g\ ml^{-1}$)	Recovered from plasma ($\mu g\ ml^{-1}$)	Recovery (%)
0.10	0.11	110
0.25	0.24	96
0.50	0.52	104

* Data from Abbott Diagnostics Division [6].

A possible source of minor differences between the two methods could be the preparation of the standard curves which involved standards and controls from a different source for each method. The FPIA calibrators and controls were from Abbott Diagnostics Division while standards for the GC method were prepared in the laboratory.

Another possible source of minor differences is the degree of interference from

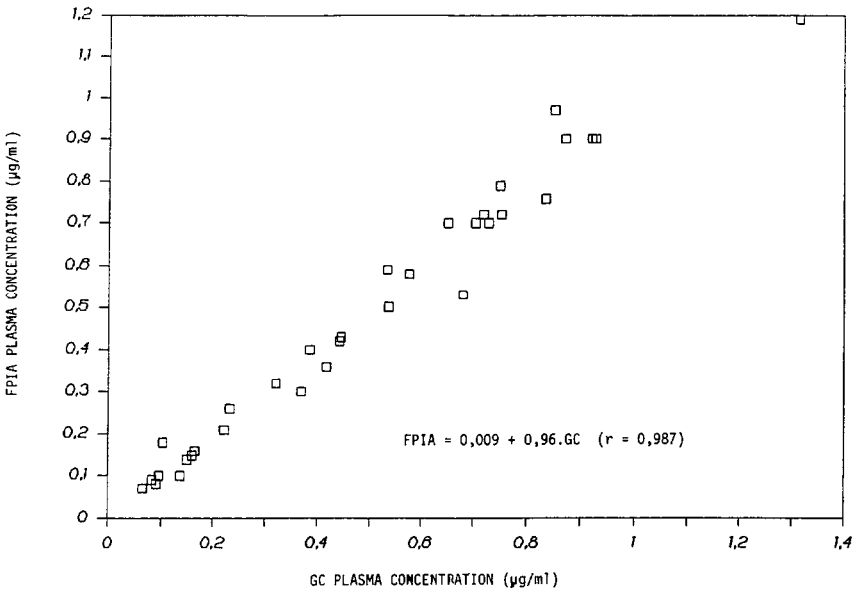


Figure 3
Correlation between FPIA and GC for the assay of flecainide acetate in human plasma.

endogenous compounds and co-medications. For the GC method, the extraction procedure and the temperature conditions eliminate all interferences from endogenous compounds. For the FPIA procedure, interferences were found to be <10% in the presence of high concentrations of protein (100 g l^{-1}), bilirubin (150 mg l^{-1}), haemoglobin (10 g l^{-1}), triglycerides (6.6 g l^{-1}) and cholesterol (2.3 g l^{-1}). Also, FPIA cross-reacts by <0.1% with several drugs that could potentially be co-administered, at concentrations between 1–100 µg ml^{-1} [6]. Only encainide showed a maximum of 0.6% cross-reactivity at 10 µg ml^{-1}. The two major metabolites of flecainide acetate, meta-*o*-dealkylated flecainide and meta-*o*-dealkylated lactam of flecainide which possess little or no detectable antiarrythmic activity, are present at very low concentrations (<0.05 µg ml^{-1}) as unconjugated metabolites in patients plasma [3] and also cross-reacted by <0.1% at concentrations between 10–0.1 µg ml^{-1}.

In conclusion, this comparison shows that both methods are suitable for the measurement of human plasma concentration of flecainide acetate. Precision and recovery for both methods are similar. However, FPIA is a faster and easier procedure. It requires a small sample volume (50 µl) and no sample preparation. Instrumentation is simple and does not require technician skill.

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