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# Short Communication

# Validation of a capillary electrophoresis method for the enantiomeric purity testing of fluparoxan

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ABSTRACT

A free solution capillary electrophoresis method has been validated for the enantiomeric purity determination of either enantiomer of fluparoxan. The method allowed determination of 1% of either enantiomer in the presence of its stereoisomer. Method validation showed adequate detector linearity over the required range. The method also gave good performance in terms of sensitivity for trace levels of the undesired enantiomers, injection precision and recovery.

## INTRODUCTION

Free solution capillary electrophoresis (FSCE) methods employing cyclodextrins as chiral recognition agents have been reported for a number of racemic pharmaceuticals [1–4]. However, few reports have considered quantitative aspects of chiral analysis and none have reported the successful validation of a method. Separation conditions have previously been reported [5] for the FSCE chiral separation of the racemic pharmaceutical fluparoxan (structure shown in Fig. 1). This paper describes the validation of a chiral FSCE method for the determination of the enantiomeric purity of both the (+)- and (−)-enantiomers of fluparoxan.

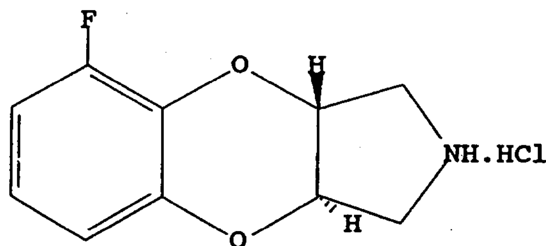


Fig. 1. Structure of fluparoxan.

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## EXPERIMENTAL

Inorganic chemicals were obtained from Aldrich (Poole, UK). Water was obtained from a Millipore Q system (Watford, UK). Capillary electrophoresis was performed on a P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, US) which was connected to a Hewlett-Packard (Bracknell, UK) data collection system. The fused-silica capillaries used were purchased from Metal Composites, Hallow, UK.

The separation conditions are given below, the method consists of five automated steps: (i) pre-separation rinse 1: 1 min with 0.1 *M* NaOH; (ii) pre-separation rinse 2: 2 min with electrolyte; (iii) set detector: UV at 214 nm; (iv) sample introduction: 5 s pressure; (v) typically +16 kV applied for 30 min. Operating temperature set at 25°C; 57 cm × 50 $\mu$m fused-silica capillary.

Sample concentration (1.25 mg/ml) dissolved in water containing 10% (v/v) electrolyte. Fluparoxan samples were obtained from within Glaxo Group Research.

Electrolyte: (10 m*M* borax, 10 m*M* Tris, 150 m*M* $\beta$-cyclodextrin, 6 *M* urea)–isopropanol (80:20, v/v), the pH of the resulting solution was adjusted to 2.5 with concentrated $H_3PO_4$.

Normalisation of peak areas to their migration times was performed prior to calculation of %area/area. If this is not performed the later eluting enantiomer is overestimated [6] as it migrates more slowly through the detector.

## RESULTS AND DISCUSSION

Capillary electrophoresis separation conditions for the chiral resolution of fluparoxan have been reported [5] which employed 100 m*M* $\beta$-cyclodextrin as the chiral selector. These conditions were shown to be capable of achieving acceptable baseline resolution of the fluparoxan racemate. This method was modified to enable enantiomeric purity detector at a 1% level for each of the single enantiomers. These variations involved use of a higher $\beta$-cyclodextrin concentration. The cyclodextrin concentration was raised to 150 m*M* which improved chiral separation. Cyclodextrin concentrations of as high as 250 m*M* have been employed elsewhere [7]. However, excessive cyclodextrin concentrations can have a deleterious effect upon certain separations [8]. A lower ionic strength electrolyte was employed to improve resolution by reducing internal heating within the capillary as resolution decreases at higher temperature [3]. The sample was prepared with dilute electrolyte to improve peak shape by increasing sample stacking [9].

The optimised method was validated for the enantiomeric purity testing of either enantiomer. The validation criteria applied were similar to those applicable to the validation of a HPLC method.

### *Specificity*

A sample of racemic fluparoxan was spiked with the (+)-enantiomer to produce a 60:40 ratio of (+)- to (−)-enantiomers. This sample was analysed to confirm the migration order. Fig. 2 shows a typical separation of this mixture with

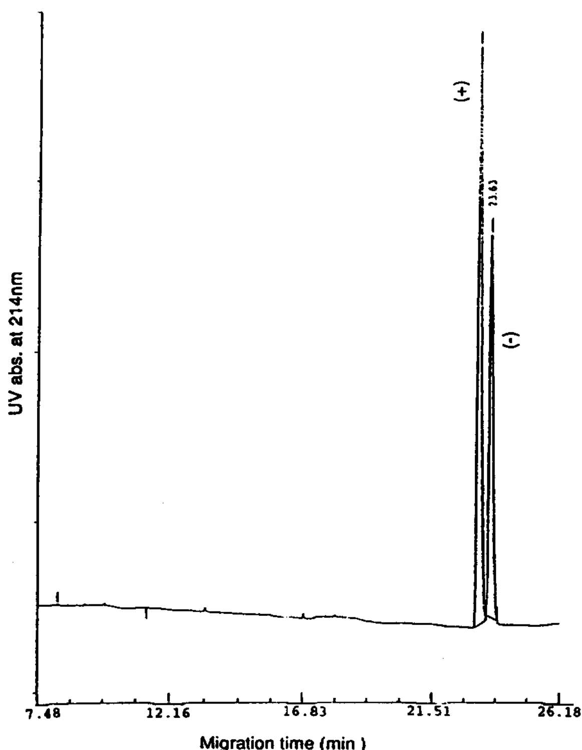


Fig. 2. Electropherogram of 60:40 (w/w) mixture of (+)- and (−)-fluparoxan enantiomers. Separation conditions: 57 cm × 50 $\mu$m fused-silica capillary (10 m*M* borax, 10 m*M* Tris, 150 m*M* $\beta$-cyclodextrin, 6 *M* urea)–isopropanol (80:20, v/v), the pH of the resulting solution was adjusted to 2.5 with concentrated $H_3PO_4$, 25°C, UV detection at 214 nm, +16 kV for 30 min.

TABLE I

QUANTITATIVE ANALYSIS OF AN ENANTIOMER MIXTURE

| | (+)-Enantiomer | (−)-Enantiomer |
|---|---|---|
| Spiking level (%, w/w) | 60.1 | 39.9 |
| % Height | 60.3 | 39.7 |
| % Normalised areas | 60.2 | 39.8 |

the (+)-enantiomer migrating first. Peak height and normalised peak area measurements quantitatively confirmed the spiking levels (Table I).

*Linearity*

Detector linearity (peak area) for the CE method was demonstrated over the range 1.5–125% of the nominal target concentration (1.25 mg/ml), a correlation coefficient of 0.994 and an intercept value of 0.2% of the nominal concentration were achieved (Table II).

TABLE II

DETECTOR LINEARITY AND DETECTION LIMITS

| Concentration range | Correlation coefficient |
|---|---|
| 1.25–125% nominal | 0.994 (intercept 0.2% of nominal value) |
| 1–8% (w/w) of spiked (−) | 0.992 |
| 1–8% (w/w) of spiked (+) | 0.970 |
| Limit of quantitation | 1.0% |
| Limit of detection | 0.3% |

In two further separate exercises solutions of each of the enantiomers were spiked with 1–8% (w/w) of their stereoisomers. In both instances acceptable levels of linearity were obtained (correlation coefficient values of 0.992 and 0.970 for the (−)- and (+)-enantiomers respectively).

*Precision of injection*

Solutions (1.25 mg/ml) of both single enantiomers were spiked with 1% of their stereoisomers and analysed 6 times. Acceptable levels of precision were obtained for the main peak and for the trace enantiomer (Table III). Good recovery was indicated by the agreement between observed enantiomer level and the % (w/w) spiking. Figs. 3 and 4 show representative separations from the two analysis sets.

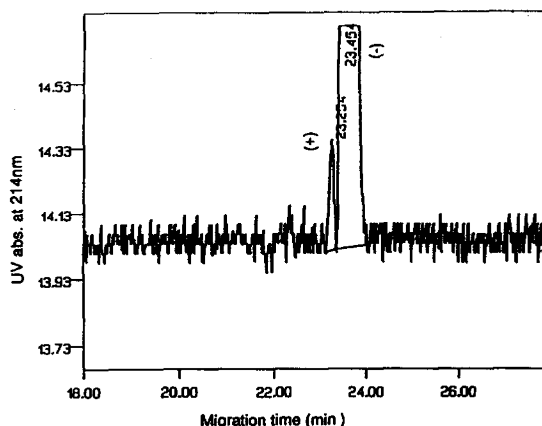


Fig. 3. Typical electropherogram of 1% (+)-enantiomer in presence of (−)-enantiomer. Separation conditions as in Fig. 2.

TABLE III

PRECISION OF INJECTION FOR 1% (w/w) SPIKED SAMPLES ($n = 6$)

| | 1% (+)-Enantiomer | 1% (−)-Enantiomer |
|---|---|---|
| Main peak (R.S.D.) for other enantiomer | 1.6 | 2.0 |
| %Area/area for trace enantiomer | 0.8 | 0.9 |
| | 0.9 | 0.9 |
| | 1.0 | 1.0 |
| | 0.9 | 1.0 |
| | 1.1 | 1.0 |
| | 0.9 | 1.2 |
| Average (%area/area) | 1.0 | 0.9 |

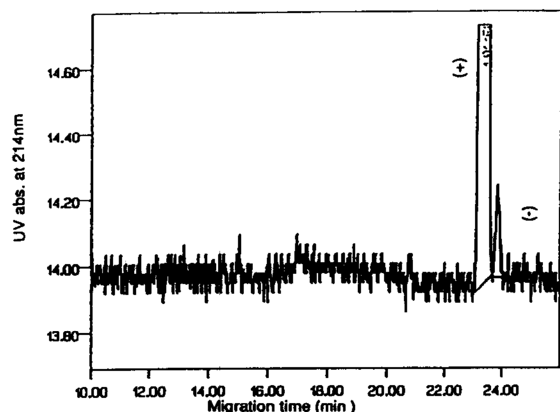


Fig. 4. Typical electropherogram of 1% (−)-enantiomer in presence of (+)-enantiomer. Separation conditions as in Fig. 2.

*Limit of detection (LOD)*

The method is capable of performing enantiomeric purity testing to the required 1% level for both enantiomers. Figs. 3 and 4 show that signal-to-noise ratios of greater than 3 were obtained for the undesired enantiomers. A limit of detection of 0.3% was calculated giving a limit of quantitation of 1.0% for the undesired enantiomer.

*Freedom from interference*

A solution of the dissolving solution was injected onto the system, in duplicate, and no interfering peaks were observed.

*Stability of sample solutions*

Solutions of both the (+)- and (−)-enantiomers were stored for 7 days at 30°C and retested by the CE method. No racemization had occurred indicating a minimum sample solution shelf-life of 7 days.

## CONCLUSIONS

A low-pH FSCE method has been validated for the enantiomeric purity determination of both the (+)- and (−)-enantiomers of fluparoxan. To maximise performance a previously reported method was modified in terms of electrolyte ionic strength, cyclodextrin concentration and the sample dissolving solution. Method validation showed good levels of performance in terms of precision, linearity, recovery and the required LOD. No interfering peaks were obtained from the dissolving solvent.

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