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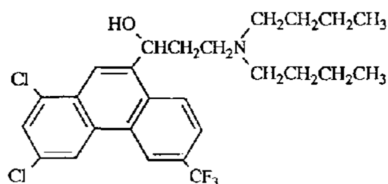
Letter to the Editor

Fluorescence detection of the enantiomers of halofantrine at picomole levels using chiral high-performance liquid chromatography

Sir,

1 - (1,3 - Dichloro - 6 - trifluoromethyl - 9 - phenanthryl) - 3 -N,N -dibutylamino-propan-1-ol (halofantrine, **1**) is an effective antimalarial agent currently marketed by SmithKline Beecham as the hydrochloride salt under the trade name of Halfan. In a previous report [1] we described a chiral high-performance liquid chromatography (HPLC) procedure for the resolution of the optical isomers of halofantrine using a Pirkle type chiral stationary phase. We have now found that fluorescence detection is far superior to UV absorbance in the analysis of very low levels of **1**.



(1)

Prior to chromatography studies fluorescence spectra of halofantrine free base were acquired in the mobile phase using a Perkin-Elmer LS-4 fluorescence spectrometer. The excitation and emission scans were performed at a scan speed of 30 nm min^{-1} and were acquired using a Nelson Analytical 960 Series analog-to-digital (A/D) interface sampling at 100 Hz for high resolution. The fluorescence excitation and emission spectra of **1** are shown in Fig. 1. As expected, the excitation spectra between 230 and 350 nm bear much resemblance to the UV spectra reported in ref. 1. The emission spectrum in Fig. 1b shows a well-defined pair of bands of approximately equal intensity at 355.2 and 371. 4 nm. The first emission band was chosen for the chiral analysis of **1**.

The HPLC method to separate the optical isomers of halofantrine was developed on a Perkin-Elmer Series 4 liquid chromatograph, equipped with a Perkin-Elmer ISS-100 autoinjector. UV detection was performed using an Applied Biosystems 783A absorbance detector operating at 260 nm, connected in series to a Perkin-Elmer LS-4 fluorescence spectrophotometer operating at an excitation

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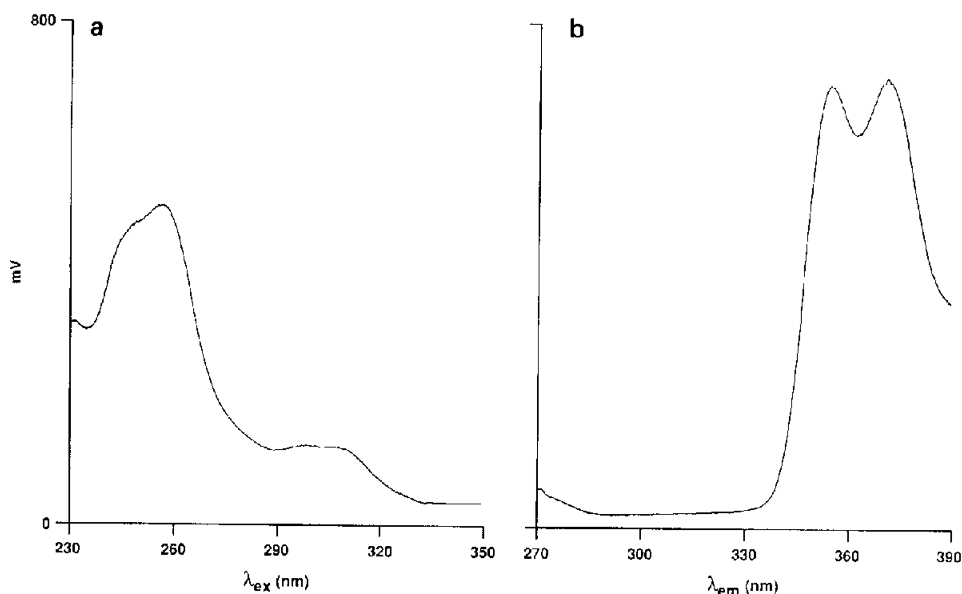

Fig. 1. Fluorescence excitation (a) and emission (b) spectra of halofantrine.

wavelength of 256 nm and an emission wavelength of 358 nm. HPLC data acquisition was performed using a Nelson Analytical 960 Series A/D interface sampling at 2 Hz.

The chiral column (250 mm × 4.9 mm I.D.) was of the Pirkle type and the chiral stationary phase consisted of L-N-(3,5-dinitrobenzyl)leucine covalently bonded to a 3-aminopropyl silica support (particle size 5 μm). This column supplied by Hichrom was operated at −10°C. The best separation of the optical isomers was achieved using *n*-hexane–chloroform–propan-2-ol (containing 1% triethylamine) in the ratio of 95:5:5 (v/v/v) flowing at a rate of 0.2 ml min^{-1}.

Fig. 2. shows a comparison of the resolution of the optical isomers of **1** (605 pmol injected) using both UV absorbance and fluorescence detection. The first and second eluted molecules have been assigned (+) and (−) as shown previously [1] by polarimetric analysis of isolated fractions. The much higher sensitivity of fluorescence compared to UV absorbance detection is clear from Fig. 2 where both methods of detection are displayed on an absolute scale. In fact, the fluorescence detector could be increased by a factor of 100 higher than that shown. The sensitivity of fluorescence detection is demonstrated in Fig. 3 when 2 fmol of halofantrine were injected and each of the optical isomers could be detected with ease.

Analysis of the linearity of the fluorescence response of the detector for injections of halofantrine in the range 10 μmol to 10 pmol showed linear behaviour in obeying eqn. 1 as derived by Scott [2]

$$y = AC^r \tag{1}$$

where y is the fluorescence response and C is the concentration of halofantrine; A is an arbitrary constant and r is the linearity index. A plot of log (response) *vs.* log

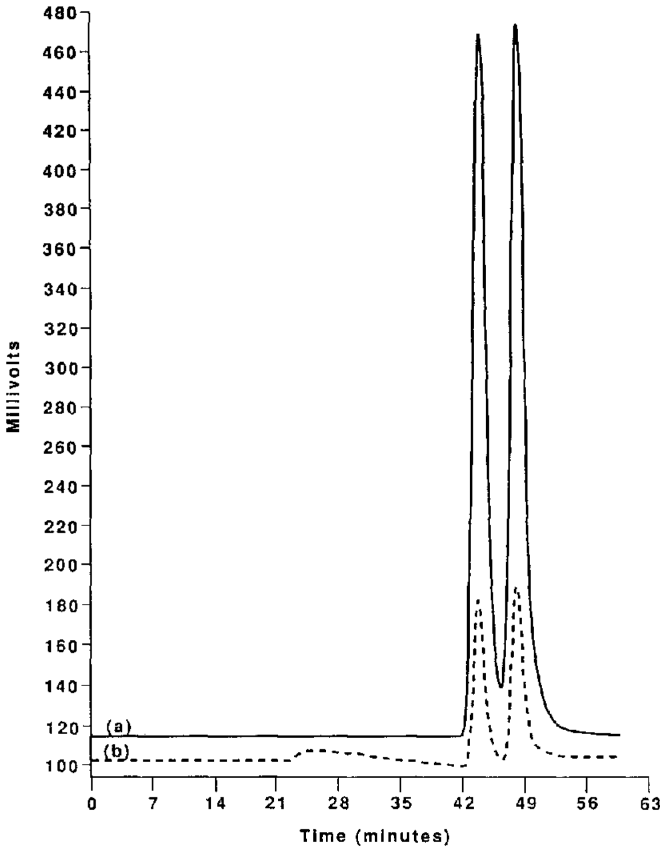

Fig. 2. Enantiomeric separation of the optical isomers of halofantrine using (a) fluorescence and (b) UV absorbance detection.

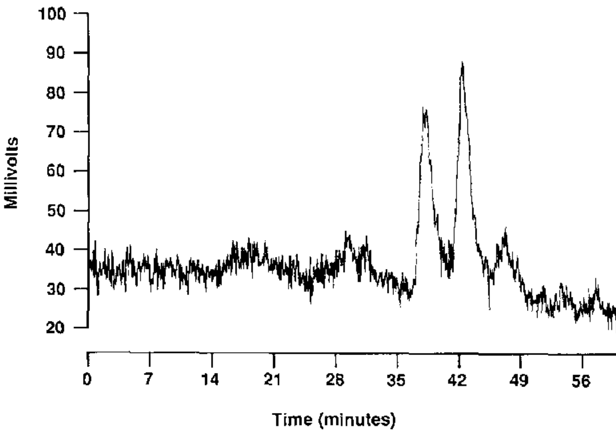

Fig. 3. Fluorescence detection of the enantiomers of halofantrine at 2.2 fmol of halofantrine injected.

[halofantrine] gave values of 1.132 ± 0.53 and 20.374 ± 0.574 for r and log A, respectively [3]. This value obtained for r is higher than that described by Scott as the definition of ideal linearity of a detector, however, it has been mentioned by this author that if the linearity index of the detector is known the use of calibration curves for quantitative analysis is possible.

The fluorescence method of detection outlined can be applied to study any physiological differences between the two enantiomers of **1** at very low concentration levels and can be used in pharmacokinetic, pharmacodynamic and metabolic studies. Moreover, this method of detection can be applied to studies utilising other methods of separation such as reversed phase HPLC, which will not necessarily involve the chirality of **1**.

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