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Letter to the Editor

Simultaneous determination of floctafenine and its main metabolites, floctafenic and hydroxyfloctafenic acid, using high-performance liquid chromatography with gradient elution

Sir,

Floctafenine, an analgesic belonging to the group of halogeno-aminoquinolines, is rapidly metabolized after oral administration to two main compounds: floctafenic acid (thought to be responsible for most of the analgesic activity) and hydroxyfloctafenic acid [1]. Numerous adverse effects attributed to floctafenine have been reported [2]. We have developed a rapid high-performance liquid chromatographic (HPLC) method allowing the assay not only of floctafenine, but also of its two main metabolites, which is convenient for both routine analyses and pharmacokinetic experiments. The method uses gradient elution, which is the solution of choice for the determination of compounds that exhibit large differences in polarity.

As we described in a previous paper [3], plasma or urine samples are extracted with ethyl acetate after alkalinization and addition of glafenine as an internal standard. After evaporating the extract to dryness, the residue is dissolved in methanol and injected into a 10-μm μPorasil (Waters) column (300 mm$\times$3.9 mm I.D.). The initial chromatographic conditions [mobile phase, *n*-hexane–ethanol (80:20, v/v); flow-rate, 1.2 ml/min] are maintained for 4 min. The final conditions [mobile phase, *n*-hexane–ethanol (50:50, v/v); flow-rate, 2.0 ml/min] are reached at the end of a linear gradient of 11 min. Hence the total analysis is performed in 15 min. Detection is effected by measuring the UV absorbance at 360 nm. Peak-area ratios are used to prepare calibration graphs and to calculate concentrations.

Fig. 1 (top) shows a typical chromatogram obtained from a plasma sample from a healthy volunteer taken after ingestion of floctafenine; the curve of the elution gradient is represented below. The retention times of floctafenine, glafenine, floctafenic acid and hydroxyfloctafenic acid are 5.65, 6.75, 10.75 and 12.05 min, respectively. The detection limits were found to be 8.5 ng/ml for floctafe-

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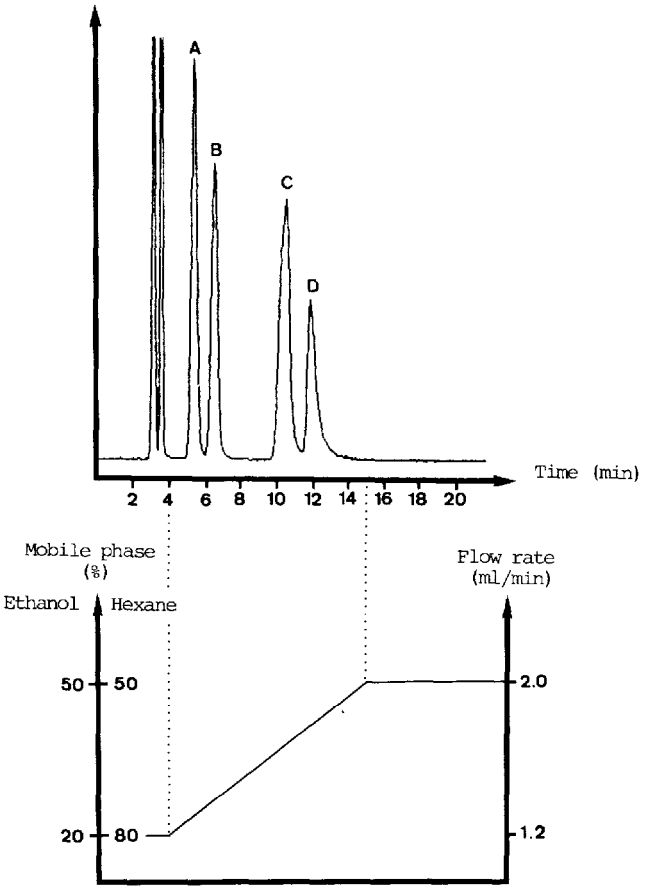

Fig. 1. Top: chromatogram of plasma extract after administration of 400 mg of floctafenine to a human volunteer. Peaks (the values in parentheses are the concentrations 1 h after ingestion): A = floctafenine (0.97 μg/ml); B = glafenine (internal standard); C = floctafenic acid (1.72 μg/ml); D = hydroxyfloctafenic acid (0.37 μg/ml). Bottom: representation of the elution gradient.

nine, 17.5 ng/ml for floctafenic acid and 26.0 ng/ml for hydroxyfloctafenic acid. The within-run and day-to-day precision, studied with plasma samples spiked with the three compounds at concentrations of 0.5 and 5.0 μg/ml, ranged from 5.7 to 7.8% and from 6.2 to 10.7% (over a period of four weeks), respectively.

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1 A.C. Moffat (Editor), Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Material, Pharmaceutical Press, London, 2nd ed., 1986, pp. 619–620.
2 G. Cheymol, M. Biour, M. Bruneel, E. Albengres and J.D. Hamel, Thérapie, 40 (1985) 45–50.
3 A. Tracqui, P. Kintz, P. Mangin, A.A. Lugnier and A.J. Chaumont, Ann. Biol., Clin., (1988) in press.

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