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Letter to the Editor

Automated high-performance liquid chromatography with column switching for on-line clean-up and analysis of diltiazem and metabolites in human plasma

Sir,

This journal recently published a new method for the determination of diltiazem, a well known calcium channel blocker [1] The method, which was developed in our laboratory, is innovative in comparison with other published techniques [2–7], because it does not need any sample manipulation before chromatography (e g extraction, organic extract transfer, evaporation, sample re-dissolution) It also allows the determination of deacetyldiltiazem, which is thought to be a predominant and important metabolite of diltiazem [8–10] More recently, pharmacological investigations have shown that another metabolite of diltiazem, N-monodemethyldiltiazem, has a coronary vasodilator and blood pressure reduction activity [11], and is the predominant metabolite in young and elderly hypertensives [12]

Therefore we wished to update and modify our previous method in order to be able to determine diltiazem and its three most important metabolites, deacetyl-N-monodemethyldiltiazem, deacetyldiltiazem, and N-monodemethyldiltiazem, in human plasma All the published methods [3,5–7] for the determination of diltiazem and its metabolites in biological fluids require an extraction and sometimes a back-extraction, therefore the possibility of avoiding all purification and manipulation steps represents a great advantage

The only relevant differences between the new proposed method and the previous concern the composition of mobile phase, the type of packing material for the pre-column and the speed of centrifugation of plasma samples An aqueous solution of 0 05 *M* potassium dihydrogenphosphate was adjusted to pH 2 9 with 1 *M* phosphoric acid, mixed with acetonitrile, acetonitrile–phosphate buffer pH 2.9 (40 60, v/v), and finally 0 1 % (v/v) triethylamine is added Perisorb® (30–40 μm) was selected as the pre-column dry-filling (E

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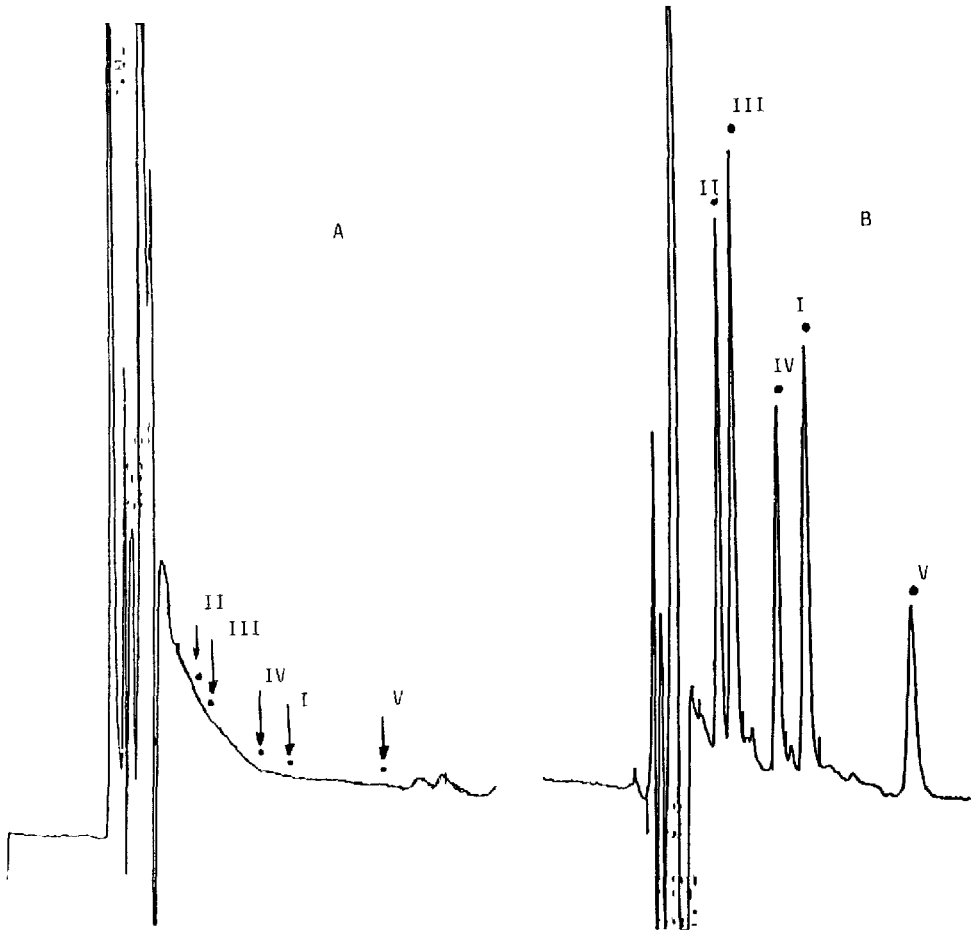

Fig 1 (A) Chromatogram of pre-dose plasma (B) Chromatogram of plasma sample from a subject, 6 h after administration of a 300-mg slow-release tablet of diltiazem (multiple administration) Peaks I = diltiazem (12 min), II = deacetyl-N-monodemethyldiltiazem (9 min), III = N-monodemethyldiltiazem (9 5 min), IV = deacetyldiltiazem (11 min), V = internal standard (16 min)

Merck, Darmstadt, F R G) The pre-column has a long life, replacement being necessary after about 300 plasma injections Plasma samples, before injection, are centrifuged in conical plastic tubes, at 11 000 *g* for 3 min on an Eppendorf centrifuge (or similar)

As far as the stability of the parent drug and its metabolites is concerned, no significant variation in drug content was found for diltiazem, deacetyldiltiazem and deacetyl-N-monodemethyldiltiazem in plasma samples maintained under laboratory conditions for 12 h in comparison with similar samples freshly prepared, N-monodemethyldiltiazem is less stable, degrading to deacetyl-N-

monodemethyldiltiazem (ca. 30% in 12 h) Therefore it is important to analyse plasma samples within 10 h after thawing; this is not a really limiting factor if one considers that the sample manipulation takes little time (it consists of thawing the plasma sample, adding the internal standard, centrifuging for 3 min and injecting).

Urine samples are treated like plasma samples, although these are sometimes diluted with water before analysis

The detection limit of the method if ca. 2 ng/ml for diltiazem and metabolites when 200-μl aliquots of plasma are processed After statistical validation, (intra- and inter-assay) the method has been used for ca. two years in our laboratory, with satisfactory results during pharmacokinetic studies on healthy subjects and patients and for drug monitoring in clinical investigations (Fig. 1A and B).

Sometimes in plasma from patients an interfering peak has been noted in the first part of chromatogram; this interference may affect the quantitative evaluation of deacetyl-N-monodemethyldiltiazem

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