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Letter to the Editor

High-performance liquid chromatographic separation and mass spectrometric identification of propafenone, 5-hydroxypropafenone and N-depropylpropafenone

Sir,

Propafenone (P) is an antiarrhythmic drug used in the treatment of supraventricular and ventricular arrhythmias [1]. Its kinetics has been extensively studied [2,3], and therapeutic plasma concentrations have been proposed, ranging from 100 to 2000 ng/ml [1,4]. Two metabolites of P have been shown to be present in plasma during chronic treatment with P: 5-hydroxypropafenone (5OHP) and N-depropylpropafenone (NDP) [3]. The first has antiarrhythmic activity comparable with that of the parent compound, whereas the second is virtually inactive [5]. Two high-performance liquid chromatographic (HPLC) methods have been published for the simultaneous assay of P and 5OHP [6] and P, 5OHP and NDP [3] in human plasma. Both seem to be sensitive and specific, even if not yet validated by mass spectrometry (MS). However, the first assay requires derivatization with dansylhydrazine and purification of the reactor products, a procedure that is time-consuming and increases variability, and the second has very long elution times, up to 48 min. We describe here a simplification of the method of Brode et al. [6], in which the compounds are analysed underivatized and the specificity is validated by MS.

EXPERIMENTAL

High-performance liquid chromatography

P, 5OHP, NDP and the internal standard, 5′-hydroxy-2′-(2-hydroxy-3-ethylaminopropoxy)-3-phenylpropiophenone (LU46532), hydrochloride salts were obtained from Knoll (Ludwigshafen, F.R.G.). All solvents were reagent grade (Carlo Erba, Milan, Italy) and filtered before use in the HPLC system. A Beckman 118 solvent delivery module, equipped with a Model 160 fixed-wavelength (254 nm) absorbance detector, was used. The chromatographic column consisted of two cartridges (Chrompack, Middelburg, The Netherlands), 100 mm × 3 mm I.D., filled with silica gel (Chromspher-Si, 5 μm particle size). P and its metab-

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olites were extracted as described [6]; the dry residue of the plasma extract was dissolved in 0.2 ml of ethyl alcohol and injected directly into the chromatograph, under the conditions already described [6]. MS analysis was performed on the fractions collected from the HPLC column eluate, under UV monitoring.

Mass spectrometry

Electron-impact (EI) mass spectra were taken with a VG 70-250 mass spectrometer (VG Analytical, Manchester, U.K.), using the direct inlet system. The electron energy was 70 eV.

RESULTS AND DISUCSSION

High-performance liquid chromatography

The chromatographic separation of P, 5OHP, NDP and the internal standard in different samples is shown in Fig. 1. Calibration curves were linear from 50 to 2000 ng/ml of each compound. Recovery of extraction from plasma, calculated by comparison with non-extracted samples by the use of external reference standards, was 80, 89 and 47%, respectively, for P, 5OHP and NDP. The assay permits quantitation of a minimum of 30 ng/ml P and 5OHP and of 100 ng/ml NDP.

Mass spectrometry

In order to confirm HPLC results, we analysed the collected fractions by MS, and compared mass spectra from these fractions with those of reference compounds. Fig. 2 presents the mass spectrum of standard P. The peak at *m/z* 342 is the protonated molecular ion. Loss of 29 u (ethyl) gives the ion at *m/z* 312. The elimination of 44 u (propane) produces the ion at *m/z* 297. Other important ions appear at *m/z* 226 (corresponding to a loss of 115 u and probably due to the fragmentation indicated in Fig. 2) and *m/z* 121 (loss of 220 u), that is the base peak. This peak probably originates from the elimination of 115 u and of CH_2–CH_2–C_6H_5. The ion at *m/z* 121 was also found in P derivatized with trifluoroacetic anhydride [7]. Fig. 3 shows the mass spectrum of 5OHP. The peak at

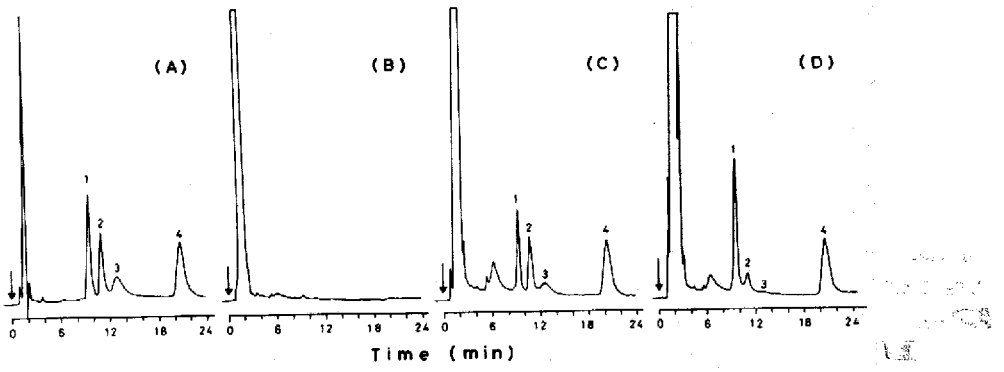

Fig. 1. HPLC of (A) an external standard, (B) a blank plasma extract, (C) a plasma extract after addition of 800 ng of each compound and (D) an extract of pooled plasma from patients chronically treated with P. Peaks: 1 = P; 2 = 5OHP; 3 = NDP; 4 = internal standard, LU46532.

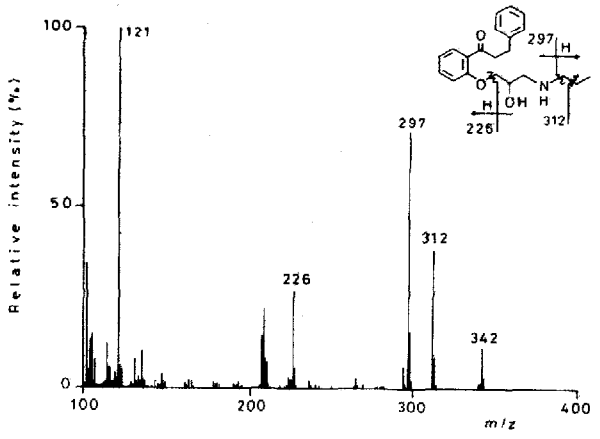

Fig. 2. Mass spectra and fragmentation patterns of P.

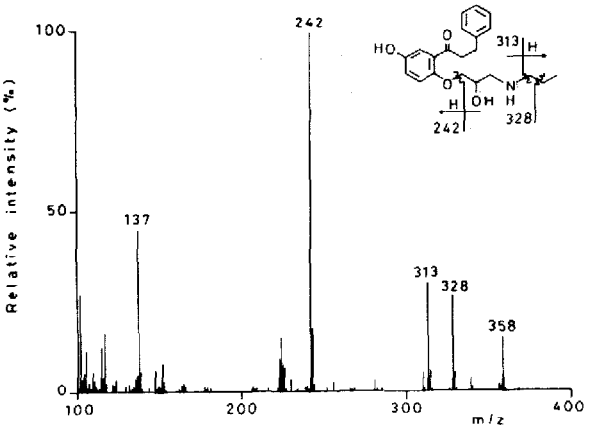

Fig. 3. Mass spectra and fragmentation patterns of 5OHP.

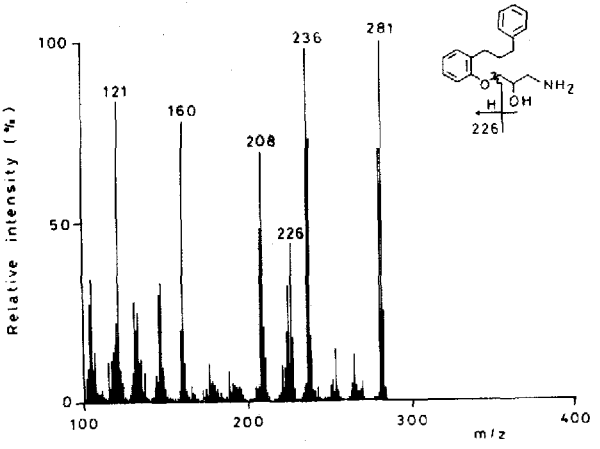

Fig. 4. Mass spectra and fragmentation patterns of NDP.

m/z 358 corresponds to the protonated molecular ion. The fragmentation of this compound is similar to that of P, confirming the previous attributions of the ions. Thus eliminations of 29, 44, 115 and 220 u give ions at *m/z* 328, 313, 242 (the base peak) and 137.

Fig. 4 presents the mass spectrum of NDP. The molecular peak does not appear at 70 eV or at 20 eV. The peak at *m/z* 281 is due to loss of water. The ion at *m/z* 236 could be produced by loss of $HO-CH_2-CH_2-NH_2$ and of hydrogen. The ions at *m/z* 226 and 121 may have the same structures as those in Fig. 2. The peak at *m/z* 160 might correspond to a charged methylated 1-benzopyran-4-one.

The El mass spectra of collected HPLC fractions were not significantly different from the mass spectra of the authentic standards. This confirms the HPLC separation findings.

In conclusion, the method described is simpler than that of Brode et al. [6] and is faster than that of Kates et al. [3]. Its specificity has been documented by MS and its sensitivity, though lower than that of a previous method [6], has proved fully satisfactory for kinetic and pharmacodynamic studies in patients and, more important, for therapeutic drug monitoring in clinical trials [8]. NDP is not active in experimental models of arrhythmias, which is why our study did not set out to quantitate it very accurately, but rather to make sure that its peak did not interfere with that of P or 5OHP, the active metabolite.

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