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Letter to the Editor

Determination of xamoterol in human plasma by high-performance liquid chromatography with electrochemical detection

Sir,

Xamoterol, (±)-N-[2-[[2-hydroxy-3-(*p*-hydroxyphenoxy)propyl]amino] ethyl]-4-morpholine carboxamide fumarate (Carwin), is a highly cardioselective, β-adrenoceptor partial agonist with potential utility as an inotropic agent [1,2] which has been shown to have clinical benefit in heart failure patients [3,4]. It is presently undergoing Phase III clinical trials. The compound is synthesized as a fumarate salt [5]. Only one method has been published for the determination of xamoterol in human physiological fluid [6]: a hand-packed cation-exchange column was used for sample clean-up followed by high-performance liquid chromatography (HPLC) with fluorescence detection, with prenalterol hydrochloride as an internal standard. Xamoterol has also been measured in plasma using radioimmunoassay [7]. Due to the ease of oxidation of xamoterol (oxidation potential ca. +0.72 V) and the demonstrated sensitivity and selectivity often observed with electrochemical detection (ED), a sensitive method based on HPLC with ED was developed. This method uses a commercially available C_{18} solid-phase extraction column for sample clean-up and does not require an internal standard. The detection limit (2 ng/ml) is comparable to that reported by Oddie et al. [6] and is sufficient to provide data for human pharmacokinetic studies. Absolute recoveries averaged 87%, while precision of duplicate pairs averaged 5.4% R.S.D. ($n=12$) within a linear range of 2–500 ng/ml of plasma.

A 1.0-ml aliquot of standard or clinical plasma sample is mixed with 2 ml pH 7.0 phosphate–citrate buffer and applied to a C_{18} Sep-Pak (Waters) cartridge that has been preconditioned with methanol and water. The cartridge is washed with 10 ml of water, followed by 5 ml of 10% methanol–water. Xamoterol (supplied by Imperial Chemical Industries PLC, U.K.) is then eluted with 2 ml methanol, evaporated to dryness at 60°C and reconstituted with 200 μl mobile phase. A 100-μl aliquot of this solution is injected onto the HPLC column (15 cm×4.1 mm I.D., 5-μm Shandon Hypersil-ODS) and eluted at ambient temperature at a flow-rate of 1.5 ml/min with a mobile phase of methanol–tetrahydrofuran–0.03 *M* perchloric acid (8:0.6:91.4). Xamoterol was detected with a Bioanalytical Sys-

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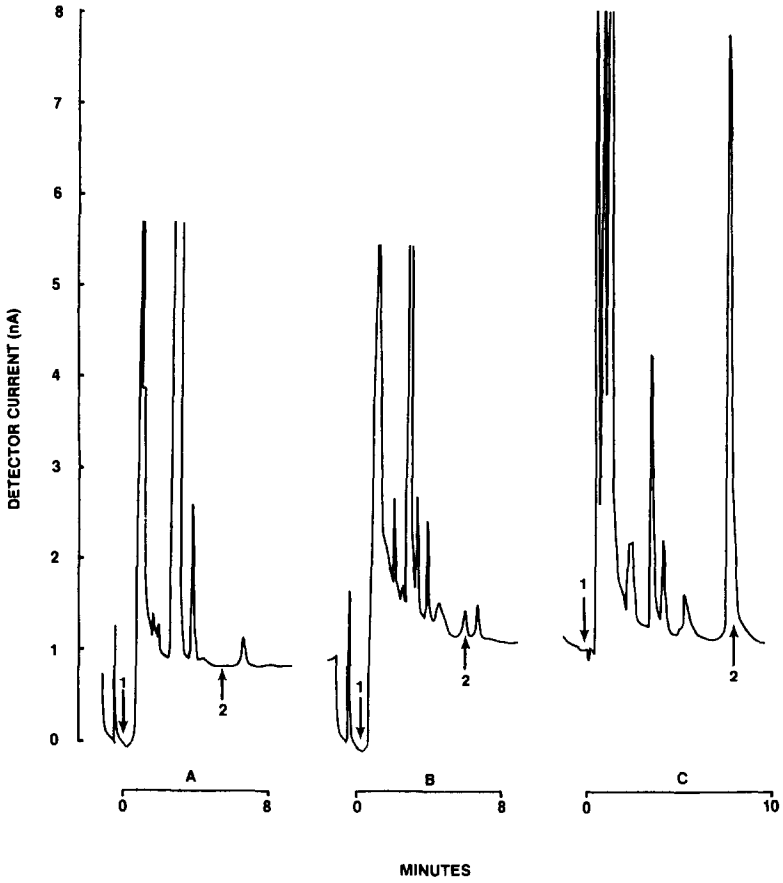

Fig. 1. Liquid chromatograms of extracted (A) drug-free plasma, (B) plasma with added xamoterol (2 ng/ml) and (C) plasma from a subject receiving 50 μg/kg xamoterol in a parenteral dose. The xamoterol peak corresponds to 55.6 ng/ml. Detector: $E = +0.85$ V, range = 10 nA full scale, chart-speed = 0.5 cm/min. Peaks: 1 = injection event; 2 = xamoterol.

tems electrochemical detector (Model LC-4A) with a thin-layer glassy carbon electrode (TL-5A), Ag/AgCl reference electrode (RE-1) and electrode compartment (RC-2) at a potential of +0.85 V and a range of 10 nA full scale.

The recovery of xamoterol from plasma was determined by comparison with a non-extracted standard over three different days. The mean concentrations found (and absolute recovery ± S.D.) from six replicates were 94.5 ng/ml (89.9 ± 4.6%) and 17.0 ng/ml (83.9 ± 5.1%) at 100 and 20 ng/ml xamoterol, respectively. The plasma standard curve is fitted to a linear regression and the assay is linear over a range of 2–500 ng/ml ($r = 0.998$). The inter-assay variability of the slope of the standard plot was less than 2% over this concentration range, with the average y-intercept being less than half the detection limit of 2 ng/ml. The intra- and inter-day assay precision data for spiked plasma samples over three days were 2.1 and 11.7% ($n = 6$), respectively, at 100 ng/ml, and 8.6 and 12.2% ($n = 6$), respectively, at 20 ng/ml.

Detection of the oxidation of the phenolic function of xamoterol provides high sensitivity and selectivity. As little as 1 ng of xamoterol injected on-column can be quantitated, with a signal-to-noise ratio in plasma of approximately 10:1. Fig. 1A shows a chromatogram of an extract of drug-free plasma. An endogenous plasma component peak elutes near xamoterol, but is baseline-resolved, as shown in a chromatogram of a plasma extract spiked at a detection limit of 2 ng/ml (Fig. 1B). The retention time of xamoterol is very sensitive to the amount of tetrahydrofuran in the mobile phase, and this concentration can be varied slightly to resolve xamoterol from the small endogenous peak. Fig. 1C shows a chromatogram of an extracted plasma sample taken from a subject who received a parenteral dose (50 μg/kg) of xamoterol. The assay was free from interference from the following drugs: spironolactone, methyldopa, caffeine, docusate sodium, crystalline warfarin sodium, flurazepam hydrochloride, acetazolamide, digoxin, triamterene, hydrochlorothiazide, sodium heparin, furosemide, chlordiazepoxide hydrochloride, meclofenamate sodium, thioridazine, simethicone, nitroglycerin, quinidine, cimetidine, acetaminophen, codeine phosphate and diazepam. However, aspirin, benzyl alcohol and hydralazine may prohibit quantitation of low levels of xamoterol. This method has been used without problems to analyze samples from seven clinical studies where xamoterol was administered orally or parenterally, resulting in over 2000 assays. One operator can perform approximately 40 assays per day. No false positive results were observed from pre-dose or from placebo-dosed subjects. The method is rugged and has adequate sensitivity to obtain the desired pharmacokinetic information.

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