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Letter to the Editor

Rapid and simple high-performance liquid chromatographic determination of saliva antipyrine for routine antipyrine test

Sir,

Antipyrine has been used extensively as a model substrate for evaluating the effects of environmental factors, co-administration of certain substances (e.g., therapeutic drugs, hormones) and disease states on hepatic oxidative drug metabolism [1,2]. In the light of increasing concern about ethical considerations in human research, it has been recommended that the routine antipyrine test preferably be performed using saliva rather than plasma [3]. Previous studies [4,5] have shown that there is no significant difference between the kinetic parameters of antipyrine obtained from monitoring saliva or plasma concentrations, while the monitoring of saliva concentrations avoids possible harm associated with multiple venipunctures or intravenous catheter placement. We report here a simple and rapid high-performance liquid chromatographic (HPLC) method, which can be easily implemented in a clinical laboratory, for the determination of antipyrine in saliva.

A 0.5-ml volume of acetonitrile and 50 μl of phenacetin (20 μg), used as an internal standard (I.S.), were added to 0.5 ml of saliva. After vortex-mixing to precipitate protein, samples were centrifuged for 1 min at 5500 *g*. The supernatant containing the analytes was passed through a 4.5 μm pore size filter membrane (Gelman Science, Tokyo, Japan) and 20 μl of filtrate were injected onto the HPLC column. The HPLC system consisted of a Model 100A pump (Altex Scientific, Berkeley, CA, U.S.A.), an Eicopack MA-ODS reversed-phase column (250 mm $\times$ 4.6 mm I.D.) packed with 7-μm particles (Eicom, Kyoto, Japan) and a Model 8000 ultraviolet (UV) absorbance detector (Toyo Soda, Tokyo, Japan) set at 254 nm. The column temperature was maintained at 40°C by a water circulator. All chromatograms were recorded and the peak heights of the respective analytes (i.e., antipyrine and phenacetin) were integrated with a Model D-2000 chromatointegrator (Hitachi, Tokyo, Japan). The mobile phase, consisting of 30% (v/v) acetonitrile in 0.02 *M* potassium di-

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phosphate buffer (pH 6.0) and 0.1% (v/v) triethylamine, was delivered at a flow-rate of 1.0 ml/min.

Under the chromatographic conditions employed, the separation of antipyrine and the I.S. was satisfactory and the supernatant prepared from drug-free saliva contained no interfering peaks for the analytes (Fig. 1). The retention times of antipyrine and the I.S. were 4.5 and 8.2 min, respectively.

The calibration graph for antipyrine in saliva was linear ($y=0.503x-0.003$, $r=0.999$, $p<0.01$) over the concentration ranges normally determined during the routine antipyrine test (i.e., 1–50 μg/ml). The detection limit of antipyrine with a signal-to-noise ratio of 5 was ca. 0.1 μg/ml.

The mean ($\pm$S.D.) extraction recoveries of antipyrine at 5 μg/ml and the I.S. at 20 μg/ml were 106.0$\pm$3.9% with a relative standard deviation (R.S.D.) of 3.7% and 101.0$\pm$1.5% with an R.S.D. of 1.4%, respectively ($n=6$). The precision and accuracy of the assay were assessed by the intra- and inter-assay R.S.D.s and relative errors, respectively. The intra- and inter-assay R.S.D.s ($n=6$) were less than 2.8 and 7.5%, respectively, at saliva antipyrine concentrations of 1, 5, 20 and 50 μg/ml. The intra- and inter-assay relative errors for determining 1, 5, 20 and 50 μg/ml antipyrine in saliva were 7.0, -11.6, -0.5, -5.0% and 7.0, 4.6, 10.5, -2.2%, respectively.

Although numerous methods have been developed for determining antipyrine in biological fluids using gas chromatography [6,7] or HPLC [8–10], these methods require an extraction procedure with various organic solvents, subsequent evaporation and reconstitution of the analytes before injecting them onto the analytical column. Overall, these procedures are tedious and therefore considered unsuitable for the routine antipyrine test performed in clinical lab-

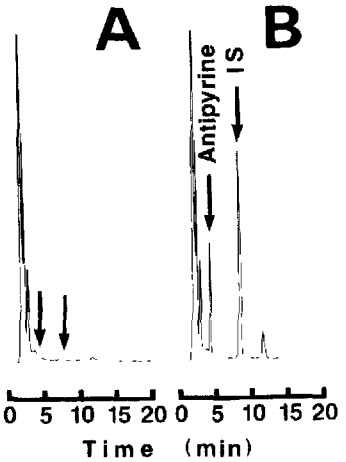

Fig. 1. Representative chromatograms of (A) drug-free saliva and (B) a 12-h post-dose saliva sample obtained from a patient with liver cirrhosis who had received an oral administration of antipyrine (10 mg/kg). The arrows in A indicate the expected appearance of antipyrine and the I.S., respectively. The saliva antipyrine concentration was measured to be 9.97 μg/ml.

oratories. In contrast, the method described here gives a more rapid and simpler sample preparation for the assay of salivary antipyrine. After brief centrifugation for 1 min and filtration, the supernatant can be directly injected onto the analytical column. In addition, the retention times for antipyrine and the I.S. were 4.5 and 8.2 min, respectively (Fig. 1). Hence a batch of fifty saliva samples can be analysed within an 8-h working day. A similar sample clean-up procedure using methanol as a protein precipitant has been applied for monitoring plasma antipyrine [11].

We performed the routine antipyrine test on a 50-year-old male with biopsy-proven liver cirrhosis using the present method. A single oral dose (10 mg/kg) of antipyrine was administered after an overnight fast and salivary antipyrine measurements were made at 2, 4, 6, 9, 12, 24 and 32 h post-dose. On analysis of the salivary antipyrine concentration–time data obtained from the patient, the total body clearance, half-life and distribution volume were calculated to be 15.04 ml/h·kg, 30.4 h and 0.66 l/kg, respectively. These values showed good agreement with those previously obtained from patients with liver cirrhosis [12].

Although we have rarely encountered unidentified co-eluting peaks with antipyrine or the I.S. during the routine antipyrine test, a simplified sample preparation method such as that described here would carry an increased risk of interference with concomitantly administered drugs and/or certain endogenous substances. Therefore, the ultimate validity of the above described sample clean-up method coupled with reversed-phase HPLC and UV detection for monitoring salivary antipyrine under a less invasive but more easily performed conditions should be more extensively evaluated in future studies with a large number of patients receiving various medications under diverse clinical conditions.

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