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Letter to the Editor

Simultaneous measurement of prednisone, prednisolone and 6β-hydroxyprednisolone in urine by high-performance liquid chromatography using dexamethasone as the internal standard[a]

Sir,

Frey and Frey [1] described a specific and reproducible high-performance liquid chromatographic (HPLC) technique for measurement of urine concentrations of prednisone, prednisolone and the metabolite, 6β-hydroxyprednisolone. However, as "no internal standard was found" in that report, the method requires radioactive internal standards which are not readily available and which require special radioactivity handling procedures. We describe here an HPLC method for routine measurement of prednisone, prednisolone and 6β-hydroxyprednisolone in urine using dexamethasone as the internal standard and a solid-phase extraction technique prior to chromatography.

EXPERIMENTAL

Urine samples of 0.1–1.0 ml, diluted if necessary to a total 1-ml volume with water, are placed in disposable tubes. Then dexamethasone (internal standard, 0.05 ml of a 10 mg/l solution in methanol) is added and the solution is vortex-mixed. The sample is poured into a Chem-Elut cartridge (Cat. No. 1003; Analytichem International, Harbor City, CA, U.S.A.) and allowed to adsorb onto the column for 5 min. The disposable tube is then rinsed with 6 ml of ethyl acetate twice (5-min interval) and the liquid is poured into the cartridge. The total eluate is collected in a disposable tube with a Teflon screw-cap and the

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eluate is washed twice with 1.0 ml of 0.2 *M* sodium hydroxide after which 1.0 g of anhydrous sodium sulfate is added to the tube to dry the organic phase for 30 min. The solution is then poured into another disposable tube and the solvent is evaporated at 30°C under a gentle stream of nitrogen. The residue is reconstituted in 250 μl of the mobile phase and a 50-μl aliquot is injected onto the column.

Chromatographic separation is accomplished using a Si column (250 mm × 4.6 mm, 5 μm, Beckman, Fullerton, CA, U.S.A.) with a mobile phase consisting of methylene chloride–methanol–tetrahydrofuran–glacial acetic acid (96.9:2.0:1.0:10.1), a flow-rate of 1.3 ml/min and UV detection at 254 nm.

RESULTS AND DISCUSSION

The retention times for prednisone, dexamethasone, prednisolone and 6β-hydroxyprednisolone are 6.4, 8.0, 12.4 and 21.0 min, respectively. With the extraction procedure above, interference from endogenous compounds excreted in urine such as cortisone and cortisol is negligible. The assay exhibits linearity from 50 to 2000 ng/ml. Standard curves yielded the following linear regression parameters for peak area versus concentration ($n=8$) for slope, intercept and correlation coefficient, respectively: prednisone, 0.00265, −0.109 and 0.999; prednisolone, 0.0026, 0.000826 and 0.998; and 6β-hydroxyprednisolone, 0.0020, 0.026 and 0.999. The lower limits for routine detection are 50 ng/ml for both prednisone and prednisolone and 60 ng/ml for 6β-hydroxyprednisolone.

The recoveries in the present analytical method (spiked urine versus methanol standard) were close to that of the method of Frey and Frey [1], over 75% for both prednisone and prednisolone and 60% for 6β-hydroxyprednisolone.

The inter-day and intra-day variabilities were determined with all three compounds over a concentration range from 200 to 2000 ng/ml. Coefficients of variation for prednisone, prednisolone and 6β-hydroxyprednisolone, respectively, ranged from 3.3 to 14.2%, 2.7 to 12.8% and 3.3 to 6.7%, characterizing the inter-day variability ($n=24$), and from 3.1 to 3.9%, 4.3 to 4.8% and 8.1 to 12.2%, characterizing the intra-day variability ($n=24$).

This assay has recently been used in human pharmacokinetic studies. The urinary excretion data from these studies are consistent with those of Frey and Frey [2]. Intravenous dosing of prednisolone (40 mg) to six healthy volunteers resulted in the following percentages of the dose excreted in urine: prednisone $3.4 \pm 1.2\%$; prednisolone $26.3 \pm 7.7\%$; and 6β-hydroxyprednisolone $9.5 \pm 2.3\%$. These values are quite close to the percentages in urine reported by Frey and Frey [2] for a 0.8 mg/kg dose: prednisone $2.77 \pm 0.25\%$; prednisolone $34.13 \pm 2.94\%$; and 6β-hydroxyprednisolone $8.19 \pm 1.41\%$, respectively.

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