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Title: New and easy extraction method for urinary free cortisol assessment

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Body: Urinary cortisol assessment is important in the diagnostic of Cushing's disease and syndrome. Even though the sensitivity of different assays has been improved over years, the extraction method has remained more of less the same, either a dichloromethane or a solid phase extraction, with recuperations that are not always optimal.

New extraction techniques have been proposed[1]. It is uses a liquid-liquid microextraction based on solidification of floating organic drop. It is based on the use of a few microliters of a specific extractant, 1-undecanol[2]. This extractant has a lower density than water, low toxicity and a melting point between 13-15°C. In this method, 10 to 25 ml of the extractant is mixed in a diluted urine sample, thus limiting possible urine interferences. The aqueous solution is mixed for a few minutes and then the droplet can be collected easily by placing the urine tube in a cold bath, thus solidifying the 1-undecanol. The frozen droplet is picked with a small spatula and allowed to dissolve at room temperature in the mobile phase to be analyzed on an HPLC system. The HPLC works with a biphenyl phase column and the mobile phase a methanol-water 46-54 mix.

The sensitivity is adequate to measure urinary concentrations lower than 40 nmol/l (less than 15 mg/l). The extraction recovery is 99-100%, and reproducible. Different situations have been tested to optimize the separation and measure of cortisol simultaneously with cortisone in the urine. Results are being correlated with a standard I-125 radioimmunoassay.

In conclusion, the method is simple, does not necessitate special equipment other than a cooled water bath and an HPLC system with standard solvents, and gives a response in 10 minutes, with an internal standard coming out in 18 minutes. All together, a result from start to end will take about 30 minutes, with new results coming out every 18 minutes in an automated injection system.

References: (1) Rezaee M et al., J Chromatogr A 2010; 1217:2342
(2) Wang Y-Y et al., Chin J Anal Chem. 2010; 38:1517