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Journal of Chromatography B, 692 (1997) 238–240

JOURNAL OF CHROMATOGRAPHY B

Technical note

Clean-up and re-use of kieselguhr (Extrelut) for liquid–liquid extraction of urinary cortisol

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Received 16 September 1996; revised 30 October 1996; accepted 4 November 1996

Abstract

Cortisol was isolated from human urine using kieselguhr (Extrelut)-filled columns. After use, Extrelut was cleaned-up once with distilled water and twice with ethanol. Before re-use, the cleaned-up kieselguhr was dried for 24 h by a warm air stream. The comparison of cortisol recovery from human urine and HPLC chromatograms of urinary extracts show that Extrelut can be repeatedly used for liquid–liquid extraction of urinary cortisol.

Keywords: Kieselguhr; Cortisol; Steroids

1. Introduction

Steroids from urine are preferably isolated by liquid–liquid extraction in which the aqueous solution is applied onto a granulate-filled column and remains as the stationary phase on the granular support material [1]. On elution with organic solvents, steroids are extracted from the aqueous phase into the eluent. This method, in which kieselguhr is usually used as the support material, offers several advantages: (1) No emulsions are formed during extraction; (2) high and reproducible recoveries; (3) compared to conventional extraction, purer extracts are obtained. A major disadvantage is that commercially available kieselguhr (Extrelut) is expensive, and no re-use of the support material is recommended by the manufacturer. The present communication describes the clean-up of Extrelut and its re-use in column extraction of urinary cortisol.

2. Experimental

2.1. Reagents and solvents

Steroids, kieselguhr (Extrelut), organic solvents and chemicals (analytical grade) were used as obtained from Merck (Darmstadt, Germany). Ethanol used for washings was obtained from the Bundesmonopolverwaltung für Branntwein (Nürnberg, Germany).

2.2. Extraction of urinary cortisol, HPLC measurement, and Extrelut clean-up

2.2.1. Cortisol extraction from human urine

Recovery: 200 μg cortisol were added to 2 l of human urine. After mixing, the sample was divided into ten portions which were immediately frozen and kept at −24°C. For every extraction test (see below),

PII S0378-4347(96)00504-X

one portion was thawed and shaken well. Then 20-ml aliquots were applied onto columns which were filled with 18 g of unused or cleaned-up Extrelut. Twenty minutes later, cortisol was eluted with 50 ml dichloromethane. The organic phase was evaporated to dryness. The residue was reconstituted in 2.0 ml of the mobile phase (methanol–water, 3:2, v/v), and 0.1 ml of each sample was assayed by HPLC.

2.2.2. Sampling and extraction of human urine

Ten healthy human males were asked to collect two consecutive 24-h urines. The samples of each individual were pooled; they were extracted as described above.

HPLC measurement. The HPLC system was comprised of a HPLC pump (Model LKB 2150; LKB, Munich, Germany), an autosampler (Series 1050; Hewlett-Packard, Waldbronn, Germany) and peak-integration software (HPLC ChemStation; Hewlett-Packard). Detection was carried out using a UV absorbance detector (254 nm, Uvicord S; LKB). Separations were carried out on a C_{18} reversed-phase column (LiChrospher 100, Merck; particle size 5 μm). The flow-rate was 0.5 ml/min, the system pressure was about 14 hPa. The injection volume was 0.1 ml. Peak quantification was carried out using peak-height values.

Extrelut clean-up. After use, Extrelut was removed from columns, and the bulk material (1 kg) was poured on to an aluminium sheet (1×1 m). After the dichloromethane had evaporated (a time span of 2

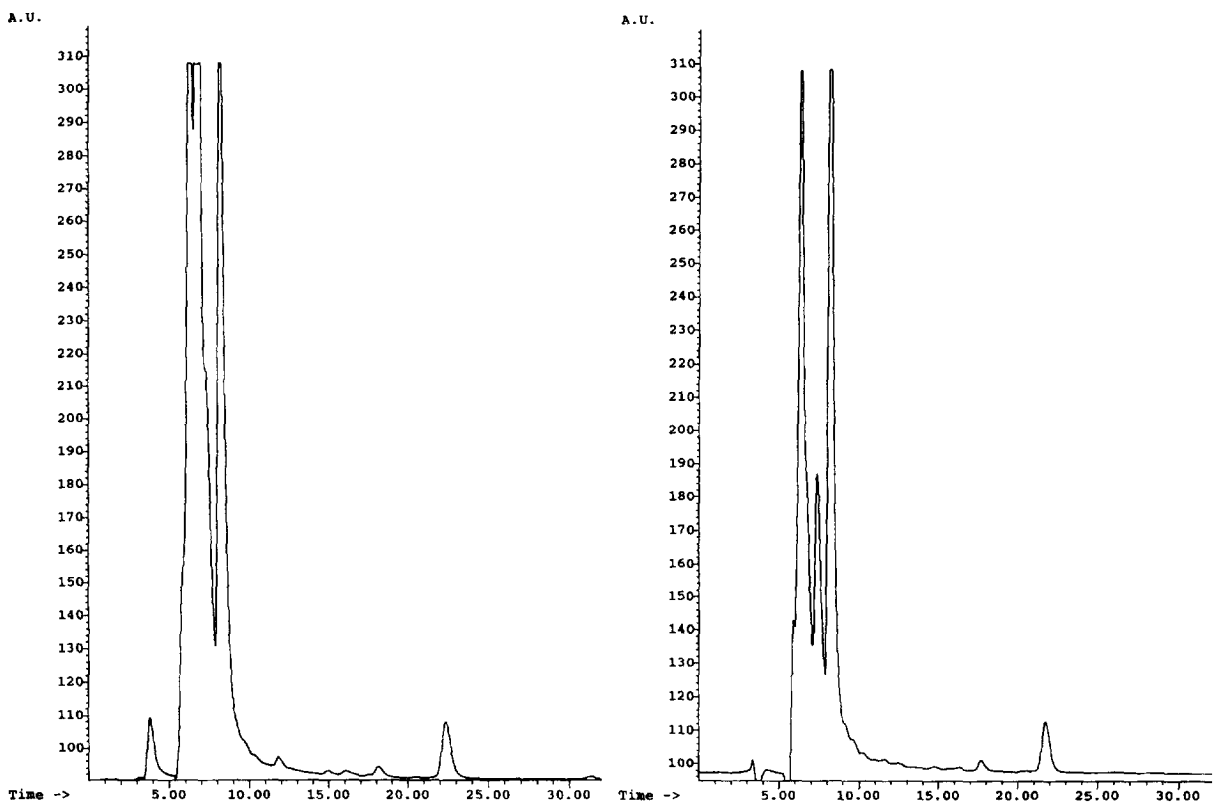

Fig. 1. HPLC chromatograms of human urine spiked with 100 μg cortisol/l of urine. Extrelut was used as supplied by the manufacturer (left) or after four clean-ups (right). Urine (20 ml) was transferred onto Extrelut-filled columns. Twenty minutes after application, samples were extracted with 60 ml dichloromethane. After evaporation of the organic phase, the residues were taken up in 2.0 ml methanol–water (3:2, v/v), and 0.1 ml of each sample was assayed by HPLC. Retention time (abscissa) is shown in minutes; UV absorbance in absorbance units (A.U.) The retention time of cortisol was about 22 min.

Table 1
Recovery of cortisol from human urine using Extrelut-filled columns

Columns filled with Extrelut	Recovery (%)
a. As supplied by the manufacturer	92±4
b. After clean-up	
1 time	93±3
2 times	93±3
3 times	93±4
4 times	94±4

Extrelut was used as supplied by the manufacturer or re-used after clean-up. Values are given as mean±SD, $n=6$.

days was used throughout), the granulate was washed with 1 l of distilled water, followed by one wash with 2 l and one wash out with 5 l of ethanol (98.9%, containing 1% petroleum ether), respectively. After each washing step, Extrelut was allowed to settle for about 10 min; then the wash solution was decanted. After the last washing, the granulate was poured onto an aluminium sheet (1×1 m) and dried by blowing a warm air stream over its surface. To refill the columns, 18 g Extrelut were filled into each column. Then a 24-mm round filter was pressed into the welt ring of the basket insert, and the latter was inserted into the column body until the filter covered the surface of the packing.

3. Results and discussion

Fig. 1 shows a chromatogram of a human urine extracted using pre-packed Extrelut-filled columns. In addition to three substances which were eluted within 10 min (they were not identified at this point), cortisol was eluted after about 22 min. When cleaned-up (1–4 times) Extrelut was used, extraction of unidentified substances (retention time: 6.2 min and 6.8 min) was significantly improved ($p<0.001$, $n=6$). Conversely, the recovery of cortisol (Table 1) and urinary excretion of cortisol (Table 2) were very similar when columns filled with unused or cleaned-up Extrelut were used. As shown in Table 2, cortisol excretion in healthy human males was about 35 μg/day, a value that is in agreement with previous work [2].

Table 2
Measurement of cortisol excretion using Extrelut-filled columns

Columns filled with Extrelut	Cortisol excretion (μg/24 h)
a. As supplied by the manufacturer	34±14
b. After clean-up	
1 time	35±15
2 times	37±16
3 times	34±16
4 times	35±17

Extrelut was used as supplied by the manufacturer or re-used after clean-up. Cortisol excretion was repeatedly determined in the urine of 10 healthy human subjects. Values are given as mean±SD.

Kieselguhr is a very frequently used column filling for the extraction of steroids. However, a major disadvantage is that commercially available kieselguhr (e.g. Extrelut) is expensive. The results of the present study show that it can be cleaned up by three washing steps and then used repeatedly. Thus, this method offers a low cost possibility for liquid–liquid extraction of urinary cortisol.

References

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[2] J. Köbberling and A. von zur Mühlen, J. Clin. Endocrinol. Metab., 38 (1974) 313.