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## Note

# Simple and rapid method for the determination of caffeine in urine using Extrelut-1 columns

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Caffeine (1,3,7-trimethylxanthine) is consumed by the population mainly as an ingredient of coffee (average 83 mg/cup) [1], cola beverages and tea. A smaller amount comes from the use of caffeine in pharmaceuticals. Indeed, besides its diuretic effect caffeine acts as a central nervous and cardiac muscle stimulant, as well as a smooth muscle relaxant [2], the usual therapeutic dose in man being 200 mg. Based on its stimulant properties, however, caffeine-containing formulations can also be used as doping agents in sportsmen. Thus, the quantity of caffeine ingested can be highly significant, especially for those seeking to improve their athletic performance.

In order to detect the abuse of caffeine in human sports, a simple and rapid method for the quantitative determination of caffeine in urine is presented in this paper. Gas chromatography with nitrogen selective detection (GC—NPD) and column extraction with Extrelut® columns are used.

## EXPERIMENTAL

### *Materials*

Caffeine was obtained from Merck (Darmstadt, F.R.G.) and the internal standard mepivacaine hydrochloride was supplied by Astra (Södertälje, Sweden). Stock solutions were prepared in double-distilled water. The concentration of the internal standard used was 100 $\mu g\ ml^{-1}$.

Extrelut-1 columns and Extrelut refilling bags were purchased from Merck.

The ammonium buffer was prepared by adjusting a saturated ammonium chloride solution to pH 9.5 with ammonium hydroxide.

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*Gas chromatography*

A Varian 3700 gas chromatograph with a nitrogen-selective detector was used and connected to a Varian CDS 111 integrator. The glass column (200 × 0.25 cm I.D.) was packed with 3% OV-7 on Chromosorb W HP. Nitrogen was used as carrier gas at a flow-rate of 25 ml $min^{-1}$. Column, injector and detector temperatures were kept at 195°C, 230°C and 330°C, respectively.

*Analytical procedure*

Urine (2 ml) was pipetted into a reaction tube and made alkaline (pH 9.3) by adding 0.2 ml of ammonium buffer. After adding 0.1 ml of the internal standard solution, the contents of the tube were briefly vortexed and 1 ml pipetted in duplicate on top of Extrelut-1 columns.

After 5 min, each column was eluted with 6 ml of methylene chloride—methanol (9:1). The eluate was collected in a conical reaction tube and evaporated under nitrogen at 40°C. The residue was redissolved in 0.2 ml of ethyl acetate and 1 $\mu$l was injected into the gas chromatograph.

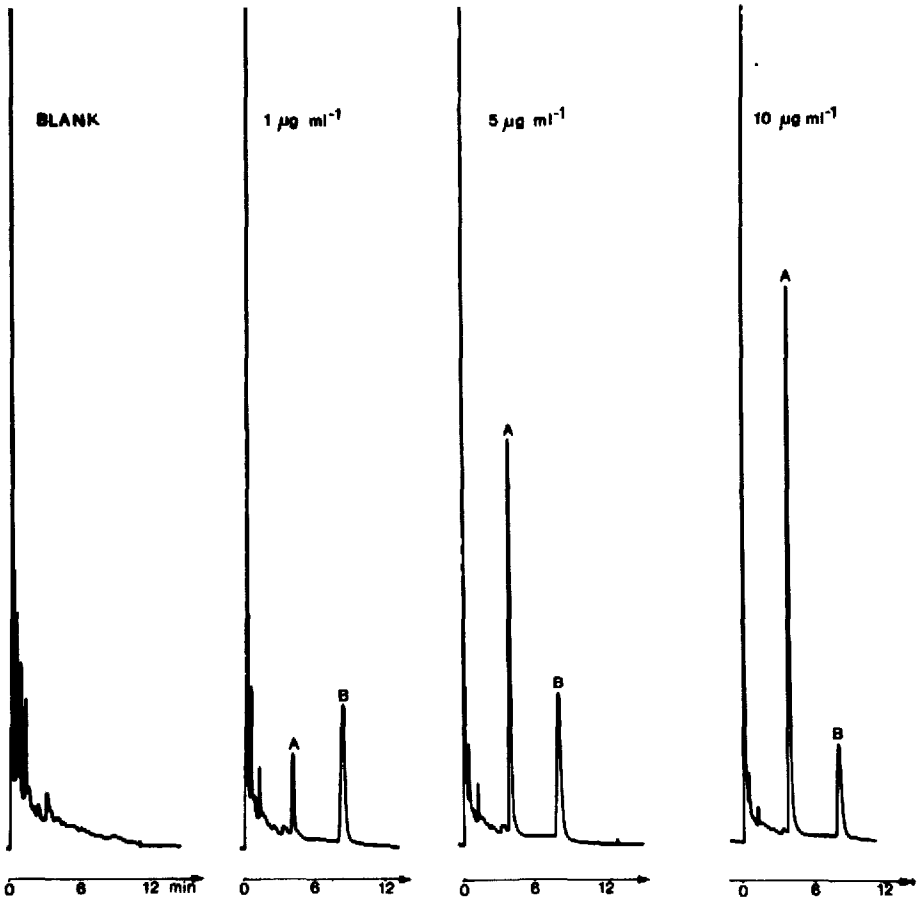


Fig. 1. Chromatograms obtained from horse urine spiked with caffeine (A = caffeine, B = mepivacaine).

## RESULTS AND DISCUSSION

Although caffeine is currently mentioned in drug screening procedures [3—6] there is a scarcity of published methods suitable for the routine quantitative determination of caffeine in urine.

Drugs such as caffeine as well as their metabolites must be extracted from body fluids before they can be determined by chromatographic procedures. The determination of caffeine in plasma using GC with NPD and conventional liquid—liquid extraction as proposed by Cohen et al. [7] requires an analysis time of at least 25 min. A recently published method [8] for the GC estimation of caffeine in the urine of dogs needs a 10-ml sample and two extraction periods of 20 min each. Liquid chromatographic (LC) determinations of caffeine in serum require a sample preparation of 30—40 min [9—12] with a high volume ratio (extraction solvent/sample) or special techniques like multidimensional LC allowing the direct injection of biological fluids without pretreatment [13].

Nevertheless, it is well known that Extrelut provides a means of achieving a rapid and precise extraction without the problems of emulsion formation and consumption of high volumes of solvent [14—16].

Under the chromatographic conditions described previously, caffeine and the

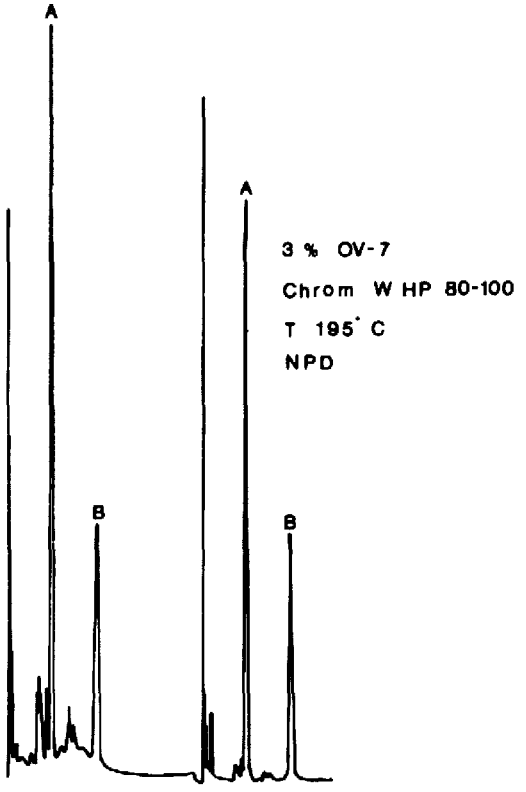


Fig. 2. Chromatograms from extracts of urine from coffee drinkers (A = caffeine, B = mepivacaine).

internal standard mepivacaine gave sharp peaks with retention times of 4.35 and 8.74 min, respectively. Typical chromatograms obtained by processing control blank and spiked horse urine containing different amounts of caffeine are presented in Fig. 1. The principal human caffeine metabolites [17] (theophylline, theobromine, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, paraxanthine and 1-methyluric acid) did not interfere under these conditions. Processing urine of coffee drinkers as described in the analytical procedure resulted in chromatograms as illustrated in Fig. 2.

The influence of the pH on the elution recovery was studied by adding caffeine in a final concentration of 20 $\mu$g ml$^{-1}$ to horse urine, brought to different pH values. Each urine sample (1 ml) was analysed in triplicate. The column residue was redissolved in 200 $\mu$l of a methanolic internal standard solution (mepivacaine 100 $\mu$g ml$^{-1}$) and 1 $\mu$l was injected and quantified.

Fig. 3 indicates that the elution recovery of caffeine in the pH range 7—10 is practically not influenced by the urinary pH. The recoveries are close to 90%. In addition, the influence of the pH on the elution of mepivacaine was also studied using caffeine as internal standard. The peak ratios, $\Delta$Mepiv./$\Delta$Caff., (mean of three determinations) as indicated in Fig. 3 show that the extractability of mepivacaine using Extrelut-1 columns is not substantially influenced by the urinary pH in the pH range 8—10.

A standard curve was obtained by adding different amounts of caffeine to horse urine and treating the samples in quadruplicate according to the procedure. The calibration graph is linear in the range 0—20 $\mu$g ml$^{-1}$ caffeine in urine ($\sigma$ = 0.9995).

Replicate determinations of caffeine in a human urine sample produced a

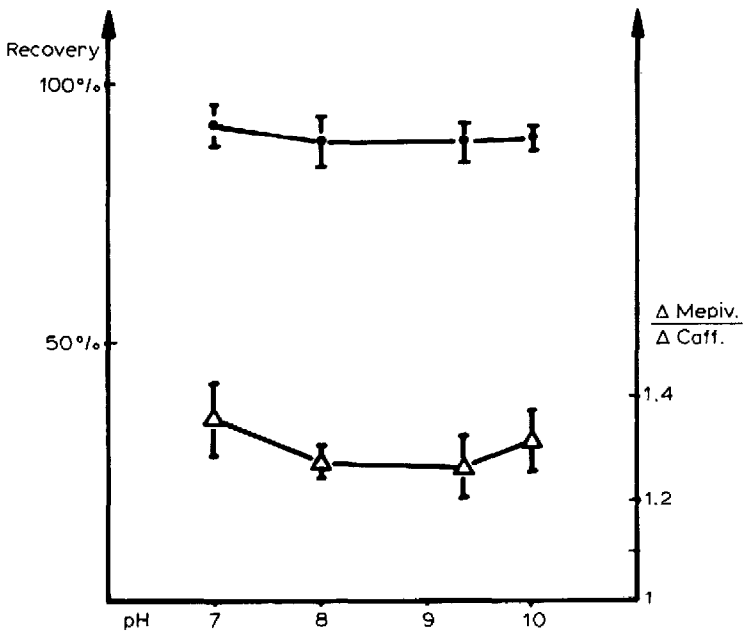


Fig. 3. Influence of the pH on the elution recovery (%) of caffeine (•) and on the extractability measured by peak ratios of mepivacaine (△).

relative standard deviation of 1.5% (1.71 ± 0.026 $\mu$g ml$^{-1}$, $n$ = 7). Starting with 1 ml of urine, the lowest concentration which allowed quantitative detection in routine analysis was 0.4 $\mu$g ml$^{-1}$ corresponding to about 1.75 ng injected. This assay is considerably more sensitive than a previously published GC-NPD procedure [7] in which a 1-ml plasma sample was required to produce a sensitivity of 10 ng injected. Notwithstanding the difference in biological matrix between plasma and urine, this improvement in detection limit can be partly attributed to a decrease in background noise seen with Extrelut-1 columns. Indeed, in agreement with our experience [14], there are reports in many papers that Extrelut eluates are cleaner than the corresponding liquid—liquid extracts. Moreover, progress has been made by introducing glass columns instead of polyethylene columns and altering the composition of the packing material of the refilling bags. It should be clear that comparable results can be obtained by using selfprepared columns filled with equivalent amounts of Extrelut.

Following this method, the urinary caffeine content of a great consumer of coffee was monitored during a 60-h period (Fig. 4). Based on the caffeine content of both brewed and instant coffee as suggested by Burg [17], the intake was estimated at 700 mg/day. The amount excreted over the 60-h period (18.2 mg) is in agreement with values from other studies [18, 19], indicating that only approximately 1% of caffeine is excreted unchanged.

In order to establish an excess level of caffeine for sporting competitions,

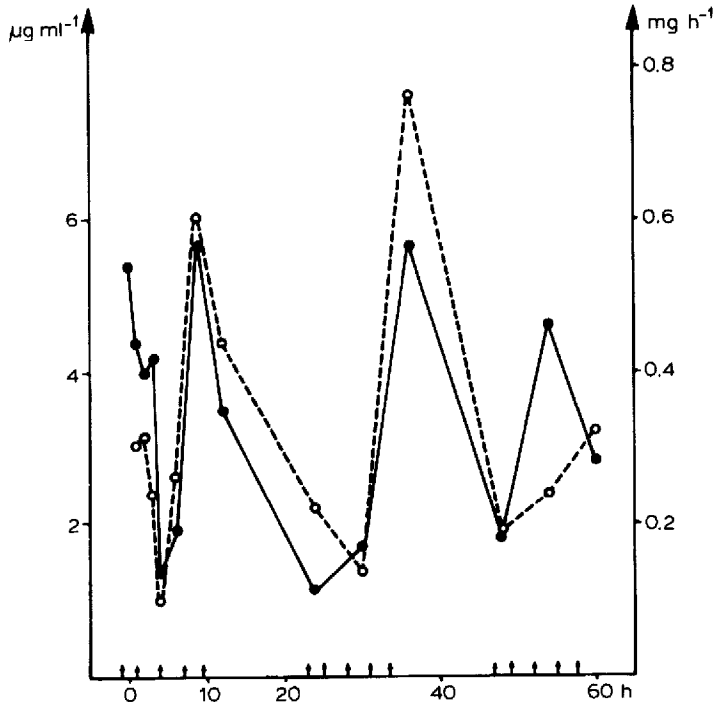


Fig. 4. Monitoring of the urinary excretion of caffeine in a great consumer of coffee. Excretion expressed as $\mu$g ml$^{-1}$ (•) and mg h$^{-1}$ (○). The intakes of coffee (approximately 140 mg) are marked by arrows.

further work with respect to the urinary caffeine content of both coffee drinkers and sportsmen is currently being investigated.

The results reported here indicate that the quantitative determination of caffeine in urine of athletes can easily and rapidly be done with low solvent consumption using Extrelut-1 columns.

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