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## Quantitative Caffeine Determination by Direct Injection of Urine

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### Direct urine injection

#### *Analytical HPLC conditions:*

HPLC: 1090 HEWLETT PACKARD, with six port column switching valve  
DAD: 272 nm  
precolumn: 'sample cleanup column' ODS-Hypersil (C<sub>18</sub>) 5 $\mu$ m, 20 mm x 4 mm  
(HEWLETT PACKARD, Böblingen, D)  
analytical column: LiChroCART<sup>R</sup>-cartridge (MERCK, Darmstadt, D):  
'LiChrospher<sup>R</sup> 100 RP-18, 5 $\mu$ m, 125 mm x 4 mm  
solvents: A = water; B = acetonitrile

The solvents are filtered and degassed through a nylon filter membrane, pore size 0.45  $\mu$ m, under vacuum.

flow: 1 ml/min  
injection volume: 5  $\mu$ l of native urine  
gradient: pre column 0.00  $\rightarrow$  3.50 min: 0% B  
3.50  $\rightarrow$  3.51 min: 0  $\rightarrow$  5% B  
3.51 min: column switch  
pre column + analytical column  
3.51  $\rightarrow$  13.0 min: 5  $\rightarrow$  25% B

HPLC-UV chromatograms are shown in Figure 1.

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## Results

Table 1: Statistical evaluation of the reproducibility of the raw data (peak height, peak area) and of the retention times: [A] caffeine standard solution ( $c = 20 \mu\text{g/ml}$ ); [B] urine sample 1; [C] urine sample 2.

		n	$\bar{x}$	s	CV [%]
[A]	peak height:	6	21.969	$\pm 0.220$	1.00
	peak area :	6	173.500	$\pm 1.200$	0.69
	R.T. (min) :	6	10.467	$\pm 0.011$	0.11
[B]	peak height:	6	20.745	$\pm 0.093$	0.45
	peak area :	6	166.700	$\pm 0.900$	0.54
	R.T. (min) :	6	10.433	$\pm 0.014$	0.13
[C]	peak height:	6	17.480	$\pm 0.246$	1.41
	peak area :	6	139.000	$\pm 2.200$	1.58
	R.T. (min) :	6	10.452	$\pm 0.018$	0.17

Out of the raw data (standard solution and urine samples) the real caffeine concentration of the urine samples can be calculated (Table 2).

Table 2: Experimental found caffeine concentration of the urine samples [B/C], calculated by the raw data of peak height and peak area.

		n	$\bar{x}$ [ $\mu\text{g/ml}$ ]	s [ $\mu\text{g/ml}$ ]	CV [%]
<u>calculated by:</u>					
[B]	peak height:	36	19.22	$\pm 0.16$	0.83
	peak area :	36	18.89	$\pm 0.19$	1.01
[C]	peak height:	36	15.92	$\pm 0.25$	1.57
	peak area :	36	16.02	$\pm 0.26$	1.62

In comparison to the results of the direct urine injection the statistical evaluation of the raw data of a quantitative caffeine determination after extraction with diethylether and ethyltheophylline as internal standard (ISTD) is shown.

## Sample preparation

To 5 ml of urine in a glass tube 20  $\mu\text{g}$  ethyltheophylline (20  $\mu\text{l}$  of a 1 mg/ml solution in methanol) as internal standard to quantify caffeine are added. After the addition of 0.5 g of a solid buffer ( $\text{NaHCO}_3/\text{K}_2\text{CO}_3$ ; 2:1, w:w; pH: about 9.5), the samples are shaken mechanically with 7 ml of diethylether (peroxidfree, freshly distilled over  $\text{CaH}_2$ ) for 15 minutes and then centrifuged. The organic layer is transferred to another glass tube and evaporated in vacuum. The dried residues will be desolved in 500  $\mu\text{l}$  methanol.

Table 3: Evaluation of the raw data of caffeine and the internal standard (ISTD) ethyltheophylline after extraction with an organic solvent.

caffeine*ISTD <sup>-1</sup>				
	n	$\bar{x}$	s	CV[%]
peak height:	10	0.972	$\pm 0.045$	4.63
peak area :	10	0.849	$\pm 0.028$	3.29

## Conclusion

With the direct urine injection and quantification by means of an external standard it is possible to reduce the coefficient of variation down to 0.83-1.57% by calculating peak heights and to 1.01-1.62% by calculating peak areas. If an extraction with diethylether is performed, the coefficient of variation was 4.63% (peak height) and 3.29% (peak area). In every positive case (12 ppm and higher) it is necessary to prove the peak purity of caffeine in the urine sample.

## References:

A.Gotzmann: Nachweis biologisch aktiver Substanzen mit Hilfe der Hochdruckflüssigkeits-Chromatographie und Normbereichsbestimmungen von Corticosteroiden.  
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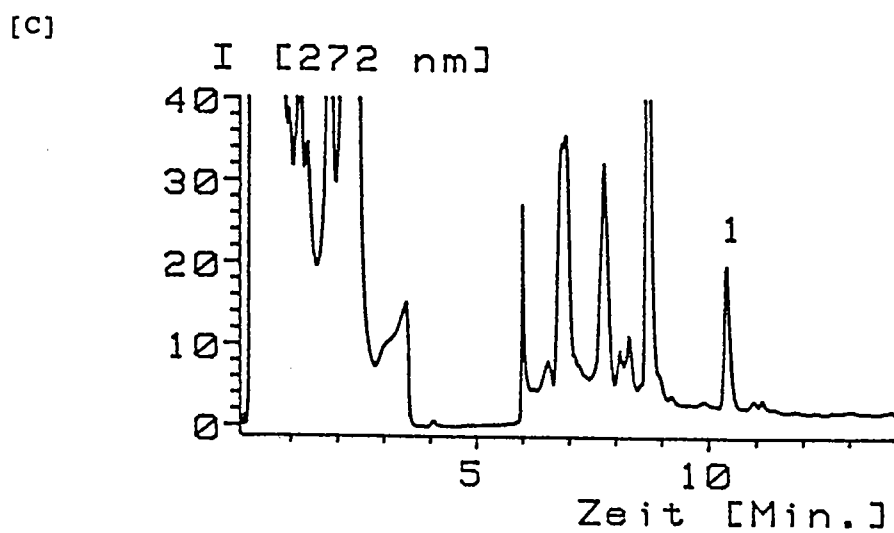
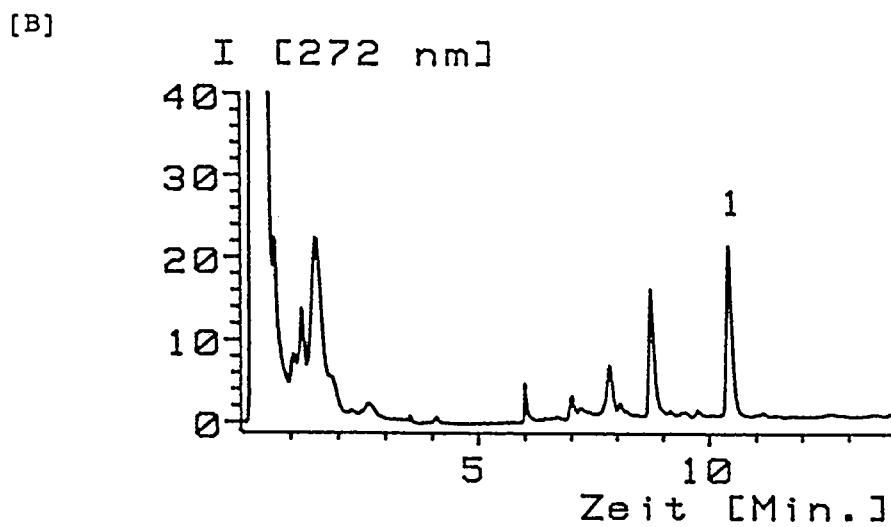
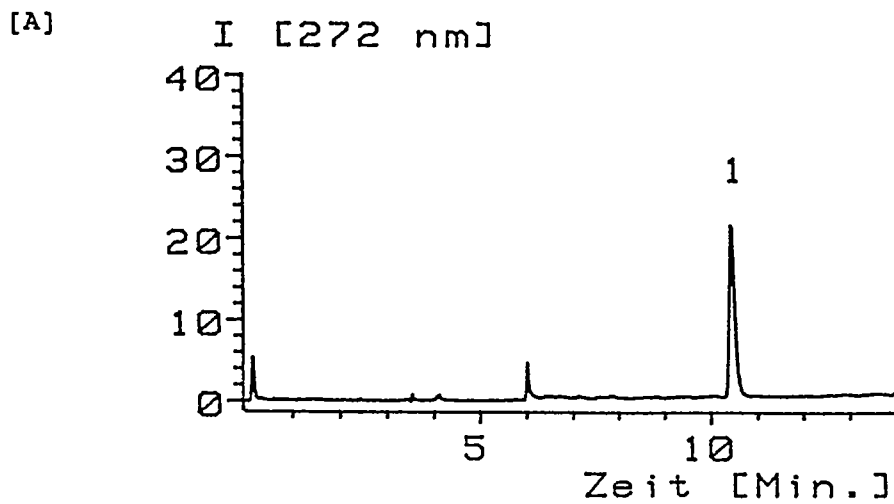


Figure 1: HPLC-UV-chromatograms:

[A] caffeine standard in aqueous solution (5  $\mu$ l) 20  $\mu$ g/ml;

[B] urine 1, direct injection (5  $\mu$ l) caffeine = 18.9  $\mu$ g/ml;

[C] urine 2, direct injection (5  $\mu$ l) caffeine = 15.9  $\mu$ g/ml.