A novel stable isotope dilution / benchtop gas chromatography - mass spectrometry (ID/GC-MS) assay for profiling estrogens, their biologically active metabolites and testosterone in human urine

P Hoffmann[1], MF Hartmann[1], T Remer[2], KP Zimmer[1] and SA Wudy[1]

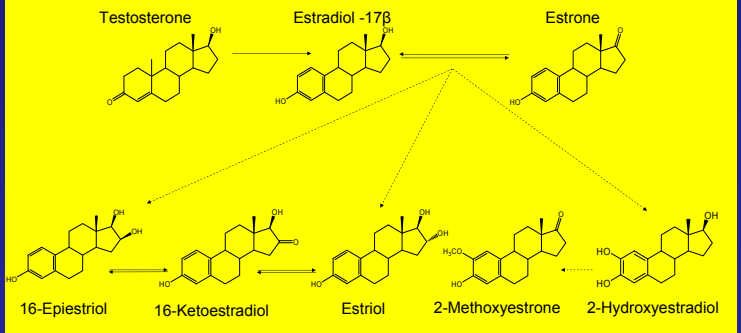
[1] Steroid Research and Mass Spectrometry Unit, Center of Child and Adolescent Medicine, Justus-Liebig-University, Giessen, Germany

[2]Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany

Background

Estrogens, such as estrone (E1), 17β-estradiol (E2), estriol (E3) and their biologically active metabolites 2-methoxyestrone (2MeOE1), 16-ketoestradiol (16-OE2), 16-epiestriol (16-epiE3), 2-hydroxyestradiol (2-OHE2) and testosterone (T) play an important role in physiological and pathological developmental processes. Stable isotope dilution/GC-MS allows for highest specificity in steroid analysis.

Origin of urinary steroid metabolites



Aims

We therefore aimed at developing an assay, based on stable isotope dilution/ benchtop GC-MS for these analytes.

Methods

The method consisted of equilibration of urine with stable isotope labeled internal standards (d_2-estrone, d_4-17β-estradiol, d_3-estriol, d_3-testosterone, d_4-2-methoxyestrone, d_5-16-ketoestradiol, d_2-16-epiestriol and d_5-2-hydroxyestradiol), solid phase extraction, enzymatic hydrolysis, re-extraction, purification by anion exchange chromatography and derivatisation (trimethylsilyl-ethers). The samples were analyzed by GC-MS (Agilent 6890N/5975).

Conclusions

We have developed a stable isotope dilution/gas chromatography - mass spectrometry (ID/GC-MS) assay to measure estrone (E1), 17β-estradiol (E2), estriol (E3), testosterone (T), and their biologically active metabolites 2-methoxyestrone (2MeOE1), 16-ketoestradiol (16-OE2), 16-epiestriol (16-epiE3) and 2-hydroxyestradiol (2-OHE2) in human urine in very low concentrations.

Results I

INTRA ASSAY - precision of GC-MS method for profiling estrogens

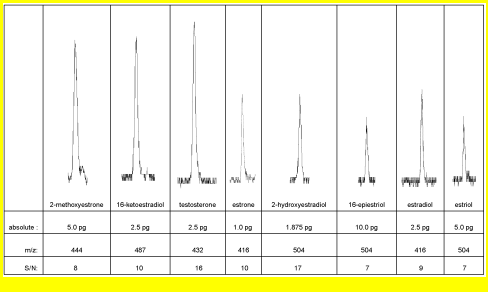
urine:	E1	2MeOE1	T	16-OE2	E2	E3	16-epiE3	2-OHE2
mean (n=7) (µg/l):	5.02	2.52	2.51	4.97	2.22	2.44	5.23	0.67
standard deviation:	0.10	0.03	0.04	0.07	0.03	0.04	0.05	0.01
CV (%):	1.92	1.36	1.48	1.49	1.37	1.63	1.00	1.71
(one sample was prepared and analyzed seven times at one day.)								

INTER ASSAY - precision of GC-MS method for profiling estrogens

urine:	E1	2MeOE1	T	16-OE2	E2	E3	16-epiE3	2-OHE2
mean (x=7) (µg/l):	4.97	2.56	2.53	4.95	2.44	2.50	5.03	0.66
standard deviation:	0.04	0.02	0.02	0.08	0.03	0.06	0.06	0.01
CV:	1.74	0.85	0.65	1.63	1.41	2.21	1.21	1.46
(one sample was prepared and analyzed at seven following days.)								

Results II

SENSITIVITY of GC-MS method for profiling estrogens



For a GC-MS assay, the method described had excellent sensitivity. The SIM recordings show the response of each analyte (absolute injection).

Results III

ACCURACY of GC-MS method for profiling estrogens

native Urine	E1	2MeOE1	T	16-OE2	E2	E3	16-epiE3	2-OHE2
measured mean (n = 7) (µg/l)	0.00	0.00	0.08	2.48	0.00	0.00	0.00	0.00
standard deviation	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00
Coefficient of variation (%)			1.53	6.45				
spiked (µg/l)	5.00	2.50	2.50	2.50	2.50	2.50	5.00	0.63
Expected mean (µg/l)	5.00	2.50	2.58	4.98	2.50	2.50	5.00	0.63
measured mean (n = 7) (µg/l)	4.93	2.54	2.50	5.16	2.29	2.24	5.22	0.69
standard deviation	0.08	0.10	0.04	0.45	0.18	0.15	0.21	0.04
Coefficient of variation (%)	3.43	3.94	1.64	8.64	7.67	6.57	3.95	5.56
relative error:	-1.49	1.63	-3.17	3.72	-8.33	-10.23	4.45	9.60
spiked (µg/l)	20.00	10.00	10.00	10.00	10.00	10.00	20.00	2.50
Expected mean (µg/l)	20.00	10.00	10.08	12.48	10.00	10.00	20.00	2.50
measured mean (n = 7) (µg/l)	19.44	9.81	9.77	12.50	9.93	9.46	19.39	2.79
standard deviation	0.40	0.25	0.14	0.28	0.34	0.17	0.27	0.08
Coefficient of variation (%)	2.07	2.57	1.46	2.24	3.43	1.79	1.40	2.94
relative error:	-2.77	-1.89	-3.15	0.15	-0.72	-5.38	-3.07	11.63

Accuracy was determined by spiking prepubertal urine with plain analytes.

Two different spike steps were made. The first spike step (lower concentrations) showed relative errors from 9.60% (for 2-hydroxyestradiol) to -10.23% (for estriol). The second spike step (higher concentrations) showed relative errors from 11.63% (for 2-hydroxyestradiol) to -5.38% (for estriol).

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