A New LC-MS/MS Assay for the Analysis of Sulfated Steroids in Human Serum: Quantification of Cholesterol Sulfate, Pregnenolone Sulfate, 17-Hydroxypregnenolone Sulfate and Androgen Sulfates

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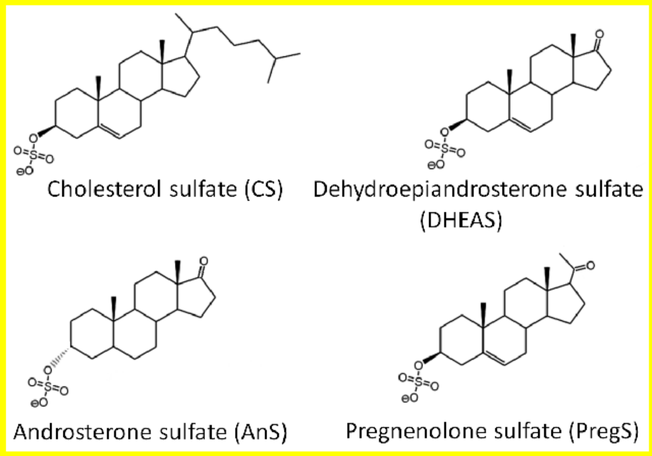
Introduction

Steroids are found in human blood predominantly as sulfated steroids. Conjugation is a physiological mechanism which increases their solubility in biological fluids, facilitating their regulation and excretion. Simultaneous analysis of an extensive number of sulfated steroids in blood is challenging because of their broad range of concentrations. Additionally, chromatographic baseline separation of some sulfated steroids is complicated but mandatory, since some of them are structurally related and provide similar signals in mass spectrometry.



Some of the sulfated steroids found in higher concentrations in human blood

Objectives

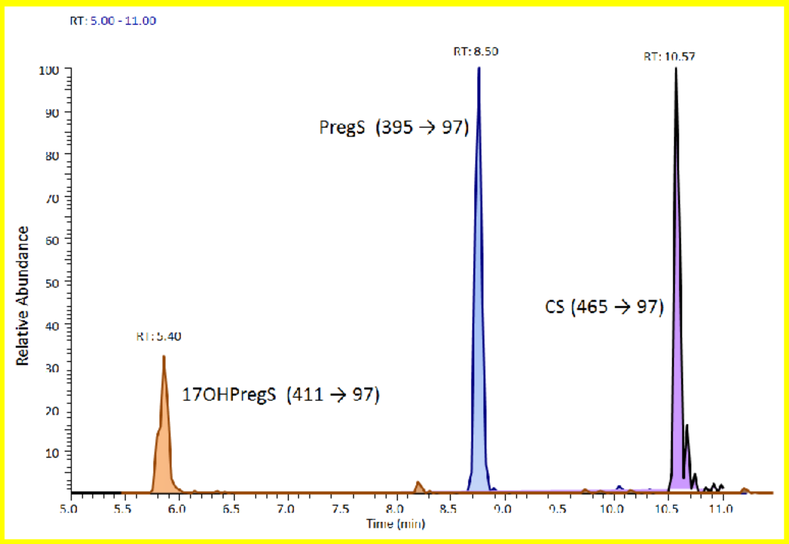
- To develop and validate a new LC-MS/MS assay to obtain the most detailed sulfated steroidome in human blood to date.
- Apply this method to study and diagnose conditions related to the metabolism of sulfated steroids, i.e. steroid sulfatase deficiency (recessive X-linked ichthyosis, RXLI).

Conclusions

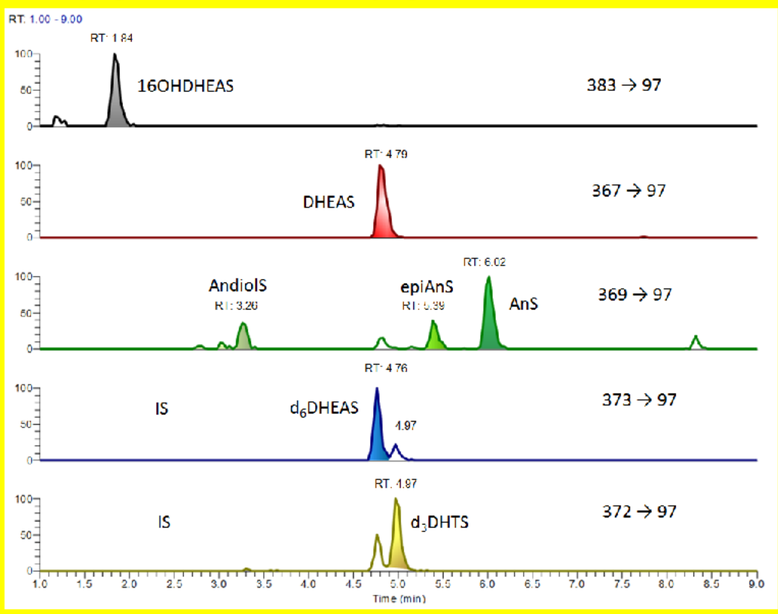
- ✓ We developed and validated a reliable method for the simultaneous quantification of 11 sulfated steroids in human serum, which requires only 300 µl of serum.
- ✓ Sample preparation allows for isolation of both unconjugated and sulfated fractions.
- ✓ The method has proved its diagnostic value to discriminate between RXLI and other types of skin ichthyoses.

Methods

- Chromatographic separations were achieved with a phenyl-reversed based fused-core column (Accucore Phenyl-X, 100x2.1 mm, 2.6 µm).
- Electrospray ionization – tandem mass spectrometry (ESI-MS/MS) was used for the identification and quantification of sulfated steroids. The MS/MS product ion for quantification was always the sulfate group HSO_4^- (m/z 97).
- Solid phase extraction required 300 µl of serum and was able to isolate both conjugated and unconjugated steroidal fractions.
- All compounds were validated at 3 different concentration levels.



17-hydroxypregnenolone sulfate (17OHPregS), PregS and CS merged chromatograms obtained from a serum sample of a 45 years male



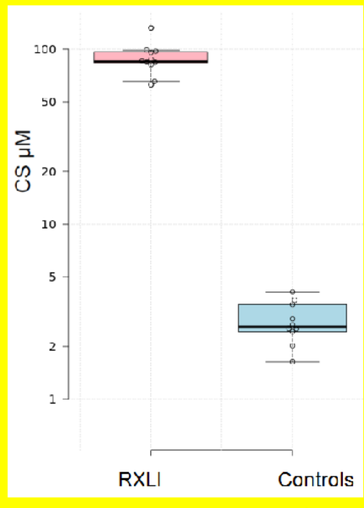
Profile of androgen sulfates detected in a real sample from a 45 years male: DHEAS, 16α-hydroxy-DHEAS (16OHDHEAS), AnS, its epimer epiAnS, and AndiolS (androstenediol-3-sulfate). Internal standards (IS) for DHEAS and dihydrotestosterone sulfate (DHTS) are depicted too

Results

- ➢ All isobaric/structurally related compounds (i.e. androgen sulfates) were baseline separated.
- ➢ DHEAS, CS, AnS, epiAnS, AndiolS, 16OHDHEAS and PregS were the sulfated steroids found in higher concentrations in human blood. The validation parameters of all these compounds met the standards of the FDA and EMA guidelines for bioanalytical evaluation.
- ➢ The sulfates of testosterone (TS), epitestosterone (eTS) and DHTS could not be detected in any sample (limits of detection LOD = 0.5 ng/ml and limits of quantification LOQ = 1 ng/ml).
- ➢ 17OHPregS presented important matrix effects which affected its analytical performance , but only at lower concentrations.
- ➢ Analytical performance was studied at 3 quality controls (QCs) for each compound. Recoveries ranged between 85.5% and 111.6%. Intra and between day accuracies were all below 20% (% relative error). Intra and between day precisions were all below 20% (% coefficient of variation), with the only exception of 17OHPregS at its lower quality control.
- ➢ The concentration of CS in RXLI patients is about 35 times higher than in healthy males and in *ichthyosis vulgaris* controls.

	CS	PregS	17OHPregS	16OHDHEAS	DHEAS	AndiolS	AnS	EpiAnS
IS	d_7CS	d_4PregS	d_{11}17OHPregS	d_6DHEAS	d_6DHEAS	d_6DHEAS	d_3DHTS	d_3DHTS
Linearity (ng/ml)	80-480000	1-500	1-250	2.5-2000	25-6000	2.5-500	10-2000	10-2000
R^2	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99	> 0.99
LOQ (ng/ml)	80.0	1.0	1.0	2.5	25.0	2.5	10.0	10.0
LOD (ng/ml)	40.0	0.5	0.5	1.0	1.0	1.0	1.0	2.5
Matrix Effects	0.99	0.80	0.59	1.01	1.06	0.99	0.95	0.94
QC1	1600	5	5	40	160	40	40	40
QC2	12800	40	40	160	400	160	400	160
QC3	32000	160	160	400	1500	400	1500	400

Some performance parameters of the LC-MS/MS method



Variation of CS levels in RXLI and controls (logarithmic scale)

Acknowledgment *This work was supported by the Selbsthilfe Ichthyose e. V., the Medical Faculty (OJ111409) of the University of Münster, and by the German Research Foundation (DFG) within DFG Research Group 1369 "Sulfated Steroids in Reproduction" to subproject 7 (S.A.W., principal investigator, WU 148/6-2).*