Sensitive quantitative LC-MS/MS method of steroid hormones determination in adipose tissue as a helpful tool in obesity research.

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INTRODUCTION

Obesity is a growing problem that affects more than 1 billion people worldwide. Estrogens deficiency play an important role in the pathogenesis of obesity. Therefore, there is a need to characterize sex hormones profile in adipose tissues in normal-weight and obese individuals to predict the effectiveness of estrogen-oriented approach in prevention and treatment of obesity. Determination of steroid hormones in human adipose tissue is challenging due to the low concentration of hormones as well as low amount and complexity of matrix. LC-MS/MS is an attractive analytical technique for development of such challenging methods because of high specificity, sensitivity and short analysis time. In this work we present LC-MS/MS method for a quantitative determination of estradiol, estrone, androstenedione and testosterone in human adipose tissue.

MATERIALS AND METHODS

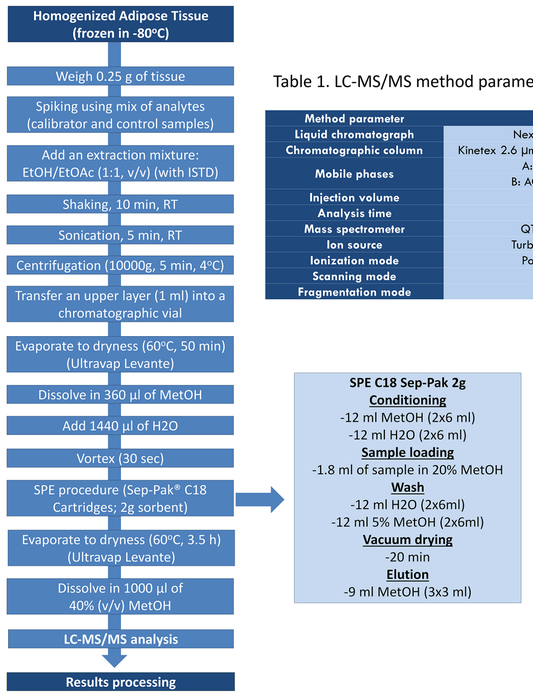

Figure 1. Schematic representation of the sample preparation procedure.

Table 1. LC-MS/MS method parameters.

Method parameter	Applied value
Liquid chromatograph	Nexera LC40 (Shimadzu)
Chromatographic column	Kinetex 2.6 µm XB-C18 100 A; 100 x 2.1 mm
Mobile phases	A: H2O LC-MS grade B: ACN/MetOH (1:1, v/v)
Injection volume	30 µL
Analysis time	8 min
Mass spectrometer	QTRAP 6500+ (Sciex)
Ion source	Turbo V™ Ion Source (ESI)
Ionization mode	Positive and Negative
Scanning mode	sMRM
Fragmentation mode	CID

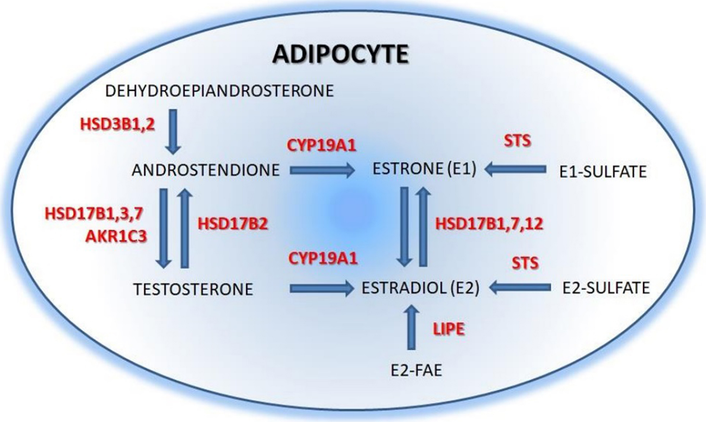

Figure 2. Synthesis of sex steroids in adipocyte (simplified version). AKR1C3 – aldo-keto reductase family 1 member C3 (17β-hydroxysteroid dehydrogenase type 5); CYP19A1 – aromatase; E2-FAE – E2 fatty acyl esters; HSD3B1,2 - 3β-hydroxysteroid dehydrogenase types 1&2; HSD17B1,3,7,12 - 17β-hydroxysteroid dehydrogenase types 1, 3, 7 & 12; LIPE – hormone-sensitive lipase, STS – steroid sulphatase.

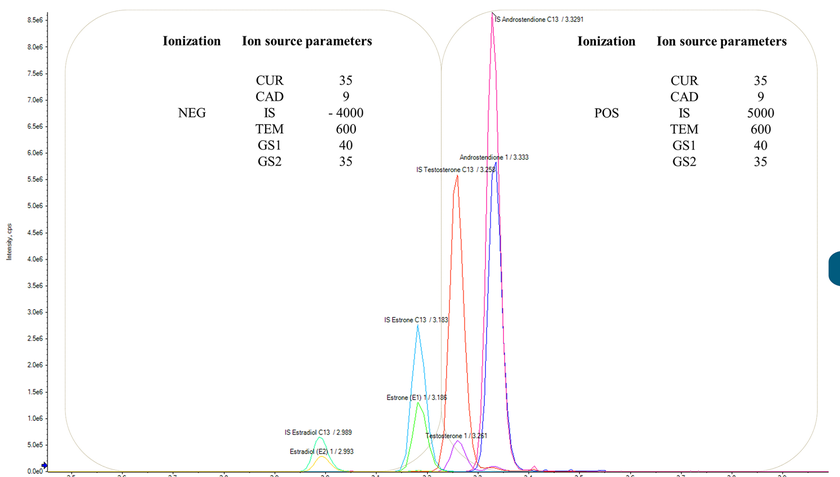

Figure 3. Chromatogram of LC-MS/MS analysis of extracted tissue sample with marked ionization parameters .

RESULTS

Table 2. Summary of method validation results depending on the adipose tissue type

Adipose Tissue	Analyte	Correlation coefficient (R^2)	Method range [ng/g of tissue]	Method precision [RSD%] (n=5)
subcutaneous (S)	Androstendione	R^2 = 0.9992	1 - 100	14.99
	Testosterone	R^2 = 0.9956	0.1 - 10	7.83
	Estradiol	R^2 = 0.9995	0.1 - 10	5.46
	Estrone	R^2 = 0.9989	0.1 - 10	8.48
visceral (V)	Androstendione	R^2 = 0.9987	1 - 100	2.80
	Testosterone	R^2 = 0.9986	0.1 -10	10.56
	Estradiol	R^2 = 0.999	0.05 - 10	4.40
	Estrone	R^2 = 0.9994	0.05 - 10	12.72

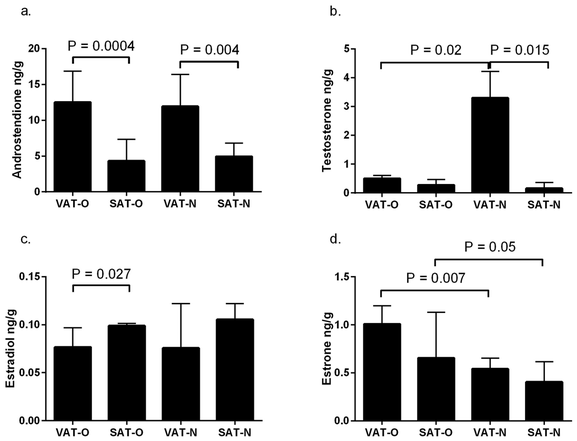

Figure 4. Charts presenting results of analysis of adipose tissue samples collected from normal-weight and obese patients. VAT-O – visceral tissue from obese patient; SAT-O – subcutaneous tissue from obese patient; VAT-N - visceral tissue from normal weight patient; SAT-N - subcutaneous tissue from normal weight patient.

The analysis was performed on pairs of visceral and subcutaneous adipose tissue from 15 obese patients (10 women and 5 men) and 5 pairs of adipose tissues from normal weight subjects (40 tissues in total). It was found that concentrations of androstenedione, testosterone and estrone were higher while estradiol lower in visceral tissues than in subcutaneous tissues, irrespective of body weight. The tissues of obese patients (both VAT and SAT) were characterized by higher concentrations of estrone. Normal weight subjects had significantly higher testosterone concentrations in visceral than in subcutaneous adipose tissue depot.

SUMMARY

- A single sample preparation and analytical method suitable for all the tested analytes with sufficiently good sensitivity was developed.
- The method was validated separately for visceral (VAT) and subcutaneous (SAT) adipose tissue and fulfilled the following criteria for each analyte: linearity (R ≥ 0.995), reproducibility (%RSD ≤ 15%)
- Developed method was successfully applied to analyze adipose tissue samples collected from normal-weight and obese patients using only 0.25 g of homogenized tissue sample.