A method for the quantification of sterols for a rapid screening of Smith-Lemli-Opitz Syndrome by Atmospheric Pressure Thermal Desorption Chemical Ionization - Mass Spectrometry (APTDCI - MS)

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INTRODUCTION

Smith-Lemli-Opitz Syndrome (SLOS) is an auto-somal recessive multiple malformation syndrome and has a very broad phenotypic spectrum. The severe form typically cause death in the perinatal period, the milder variant distinct behavioral and learning problems, growth failure and intellectual disability. The deficiency of the DHCR7 enzyme activity, which catalyzes the last step of cholesterol biosynthesis, results in low plasma cholesterol (Chol) and increased 7- and 8-dehydrocholesterol (7-DHC, 8-DHC) levels.

GC-MS Chol and DHCs determination in blood, tissues or cells is the reference method for the diagnosis of SLOS.

EXPERIMENTAL SECTION

Figure 1. APTDCI analytical procedure steps.
Ambient desorption ionization (ADI) techniques permit to analyze many compounds by direct sampling/ionization of molecules from raw samples. The figure depicts a new rapid and sensitive ADI technique for SLOS screening. Analytes are desorbed by heated nitrogen gas flow and the ionization induced by a corona discharge (2 µA) without any solvent spray.

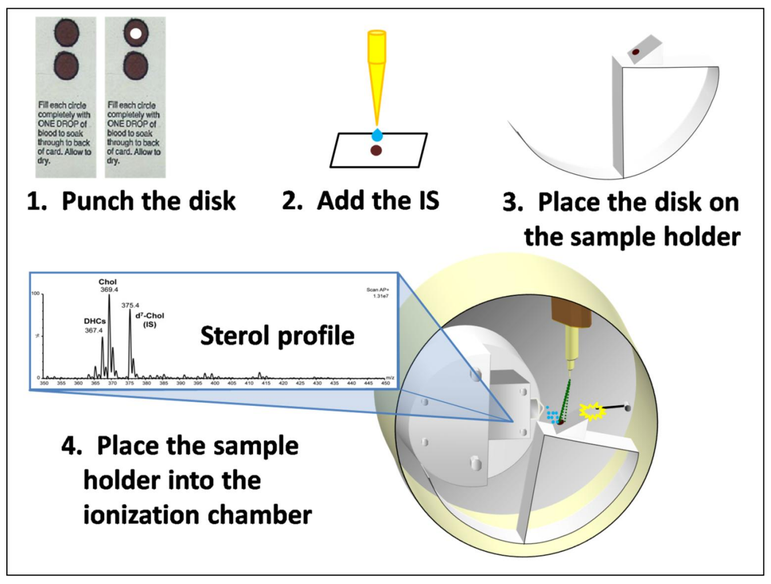

Table 1. Optimal APTDCI parameters.

Parameters	Optimal values
Sprayer-surface distance	14 mm
MS inlet-sample distance	1 mm
Collection angle	20°
Incidence angle	82°
Cone voltage	20 V
Source Temperature	150°C
Desolvation Gas Temperature	500°C
Desolvation Gas Flow	450 L/h
Nebulizer gas pressure	80 psi
Ion mode	Positive
Mass range scan	350-450 *m/z*

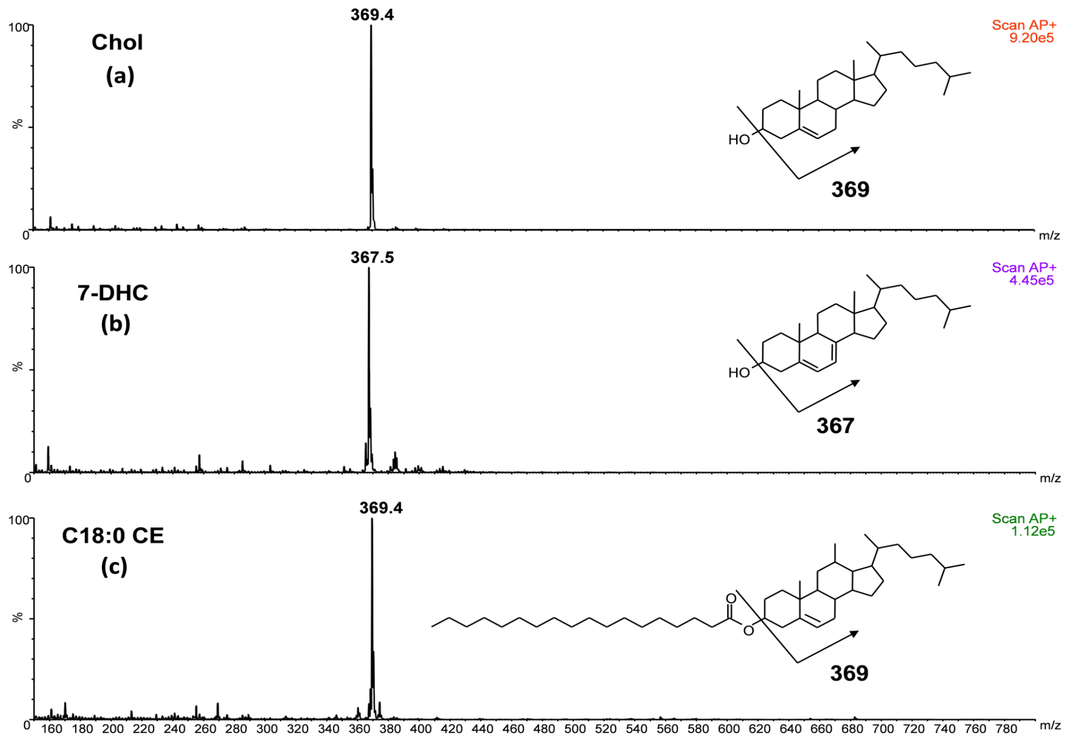

Figure 2. APTDCI-MS spectra obtained spotting 2 µL of standard solutions onto glass slides. The thermal desorption mechanism promotes the formation of cholestadiene, suddenly protonated by a corona discharge (*m/z* 369), from Chol and C18:0 CE by the loss of water and fatty acid, respectively; 7-DHC produces the ion at *m/z* 367. This mechanism also causes the desorption/ionization of other sterols and cholesteryl esters. Monitoring of cholestadiene ion allows the determination of total cholesterol without the pre-analytical conversion of cholesteryl esters into free Chol.

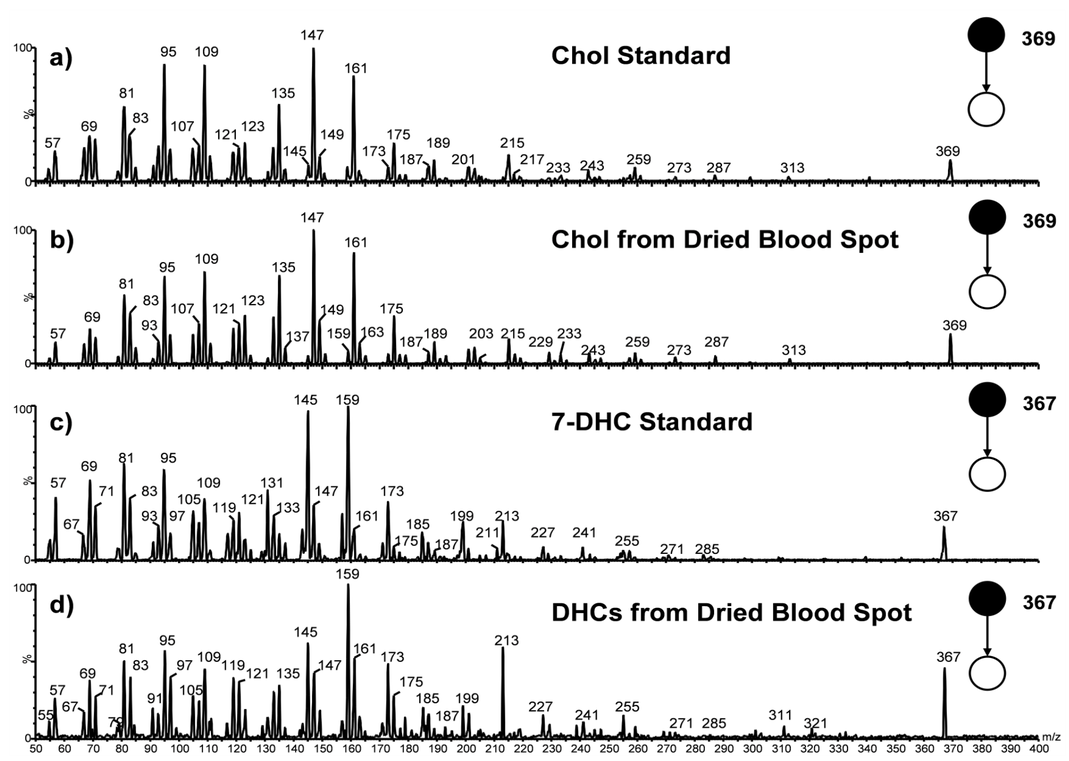

Figure 3. Chol and DHCs specificity study.
Collision induced dissociation study (mass range: *m/z* 50-400; collision energy: 25 eV) to confirm the identity of Chol and DHCs. The product ion spectra of *m/z* 369 and 367 from standards (panels a and c) compared to those from SLOS dried blood spot (panels b and d) are well overlapped.

RESULTS

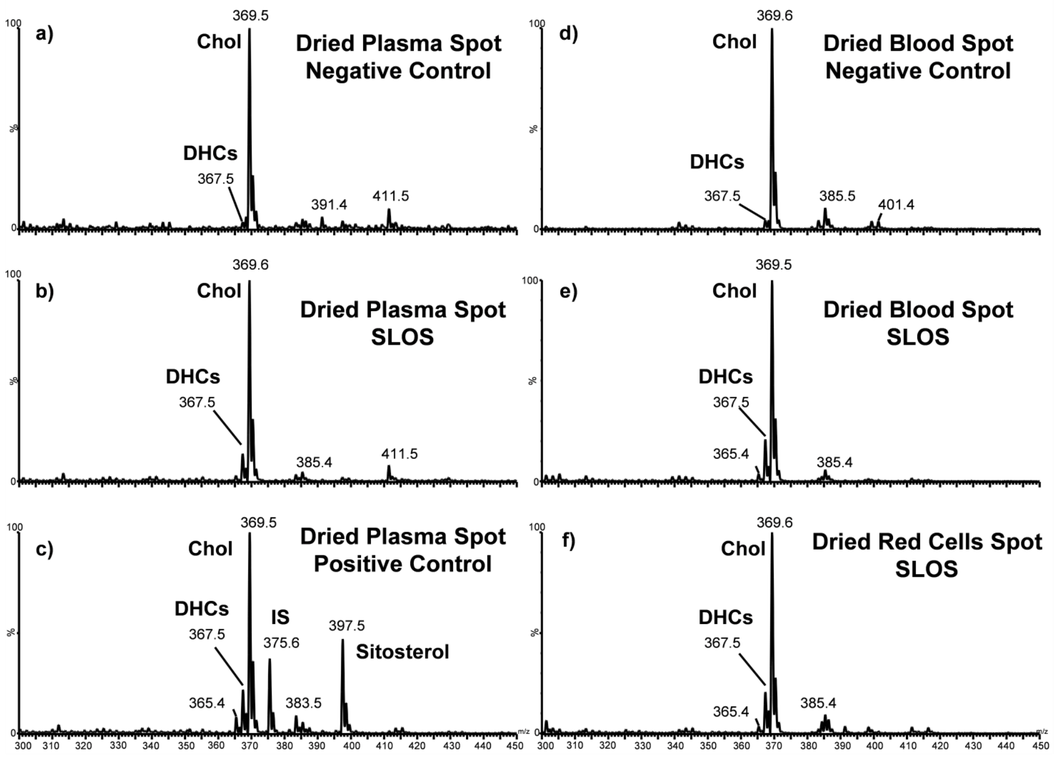

Figure 4. APTDCI-MS qualitative response of diagnostic sterols in plasma, washed red cells and whole blood of normal and SLOS patients spotted on paper.
In SLOS samples the signal of DHCs is increased compared to Chol signal (20%), while in control DHCs is less than 2%. Therefore in SLOS patients is noticed an increase of DHCs/Chol ratio which could improve the diagnostic sensitivity.
Panel c depicts a spectrum obtained from a plasma spot enriched with DHCs (367 *m/z*), Internal standard d6-Chol (375 *m/z*) and sitosterol (397 *m/z*).

	DHCs/Chol[a]	
	Range (APTDCI/MS)	Range (GC/FID)
Normal (n=23)	0.01 - 0.17	0 - 0.002
SLOS (n=9)	0.26 - 1.59	0.07 - 0.71

[a] Data referred to dried plasma spots

Table 3. Normal and SLOS DHCs/Chol ratio ranges by APTDCI-MS and GC/FID.
Ranges are not overlapped; this allow us to distinguish affected patients from normal subjects.
APTDCI-MS DHCs/Chol ratio is influenced by the baseline noise and the dynamic range of GC-FID is narrower than of MS method.

Table 2. Comparison between DHCs/Chol ion intensity ratio of two SLOS patients by APTDCI-MS and GC/FID.
Patient #1 provides similar APTDCI-MS and GC/FID ratios while the APTDCI-MS ratios of patient #2 are lower than GC/FID ratios.

		DHCs/Chol [a]			
	Matrix	APTDCI-MS	CV %	GC/FID	CV %
SLOS sample (#1)	Dried Blood Spot	0,22	10	0,28	10
	Dried Red Cells Spot	0,19	8	0,27	4
	Dried Plasma Spot	0,20	7	0,26	10
SLOS sample (#2)	Dried Blood Spot	0,31	2	0,63	10
	Dried Red Cells Spot	0,36	10	0,72	9
	Dried Plasma Spot	0,53	2	0,81	5

[a] The ratio DHCs/Chol was calculated using the signal intensity (n=3) of the ions obtained from the spectra

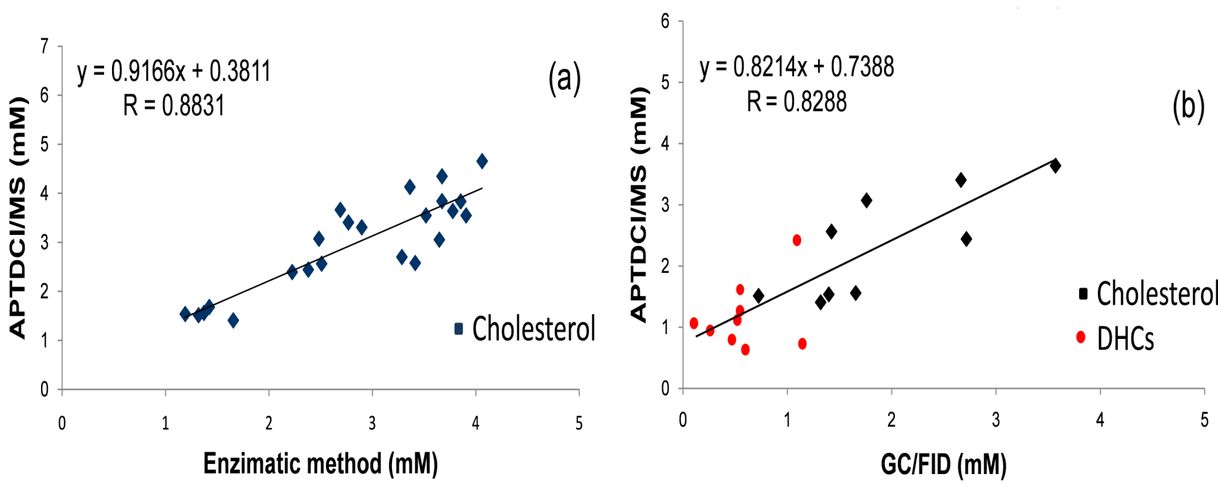

Figure 5. Methods comparison.
(a) Chol concentrations from normal plasma samples (n=23) by APTDCI-MS compared to the enzymatic method show a significant linear correlation. The high baseline noise of full scan spectra by APTDCI-MS influences positively the concentrations. (b) Chol and DHCs concentrations from SLOS plasma samples (n=9) by APTDCI-MS compared to GC/FID. Although the number of data pairs are few, the correlation is significant and the value of intercept is positive.

DISCUSSION. The proposed APTDCI-MS method is an ADI technique able to ionize more lipophilic compounds. It is a potentially useful tool to screen SLOS by determining the DHCs and Chol levels and the DHCs/Chol ratio in proband patients.
In addition the method is able to detect other metabolic disorders of sterols such as sitosterolemia.
The quantification of Chol is well correlated with the enzymatic method then it is able to screen population for hypercholesterolemia using dried blood spot.

CONCLUSION. Here is proposed for the first time a method by which sterols are quickly analysed directly on dried plasma/blood spot, without sample preparation, providing qualitative and quantitative results acceptable for the screening of SLOS in less than 3 minutes.

References

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