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Rapid LC-MS/MS Assay for CER1, an Essential Omega-O-acylceramide in Primary Human Keratinocytes

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PURPOSE

Ceramides (CERs) are essential components of stratum corneum and critical to the barrier and permeability properties of human skin. Recent studies have demonstrated that the ceramide expression is closely related with atopic dermatitis, Stargardt disease-3, and other pathologies. CER1 is a critical very long-chain Omega- O-acylceramide and is often used as a marker molecule during dermatology studies.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has become the main workhorse in endogenous biomarker analysis characterized by structural specificity, sensitivity, and quantitative precision. The purpose of the current study is to develop an LC-MS/MS assay and sample extraction procedure for the quantification of CER1 and Cholesterol in primary human keratinocytes as an efficacy model for skin disease treatment.

OBJECTIVES

- Develop an LC-MS/MS assay for the quantification of CER1 and Cholesterol in primary human keratinocytes.
- Evaluate Cholesterol as an endogenous reference for relative composition of CER-1 in the cell membrane.

METHOD

A 0.2 mL aliquot of primary human keratinocyte suspension was extracted with 0.5 ml chloroform and 1 ml methanol. After vortexing and centrifugation, the aqueous layer was further extracted by 1.4 ml chloroform and 1 ml methanol. Two organic layers were combined and then washed with 3 ml 0.88% KCl and 3 ml water, sequentially. The extract was evaporated under nitrogen and reconstituted with methanol for LC-MS/MS analysis.

The stable isotope labeled CER1 (d18:1/26:0/18:1(d9)) and Cholesterol-d7 were used as internal standards and were added into cell samples prior the extraction. The reconstituted lipid fraction was separated using an ACQUITY UPLC BEH Amide 1.7 µm, 50 × 1.0 mm column. The extracted CER1 and Cholesterol were quantified by monitoring m/z 959.1> 264.1 and 369.6>161.1 on a Thermo Scientific TSQ Vantage triple quadruple mass spectrometer equipped with Thermo Ultimate 3000 UHPLC system. The LC-MS/MS system was operated in positive ion mode using a Thermo Ion Max APCI Probe. During initial CER1 determination, the UHPLC mobile phases consisted of 0.05% Formic Acid in acetonitrile (A) and 200 mM ammonium formate at pH=3 (B). The flow rate is 0.3 mL/min. To simultaneously quantify CER1 and Cholesterol, the UHPLC mobile phases consisted of 5 mM ammonium formate in 95% acetonitrile (A) and ammonium formate in water (B). The flow rate is 0.4 mL/min.

RESULTS

Scheme 1: Molecular structure of CER1

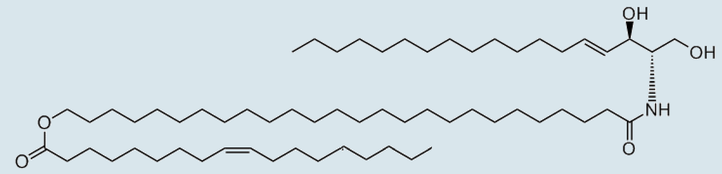


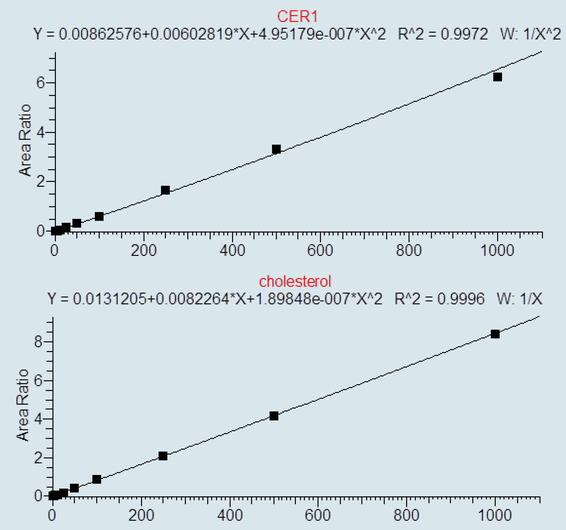
Table 1: Accuracy and reproducibility of CER 1 LCMSMS assay

Conc. (ng/mL)	QC Samples	Calculated Conc. (ng/ml)	% Error	%RSD
4.17	Replicate 1	4.498	7.87%	6.43%
	Replicate 2	3.898	-6.52%	
	Replicate 3	3.864	-7.35%	
	Replicate 4	3.956	-5.13%	
	Replicate 5	3.995	-4.20%	
417	Replicate 1	420.887	0.93%	1.84%
	Replicate 2	431.306	3.43%	
	Replicate 3	412.358	-1.11%	
	Replicate 4	426.876	2.37%	
	Replicate 5	430.062	3.13%	
4170	Replicate 1	4083.161	-2.08%	2.36%
	Replicate 2	4083.820	-2.07%	
	Replicate 3	4241.632	1.72%	
	Replicate 4	4294.065	2.98%	
	Replicate 5	4244.073	1.78%	

Table 2: CER 1 concentrations in cell samples with different treatments

Conc. (ng/mL)	Peak Area Ratio		Conc. in Cell Suspension (ng/ml)
	Average 3 injections	%RSD	
A1	0.000761	8.9%	14.3
A2	0.000888	9.1%	18.6
A3	0.000998	3.6%	22.3
B1	0.000921	3.7%	19.7
B2	0.000901	4.2%	19.0
B3	0.000859	6.7%	17.6
C1	0.001248	2.9%	31.0
C2	0.001077	1.5%	25.0
C3	0.000853	8.2%	17.4
D1	0.000808	3.6%	15.9
D2	0.000901	4.1%	19.0
D3	0.000797	7.1%	15.5

Figure 1: Graphic representation of calibration curve for simultaneously quantifying CER1 and Cholesterol between 1 to 1,000 ng/mL



CONCLUSION

Calibration curve was established for concentrations across a wide range of CER1 and Cholesterol, with a coefficient of determination (R2) of 0.99. The expression of CER1 level in primary human keratinocytes under different treatments was analyzed.

REFERENCES

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