

OVERVIEW

Purpose

- Characterization of different chemical constituents in a Quinoa seed extract.
- Development of a chemical identification and/or quantification assay of linoleic acid as a characteristic marker in a Quinoa seed extract for quality control purpose.

Method

- 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine (PC) and Linoleic acid (LA) were spiked into a Quinoa seed extract.
- PC extraction method :
 - Solid phase extraction (SPE-HLB)
 - Nominal PC calibration range: 20 to 800 ng/ml
- LA extraction :
 - Liquid-liquid extraction with Ethyl-acetate in acid pH condition
 - Nominal LA calibration range: 1 to 50 µg/ml
- LDTD-APCI-MS/MS analysis: Laser Diode Thermal Desorption coupled with triple quadrupole mass spectrometer.

INTRODUCTION

Quinoa seed extract is used as an active ingredient in cosmetics. In addition to the identification of the plant material (Quinoa seeds) by qualified and trained personnel, samples of the active ingredient (Quinoa seed extract) must also be tested to confirm the identity of the plant material used. Two chemical constituents were characterized in this Quinoa seed extract: 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine (one of the main phospholipids in Quinoa seed extract; PC; Figure 2) and linoleic acid (LA; Figure 3). A method was then developed to identify and/or quantify linoleic acid as a characteristic marker in this Quinoa seed extract.

INSTRUMENTATION

Instrumentation (Figure 1)

- LDTD model T-960, Phytronix Technologies.
- TSQ® Vantage, ThermoFisher Scientific.



Figure 1 LDTD-MS/MS analytical system.

MS Parameters

	PC	LA
Mode	APCI (+)	APCI (-)
Collision gas	1.5 mTorr (Argon)	1.5 mTorr (Argon)
Collision energy	25 V	40 V
S-lens	165 V	100 V
Scan time	0.020 s	0.020 s
Needle current	3 µA	3 µA
Q1 width	0.70 amu	0.70 amu
Q3 width	0.70 amu	0.70 amu
SRM transition	599-> 263 amu	279-> 279amu
SRM transition(IS)	603-> 267 amu PC (18:0/18:2)	288-> 288 amu Octadecanoic-d5 Acid



Figure 2 PC structure (MW = 782.08 g/mol)



Figure 3 LA structure (MW = 280.45 g/mol)

LDTD Parameters

	PC	LA
Laser power pattern	0 to 55 % in 2.0 s	0 to 35 % in 2.0 s
Carrier gas flow	3.0 L/min (Air)	3.0 L/min (Air)

METHOD (Extraction)

Standard/QC Preparation

- Standard / QC preparation
 - 100 µL working solution in MeOH
 - 1000 µL of Quinoa seed extract solution (Blank extract)
 - Vortex 1 min.
- QC in seed extract matrix
 - 100 µL working solution in MeOH
 - 1000 µL of Quinoa seed extract solution
 - Vortex 1 min.

Sample Preparation for PC

- Solid phase extraction (Oasis, HLB, 1cc)
 - Activation: 1 mL Methanol and 1 mL Water by gravity
 - Loading: 1 mL Water + 200 µL STD/QC + 20 µL IS
 - Wash 1: 2 X 1 mL Water (centrifuge, 3000 rpm, 2 min)
 - Wash 2: 1 mL Water:Methanol (1:1) (centrifuge, 14000 g, 2 min)
 - Elution: 1 mL Methanol: Chloroform (2:1) (centrifuge, 14000 g, 2 min)
 - Evaporate to dryness
 - Reconstitute with 100 µL Stearic acid solution (4 mg/ml in Ethyl acetate)
 - Transfer Manually 2.0 µL of reconstitute solution onto LazWell™
 - Evaporate solvent at room temperature
 - Perform LDTD-MS/MS analysis

Sample Preparation for LA

- Liquid-Liquid extraction
 - 50 µL STD or QC in eppendorf tube (1.5 ml)
 - 100 µL of HCl solution (1 N)
 - Vortex 1 min.
 - 150 µL Ethyl acetate (Blank) or IS solution (5 µg/ml in Ethyl acetate)
 - Vortex 1 min and centrifuge 5 min at 14000 g.
 - Mix 25 µL of organic phase + 25 µL Methanol + 25 µL EDTA solution*
 - Transfer 2.0 µL onto LazWell™
 - Evaporate solvent at room temperature
 - Perform LDTD-MS/MS analysis
- *EDTA solution: MeOH/NH4OH/ EDTA (9mg/ml in water), (1/1/1)

RESULTS for PC

Calibration Curve

The calibration curve for PC was evaluated over a nominal range of 20 to 800 ng/ml (Figure 4) with a R² > 0.99 (Table 1).

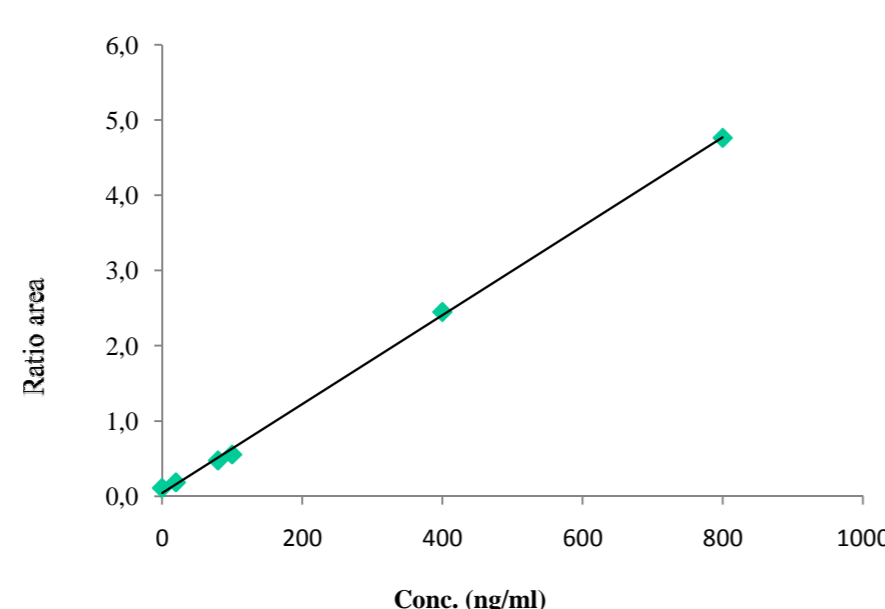


Figure 4 Calibration curve for PC

Table 1 Calibration curve parameters.

	PC
R ²	0.9994
Slope (ratio area / concentration)	0.005854
y-Abciss	0.04515

Weighting factor 1/x

Within-run Accuracy and Precision

The within-run accuracy and precision were evaluated on two different QC's at 100 ng/mL : a spiked extraction blank (QC-B), and a spiked Quinoa seed extract (QC-M). The LDTD shown an excellent accuracy of 94 % to 102.0 % and a precision < 15% (Table 2).

Table 2 Within-run accuracy and precision for PC.

	QC-B*	QC-M**
Nominal conc. (ng/ml)	100	100
N	3	3
Mean (ng/ml)	101.6	94.9
RSD (%)	15.0	10.7
%Nominal conc.	101.6	94.9

* Spiking in Blank matrix
** Spiking in plant extract (Quinoa extract)

Bench top stability

The bench top stability of spiked solution of PC in blank extraction solution (QC-B) and in Quinoa seed extract (QC-M) were evaluated. The results show that after 72h at room temperature and at 4°C more than 67 % of the PC have been degraded (Table 3).

Table 3 Bench top stability result for PC

Stability conditions	QC-B*		QC-M**	
	72h - RT	72h - 4°C	72h - RT	72h - 4°C
Nominal conc. (ng/ml)	100	100	100	100
Stability concentration (ng/ml)	< 20	32.3	< 20	32.6
%Difference	NA	(-67.7)	NA	(-67.4)

* Spiking in Blank matrix
** Spiking in plant extract (Quinoa extract)

PC Degradation Product

We have investigated into which compound PC undergo degradation. Q1-scans were performed in APCI(-) and (+). No degradation product was observed in the APCI(+) scan. However, we found a new peak at 279.15 m/z (Figure 5). This compound might be associate to the presence of Linoleic acid (MW=280.45) which is part of the PC structure (Figure 2).

Therefore, we propose to use the produced Linoleic acid (the PC degradation product) to develop a method for the quality control of the Quinoa seed extract production.

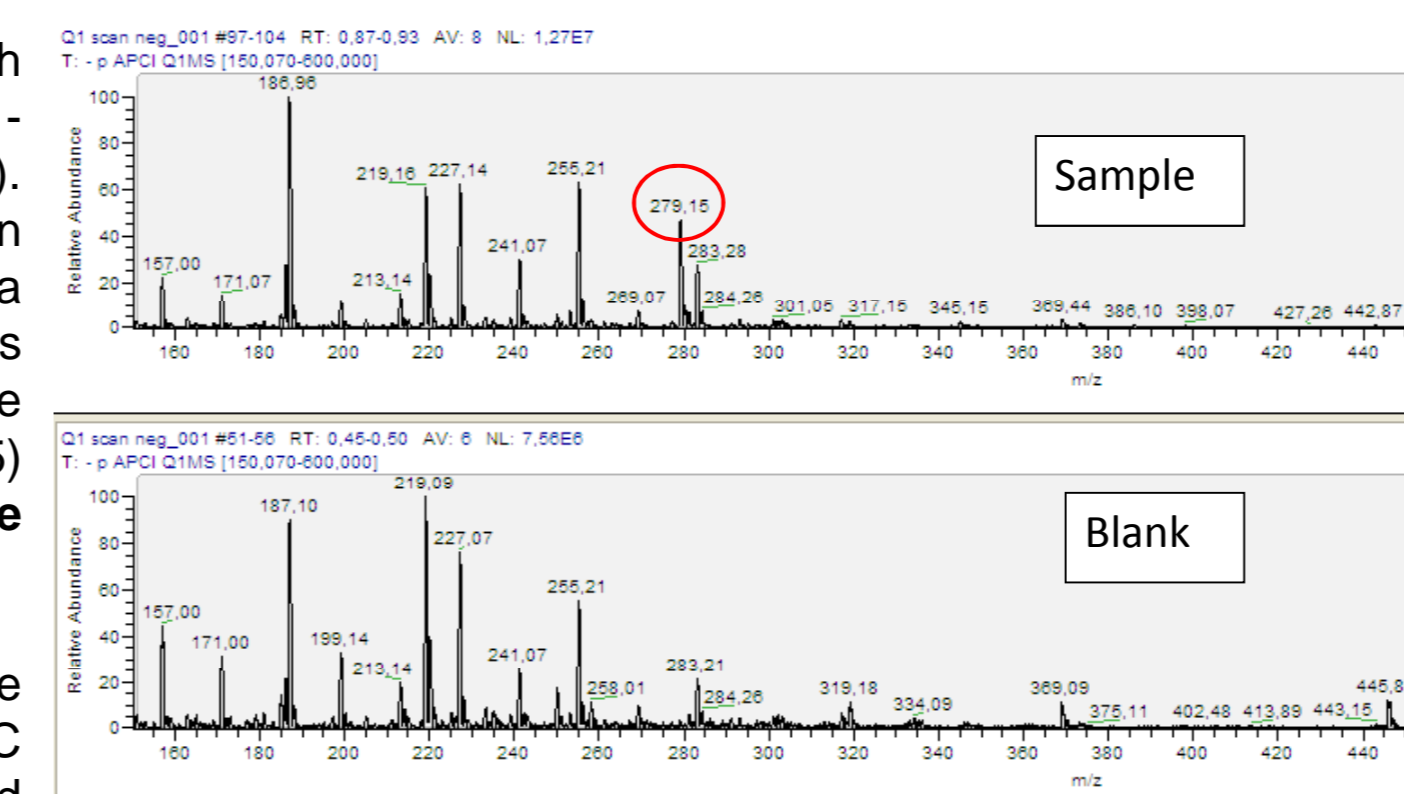


Figure 5 Q1-scan in negative APCI mode of PC degradation product

RESULTS for LA

Calibration Curve for LA

The calibration curve for LA was evaluated over a nominal range of 1 to 50 µg/ml (Figure 6) with a R² > 0.99 (Table 4).

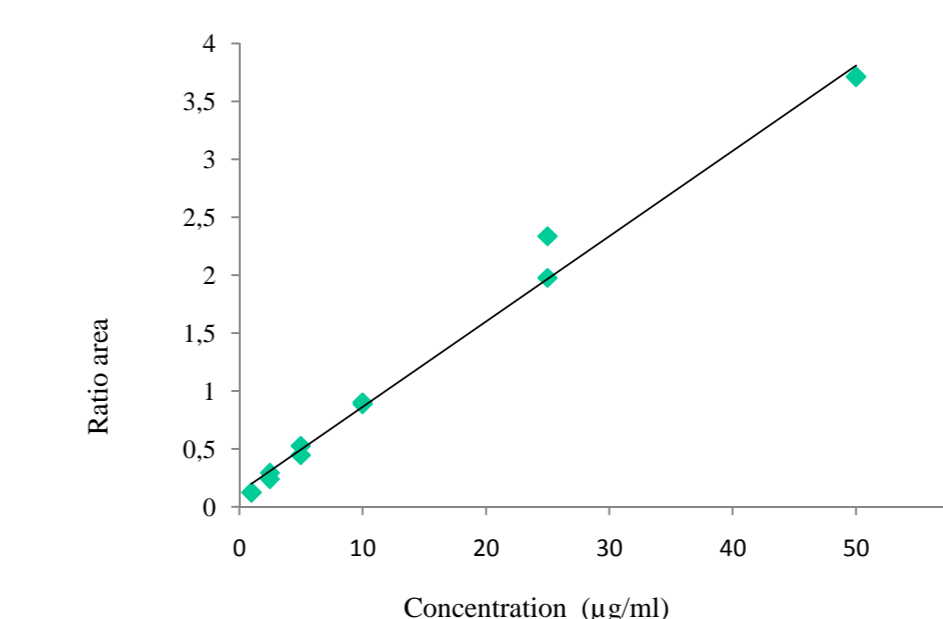


Figure 6 Calibration curve for LA

Table 4 Calibration curve parameters

	LA
R ²	0,9910
Slope (ratio area / concentration)	0,0776
y-Abciss	0,0636

Weighting factor 1/x

Within-run Accuracy and Precision

The within-run accuracy and precision were evaluated on two different QC's at 100 ng/mL : a spiked extraction blank (QC-B), and a Quinoa seed extract (QC-M). The LDTD shown an excellent accuracy of 99 % to 109% and a precision < 15% (Table 5).

Table 5 Within-run accuracy and precision for LA.

	QC-B*	QC-M**
Nominal conc. (µg/ml)	5	8,95
N	2	2
Mean (µg/ml)	5.5	8.9
RSD (%)	13.4	0.7
%Nominal conc.	109.0	99.2

* Spiking in Blank matrix
** Spiking in plant extract (Quinoa extract)

Bench top stability

The bench top stability of spiked solution of LA in blank extraction solution (QC-B) and in Quinoa seed extract (QC-M) was verified. In both matrix, good stability was obtain after six days at room temperature or at 4°C (Table 6).

Table 6 Bench top stability result for LA

Stability conditions	QC-B*		QC-M**	
	6 days - RT	6 days - 4°C	6 days - RT	6 days - 4°C
Nominal conc. (µg/ml)	5	5	8.95	8.95
Stability concentration (µg/ml)	5.0	5.2	7.8	8.1
%Difference	0.4	3.4	-12.6	-9.6

* Spiking in Blank matrix
** Spiking in plant extract (Quinoa extract)

CONCLUSION

A fast, precise and reproducible method was developed for the identification and/or quantification of Linoleic acid as a characteristic marker in a Quinoa seed extract. Such method can be used for quality control purpose to confirm the identity of the plant material used to manufacture cosmetic ingredient.