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Nota applicativa

Quantitative Analysis of Phosphatidylethanolamine and Phosphatidylcholine from Rice Oil Lecithin and Sunflower Oil Lecithin by ACQUITY UPLC H-Class Plus System with PDA Detection

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Abstract

In this application note, we have developed a 15 minutes method for quantitative analysis of PE and PC on the ACQUITY UPLC H-Class Plus System with a PDA Detector.

Benefits

Quantification of PE and PC in rice and sunflower oil lecithin within 15 minutes run time on the ACQUITY UPLC H-Class Plus System with a PDA Detector.

Introduction

Phospholipids are major constituents of cell membrane and are found in all tissues and subcellular compartments as mixtures of various molecular species such as phosphatidylcholine (PC),

phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (SM), and lysophosphatidylcholine (LPC) depending on the type of polar head groups and the degree of unsaturation of the acyl chains. Among these phospholipids, PC and PE represents a major constituent of cell membranes. The demand for lecithin with high PC and PE content from vegetable or cereal source is increasing these days, particularly in pharmaceutical, cosmetic, food, and other applications due to their emulsifying properties and nonantigenic nature. The application of lecithins in pharmaceutical and cosmetics domain depends mainly on the PC and PE with its saturated or unsaturated fatty acid content.

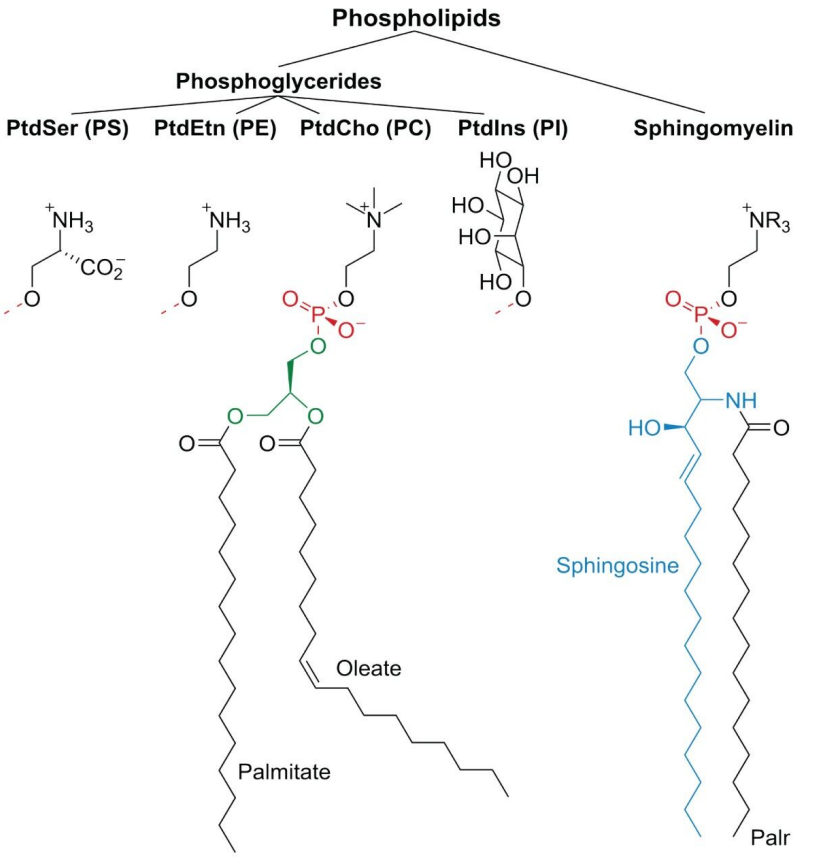

Figure 1. Classification of phospholipids.

The present method of UltraPerformance Liquid Chromatography (UPLC) with UV detection offers advantages of high speed, resolution and simplicity for the separation and detection of phospholipids including phosphatidylcholine and phosphatidylethanolamine from rice and sunflower oil lecithin. The ultraviolet/visible spectrometer coupled with the UPLC system for the phospholipids detection has greater sensitivity over refractive index or flame-ionization detection. However, the UV detection restricts the use of common chromatographic solvents that are not transparent in 200 nm to 210 nm regions wherein

phospholipids have tendency of absorbing the light energy. The Reverse Phase (RP) UPLC-UV system however can successfully engage solvents such as acetonitrile, ethanol, methanol, iso-propanol, and water. The significance of UV detection is that it has multiple choices of compositions of mobile phase to advance the isocratic or gradient elution. The available reverse phase methods are complex, laborious, and time-consuming, while this UPLC method is simple and short.

In this application note, we have developed a 15 minutes method for quantitative analysis of PE and PC on the ACQUITY UPLC H-Class Plus System with a PDA Detector.

Experimental

LC method parameters

Instrument:	ACQUITY UPLC H-Class Plus System with PDA Detector
Column:	ACQUITY UPLC BEH HILIC 1.7 µm, 100 mm × 2.1 mm
Flow rate:	0.2 mL/min
Mobile phase A:	10 mM ammonium formate and 0.05% ammonia solution in water
Mobile phase B:	Acetonitrile with 0.05% ammonia solution
Column temp.:	80 °C
Sample temp.:	15 °C
Injection volume:	10 µL

Sample concentration:	100 µg/mL for PE and 10 µg/mL for PC
Wash and purge solvent:	20:80 (Water:IPA)
Seal wash:	9:1 (water:methanol)
Diluent:	1:1 (chloroform:IPA)
PDA fixed wavelength:	205 nm
Data acquisition rate:	2 pts/sec

Figure 2. ACQUITY UPLC H-Class Plus System with an ACQUITY PDA Detector.

Time (min)	Flow rate (mL/min)	Mobile phase %A	Mobile phase %B
Initial	0.200	5.00	95.0
1.00	0.200	5.00	95.0
6.00	0.200	15.0	85.0
10.0	0.200	15.0	85.0
10.1	0.200	80.0	20.0
10.5	0.200	80.0	20.0
10.6	0.200	5.00	95.0
15.0	0.200	5.00	95.0

Table 1. Gradient program.

Standard and sample details		
Sr. no.	**Sample details**	**Code**
1	Sunflower Oil Lecithin	SFOL
2	Sunflower Oil Lecithin Enriched	SFOLE
3	Rice Oil Lecithin	ROL
4	Phosphatidylcholine Standard (75% pure)	PC
5	Phosphatidylethanolamine standard 10 mg/mL (97% pure)	PE

Table 2. Standard and sample details.

Standard solution preparation

Accurately weighed 100 mg of PC standard (75% pure) and dissolved in 10 mL of diluent as a standard stock solution I of 10,000 µg/mL concentration.

Working standard was prepared by mixing 100 µL of standard stock solution I and 100 µL of PE standard labelled as 10 mg/mL (97% pure) and making up the volume to 1000 µL to make concentration 1000 µg/mL of each component mix.

Further dilutions for linearity were prepared by appropriate dilutions from the standard mix.

Sample preparation

10,000 µg/mL stock solutions were prepared by weighing 100 mg of each sample and dissolved in 100 mL of diluent. Two separate concentrations were prepared by further diluting the stock solution to 100 µg/mL and 10 µg/mL for PE and PC analysis respectively.

Results and Discussion

Due to the huge difference in the concentration of PE and PC present in oil lecithin samples, separate sample dilutions were prepared for PE and PC. 1000 µg/mL stock solution of samples were diluted to 100 µg/mL (10 times) and 10 µg/mL (100 times) for PE and PC respectively and quantified against the calibration curve plotted from 1 ppm to 25 ppm.

Sr. no.	Sample details* (mg/mL)		
	SFOL	SFOLE	ROL
PE	0.0299	0.0327	0.0123
PC	0.1731	0.1573	0.0816

Table 3. Sample analysis results.
**All concentrations reported are with respect to 1000 ppm sample concentration and adjusted by dilution factor.*

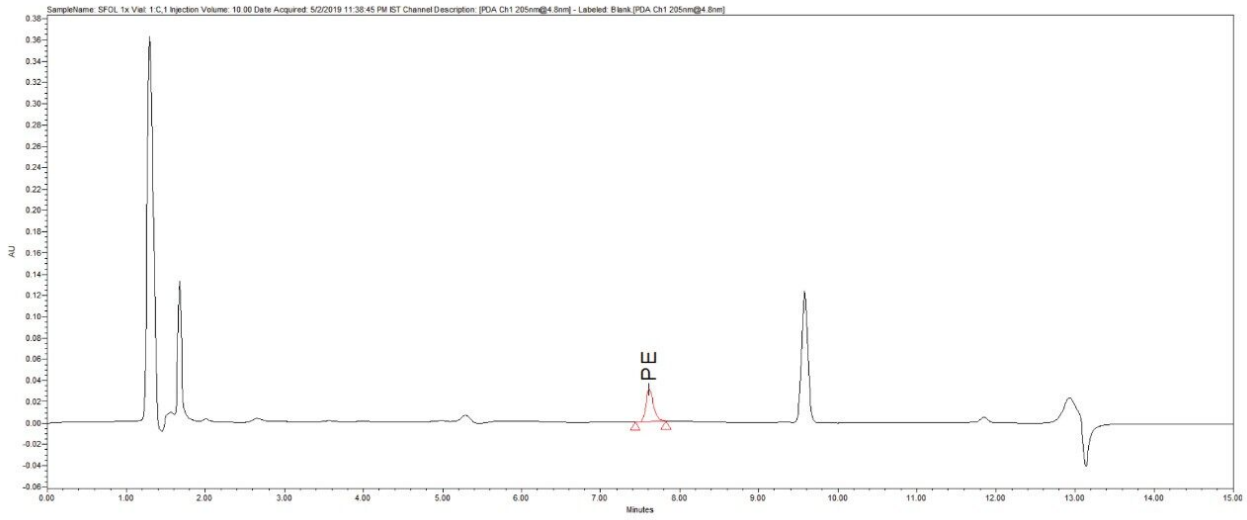

Figure 3. SFOL sample chromatogram for PE.

Component summary table Name: PE								
	Sample name	**Vial**	**Inj**	**Channel**	**Name**	**RT**	**Area**	**Amount**
1	SFOL 1x	1:C,1	1	PDACh1 205 mm @ 4.8 nm	PE	7.613	210871	29.580
2	SFOL 1x	1:C,1	2	PDACh1 205 mm @ 4.8 nm	PE	7.586	215209	30.218
3	SFOL 1x	1:C,1	3	PDACh1 205 mm @ 4.8 nm	PE	7.631	213200	29.922
Mean						7.610	213093.386	
Std. dev.						0.023	2171.024	
%RSD						0.3	1.0	

Table 4. Three replicate injection result table for PE in SFOL.

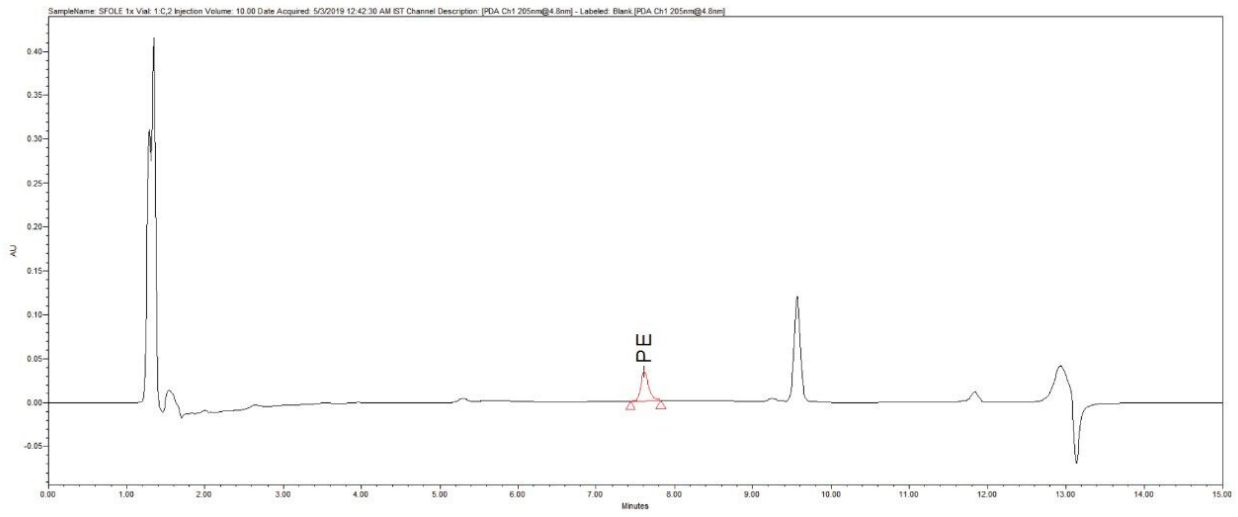

Figure 4. SFOLE sample chromatogram for PE.

Component summary table Name: PE								
	Sample name	Vial	Inj	Channel	Name	RT	Area	Amount
1	SFOLE 1x	1:C,2	1	PDACh1 205 mm @ 4.8 nm	PE	7.622	229566	32.331
2	SFOLE 1x	1:C,2	2	PDACh1 205 mm @ 4.8 nm	PE	7.611	234381	33.040
3	SFOLE 1x	1:C,2	3	PDACh1 205 mm @ 4.8 nm	PE	7.610	213714	32.647
Mean						7.614	231886.964	
Std. dev.						0.007	2412.121	
%RSD						0.1	1.0	

Table 5. Three replicate injection result table for PE in SFOLE.

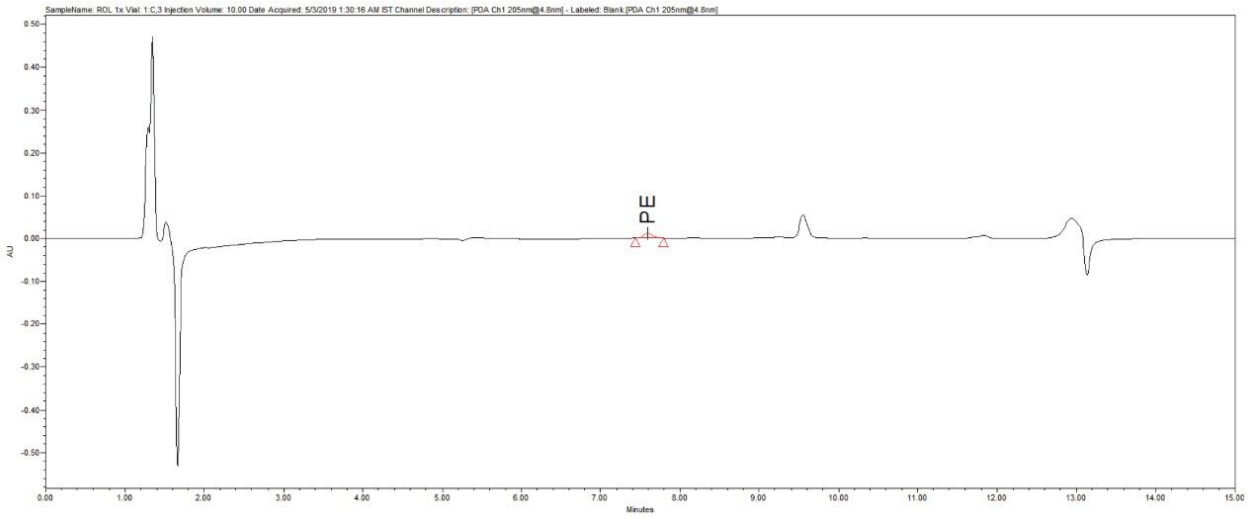

Figure 5. ROL sample chromatogram for PE.

Component summary table Name: PE								
	Sample name	Vial	Inj	Channel	Name	RT	Area	Amount
1	ROL 1x	1:C,3	1	PDACh1 205 mm @ 4.8 nm	PE	7.593	92425	12.146
2	ROL 1x	1:C,3	2	PDACh1 205 mm @ 4.8 nm	PE	7.593	94059	12.387
3	ROL 1x	1:C,3	3	PDACh1 205 mm @ 4.8 nm	PE	7.557	94384	12.435
Mean						7.581	93622.787	
Std. dev.						0.021	1050.311	
%RSD						0.3	1.1	

Table 6. Three replicate injection result table for PE in ROL.

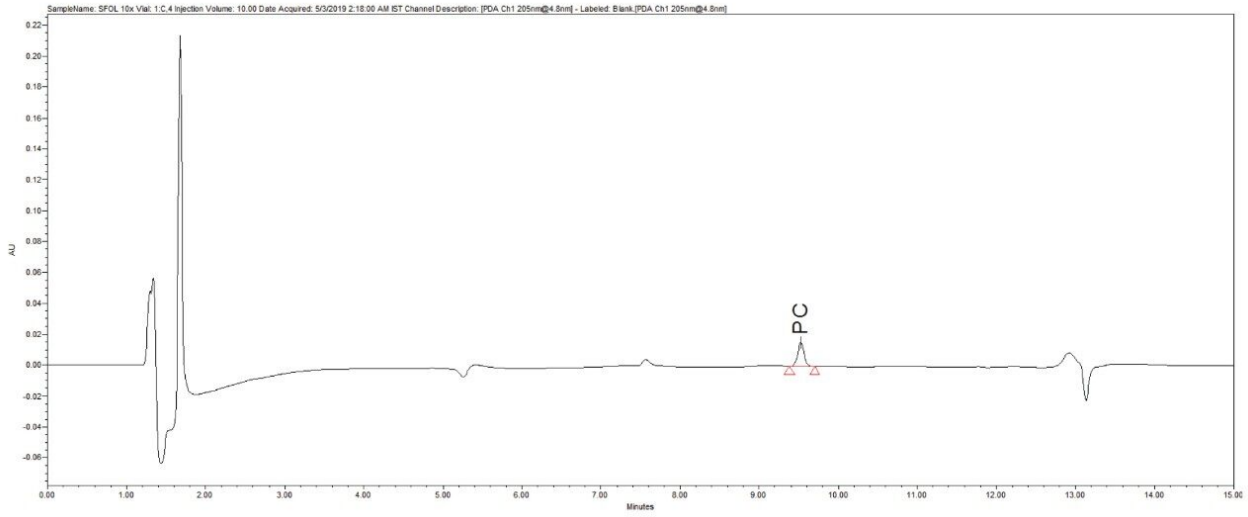

Figure 6. SFOL sample chromatogram for PC.

Component summary table Name: PC								
	Sample name	Vial	Inj	Channel	Name	RT	Area	Amount
1	SFOL 10x	1:C,4	1	PDACh1 205 mm @ 4.8 nm	PC	9.529	82969	171.343
2	SFOL 10x	1:C,4	2	PDACh1 205 mm @ 4.8 nm	PC	9.520	84079	173.640
3	SFOL 10x	1:C,4	3	PDACh1 205 mm @ 4.8 nm	PC	9.535	84350	174.200
Mean						9.528	83799.390	173.1
Std. dev.						0.008	731.609	1.5
%RSD						0.1	0.9	0.9

Table 7. Three replicate injection result table for PC in SFOL.

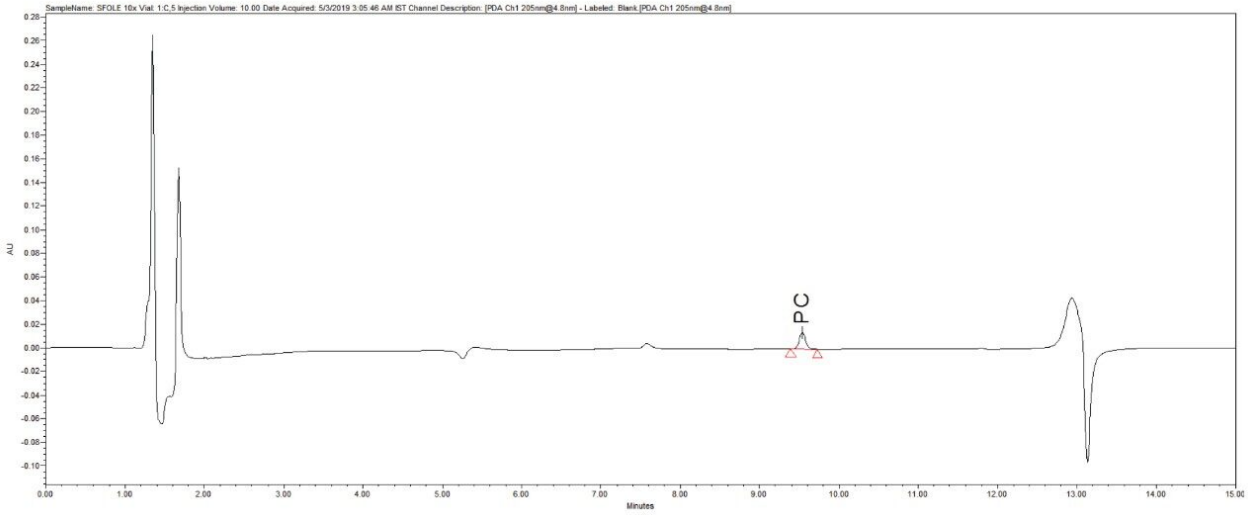

Figure 7. SFOLE sample chromatogram for PC.

Component summary table Name: PC								
	Sample name	Vial	Inj	Channel	Name	RT	Area	Amount
1	SFOLE 10x	1:C,5	1	PDACh1 205 mm @ 4.8 nm	PC	9.541	75752	156.406
2	SFOLE 10x	1:C,5	2	PDACh1 205 mm @ 4.8 nm	PC	9.533	76646	158.256
3	SFOLE 10x	1:C,5	3	PDACh1 205 mm @ 4.8 nm	PC	9.532	76202	157.337
Mean						9.535	76199.850	157.3
Std. dev.						0.005	447.072	0.9
%RSD						0.1	0.6	0.6

Table 8. Three replicate injection result table for PC in SFOLE.

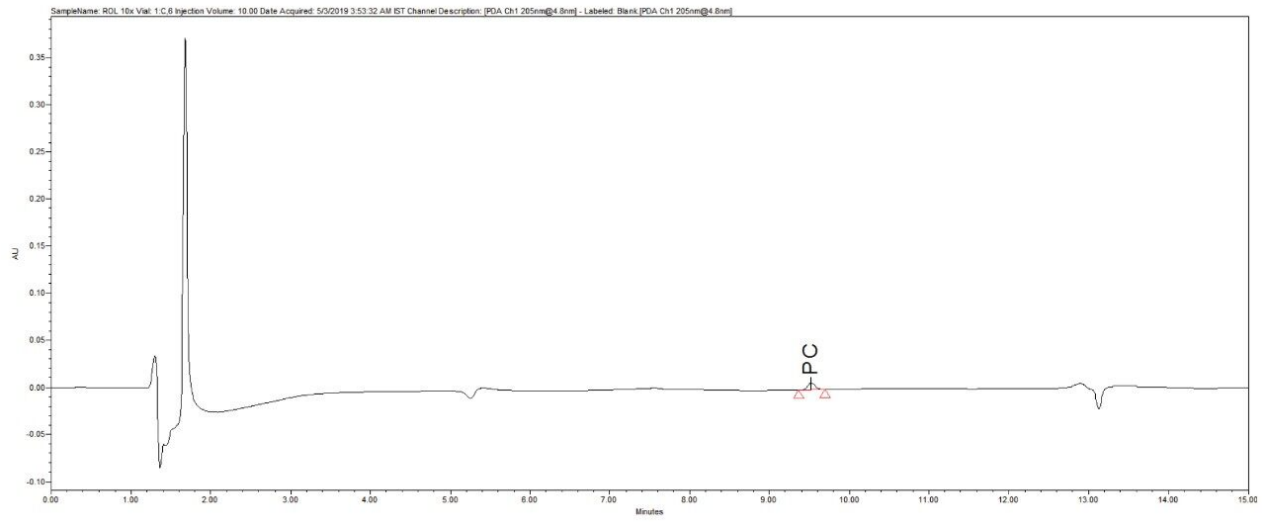

Figure 8. ROL sample chromatogram for PC.

Component summary table Name: PC								
	Sample name	Vial	Inj	Channel	Name	RT	Area	Amount
1	ROL 10x	1:C,6	1	PDACh1 205 mm @ 4.8 nm	PC	9.506	39663	81.509
2	ROL 10x	1:C,6	2	PDACh1 205 mm @ 4.8 nm	PC	9.509	39626	81.431
3	ROL 10x	1:C,6	3	PDACh1 205 mm @ 4.8 nm	PC	9.504	39799	81.996
Mean						9.506	39629.156	81.6
Std. dev.						0.003	148.025	0.3
%RSD						0.0	0.4	0.4

Table 9. Three replicate injection result table for PC in ROL.

Reproducibility

Reproducibility test was performed with 1 µg/mL (LOQ) concentration for PE and PC. %RSD of area observed in six replicate injections for both the components were within the limit.

Component summary table Name: PC							
	Sample name	Vial	Inj	Channel	Name	RT	Area
1	1 PPM	1:A,2	1	PDACh1 205 mm @ 4.8 nm	PC	9.569	38271
2	1 PPM	1:A,2	2	PDACh1 205 mm @ 4.8 nm	PC	9.586	36831
3	1 PPM	1:A,2	3	PDACh1 205 mm @ 4.8 nm	PC	9.567	37480
4	1 PPM	1:A,2	4	PDACh1 205 mm @ 4.8 nm	PC	9.591	38775
5	1 PPM	1:A,2	5	PDACh1 205 mm @ 4.8 nm	PC	9.574	38741
6	1 PPM	1:A,2	6	PDACh1 205 mm @ 4.8 nm	PC	9.557	36371
Mean						9.574	37744.797
Std. dev.						0.013	1012.182
%RSD						0.1	2.7

Table 10. Six replicate injection result table for PC LOQ.

Component summary table Name: PE							
	Sample name	Vial	Inj	Channel	Name	RT	Area
1	1 PPM	1:A,2	1	PDACh1 205 mm @ 4.8 nm	PE	7.621	78104
2	1 PPM	1:A,2	2	PDACh1 205 mm @ 4.8 nm	PE	7.611	74747
3	1 PPM	1:A,2	3	PDACh1 205 mm @ 4.8 nm	PE	7.606	77502
4	1 PPM	1:A,2	4	PDACh1 205 mm @ 4.8 nm	PE	7.621	76714
5	1 PPM	1:A,2	5	PDACh1 205 mm @ 4.8 nm	PE	7.610	74628
6	1 PPM	1:A,2	6	PDACh1 205 mm @ 4.8 nm	PE	7.587	78613
Mean						7.609	76718.071
Std. dev.						0.013	1695.895
%RSD						0.2	2.2

Table 11. Six replicate injection result table for PE LOQ.

LOQ for PE and PC

1 µg/mL (LOQ) concentration of the standard mix was injected and observed that the signal-to-noise ratio value is 30 for PE peak and 17 for PC peak.

Name	RT (min)	S/N
PE	7.596	30
PC	9.560	17

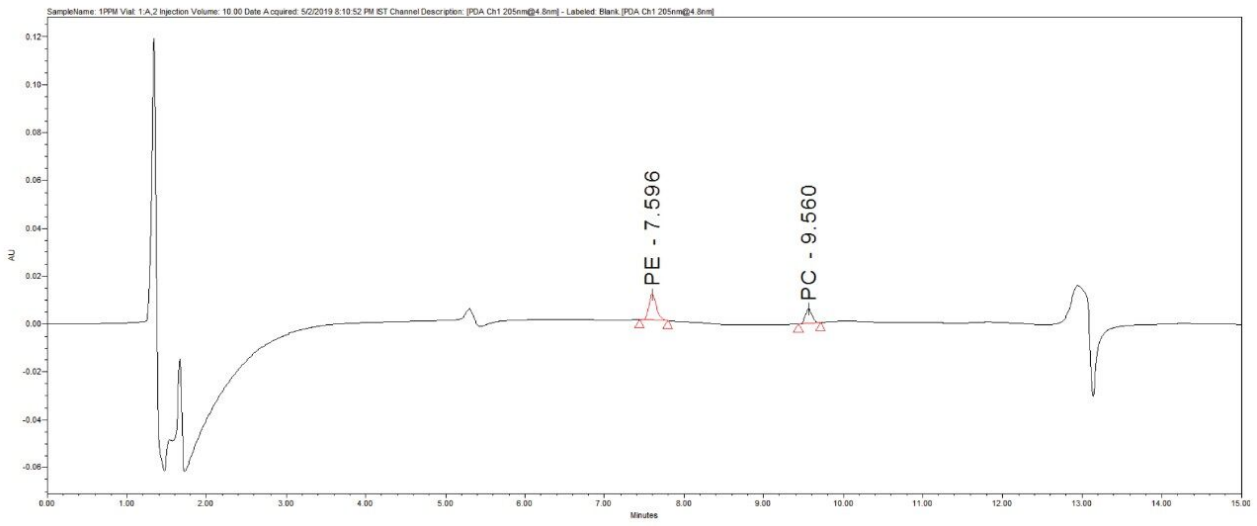

Figure 9. LOQ chromatogram PE and PC standard mix at 205 nm.

Recovery study for PE and PC

Recovery study was performed for PE and PC by injecting 2, 10, and 15 µg/mL concentration of standard mix in neat solution and spiked 2, 10, and 15 µg/mL of PE and PC standard mix with final concentration of three samples and observed the recovery.

$$\textbf{\% Recovery} = \frac{(\text{Component peak area in spiked solution} - \text{Component peak area in sample})}{\text{Area in std}} \times 100$$

% Recovery			
Matrix	**2 ppm**	**10 ppm**	**15 ppm**
SFOL	108.8	101.2	104.4
SFOLE	99.2	105.3	102.6
ROL	108.1	107.9	107.4

Table 12. % Recovery for PE in three matrices at three different concentrations.

% Recovery			
Matrix	2 ppm	10 ppm	15 ppm
SFOL	99.6	106.9	107.8
SFOLE	100.6	107.9	107.6
ROL	104.3	96.1	107.9

Table 13. % Recovery for PC in three matrices at three different concentrations.

Linearity for PE and PC

Prepared Linearity solutions of PE (97% pure) from 1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL solutions and PC (75% pure) standard with different concentrations of 0.75 µg/mL, 1.5 µg/mL, 3.75 µg/mL, 7.5 µg/mL, 11.25 µg/mL, 15 µg/mL, and 18.75 µg/mL solutions and plotted calibration curve.

	Calibration curve properties	
	Linearity range	R^2
PE	1 ppm–25 ppm	0.999183
PC	1 ppm–25 ppm	0.998547

Table 14. Calibration curve properties.

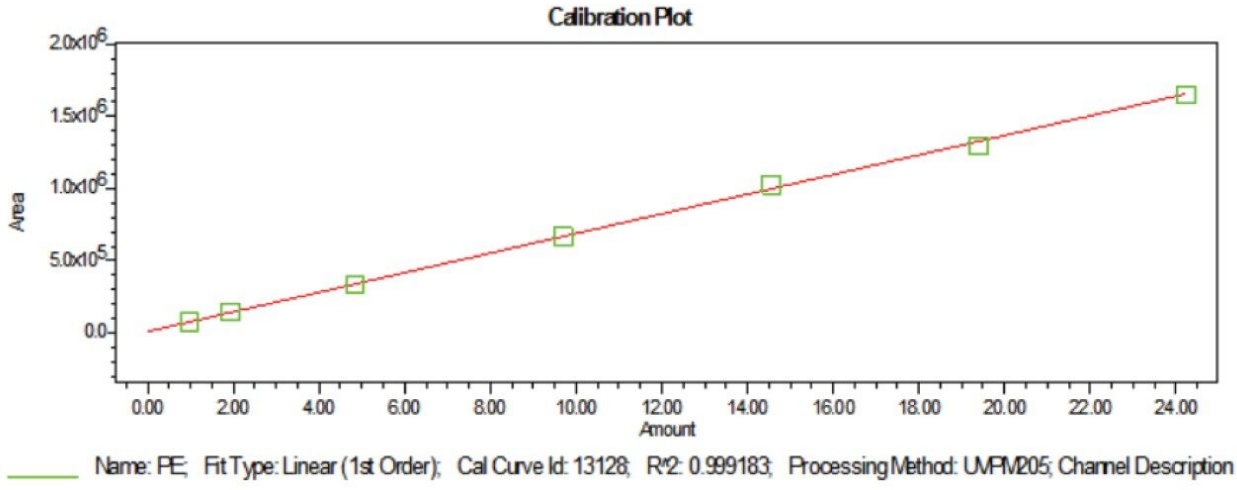

Figure 10. Calibration curve for PE.

Peak PE							
	Name	Area (µVsec)	X Value	Calc value	% Deviation	Manual	Ignore
1	PE	74817	0.970000	0.955464	-1.499	No	No
2	PE	146586	1.940000	2.011776	3.700	No	No
3	PE	333192	4.850000	4.758327	-1.890	No	No
4	PE	667756	9.70000	9.682566	-0.180	No	No
5	PE	1025902	14.550000	14.953889	2.776	No	No
6	PE	1296176	19.40000	18.931882	-2.413	No	No
7	PE	1649357	24.250000	24.130142	-0.494	No	No

Table 15. Calibration curve table for PE.

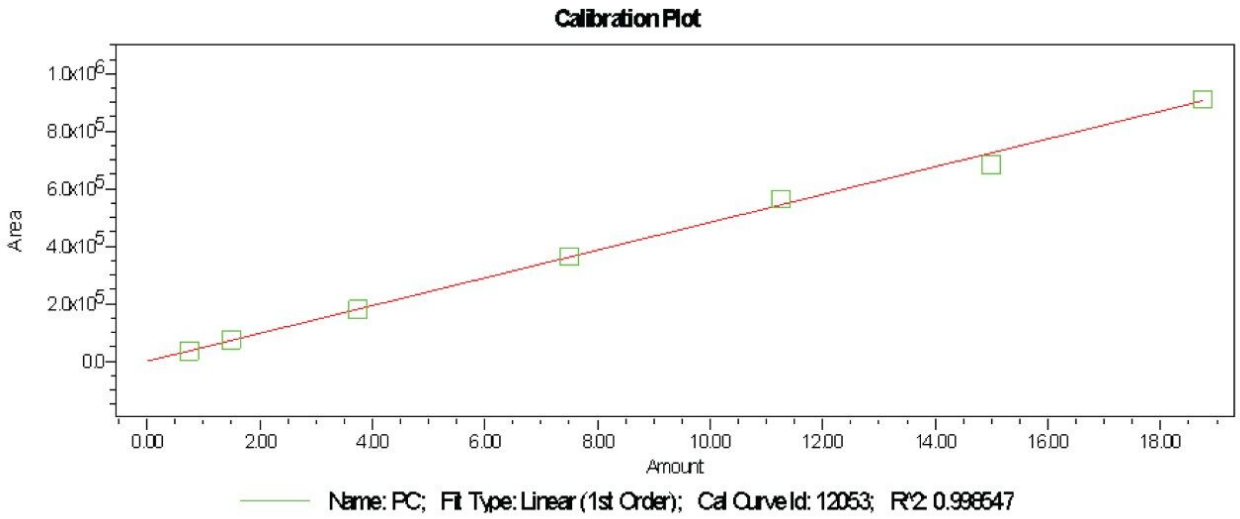

Figure 11. Calibration curve for PC.

Peak PC							
	Name	Area (µVsec)	X Value	Calc value	% Deviation	Manual	Ignore
1	PC	35897	0.750000	0.739215	-1.438	No	No
2	PC	74830	1.500000	1.544967	2.999	No	No
3	PC	108675	3.750000	3.735560	-0.385	No	No
4	PC	363996	7.500000	7.529621	0.395	No	No
5	PC	563597	11.250000	11.660606	3.650	No	No
6	PC	682565	15.000000	14.122779	-5.848	No	No
7	PC	911826	18.750000	18.867631	0.627	No	No

Table 16. Calibration curve table for PC.

Conclusion

- Quantitative analysis of Phosphatidylethanolamine (PE) and Phosphatidylcholine (PC) was performed using ACQUITY UPLC H- Class Plus with PDA Detector at wavelength 205 nm.
- UV Lowest Limit of Quantification has been set at 1ppm in neat solution (without considering standard purity) for PE and PC standards.
- Due to the huge difference in the concentrations of PE and PC, separate sample dilutions were used for quantifying both compounds.
- The reproducibility, accuracy, and recovery has been observed within the accepted limits.
- All the samples have been analyzed and the concentrations are reported.

UPLC technology coupled with UV detection provides a unique solution for quantitative analysis of phosphatidylethanolamine and phosphatidylcholine from rice and sunflower oil lecithin.

Acknowledgement

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