Quantitative analysis of four long-chain lysophosphatidylcholines in dried blood spots using Liquid Chromatography Tandem Mass Spectrometry for clinical research

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INTRODUCTION

Historically, very long chain fatty acids have been quantified using direct transesterification and gas chromatography methods which involve time-consuming and labor-intensive sample preparation and analysis. Consequently, simpler LC-MS/MS methods have been reported for the measurement of C26:0 among other lysophosphatidylcholines (LPCs) in dried blood spots (DBS). Interferences have been reported using positive electrospray ionisation (ESI) and so researchers have developed methodologies utilising negative ESI to circumvent isobaric interferences when there is lack of chromatographic resolution. Thus, we describe a UHPLC-MS/MS method using acetate adducts for the reliable analysis of four long-chain LPCs in DBS for clinical research.

METHODS

Materials:
C20:0-LPC-d4, C22:0-LPC-d4 and C24:0-LPC-d4 were purchased from Cambridge Isotope Laboratories, whereas C26:0-LPC-d4 was supplied by Avanti Polar Lipids. LC-MS/MS grade reagents, methanol and acetonitrile were purchased from Honeywell, isopropanol from Merck and formic acid from Fisher Scientific. Deionized water was used for the preparation of LC washes and mobile phases.

DBS Samples:
DBS material from different lot numbers was kindly supplied by the Center of Disease Control (CDC). Lot 2110 acted as calibrators and Lot 2215 were used as quality controls. Linearity was tested on Lot 2019.

Assay procedure:
A single 3.2mm punch from each DBS material was placed into a 96-well plate. The internal standard extraction working solution containing all four LPCs was prepared to a final concentration of 0.04 µmol/L, respectively. 100 µL of this extraction solution were added to the DBS samples and incubated for 30 minutes at room temperature with shaking at 500 rpm. The extract was removed and transferred to a clean 96-well and 5 µL was injected for analysis.

Instrumentation
All samples were analysed using the Xevo™ TQ-S micro MS (Figure 1) operated in negative ESI (see Table 1 for MRM transitions) and coupled to the ACQUITY™ UPLC™ I-Class PLUS System. Data was processed and reviewed on TargetLynx™ Software v4.2 SCN1045 and QUAN Review. The mobile phase composition consisted of a mixture of water, acetonitrile and isopropanol with ammonium acetate as a modifier. Chromatographic separation was achieved on a Waters ACQUITY Premier CSH™ C_{18} Column which provided baseline resolution of endogenous isobaric interferences for the saturated forms of the targeted LPCs.

Figure 1: Xevo TQ-S micro MS

Analyte	Parent (m/z)	Daughter (m/z)	Cone (V)	Collision (V)
C20:0-LPC (Quan)	610.6	311.1	20	35
C20:0-LPC (Qual)	610.6	536.2	20	20
C20:0-LPC-D4 (Quan)	614.4	315.1	20	35
C20:0-LPC-D4 (Qual)	614.4	540.5	20	20
C22:0-LPC (Quan)	638.5	339.2	20	35
C22:0-LPC (Qual)	638.5	564.5	20	20
C22:0-LPC-D4 (Quan)	642.5	343.3	20	35
C22:0-LPC-D4 (Qual)	642.5	568.5	20	20
C24:0-LPC (Quan)	666.4	367.4	20	35
C24:0-LPC (Qual)	666.4	592.5	20	20
C24:0-LPC-D4 (Quan)	670.7	371.4	20	35
C24:0-LPC-D4 (Qual)	670.7	596.4	20	20
C26:0-LPC (Quan)	694.5	395.5	20	35
C26:0-LPC (Qual)	694.5	620.5	20	20
C26:0-LPC-D4 (Quan)	698.5	399.5	20	35
C26:0-LPC-D4 (Qual)	698.5	624.5	20	20

Table 1: MRM parameters for the detection of C:20:0, C22:0, C24:0 and C26:0 LPCs .

RESULTS

Acetate adducts $[M+CH_3COO]^-$ were the most abundant parent ion masses for each LPC, generated under negative ESI using acetate as modifier in the mobile phase. These detection conditions combined with the chromatographic resolution and retention time comparison to a matched internal standard allowed unambiguous identification (Figure 2). The elution order depended on the length of the carbon chain starting with C20:0-LPC and ending with C26:0-LPC. The detection of the 2-acyl isomer could be postulated for the adjacent peak in each of the LPC chromatograms as these isomers may form readily in a pH dependent fashion. In addition, there might be a contribution on the presence of isomers attributed to the purity of the synthetic internal standards which was reported to be 95% for each LPC.

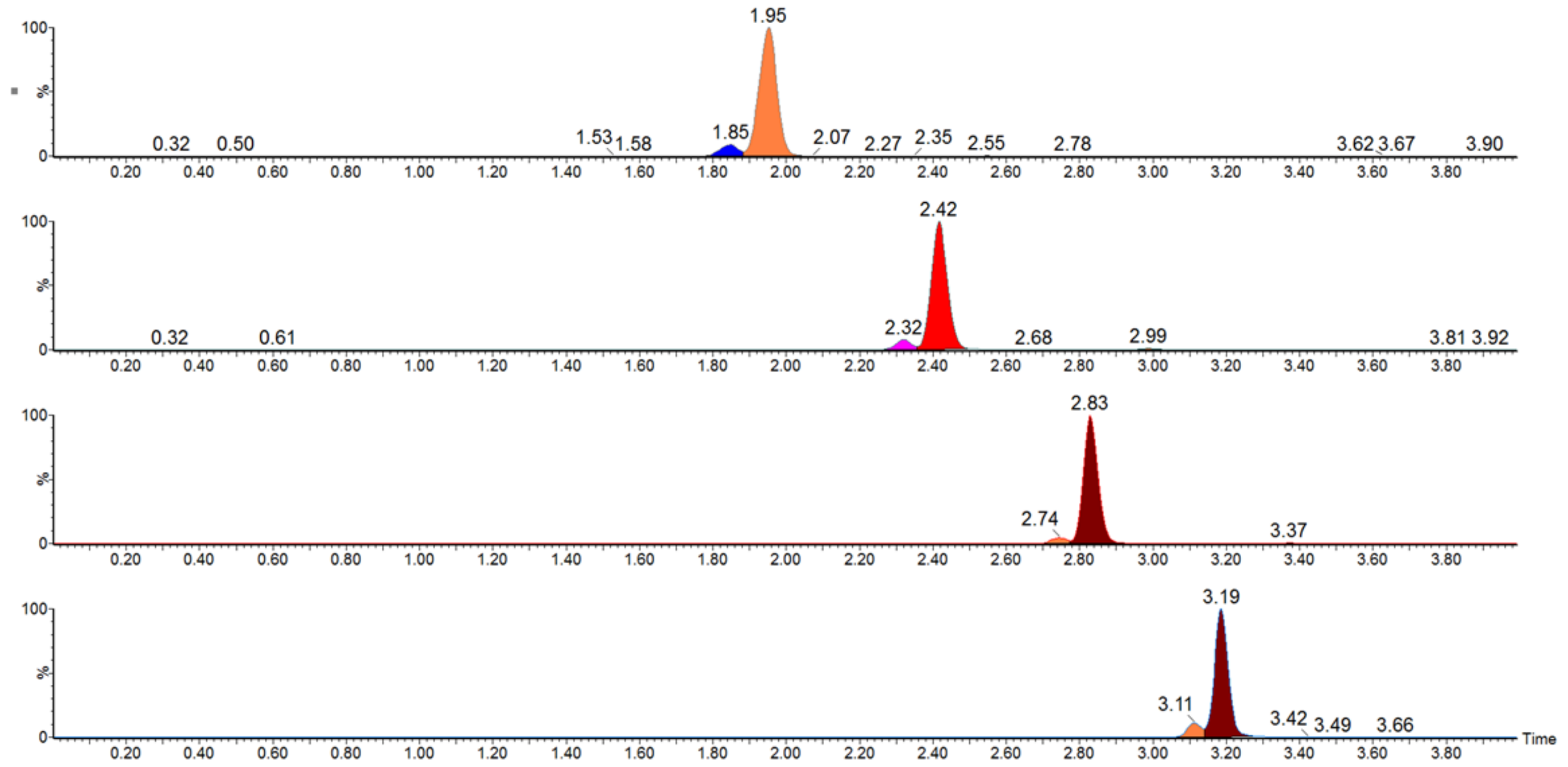

Figure 2: Representative chromatograms for C20:0-LPC eluting at 1.95 min, C22:0-LPC at 2.42 min, C24:0-LPC at 2.83 min and C26:0-LPC at 3.19 min.

Analytical validation parameters
Carryover, linearity, precision and bias of the LC-MS/MS method reported herein were evaluated. No significant carryover into blank samples was observed after analyzing alternate injections of the 2 µmol/L DBS samples. The clinical research method was shown to be linear from 0.1–10 µmol/L. However, calibration curves with $r^2 \geq 0.99$ across all five analytical runs were constructed using only 4 calibrator points (Figure 3).
Table 2 shows the precision determined over 5 days at three different concentrations and the bias for all three QC concentration levels with respect to their assigned values by CDC.

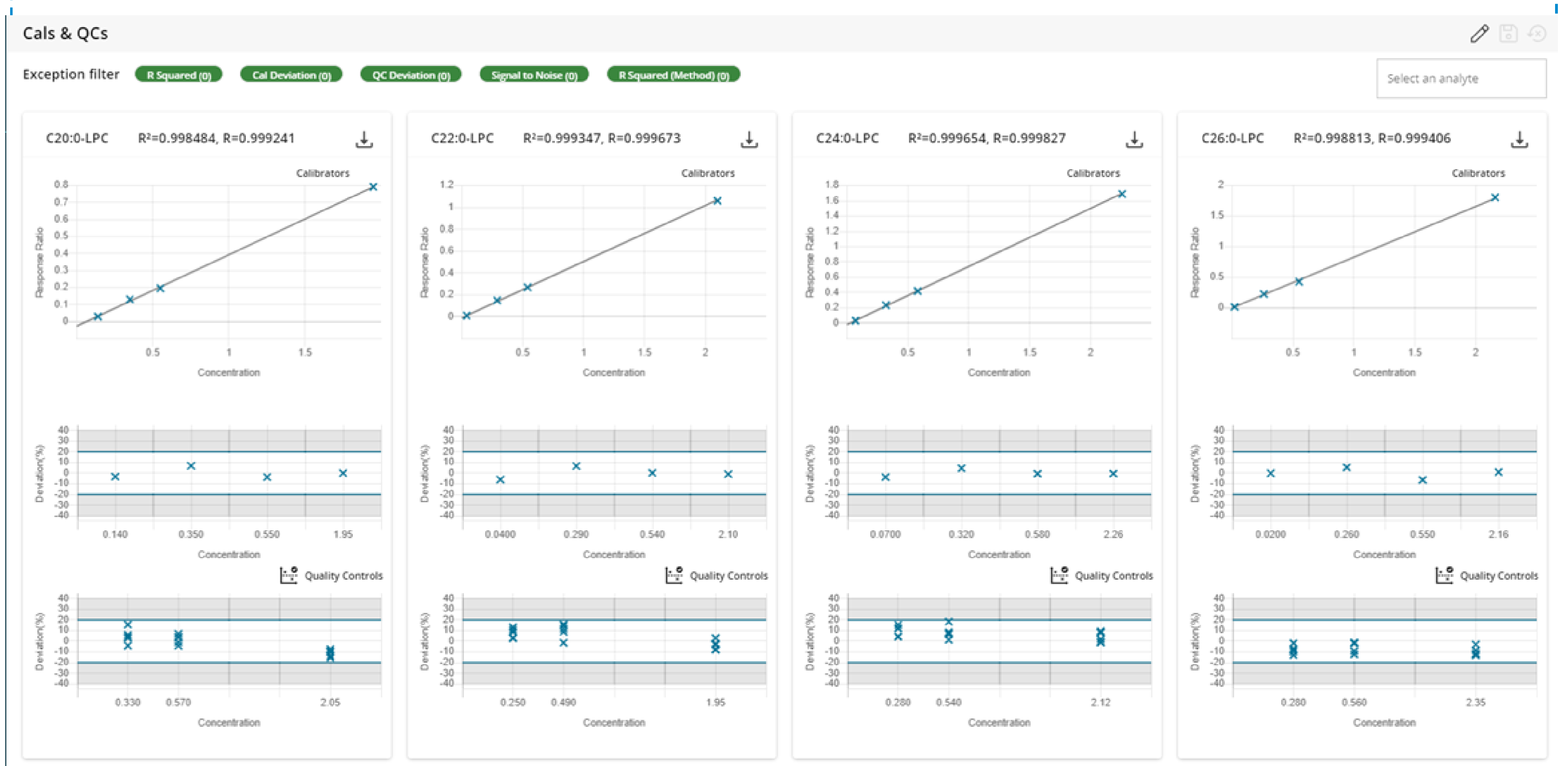

Figure 3: Example calibration curves for C20:0-LPC, C22:0-LPC, C24:0-LPC and C26:0-LPC.

	C20:0-LPC QC conc. (µmol/L)		
Assigned	0.33	0.57	2.05
Mean	0.33	0.59	1.91
% Bias	1.3%	4.1%	-6.6%
Precision			
Within day %CV	5.4	4.6	5.4
Total %CV	7.2	7.9	7.5

	C22:0-LPC QC conc. (µmol/L)		
Assigned	0.25	0.49	1.95
Mean	0.26	0.52	1.90
% Bias	3.2	6.5	-2.8
Precision			
Within day %CV	5.5	5.4	5.4
Total %CV	5.9	6.7	6.4

	C24:0-LPC QC conc. (µmol/L)		
Assigned	0.28	0.54	2.12
Mean	0.27	0.54	2.03
% Bias	-2.6	-0.1	-4.2
Precision			
Within day %CV	6.7	4.1	5.2
Total %CV	8.5	8.7	7.8

	C26:0-LPC QC conc. (µmol/L)		
Assigned	0.28	0.56	2.35
Mean	0.24	0.53	2.12
% Bias	-12.9	-5.9	-9.9
Precision			
Within day %CV	6.3	6.3	5.3
Total %CV	6.3	8.9	5.9

Table 2: %Bias and Precision (within-day and across five days) for C:20:0, C22:0, C24:0 and C26:0 LPCs.

CONCLUSION

Method performance characterization indicated no significant carryover from a concentration of 2 µM into a consecutive blank injection. Linearity was demonstrated over the range 0.1-10 µM, with a precision of ≤15% CV and bias within 20%.
This method offers a simple extraction procedure and reliable quantification of C20:0, C22:0, C24:0 and C26:0 LPCs in DBS for clinical research.

Note: ACQUITY, UPLC, Xevo, CSH, TargetLynx and Waters are trademarks owned by Waters Corporation.

KEY HIGHLIGHTS

- **This LC-MS/MS clinical research method for measuring C20:0, C22:0, C24:0 and C26:0 LPCs with the ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S micro Mass Spectrometer offers good precision, selectivity and analytical sensitivity in negative mode.**
- **This method can analyze 220 samples in 24 h.**

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