Scheduled data-dependent acquisition MS provides enhanced identification and sensitivity in clinical lipidomics

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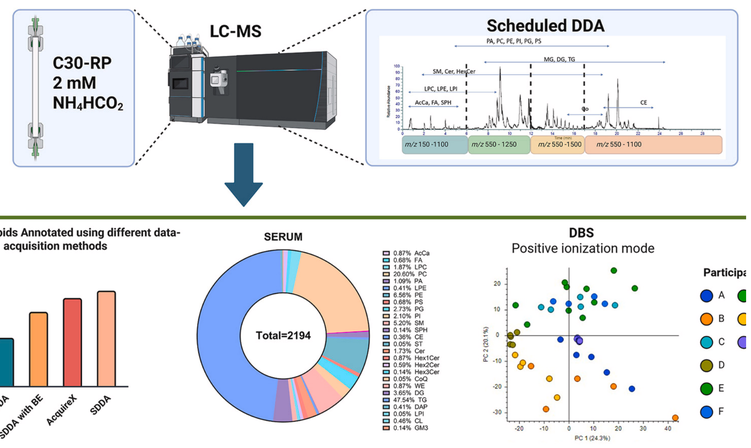
Introduction

Lipidomics can provide critical insight into metabolic changes in health and disease, but faces challenges in sensitivity, lipid coverage, and annotation accuracy. To address these limitations, we optimized a liquid chromatography–mass spectrometry (LC-MS) method combining scheduled data-dependent acquisition (SDDA) and C30 column-based separations, aimed at improving global lipidomics for clinical diagnostics.

Highlights

- Optimized ion source and LC improves sensitivity and isomer separation.
- SDDA doubles lipid coverage and annotation confidence over DDA.
- High repeatability across clinical matrices; serum, plasma, and DBS.



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Method

Sample preparation: To 30 µL of sample (serum, EDTA plasma, or heparin plasma), 90 µL of cold (4 °C) isopropanol (IPA) was added and vortexed for ~20 s. Samples were centrifuged (10 min, 21,100 RCF, 4 °C), and supernatants were transferred to HPLC vials with glass inserts. Global lipidomics was performed using a Vanquish Horizon UHPLC coupled to a Fusion Orbitrap Tribrid MS (Thermo Fisher Scientific)

Liquid Chromatography Settings: Lipids were separated on an Accucore C30 column (150 × 2.1 mm, 2.6 µm, 5% carbon load). Mobile phase A: 60% ACN with 2 mM ammonium formate. Mobile phase B: 9.5% ACN, 85.5% IPA with 2 mM ammonium formate. Flow rate: 0.4 mL/min; Injection volume: 2 µL. Preheater and column oven temperature: 55 °C. Total run time: 40 min.

Mass Spectrometry Acquisition Parameters: Data were acquired in positive and negative ionization modes (150–1500 m/z) using separate injections. Resolution was set to 120,000 (Full MS) and 30,000 (SDDA) at 200 m/z.Ion source settings: 3.8 kV (positive), 2.8 kV (negative); sheath gas: 40; auxiliary gas: 10; sweep gas: 2; S-Lens RF: 60; ion transfer tube: 350 °C; vaporizer: 400 °C. Stepped collision energies: 20/40/100 (positive), 40/60/130 (negative). Maximum injection time: 50 ms; isolation width: 3.0 m/z.Scheduled DDA m/z ranges: 0–6 min: 150–1100, 6–12 min: 550–1250, 12–17 min: 550–1500, 17–30 min: 550–1100. Regular DDA and AcquireX used a fixed range of 150–1500 m/z.

C30 separation allows for inter- and intra-class separation

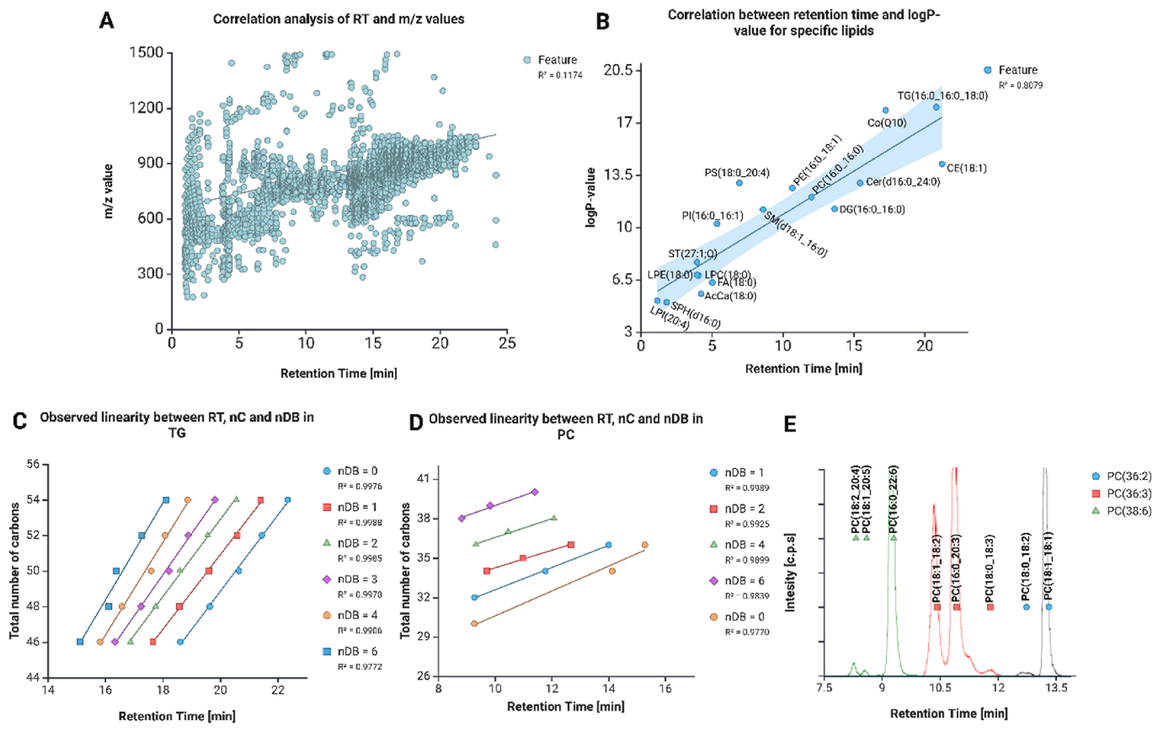

Fig. 1. C30 lipid separation. A) Correlation between *m/z* and retention time (minutes) for all detected features (R^2 = 0.1174). **B)** Correlation between logP-value and retention time (minutes) for selected lipids across different lipid subclasses (R^2 = 0.8097). **C)** Linearity observed between the total number of carbons on the fatty acid side chains, retention time (RT) in minutes and number of double bonds (nDB) for triacylglycerols (TG). **D)** Observed linearity between total number of carbons on the fatty acid side chains, retention time (RT) in minutes and number of double bonds (nDB) for phosphatidylcholines (PC). **E)** Chromatographic separation between isomers of PC(36:2), PC(36:3) and PC(38:6).

Scheduled DDA increased lipid coverage

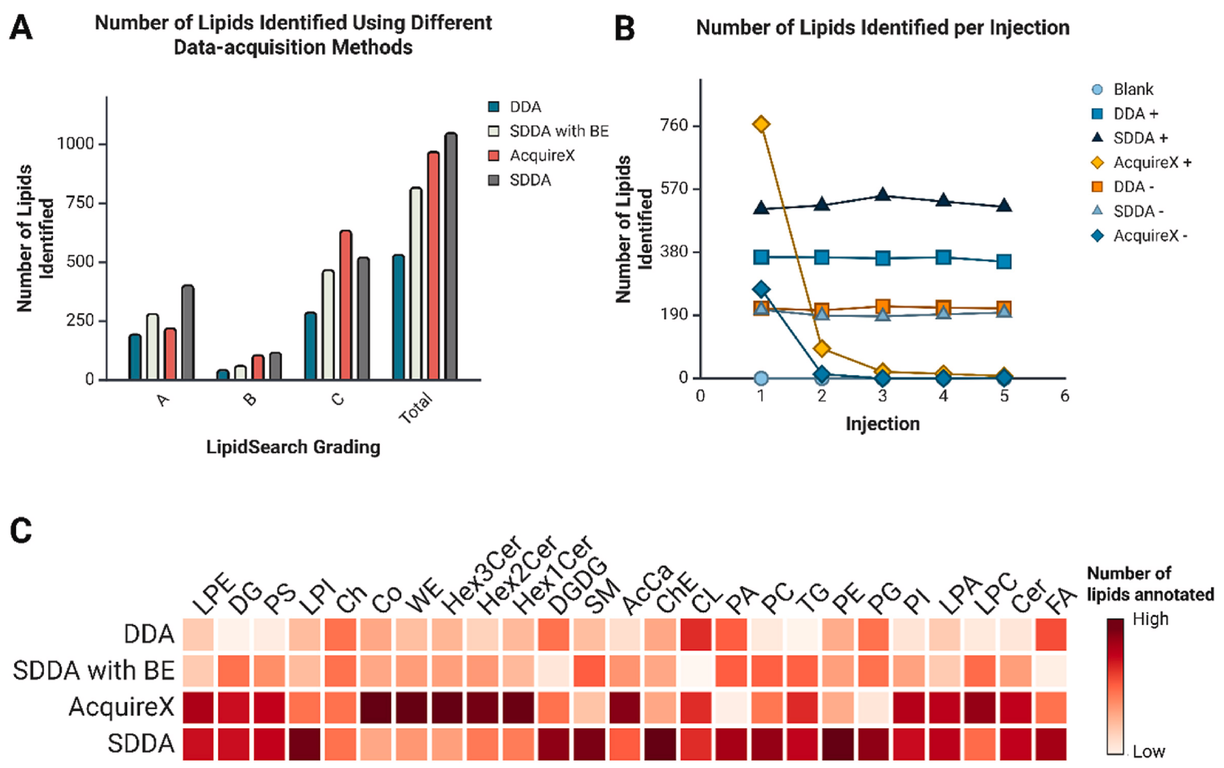

Fig. 2. Comparison of Lipid Identification Across Data Acquisition Methods. A) Number of identified lipids (LipidSearch grades A–C and total) using DDA, scheduled DDA (SDDA), SDDA with blank exclusion (SDDA + BE), and AcquireX deep scan. Five injections per method and ionization mode.**B)** Lipid identifications from single injections (total 5) across methods in both positive (+) and negative (–) ionization modes.**C)** Heatmap showing lipid subclass coverage across methods. Color intensity reflects normalized annotation count per subclass, highlighting relative performance per method.

Cholestryl ester separation and identification

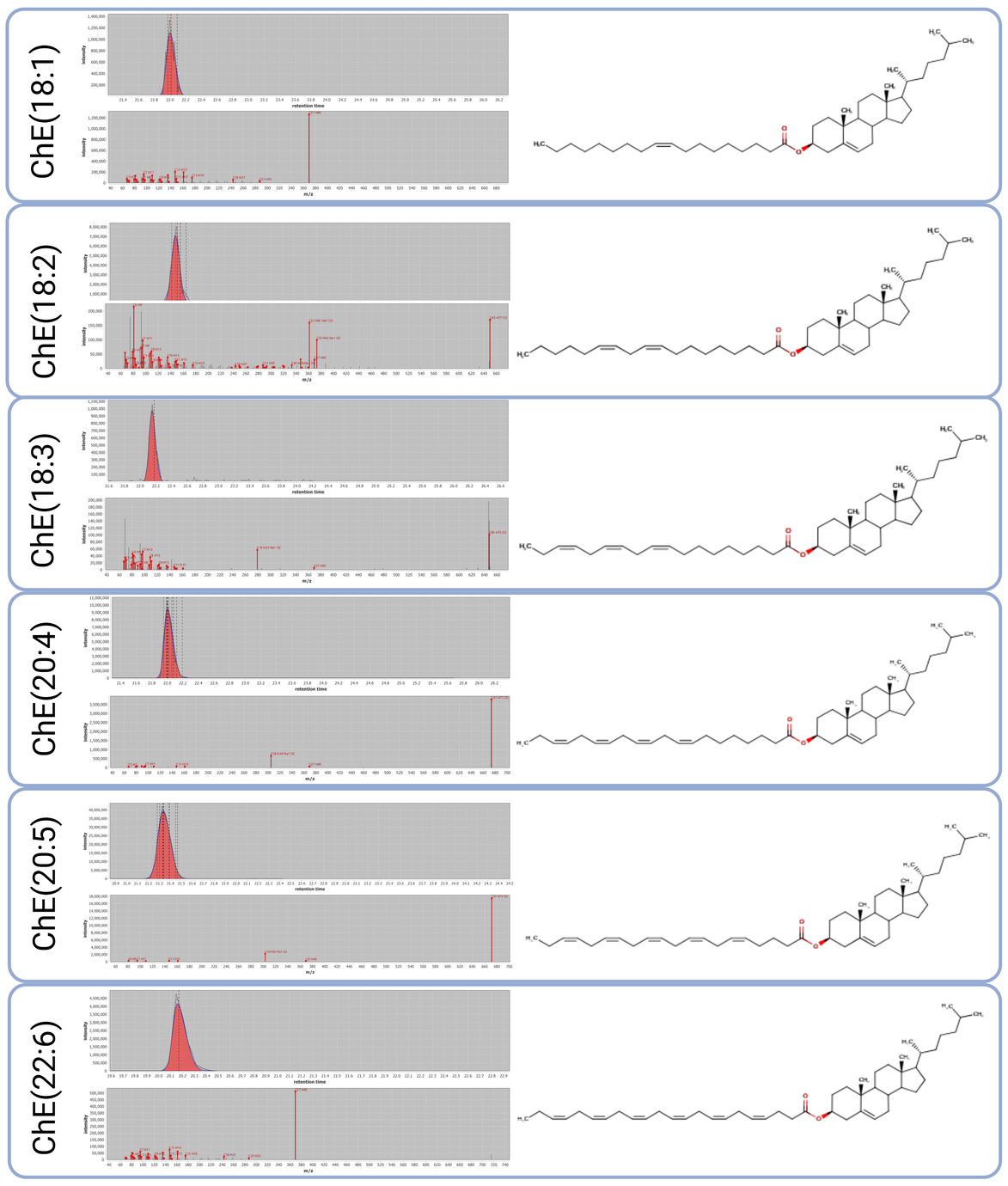

Fig. 3. Extracted Ion Chromatograms (EICs) of Cholesteryl Esters. EICs of selected cholesteryl esters detected in EDTA plasma, showing retention time profiles, relative intensities and fragmentation spectra. Each trace corresponds to a distinct cholesteryl ester species, illustrating chromatographic separation and signal quality across the lipid class.

Conclusion

This study presents an optimized high-coverage and robust global LC-MS lipidomics method, utilizing Scheduled Data-dependent acquisition (SDDA) and C30 chromatography to enhance lipid coverage and identification. The optimized MS-parameters and mobile phase composition provided enhanced sensitivity and intra- and inter-class separation. Compared to DDA and AcquireX, SDDA demonstrated improvement in lipid coverage and annotation confidence. This is due to the shorter scan duration and cycle time that SDDA can achieve.