

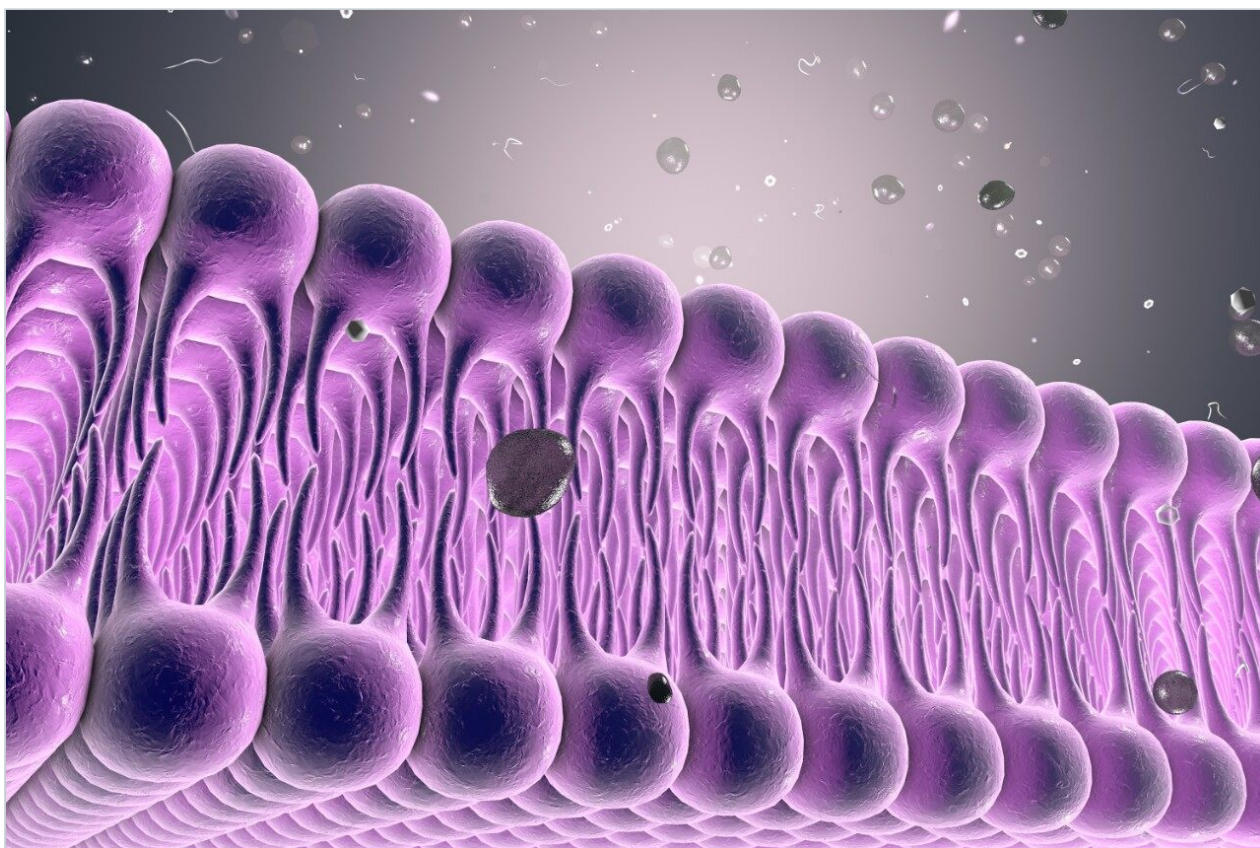
Application Note

## Targeted Lipidomics Using the ionKey/MS System

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## Abstract

This application note demonstrates the use of novel ionKey/MS System, which utilizes the iKey Separation Device packed with 1.7  $\mu\text{m}$  particles for fast and robust chromatographic separation. By integrating microscale LC components into a single platform design, the device avoids problems associated with capillary connections, including manual variability, leaks, and excessive dead volume. This integrated microfluidic device is suitable for lipidomics analyses with considerable advantages when compared to analytical scale LC/MS analysis.

### Benefits

The ionKey/MS System allows for fast and robust LC-MS lipidomics analyses with considerable reduction in solvent consumption and increase in sensitivity when compared to 2.1 mm I.D. chromatography. Potential applications include large-scale lipid profiling and low-abundance lipids analyses in biological materials.

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## Introduction

Lipidomics is the comprehensive analysis of hundreds of lipid species in biological samples. Lipids play prominent roles in the physiological regulation of many key biological processes such as inflammation and neurotransmission. Alterations in lipid pathways have been associated with many diseases including cardiovascular diseases, obesity, and neurodegenerative disorders.

The ability to measure the wide array of lipid species in biological samples could help our understanding of their roles in health and disease. The need for a fast, comprehensive, and sensitive analysis of the hundreds of lipid species challenges both the chromatographic separation and mass spectrometry.

Here we used the novel ionKey/MS System, which utilizes the iKey Separation Device packed with 1.7  $\mu\text{m}$  particles for fast and robust chromatographic separation. By integrating microscale LC components into a single platform design, the device avoids problems associated with capillary connections, including manual variability, leaks, and excessive dead volume. This integrated microfluidic device is suitable for lipidomics analyses with considerable advantages when compared to analytical scale LC-MS analysis.

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## Experimental

### LC conditions

LC system:	ACQUITY UPLC M-Class System
Sample loop:	1 $\mu$ L
Separation device:	iKey CSH C <sub>18</sub> Separation Device, 1.7 $\mu$ m, 150 $\mu$ m x 100 mm (p/n 186007245)
iKey temp.:	55 °C
Flow rate:	2 $\mu$ L/min
Mobile phase A:	Acetonitrile/water (60/40) with 10 mM ammonium formate + 0.1% formic acid
Mobile phase B:	Isopropanol/acetonitrile (90/10) with 10 mM ammonium formate + 0.1% formic acid
Volume injected:	0.2–0.5 $\mu$ L

### Gradient:

Time (min)	%A	%B	Curve
Initial	55.0	45.0	Initial
1.00	40.0	60.0	6
10.00	1.0	99.0	6
16.00	1.0	99.0	6

Time (min)	%A	%B	Curve
16.01	55.0	45.0	6
18.00	55.0	45.0	6

## MS conditions

Mass spectrometer:	Xevo TQ-S
Acquisition mode:	MRM
Ionization mode:	ESI positive
Capillary voltage:	3.0 KV
Source temp.:	120 °C

## Materials

Lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL) and Nu-Chek Prep (Elysian, MN). Total lipid extract from bovine brain was purchased from Avanti Polar Lipids. Mouse plasma (10 µL) was extracted with isopropanol (490 µL). The solution was then allowed to stand for 30 minutes in ice, vortexed, and then centrifuged (10,000 x g, at 4 °C for 10 min). The supernatant was collected in a new vial, evaporated to dryness under vacuum, and kept at -80 °C until further analysis. Immediately prior to analysis, all lipid extracts were re-suspended in isopropanol/acetonitrile/water (50/25/25, 250 µL).

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## Results and Discussion

For the analysis of lipids, we used the ionKey/MS System, comprised of the Xevo TQ-S Mass Spectrometer, the ACQUITY UPLC M-Class System, the ionKey Source, and the iKey Separation Device. The iKey Separation Device contains the fluidic connections, electronics, ESI interface, heater, e-Cord, and the chemistry, permitting operation at high pressure with sub-2-micron particles, leading to highly efficient LC

separations of lipid molecules. By integrating microscale LC components into a single system design, we avoided problems associated with capillary connections, including manual variability, leaks, and excessive dead volume. Lipidomics analyses were conducted using small volumes of lipid standards and lipid extracts from typical biological samples including plasma and brain tissues (0.2  $\mu\text{L}$ ). We separated lipids at flow rates of 2  $\mu\text{L}/\text{min}$  using a ACQUITY UPLC M-Class engineered with 150  $\mu\text{m}$  I.D. x 100 mm ceramic channel packed with CSH C<sub>18</sub>, 130 Å, 1.7  $\mu\text{m}$  particles size (Fig. 1). The small column diameter (150  $\mu\text{m}$ ) of the iKey Separation Device allows low injection volumes (0.5  $\mu\text{L}$ ) and low flow rates (2  $\mu\text{L}/\text{min}$ ) increasing up to 10x the sensitivity compared to regular analytical columns (e.g. 2.1 mm I.D.) (Fig.1). Mobile phase consumption was reduced compared to 2.1 mm I.D. chromatography albeit maintaining comparable chromatographic resolution and analysis times (Fig. 2)<sup>1</sup>.

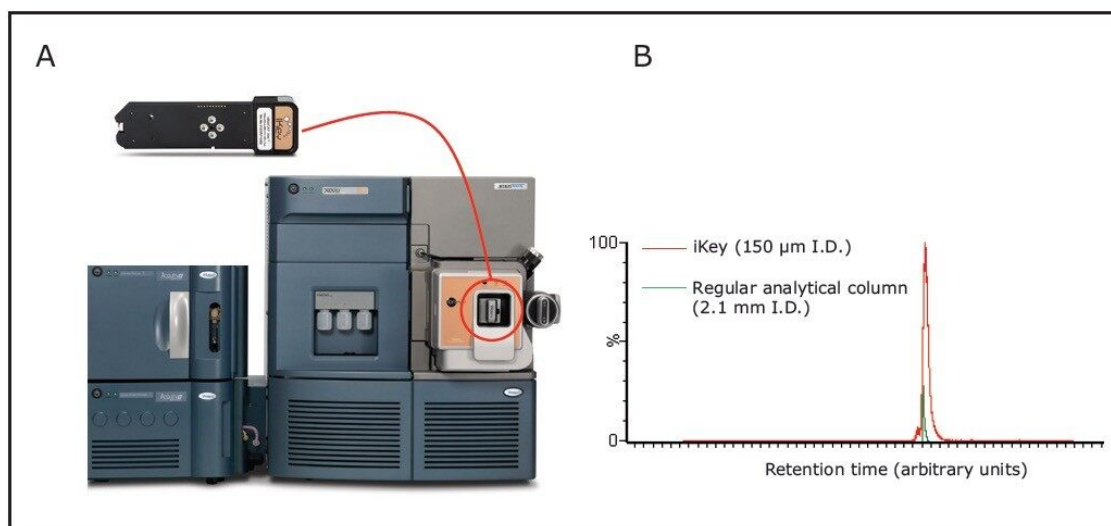


Figure 1. (A) The ionKey/MS System: comprised of the Xevo TQ-S, the ACQUITY UPLC M-Class, the ionKey Source and the iKey Separation Device. (B) Representative analysis of phosphatidylcholine (14:0/14:0) using the ionKey/MS System (red line) as compared to regular UPLC-MS1 (green line).

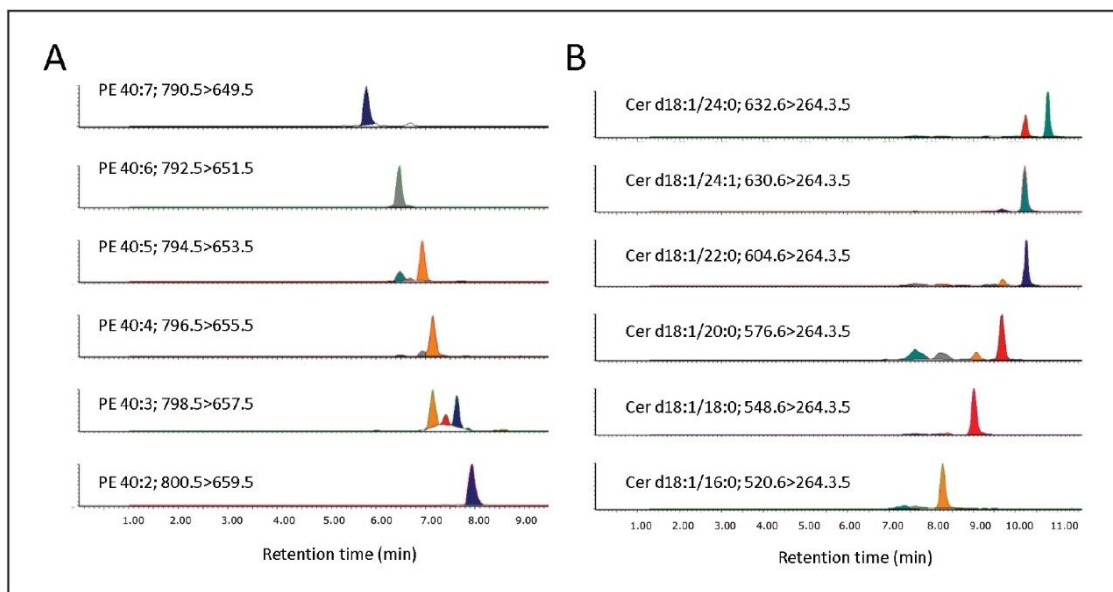


Figure 2. Representative extracted ion chromatograms of A) glycerophospholipids (e.g. phosphatidylethanolamines, PE) extracted from bovine brain and B) sphingolipids (e.g. ceramides, Cer) extracted from mouse plasma. Samples were analyzed using the ionKey/MS System.

We conducted targeted lipidomic analyses using Xevo TQ-S in MRM mode and monitored 215 lipid species belonging to various lipid classes including phosphatidylethanolamines (PE), lyso PE, phosphatidylcholines (PC), lyso PC, ceramides (Cer), sphingomyelins, hexosylceramides, lactosylceramides, and cholesteryl esters (Table 1). Targeted lipids were measured over approximately five orders of dynamic range (Fig. 3 and 4). Lipids were separated according to acyl chain length and number of double bonds. Quantification was performed using TargetLynx Application Manager (Fig. 5). Initial reports in peer reviewed journals showed the advantages of using the ionKey/MS System in real world applications dealing with the analysis of low abundance lipids.<sup>2,3</sup>

Lipid class	No. MRMs	Cone voltage	Collision energy
PE	45	26	18
Lyso PE	18	26	18
PC	44	42	26
Lyso PC	19	42	26
Ceramide	19	20	30
Sphingomyelin	20	36	24
Hexosyl Ceramide	19	20	26
Lactosyl Ceramide	16	20	30
Cholesteryl Ester	15	36	24

Table 1. Overview of the MRM method used.

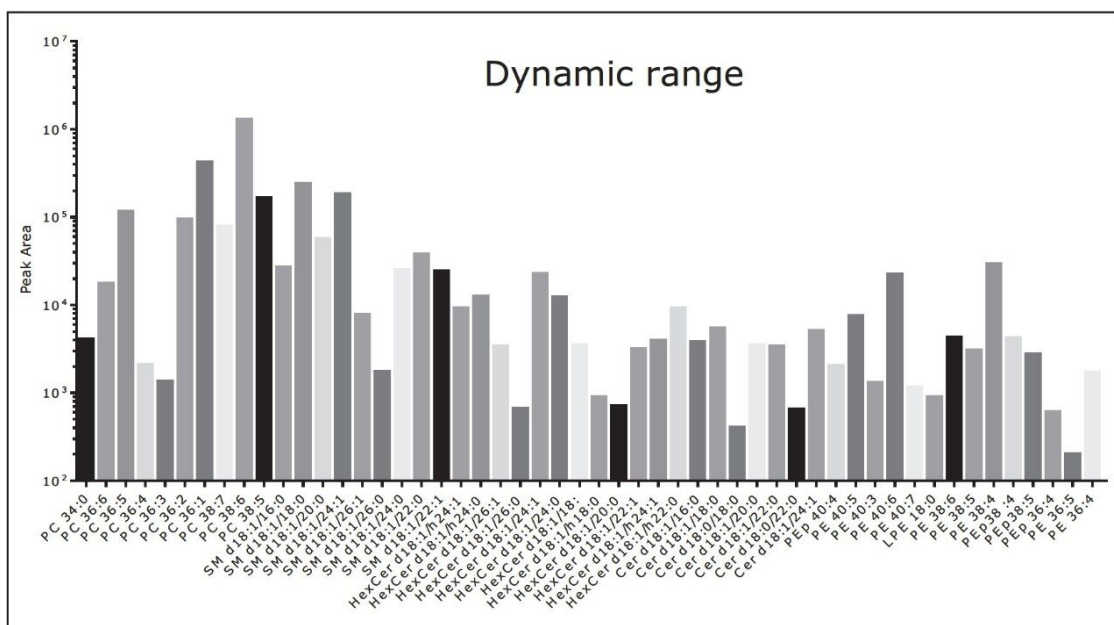


Figure 3. Intensities of selected lipids extracted from bovine brain.

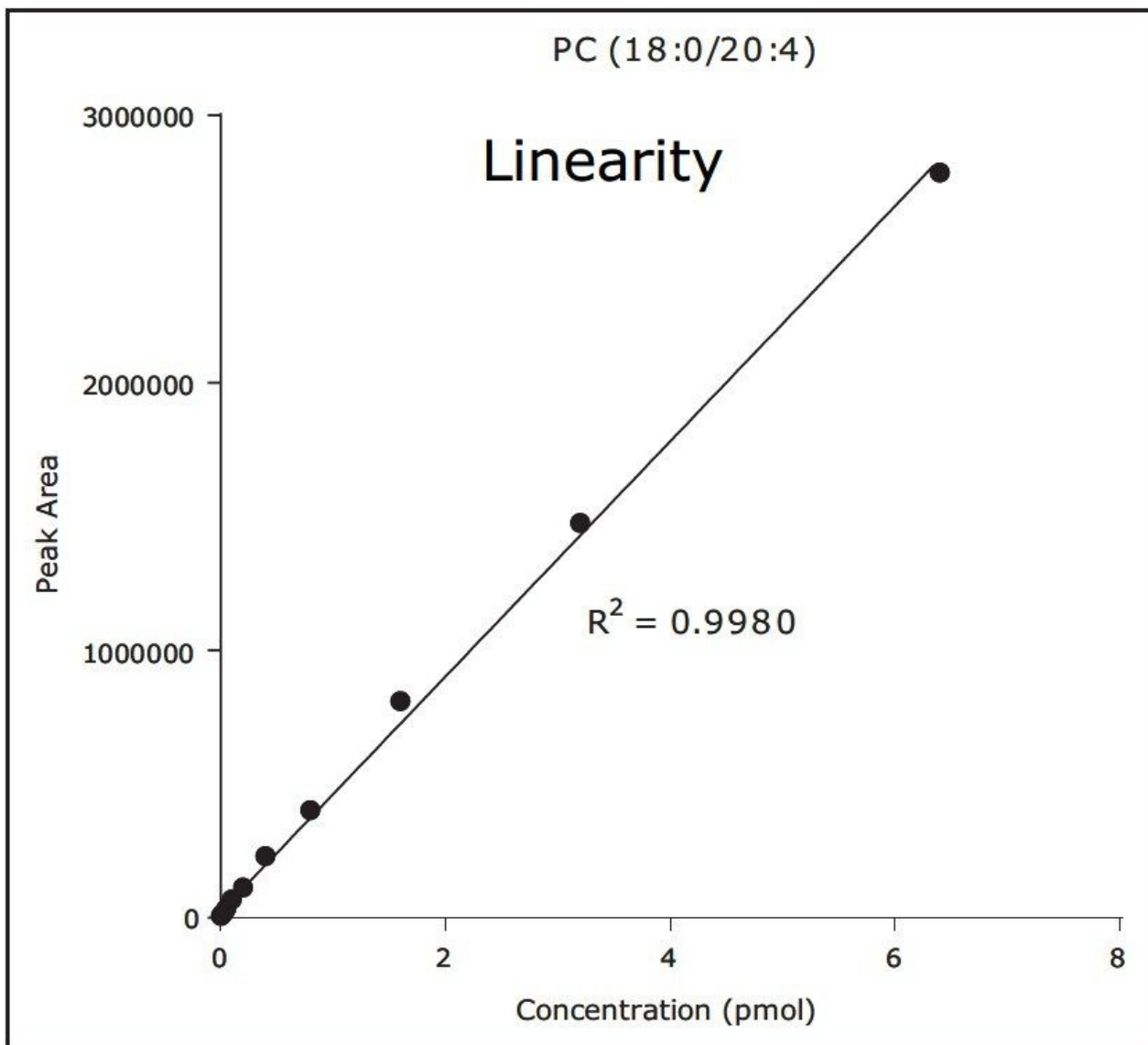


Figure 4. Linearity of response for a selected phosphatidylcholine species (PC).



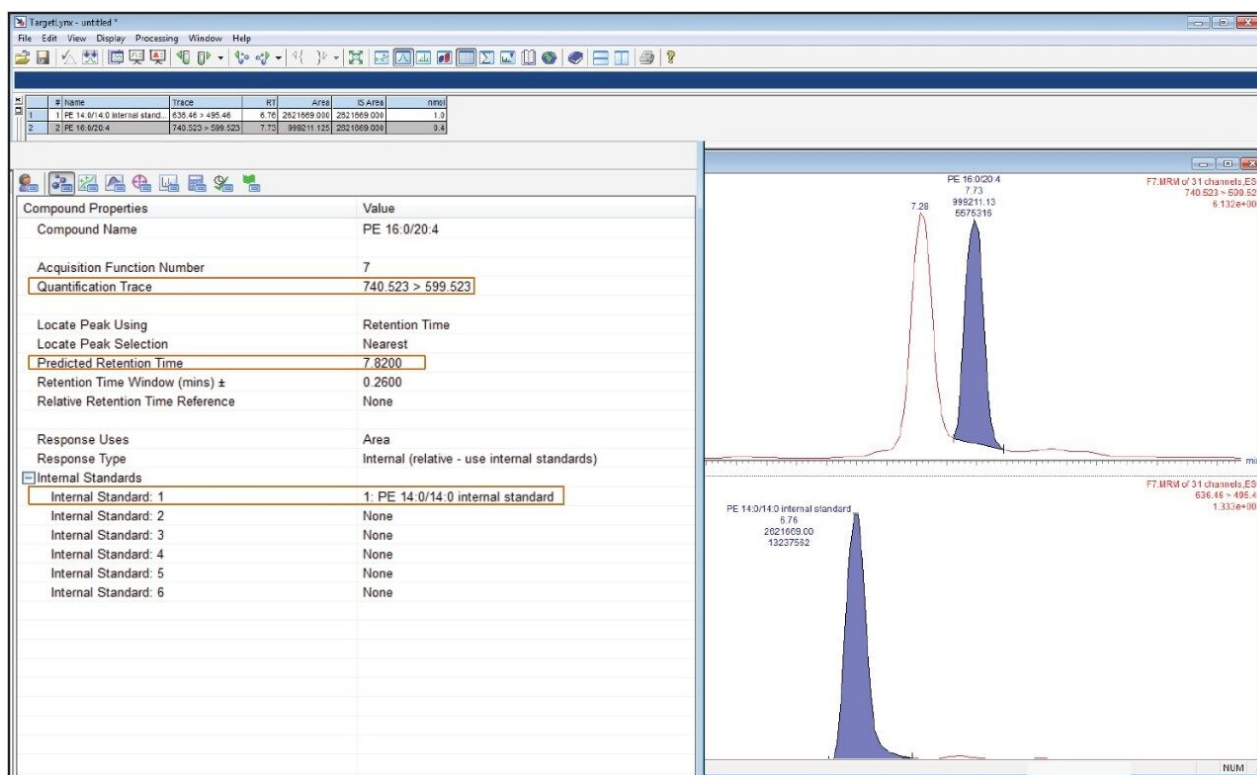


Figure 5. Quantification can be performed using TargetLynx. MRM and retention times are automatically extracted and normalized by comparison to selected internal standard.

## Conclusion

The ionKey/MS System is a novel microfluidics-MS platform that leads to highly efficient LC separation of lipids with comparable resolution to analytical scale LC-MS analysis.<sup>1</sup> The use of the 150  $\mu\text{m}$  iKey Separation Device enables the development of low flow MRM methods, bringing three major advantages over standard flow rate analysis<sup>1</sup>:

1. up to 200x decrease in solvent consumption, making it convenient for the large-scale analysis and screenings of hundreds or thousands samples;
2. up to 10x increase in sensitivity, which could facilitate the detection of low abundance metabolites;<sup>2,3</sup>
3. low volumes injection (e.g. 0.2  $\mu\text{L}$ ), which makes it ideal when sample limited studies or when multiple injections are required. Potential applications include large-scale lipid profiling and low-abundance lipids

analyses in biological materials.<sup>2,3</sup>

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## References

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