

## Analysis of Eicosanoids in Blood Using the Same Sample Preparation Method as for Primary Metabolite Analysis

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### User Benefits

- ◆ The same sample solution can be used to analyze both primary metabolites and eicosanoids.
- ◆ Good peak shapes and high sensitivity can be achieved for eicosanoids such as thromboxane that are difficult to analyze by LC-MS.
- ◆ Low analyte concentrations of around 10 ng/mL can be analyzed.

### Introduction

Eicosanoids are lipid mediators causing physiological processes such as inflammation and blood coagulation. In vivo, eicosanoids are synthesized from arachidonic acid in response to stimuli such as physical trauma or stress. Eicosanoids are considered as important as primary metabolites for elucidating physiological function and searching for biomarkers.

This study used derivatization including methoximation and trimethylsilylation to quantify seven eicosanoids: prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), 11β-prostaglandin E<sub>2</sub> (11β-PGE<sub>2</sub>), 8-iso-prostaglandin E<sub>2</sub> (8-iso-PGE<sub>2</sub>), 6-keto-prostaglandin F<sub>1α</sub> (6-keto-PGF<sub>1α</sub>), prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and thromboxane B<sub>2</sub> (TXB<sub>2</sub>) using the GCMS-TQ8050 NX.

### Sample Preparation

Samples were prepared by the same procedure used to analyze primary metabolites with the Smart Metabolites Database™ (see Fig. 1). First, plasma samples were deproteinated using a methanol/water/chloroform solution. The resulting supernatant was then dried with a centrifugal evaporator. Eicosanoids include PGE<sub>2</sub>, TXB<sub>2</sub>, and many other compounds that cause keto-enol tautomerism. For this reason, ketone groups were protected by methoximation with methoxyamine hydrochloride. Next, MSTFA was used to perform trimethylsilylation. This preparation method improves analyte volatility and separation.

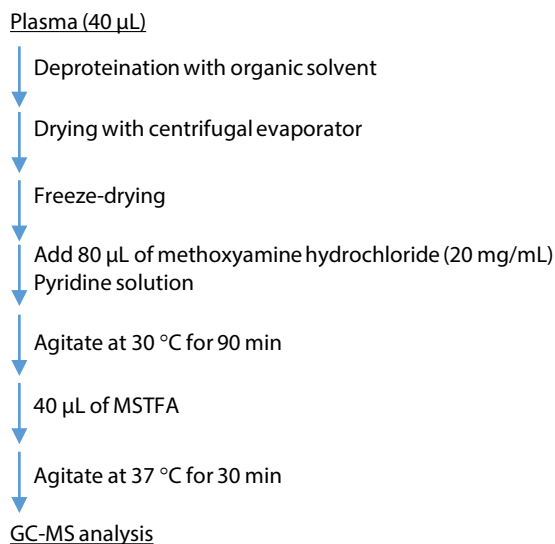


Fig. 1 Sample Preparation Flowchart

### Analytical Conditions

Table 1 shows the instrument and analytical conditions used in this article.

Table 1 Instrument and Analytical Conditions

Model:	GCMS-TQ8050 NX
<b>GC</b>	
Vaporization Chamber Temp.:	300 °C
Injection Method:	Splitless (1.5 min)
Carrier Gas:	He
Carrier Gas Control:	Linear velocity (40 cm/sec)
Column:	DB-5MS (30 m × 0.25 mm, I.D. 0.25 μm)
Column Temp.:	150 °C → (20 °C/min) → 200 °C → (5 °C/min) → 300 °C
<b>MS (Electron Ionization, MRM)</b>	
Ion Source Temp.:	230 °C
Interface Temp.:	300 °C
Tuning Mode:	Standard
Measurement Mode:	MRM
Transitions	
PGF <sub>2α</sub> -4TMS:	191.0 > 73.0 (CE 12 V) 391.0 > 73.0 (CE 39 V) 191.0 > 91.0 (CE 15 V)
8-iso-PGE <sub>2</sub> -3TMS:	225.0 > 73.0 (CE 21 V) 225.0 > 91.0 (CE 15 V) 566.0 > 73.0 (CE 36 V)
PGD <sub>2</sub> -3TMS:	526.0 > 296.0 (CE 12 V) 436.0 > 73.0 (CE 36 V)
11β-PGE <sub>2</sub> -3TMS:	225.0 > 73.0 (CE 18 V) 225.0 > 91.0 (CE 15 V) 353.0 > 131.0 (CE 12 V)
PGE <sub>2</sub> -3TMS:	225.0 > 73.0 (CE 18 V) 225.0 > 91.0 (CE 18 V) 225.0 > 155.0 (CE 9 V)
TXB <sub>2</sub> -4TMS:	211.0 > 73.0 (CE 12 V) 301.0 > 211.0 (CE 9 V) 301.0 < 73.0 (CE 21 V)
6-keto-PGF <sub>1α</sub> -4TMS:	436.0 > 205.0 (CE 9 V) 436.0 > 73.0 (CE 24 V) 476.0 > 73.0 (CE 24 V)
Event Time:	0.3 sec

## ■ Analysis of Standard Samples

Standard samples were diluted with pyridine to prepare calibration curve standards of 10, 50, 100, and 500 ng/mL. Fig. 2 shows MRM chromatograms of 100 ng/mL standard samples. Calibration curves appeared as gentle second-order curves and all compounds showed good results with  $R^2$  of 0.995 or higher. Good sensitivity as indicated by an S/N ratio of 53 at 10 ng/mL was even achieved for TXB<sub>2</sub>, which tends to give poor peak shapes during LC-MS analysis.

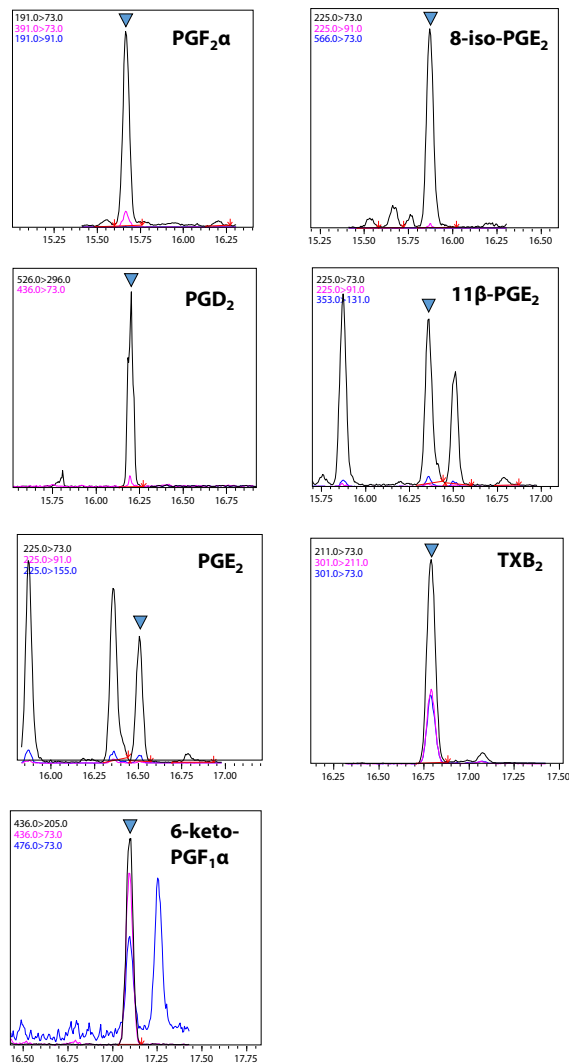


Fig. 2 MRM Analysis of Standard Samples (100 mg/mL)

## ■ Analysis of Biological Samples

As an example of a biological sample, standard human plasma was analyzed. Given that no eicosanoids were detected in the standard plasma, the standard plasma was spiked with standard samples to a final concentration of 200 ng/mL. Fig. 3 shows the MRM chromatograms of PGE<sub>2</sub> and 11β-PGE<sub>2</sub>, eicosanoids that are difficult to separate, and TXB<sub>2</sub>, which is relatively difficult to analyze on other analytical instruments. Good recovery was obtained by this method, with recovery results ranging from 80.4 % to 119.3 % for the seven eicosanoids (Table 2).

Table 2 Eicosanoids Recovery from Plasma

Compound	Recovery (%)
(+)-PGF <sub>2</sub> α-4TMS	104.9
8-iso-PGE <sub>2</sub> -3TMS	80.4
PGD <sub>2</sub> -3TMS	92.2
11β-PGE <sub>2</sub> -3TMS	83.3
PGE <sub>2</sub> -3TMS	119.3
TXB <sub>2</sub> -4TMS	105.3
6-keto-PGF <sub>1</sub> α-4TMS	114.0

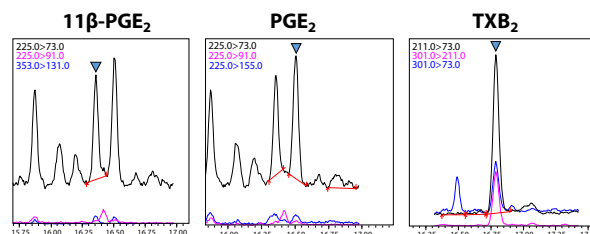


Fig. 3 MRM Analysis of Plasma Spiked with Standard Samples (200 mg/mL)

## ■ Conclusion

Seven eicosanoids were identified by GC-MS using methoximation and trimethylsilylation. Although more stable derivatives can be obtained by methylating carboxyl groups before methoximation, the method used in this article has sufficient sensitivity.

## ■ Using the Smart Metabolites Database

The samples prepared in this article can also be used for primary metabolite analysis with the Smart Metabolites Database. The same column and insert can even be used to analyze both primary metabolites and eicosanoids. By combining this analysis with Garuda or other analysis software, the user can perform multivariate analysis and create metabolite maps.

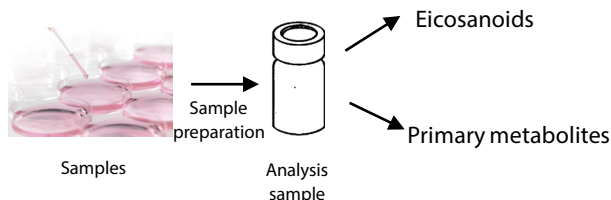
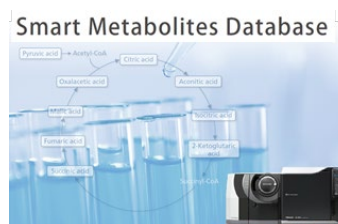


Fig. 4 Using the Smart Metabolites Database™

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