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LCMS™-8060 High Performance Liquid Chromatography Mass Spectrometer

Application News

Development of a Quantitative Method for Epoxy-eicosanoids in Mouse Brain Microorgan

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User Benefits

- Enables highly sensitive analysis of epoxy-eicosanoids
- Enables 15-minute analysis cycle time
- Use to search for new biomarkers or explore new physiological functions

■ Introduction

Epoxy-eicosanoids (epoxy-eicosatrienoic acids or EETs) are known as a group of bioactive lipids derived from arachidonic acid, in addition to prostaglandins and leukotrienes. EETs are formed from arachidonic acid by cytochrome-P450 (CYP) (Fig. 1). 5(6)-EET has been reported as a lipid mediator with physiological functions such as vasorelaxation.[1),2)]

The LC/MS/MS Method Package for Lipid Mediators Ver. 3 enables comprehensive analysis of 196 metabolites of polyunsaturated fatty acids. EETs elute after 18 minutes of the 20-minute linear gradient time specified in the comprehensive method (Fig. 2A).[3)]

This article describes the development of a method for quantitative analysis of EETs in tiny mouse brain tissue (organum vasculosum laminae terminalis, or OVLT). A heptacosyl group-based column was used to develop the method, which elutes EETs in 8 to 9 minutes. The peak resolution of EETs was significantly improved by using a curved gradient (Fig. 2B). Using this method, we were able to quantitatively analyze 5(6)-EET and 8(9)-EET in less than 1 mg of the OVLT of mice brain tissue samples.

■ Analytical condition

Table 1 HPLC and mass spectrometry conditions

HPLC conditions (*Nexera*)	
Column:	CAPCELL CORE C27*[1] (75 mm x 2.1 mm I.D., 2.7 µm)
Mobile phases:	A) 0.1% Acetic acid in water B) Methanol
Gradient program:	B conc. 20 % (0 min) – 20 % (1.0 min) – 40 % (2.0 min) – 80 % (10.0 min) – 95 % (10.1 min) – 95 % (12.0 min) – 20 % (12.1 min) – stop (15 min), curve – 3 (2.0 min)
Flowrate:	0.4 mL/min
Column temp.:	40 °C
Injection volume:	5 µL
MS conditions (LCMS-8060)	
Ionization:	ESI (negative mode)
Probe voltage:	-3 kV
MRM mode:	*m/z* 319.2 > 191.1, 5(6)-EET (pink in Fig. 2B, Fig. 5) *m/z* 319.2 > 179.2, 8(9)-EET (blue in Fig. 2B, Fig. 5)
Nebulizing gas flow:	2.5 L/min
Drying gas flow:	10 L/min
Heating gas flow:	10 L/min
DL temp:	250 °C
Heat block temp:	400 °C
Interface temp:	300 °C

*1 Currently CAPCELL CORE AQ (Osaka Soda)

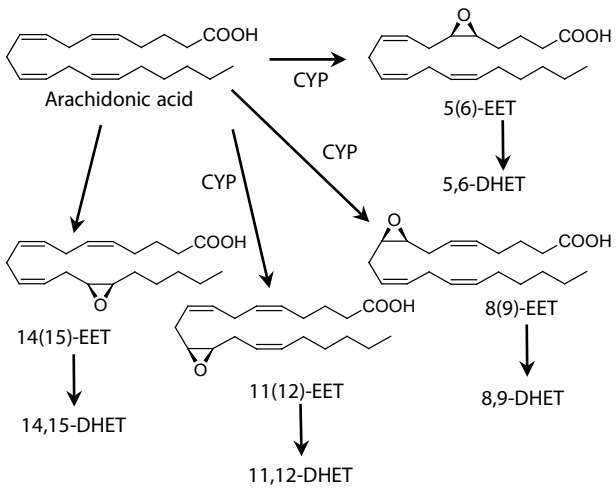

Fig. 1 Chemical structures of EETs and their metabolic cascades

■ Optimization of separation parameters

The MRM chromatograms obtained by injecting 5 pg of 5(6)-EET and 8(9)-EET are shown in Fig. 2. Using the Method Package for Lipid Mediators,[3)] 5(6)-EET and 8(9)-EET were eluted in 18.28 and 18.24 minutes, respectively, with the resolution *R* being approximately 0.3 (Fig. 2A). Separation parameters were optimized using a heptacosyl group(C27)-based core-shell column. The MRM chromatograms obtained under the conditions in Table 1 are shown in Fig. 2B. 5(6)-EET and 8(9)-EET were eluted at 8.63 and 8.44 minutes with good separation and a resolution *R* value of 2.3.

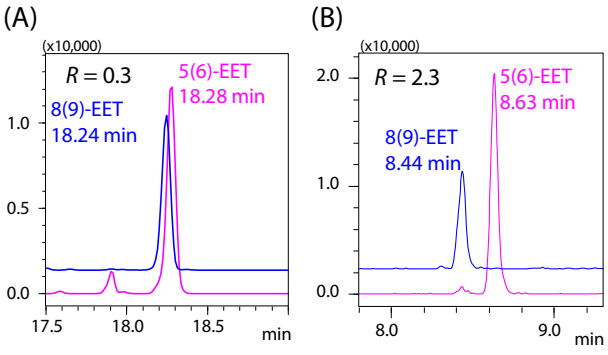

Fig. 2 The MRM chromatograms in (A) for 5(6)-EET and 8(9)-EET were obtained with the Method Package for Lipid Mediators using a Kinetex® C8 150 mm x 2.1 mm I.D., 2.7 µm (Phenomenex) column and 0.1 % water formate as eluent A and acetonitrile as eluent B, a linear gradient. The chromatogram (B) was obtained under the analytical conditions in Table 1

■ Sensitivity and lower limits of quantification for EETs

The MRM chromatograms for 0.5 pg of 5(6)-EET and 8(9)-EET are shown in Fig. 3. The chromatograms obtained from three repetitions were overlaid. Linearities of R^2 > 0.999 were obtained for the range of 0.5 to 500 pg for both 5(6)-EET and 8(9)-EET (Fig. 4).

The Lipid Mediator LC/MS/MS Method Package Ver. 3 uses 0.1% formic acid water as eluent A. By using 0.1% aqueous acetic acid for eluent A, as in this method, EET sensitivity was improved by about 10 times.

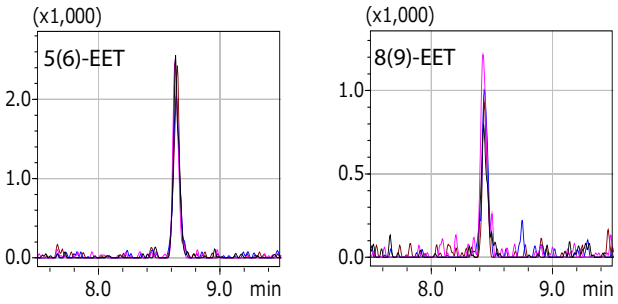

Fig. 3 MRM chromatograms for 5 pg injections of 5(5)-EET and 8(9)-EET

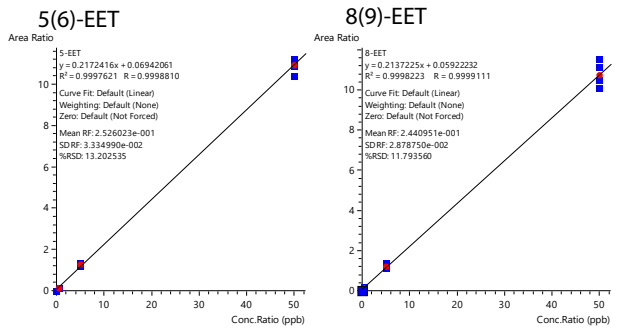

Fig. 4 Calibration curves for 5(5)-EET and 8(9)-EET

■ Results of mouse brain microorgan analysis

Fig. 5 shows the results of analyzing lipids extracted from the organum vasculosum laminae terminalis (OVLT) of mice brains. Lipid extraction was performed by adding 500 µL of 0.1% formic acid in methanol to OVLT tissue samples (less than 1 mg) collected from 5 mice. The supernatant after centrifugation was subjected to solid-phase extraction, the lipid extract was concentrated to 50 µL, and 5 µL was subjected to LC/MS analysis. Some unknown peaks,* as shown in Fig. 5, were detected in each chromatogram, but the target and unknown peaks were separated with good resolution. Concentrations of 5(6)-EET and 8(9)-EET were estimated by the internal standard method.[4)]

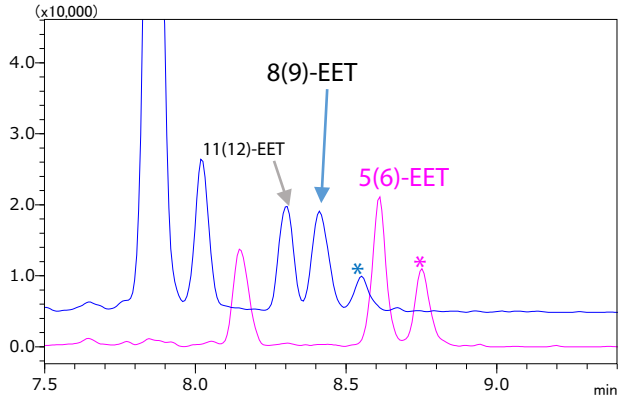

Fig. 5 MRM chromatograms of 5(5)-EET and 8(9)-EET in extract from organum vasculosum laminae terminalis
* Unknown peaks

■ Conclusion

A high-speed and highly sensitive detection method for EETs was developed. The lower limit of quantification for 5(6)-EET and 8(9)-EET was 0.5 pg. Separation was improved by using a column based on a heptacosyl group and utilizing a curved gradient. By improving the separation between 5(6)-EET and 8(9)- EET, it was possible to distinguish unknown peaks detected in the analysis of brain tissue samples. The analysis cycle time of 15 minutes, including column cleaning and initialization, enabled development of a highly sensitive and high throughput epoxy eicosanoid analysis method.

Using the novel EET analytical method, 5(6)-EET and 8(9)-EET were detected and quantitatively analyzed in less than 1 mg of the mouse brain organ (OVLT) samples. The results of this analysis revealed that 5 (6)-EET and 8(9)-EET act as lipid mediators that transmit a “thirst” signal in the brain.[4)]

Given that an ELISA method has not been developed for EETs, the physiological and pathophysiological functions of EETs are presumably less understood than for prostaglandins and leukotrienes. Use this method to search for disease biomarkers and to analyze new physiological functions.

■ References

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3. Yamada M. et al., *J. Chromatography B*, 995, 74-84 (2015).
4. Sakuta, H. et al., *Neuroscience Res.* 154, 45 – 51 (2020).

■ Acknowledgements

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