Analysis of nonvolatile molecules in supercritical carbon dioxide using proton-transfer-reaction ionization time-of-flight mass spectrometry

Project Research Center for fundamental Sciences, Graduate School of Science

Professor Michisato Toyoda Researchmap https://researchmap.jp/toyodam

Researcher Toshinobu Hondo Researchmap hhttps://researchmap.jp/toshi-hondo

Abstract

Proton transfer reaction (PTR) mass spectrometry (MS) has been applied to the real-time quantitative analysis of trace-level volatile organic compounds in gaseous samples. The principle of the PTR-MS should be able to detect nonvolatile organic compounds if we can transfer molecules into a PTR flow tube. Supercritical fluid extraction (SFE) and chromatography (SFC) is a suitable candidate method. We have found that the detection of ions up to ivermectin (molecular weight 875) was confirmed, and an extremely low quantification limit of 10-200 attomole was obtained for fatty acids.

Background & Results

Supercritical fluid extraction (SFE) was introduced by Zosel et al. in the late 1960 to 1970s. A well-known application of SFE is that it selectively removes caffeine from coffee beans without changing another component (US Pat. 3969196). This example shows that SFE is a separation technique that selectively separates a component from a mixture by manipulating temperature and pressure rather than a simple extraction. In addition, the SFE process can be completed in a short period than extraction using organic solvents. We have developed a small-scale SFE-PTR ionization system prototype that uses a column in-line filter as an SFE vessel (1 μL volume). A sample was applied on the stainless steel flit and then placed into a column filter, then performing SFE at 25 MPa that introduced the solute directory into a PTR tube in a vacuum.

The PTR-MS is capable of high detection sensitivity in fast response time, the ability to ionize a wide range of organic compounds, and the ease of interpretation of the resulting mass spectrum. Although the PTR ionization has been investigated mainly for the formation of protonated ions, the deprotonated reaction that the high sensitivity obtained for fatty acids.

To explore the method's applicability, we tried to measure various substances shown in Table 1 and obtained low limit of quantitation (LOQ) for most of the tested compounds. In particular, we noted that the low LOQs were obtained for fatty acids. Fat and fatty acids have a high affinity for $scCO_2$ and are easy to extract from cellular organisms by SFE. We expect the technique to be applied for trace level fatty acids monitoring *in-situ* on the biological tissues. As summarized in the next section, the measurement of reaction intermediates known as arachidonic acid cascade by SFE/SFC-PTR-MS *in-situ* in localized cells such as diseased cite is expected to lead to the development of physiology and to help cancer patients in the future.

Significance of the research and Future perspective

Since the beginning of this study, we have been supposed to obtain a high sensitivity for nonvolatile organic compounds using supercritical carbon dioxide ($scCO_2$) as a carrier fluid, which is inert to the PTR. However, the obtained result of LOQ for fatty acids was way lower (better) than our expectations, possibly being applied to the lipid metabolism study.

For example, a series of metabolites derived from arachidonic acid, a type of fatty acid produced in the body, has been studied in relation to pain, fever, cancer, and manic depression. Although these fatty acids are widely distributed in the body, the amount of fatty acids present in the body is small, and the extraction of fatty acids from tissues is complicated, so it is not easy to measure changes in the amount. If these fatty acids can be rapidly and sensitively quantified, *in-situ* analysis of diseased sites will be possible. We hope this will contribute to the development of physiology.

Table 1: SFE-DI-PTR Limit of Quantitation

Compound	mass	$log(P)$[1]	positive ion mode		negative ion mode	
Acetaminophen	151.063	2.0	4.9 fmol	$[M+H]^+$	274 fmol	$[M-H]^-$
Phenacetin	179.095	2.0	1.4 fmol	$[M+H]^+$	203 fmol	$[M-H]^-$
Caffeine	194.080	-1.0	0.008 fmol	$[M+H]^+$	–	
Pyrene	202.078	4.6	9.5 fmol	$[M+H]^+$	–	
Oleic acid	282.256	6.1	–		0.048 fmol	$[M-H]^-$
Stearic acid	284.272	6.3	–		10 fmol	$[M-H]^-$
Linolic acid	280.240	5.9	–		0.2 fmol	$[M-H]^-$
Arachidic acid	312.303	7.1	–		27 fmol	$[M-H]^-$
Arachidonic acid	304.240	6.2	–		19 fmol	$[M-H]^-$
Tetracosanoic acid	368.365	8.7	–		17 fmol	$[M-H]^-$
α-Tocopherol	430.381	8.8	63 fmol	$[M+H]^+$	4 fmol	$[M-H]^-$
Vitamine K_1	450.350	9.2	406 fmol	$[M+H]^+$	41 fmol	$[M]^-$
γ-Oryzanol A	602.434	10.2	–		530 fmol	$[M-H]^-$
Reserpine	608.273	4.2	8.3 fmol	$[M+H]^+$	8 pmol	$[M-H]^-$
Ivermectin (B_{1a})	874.508	5.6	2.3 fmol	$[M+NH_4]^+$	–	

References

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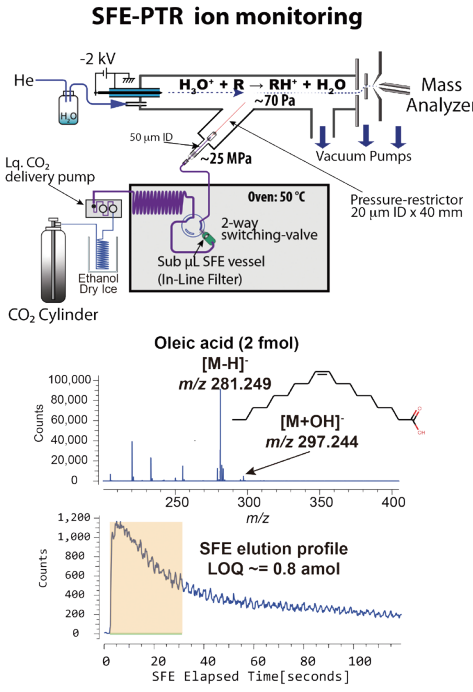

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