

Confirmation of Mass Information by Online Solvent Extraction of Components Separated by TLC

In order to confirm the mass information of the components separated by thin layer chromatography (TLC), a time-consuming manual procedure consisting of (1) removal of the spot, (2) extraction, (3) analysis by mass analyzer, is often performed. By using the CAMAG TLC-MS Interface 2 (CAMAG) which automates the online solvent extraction for the above procedure, the extraction and introduction of a TLC spot to the mass analyzer can be performed easily.

In this study, analysis of various compounds was performed by using CAMAG in combination with the 5610 MS Detector. Target spots were directly extracted from TLC plates and analyzed, and the results are presented here.



5610 MS Detector

TLC-MS Analysis Result (1) -Chlorpromazine, Acetaminophen-

Analytical Conditions (1)

Table 1 MS Detector Settings

Ionization method	ESI
Ionization mode	Positive
Ionization voltage	2600 V
Measurement mode	Scan (m/z 100-400)

Table 2 LC Pump Settings

Mobile phase	CH ₃ OH
Flow rate	0.2 mL/min (Split ratio =1:50)

Table 3 TLC-MS Interface 2 Setting

N ₂ gas	400 kPa
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Table 4 TLC Conditions

Sample	Chlorpromazine: MW 318.9	Acetaminophen: MW 151.2
Thin layer plate	Silica gel	Silica gel
Developing solvent	Acetone / 28% Ammonia =99 / 1	Ethyl acetate
Weight of spot	2 µg	2 µg

TLC-MS Analysis

The online solvent extraction was performed by placing the elution head of the TLC-MS interface 2 in close contact with the spot obtained after TLC development and the mass analysis was conducted. As a result, the characteristic ions of 319 [M+H]⁺ for chlorpromazine and at 174 [M+Na]⁺ and 325 [2M+Na]⁺ for acetaminophen were detected.

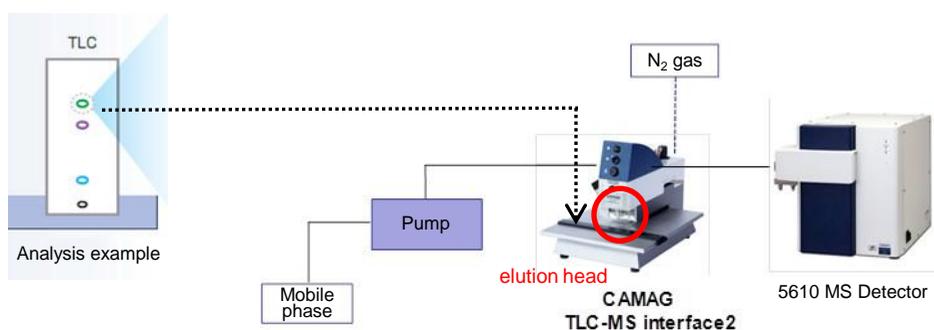


Figure 1 Example of TLC-MS System Configuration

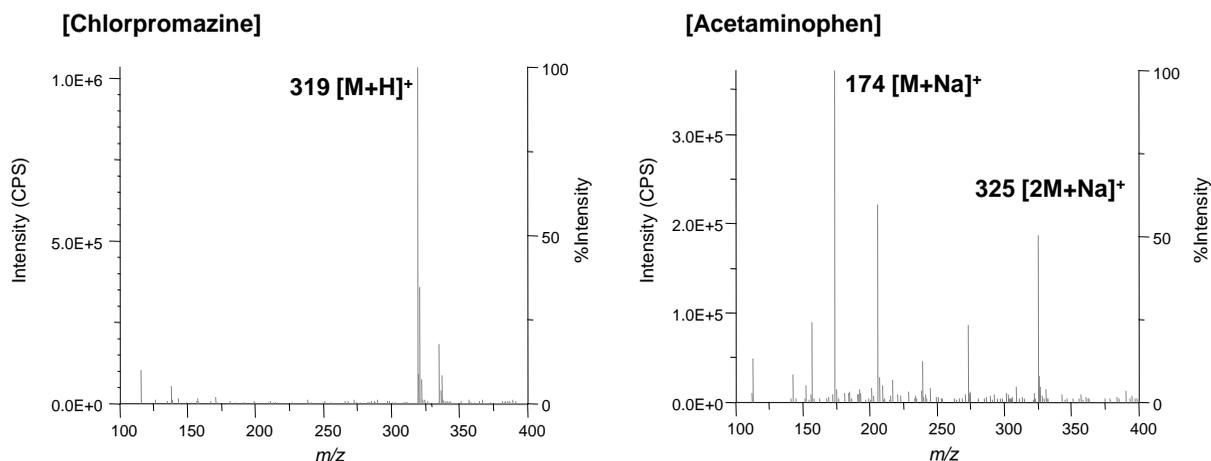


Figure 2 Mass Spectra of Each Spot Extracted From TLC Plate

TLC-MS Analysis Result (2) -3 Flavonoids-

Analytical Conditions (2)

Table 5 MS Detector Settings

Ionization method	ESI
Ionization mode	Positive
Ionization voltage	2500 V
Measurement mode	Scan (<i>m/z</i> 310-450)

Table 8 MS Detector Settings

Sample	Naringenin acetic ester
Thin layer plate	Silica gel
Developing solvent	Hexane/Ethyl acetate = 1/1
Weight of spot	2 µg

Table 6 LC Pump Settings

Mobile phase	CH ₃ OH
Flow rate	0.2 mL/min (Split ratio = 1:50)

Table 7 TLC-MS Interface 2 Settings

N ₂ gas	400 kPa
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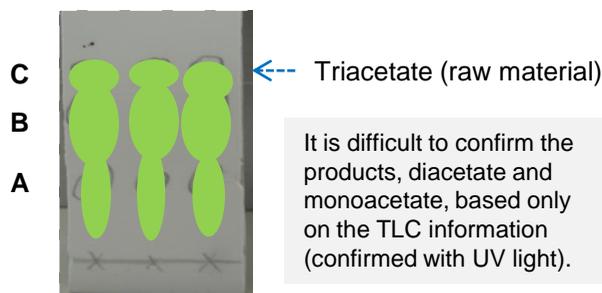


Figure 3 Image of TLC Development

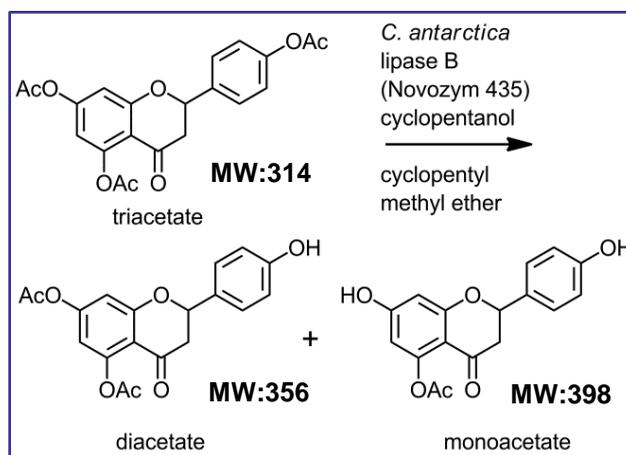


Figure 4 Synthesis Route for Naringenin Acetic Ester (Deacetylation)

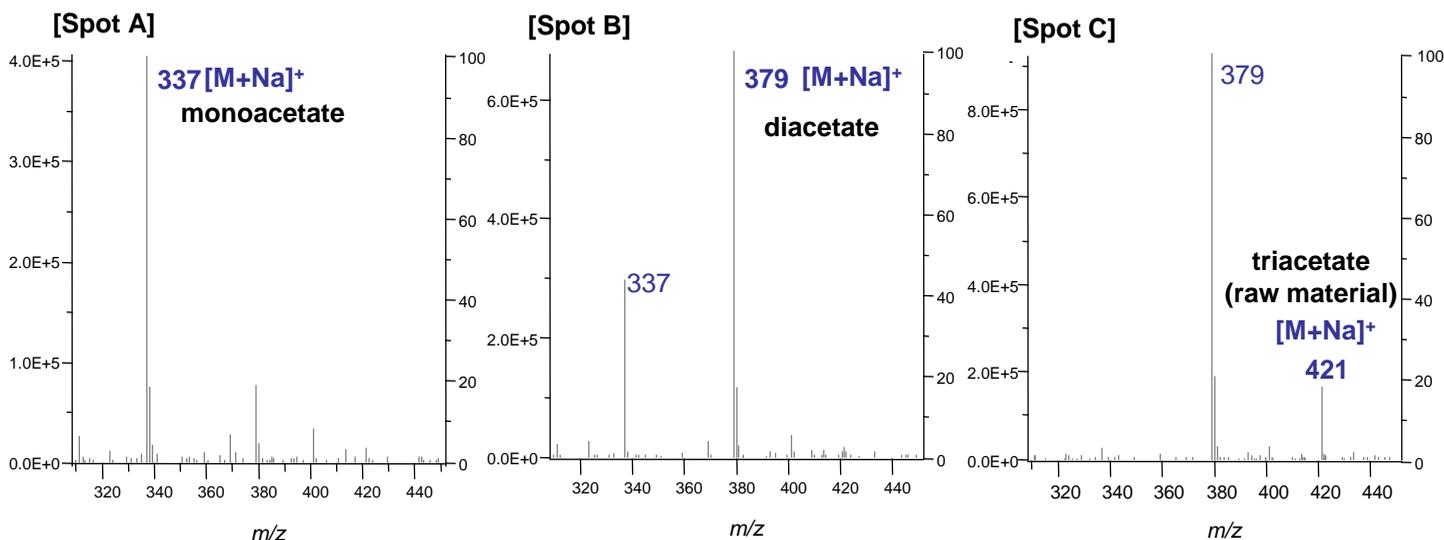


Figure 5 Mass Spectrum of Each Spot Extracted from TLC Plate

By directly extracting the target spot from the TLC plate and obtaining mass information, triacetate, diacetate, and monoacetate, for which identification was difficult due to insufficient separation only by TLC, could be easily identified. In many cases, there is no reference standard available for a product formed during the synthesis process and the mass information is useful for compound identification.

*The data introduced here were analyzed with the support of EKO Instruments

** The sample shown here was provided by Organic and Biocatalytic Chemistry Laboratory, Keio University Faculty of Pharmacy (Sugai, T., et al., J. Mol. Catal. B: Enz. 2013, 92, 14.)

<Main system configuration> Chromaster 5110 Pump, 5610 MS Detector, CAMAG TLC-MS Interface 2

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.