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Application News

No. B109

Probe Electrospray Ionization Method

Quick Screening and Quantitative Analysis of Carcinogens in Medicinal Plants Using DPiMS™-8060

From the viewpoint of preventing health damage, it is necessary to establish rapid and effective screening and quantitation methods for harmful substances which have serious impacts on human health. Harmful substances contained in plants are analyzed by LC and LC/MS, but these techniques require separation using a column and removal of contaminants. Among the problems of these techniques, in addition to the time required for pretreatment and analysis, consumption of organic solvents is also large in some cases.

These problems can be solved by the probe electrospray ionization method (PESI), which is one ultra-high speed screening platform supplied by Shimadzu. PESI has already been adopted in analysis of narcotics, pharmaceutical products, pesticides, biotoxins and metabolites.

Aristolochic acid, which is the target of this analysis, is found in plants of the genus *Aristolochia*, and is classified as a carcinogen, including their plant forms. This article introduces an internal standard method using naproxen for rapid screening and quantitative analysis of four types of aristolochic acid contained in *Aristolochia debilis* and *Asiasarum sieboldii* and aristolactam I, which is one of its metabolites, using the Shimadzu DPiMS-8060 direct probe ionization mass spectrometer kit.

Z. Chen, K. Waki

Features of DPiMS-8060

The DPiMS-8060 system features a combination of an ion source utilizing the probe electrospray ionization method (PESI) installed in a triple quadrupole mass spectrometer (LCMS™-8060), as shown in Fig. 1. The PESI ion source can be coupled directly with Shimadzu LCMS-8045 mass spectrometer and higher models in the LCMS series. The PESI analysis method does not require a mobile phase, as the probe is thrust into the sample on the sample plate, and sample molecules are ionized by applying a voltage to the sample adhering to the probe surface (Fig. 2). Rapid analysis without using a separation column is possible with only simple pretreatment.

Fig. 1 Appearance of DPiMS™-8060

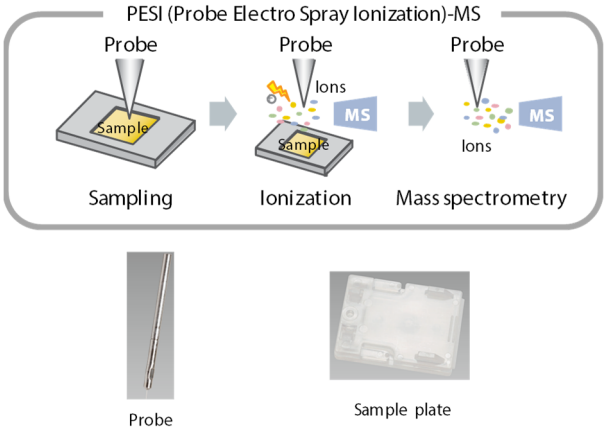

Fig. 2 Principle of Probe Electrospray Ionization Method
The probe is thrust into the sample on the sample plate, and sample molecules are ionized by applying a voltage to the sample adhering to the probe surface.

Sample Preparation

The following two types of solutions were prepared for use in sample preparation.

- Methanol/water (70/30, v/v)
- Ethanol/water (60/40, v/v) : 2 mM ammonium formate, including 10 ppb naproxen (internal standard)

The samples were prepared by the procedure in Fig. 3. Ultra-high speed analysis of 10 µg/L of the standard sample solutions of the target compounds was conducted under the conditions in Table 1 and Table 2, and required only about 20 s/sample. Fig. 4 shows the MRM data of the standard substances.

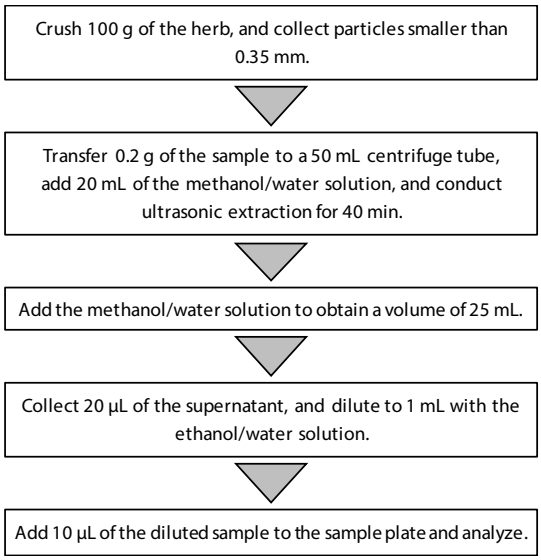

Fig. 3 Workflow of Sample Preparation

Linearity of Calibration Curve

Table 3 shows the linearity of the calibration curves analyzed under the conditions in Table 1 and Table 2. The calibration curves were prepared for the range of 0.05 to 50 µg/L. Satisfactory linearity was obtained, as the coefficient of determination R^2 = 0.999 or higher for all components.

Repeatability

Table 4 shows the relative standard deviation (RSD (%), n = 6) of the measured values of the standard substances, which is an index of repeatability. Excluding the cases of 0.2 µg/L for aristolochic acid C and aristolochic acid D, RSD was 15% or less for all compounds. Considering the PESI was used as the sample measurement method, repeatability is thought to be within the permissible range.

Table 1 Measurement Conditions

Sampling stop time	: 50 ms
Sampling position	: -46.0 mm
Ionization stop time	: 220 ms
Heat block temp.	: 30 °C
Interface voltage	: 2.3 kV (+) / -3.0 kV (-)
Probe cleaning time	: 0.05 min (+) / 0.05 min (-)
Probe cycle speed	: 2.78 Hz
DL temp.	: 250 °C

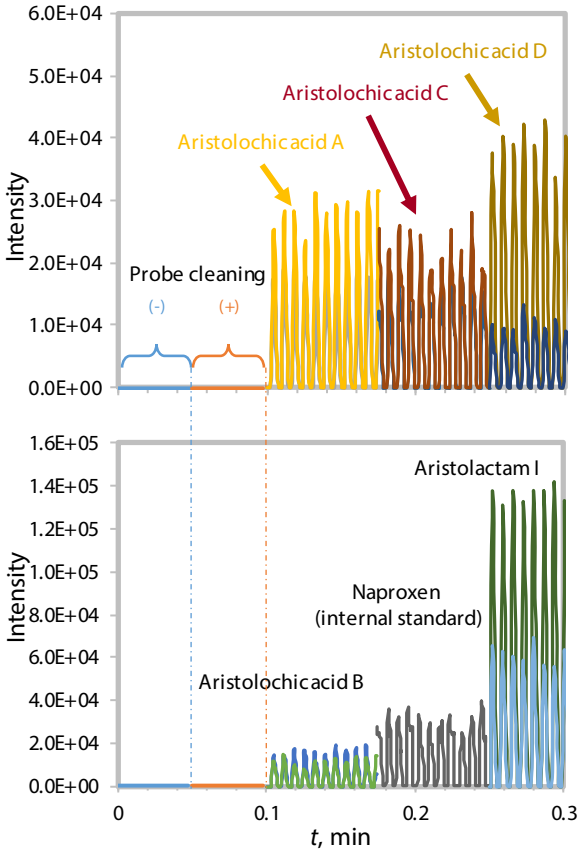

Fig. 4 MRM Data of Standard Substances

Table 2 MRM Analysis Parameters

Compound	*m/z*	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Aristolochic acid A	359.05>296.15*	-25.0	-18.0	-22.0
	359.05>324.05	-25.0	-21.0	-20.0
Aristolochic acid B	329.05>268.15*	-12.0	-12.0	-10.0
	329.05>294.10	-13.0	-15.0	-15.0
Aristolochic acid C	345.00>284.05*	-24.0	-13.0	-14.0
	345.00>282.05	-26.0	-20.0	-32.0
Aristolochic acid D	375.00>312.10*	-14.0	-15.0	-24.0
	375.00>297.05	-14.0	-34.0	-22.0
Aristolactam I	294.05>279.10*	-12.0	-27.0	-29.0
	294.05>251.05	-12.0	-35.0	-18.0
Naproxen	231.10>185.15*	-16.0	-15.0	-24.0
	231.10>170.15	-16.0	-26.0	-16.0

* Quantitative ion

Table 3 Calibration Curve Linearity and Range, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Compound	Calibration curve	R^2	Linearity range (µg/L)	LOD	LOQ
Aristolochic acid A	Y = 0.843484X + 0.0184829	0.999	0.05-50	0.09	0.27
Aristolochic acid B	Y = 0.382409X + 0.0029026	0.999	1.0-50	0.66	1.99
Aristolochic acid C	Y = 0.833272X - 0.0366437	0.999	0.05-50	0.06	0.19
Aristolochic acid D	Y = 1.33628X - 0.0340039	0.999	0.05-50	0.14	0.41
Aristolactam I	Y = 4.28566X - 0.0844784	0.999	0.05-50	0.04	0.13

Recovery Rate

The recovery rate in the spike and recovery test is an index of accuracy. Table 5 shows the recovery rates (n = 4) for the target standards in the extracts of *Herba aristolochiae* and *Asarum sieboldii*. With both herbs, the recovery rate ranged from 85.5% to 146% with low concentration addition (1.0 µg/L) and from 60.1% to 151% with high concentration addition (20 µg/L). These are satisfactory recovery rates for general applications. As the estimated reason for the low recovery rate (60%) of aristolactam I from *H. aristolochiae*, it is thought that the internal standard could not be reflected correctly due to the matrix effect, as aristolactam I has an amide structure, whereas naproxen contains a carboxy group.

Measurement of Actual Samples

Fig. 5 shows the data when *H. aristolochiae* and *A. sieboldii* samples were prepared by the procedure in Fig. 3, and the aristolochic acids and their metabolites were measured. Table 6 shows the contents of aristolochic acids A to D and aristolactam I. High contents of aristolochic acid D were found in the *H. aristolochiae* samples, and high contents of both aristolochic acid D and aristolactam I were detected in the *A. sieboldii* samples.

Table 4 Repeatability of Peak Area

Compound	Concentration (µg/L)	No. of measurements						Average (µg/L)	RSD (%)
		1	2	3	4	5	6		
Aristolochic acid A	0.2	0.23	0.28	0.22	0.25	0.29	0.26	0.25	11.5%
	10	9.92	9.67	8.84	9.24	9.36	9.22	9.37	4.02%
	50	49.4	48.9	43.6	48	51.3	50.9	48.7	5.68%
Aristolochic acid B	1	1.46	1.35	1.19	1.32	1.35	1.38	1.34	6.59%
	10	8.23	9.83	7.8	8.61	9.46	9.43	8.89	9.01%
	50	50.7	50.5	44.3	50	52.6	51.5	50.0	5.81%
Aristolochic acid C	0.2	0.2	0.17	0.21	0.13	0.27	0.15	0.19	26.6%
	10	7.37	8.53	6.98	7.22	8.01	7.98	7.68	7.64%
	50	47.9	50.5	43.6	49.1	51.8	46.6	48.2	6.07%
Aristolochic acid D	0.2	0.12	0.16	0.24	0.21	0.17	0.14	0.17	26.7%
	10	7.58	8.34	7.35	8.28	8.44	8.83	8.14	6.87%
	50	49.3	50.5	45.6	50	53	45.3	48.9	6.10%
Aristolactam I	0.2	0.2	0.21	0.17	0.18	0.21	0.21	0.2	8.39%
	10	8.2	8.9	7.7	8.42	9.07	8.92	8.54	6.19%
	50	48.4	46.7	45.2	47.8	49.7	46.1	47.3	3.47%

Table 5 Recovery Rates of *H. aristolochiae* and *A. sieboldii*

Addition (µg/L)	Plant name	Compound	Recovery rate (%)			
			1	2	3	4
1.0	*H. aristolochiae*	Aristolochic acid A	129	125	108	95.4
		Aristolochic acid B	109	134	85.7	120
		Aristolochic acid C	113	115	106	105
		Aristolochic acid D*	——	——	——	——
		Aristolactam I	112	101	85.5	101
1.0	*A. sieboldii*	Aristolochic acid A	134	140	119	120
		Aristolochic acid B	132	119	146	133
		Aristolochic acid C	93.6	101	93.5	101
		Aristolochic acid D*	——	——	——	——
		Aristolactam I	——	——	——	——
20.0	*H. aristolochiae*	Aristolochic acid A	74	87.4	71	79.8
		Aristolochic acid B	86.5	78.5	91.9	74.6
		Aristolochic acid C	85	88.1	80.4	81.6
		Aristolochic acid D	118	151	127	119
		Aristolactam I	65.4	60.1	65.6	60.5
20.0	*A.sieboldii*	Aristolochic acid A	94.1	97.7	106	110
		Aristolochic acid B	97.3	93.5	96.7	104
		Aristolochic acid C	97.8	101	94.4	104
		Aristolochic acid D	104	100	105	102
		Aristolactam I	86.0	72.5	87.9	99.0

* Not identified.

Conclusion

This technique made it possible to determine the contents of aristolochic acid and its metabolite aristolactam in herbs in a time of approximately 20 s per sample with only simple pretreatment. This measurement technique using an internal standard is also applicable to other substances. Moreover, the principle of ionization in PESI is ESI, suggesting the possibility that substances which can be measured by ionization with the ESI probe in LC/MS can also be measured by PESI. In addition, because simple switching with Shimadzu triple quadrupole-type LCMS Series (models LCMS-8045 and higher) is possible, comparison of the data between the LCMS-8060 and DPiMS, and analysis by DPiMS by customizing analysis methods prepared with Shimadzu LCMS are also possible.
Based on these points, analysis by DPiMS is expected to become a useful technique in situations where rapid screening and quantitation of harmful substances are necessary.

Table 6 Contents of Target Compounds in *H. aristolochiae* and *A. sieboldii* (n = 4)

Plant name	Compound	Average (μg/g)	RSD (%)
H. aristolochiae	Aristolochic acid A	11.6	2.0%
	Aristolochic acid B	8.17	9.4%
	Aristolochic acid C	7.49	10.3%
	Aristolochic acid D	90.4	4.8%
	Aristolactam I	5.89	4.2%
A. sieboldii	Aristolochic acid A	6.39	4.0%
	Aristolochic acid B	6.35	9.5%
	Aristolochic acid C	1.55	3.4%
	Aristolochic acid D	36.5	4.3%
	Aristolactam I	82.0	3.7%

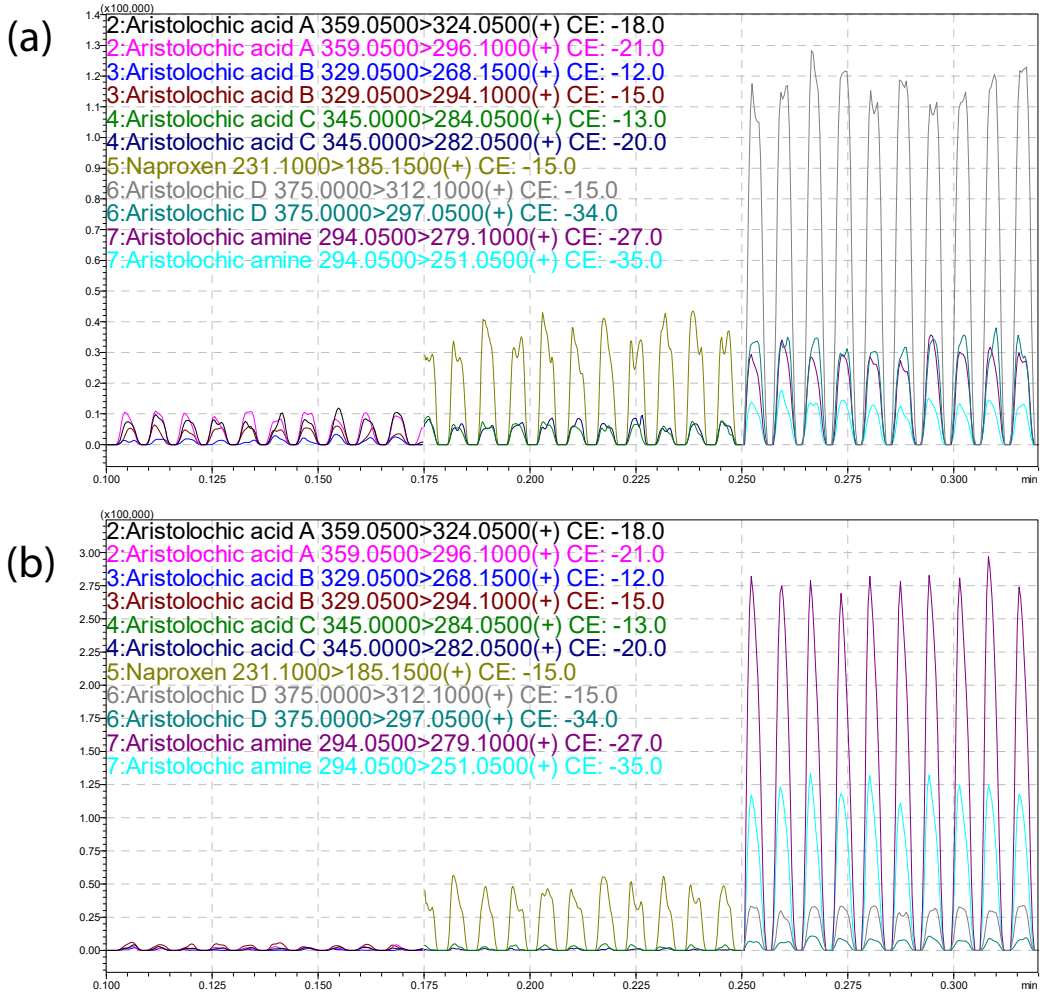

Fig. 5 MRM Data from Measurement of Actual Samples
(a): *A. sieboldii*, (b): *H. aristolochiae*

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First Edition: May 2020

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