SHIMADZU
Excellence in Science

Liquid Chromatograph Mass Spectrometer LCMS-8060

Application News

LC-MS/MS Method for Analysis of Pyrrolizidine Alkaloids (PAs) in Herbal Teas

Jihyun Lee
Shimadzu Scientific Korea

User Benefits

- This analytical method for simultaneous analysis of 28 pyrrolizidine alkaloids in herbal teas is capable of qualitative and quantitative analysis.
- The verification for established method was evaluated in terms of linearity, LOQ, recovery rate and the reliable results were obtained.

■ Introduction

Pyrrolizidine alkaloids (PAs) are natural toxic substances produced by plants to protect themselves from the outside and are known to damage the human liver.

The International Agency for Research on Cancer (IARC) classified some of the pyrrolizidine alkaloids into Group 2B (possibly carcinogenic to humans) and Group 3 (unclassifiable as to its carcinogenicity to humans). The basic structure of pyrrolizidine alkaloids is two aromatic pentanes containing the nitrogen. The structures for some compounds are as shown in Fig. 1.

Currently, there are no safety standards for pyrrolizidine alkaloids in Korea, except for bee pollen products. However, detection cases of pyrrolizidine alkaloids have been reported both domestic and foreign for herbal teas, plants in the Asteraceae, honey and some herbal medicines.

Therefore, this newsletter introduces the LC-MS/MS analysis method for detecting pyrrolizidine alkaloids in herbal teas and aims to help establish safety standards.

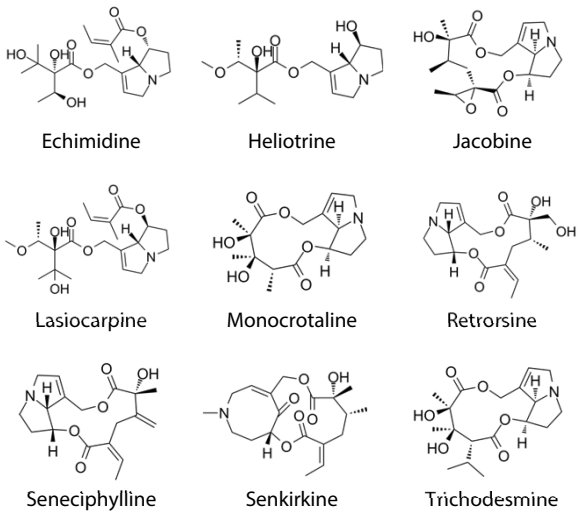

Fig. 1 Examples of Structures of Pyrrolizidine Alkaloids

■ Measurement Conditions

In this analysis, a Shimadzu liquid chromatography mass spectrometer LCMS-8060 and a Shim-pack™ GIST C18-HP (2.1×150 mm, 3 µm) column were used for optimization of 28 pyrrolizidine alkaloids. The instrumental conditions and MRM conditions are as shown in the Table 1 and Table 2, respectively. The chromatogram of the pyrrolizidine alkaloid mixed standard solution is as shown in Fig. 2.

Table 1 Instrumental Conditions

Liquid chromatograph	Nexera™ X3
Column	: Shim-pack™ GIST C18-HP (2.1 mm I.D. × 100 mm, 3 µm; P/N: 227-30039-04)
Flow rate	: 0.3 mL/min
Mobile phase (A)	: Water containing 0.1 % formic acid and 5 mM ammonium formate
Mobile phase (B)	: Methanol containing 0.1 % formic acid and 5 mM ammonium formate
Oven temp.	: 30 ˚C
Injection volume	: 10 µL
Gradient	: 1 % B (0.0 - 1.5 min) - 15 % B (3.0 min) 30 % B (18.0 min) - 95 % B (19.0 - 21.0 min) 1 % B (21.0 - 25.0 min)
Mass spectrometer	LCMS-8060
Interface	: ESI
Data acquisition	: MRM mode, positive mode
Interface temp.	: 400 ˚C
DL Temp.	: 300 ˚C
Heat block Temp.	: 400 ˚C
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Flow	: 5.0 L/min
Heating Gas Flow	: 15.0 L/min

Table 2 Multiple Reaction Monitoring (MRM) Conditions

Compound name	Precursor ion (m/z)	Product ion(1) (m/z)	Product ion(2) (m/z)
Echimidine	398	120	220
Echimidine-N-oxide	414	254	352
Erucifoline	350	120	138
Erucifoline-N-oxide	366	94	119
Europine	330	138	156
Europine-N-oxide	346	172	111
Heliotrine	314	138	156
Heliotrine-N-oxide	330	172	111
Intermedine	300	94	138
Intermedine-N-oxide	316	172	94
Jacobine	352	120	155
Jacobine-N-oxide	368	296	120
Lasiocarpine	412	120	336
Lasiocarpine-N-oxide	428	254	94
Lycopsamine	300	94	138
Lycopsamine-N-oxide	316	172	94
Monocrotaline	326	120	94
Monocrotaline-N-oxide	342	137	119
Retrorsine	352	120	138
Retrorsine-N-oxide	368	94	118
Senecionine	336	120	94
Senecionine-N-oxide	352	94	118
Seneciphylline	334	120	94
Seneciphylline-N-oxide	350	120	94
Senecivernine	336	120	308
Senecivernine-N-oxide	352	118	94
Senkirkine	366	168	122
Trichodesmine	354	222	120

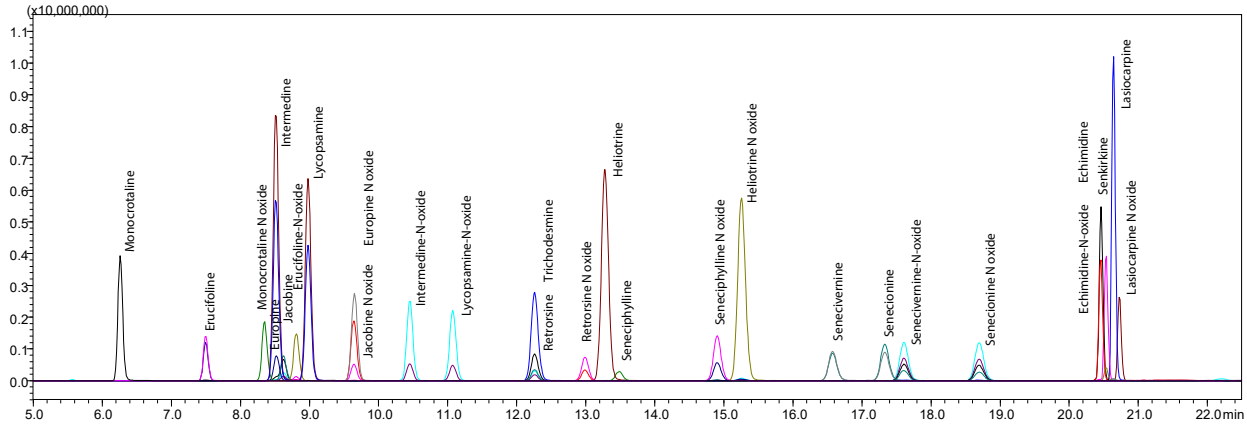

Fig. 2 MS Chromatogram of 28 Pyrrolizidine Alkaloids (concentration: 10 ng/mL)

■ Sample Pretreatment

The herbal tea sample was pulverized to homogenize and then the extraction, purification and concentration processes were performed. The details of the pretreatment process are as shown in Fig. 3 [1].

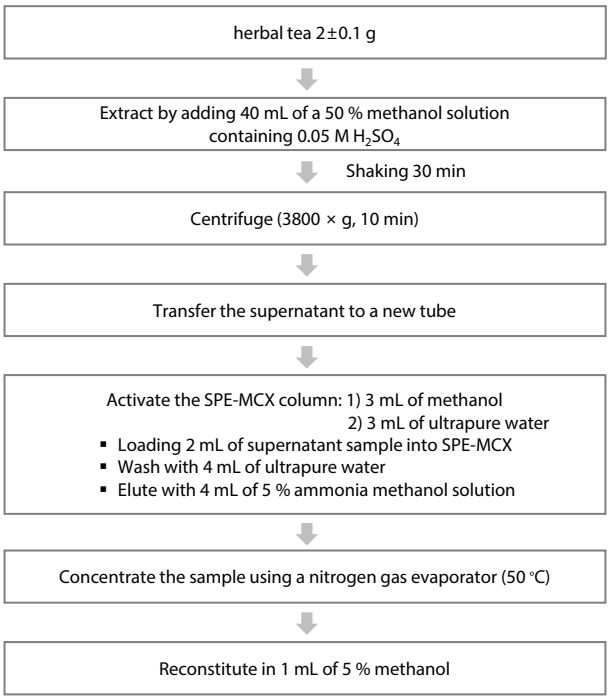

Fig. 3 Sample Preparation Protocol

■ Results and Discussion

Calibration curve

The analytical method was validated using green tea leaves among herbal teas. Quantification was performed using matrix-matched calibration. The range of the calibration curve was set differently for each compound in consideration of the limit of quantification (LOQ). The coefficients of determination (r^2) of the calibration curve for all compounds were excellent at 0.99 or more.

Recovery rate and Limit of quantification

A mixed standard solution of pyrrolizidine alkaloids was added to the herbal tea sample at low, medium, and high concentration levels within the range of the calibration curve. Five samples were prepared by pretreating for each concentration. The recovery rate results ranged from 75 % to 115 %, and the relative standard deviation (%RSD) was within 17 %. The limit of quantification (LOQ) was calculated using Labsolutions™ software as S/N=10. The obtained limit of quantification (LOQ) ranged from 0.1 ng/g to 8.5 ng/g depending on the compounds (Table 3).

Table 3 Validation Results of Pyrrolizidine Alkaloid Analysis Method

Compound name	Recovery rate (%) ± %RSD, *n*=5			LOQ ng/g (S/N=10)
	Low level	Medium level	High level	
Echimidine	86±9	89±11	103±7	0.1
Echimidine-N-oxide	100±9	104±12	115±5	0.2
Erucifoline	75±8	79±10	76±5	1.1
Erucifoline-N-oxide	89±15	98±12	96±9	2.2
Europine	77±11	82±12	79±9	0.2
Europine-N-oxide	85±10	92±11	92±10	1.3
Heliotrine	91±6	92±14	87±11	0.3
Heliotrine-N-oxide	90±10	101±14	106±8	0.3
Intermedine	80±6	91±12	94±13	0.8
Intermedine-N-oxide	75±7	81±10	83±11	0.7
Jacobine	82±13	85±15	88±9	1.0
Jacobine-N-oxide	102±13	102±9	99±11	0.3
Lasiocarpine	87±15	89±11	98±10	0.2
Lasiocarpine-N-oxide	100±4	112±7	115±6	3.3
Lycopsamine	78±12	86±13	87±8	2.2
Lycopsamine-N-oxide	99±8	109±10	102±9	2.2
Monocrotaline	92±9	93±13	89±10	1.1
Monocrotaline-N-oxide	82±7	93±9	91±11	0.9
Retrorsine	79±17	82±15	83±8	1.2
Retrorsine-N-oxide	98±10	103±11	109±9	8.2
Senecionine	78±11	78±13	84±7	1.5
Senecionine-N-oxide	97±12	102±7	109±8	2.1
Seneciphylline	79±8	78±13	79±9	8.5
Seneciphylline-N-oxide	75±12	77±11	77±9	1.3
Senecivernine	78±12	77±9	78±13	1.4
Senecivernine-N-oxide	98±7	105±8	110±8	0.7
Senkirkine	115±13	110±6	104±4	2.4
Trichodesmine	76±3	88±13	98±9	1.0

■ Conclusion

Simultaneous analytical method of 28 pyrrolizidine alkaloids in herbal tea was established using the Shimadzu mass spectrometer LCMS-8060. Pyrrolizidine alkaloids were quantified using a matrix-matched calibration. The calibration curves were obtained with a coefficient of determination r^2 more than 0.99. The results of recovery rate were with the range from 75 % to 115 % at three concentration levels. The limit of quantification was at the level from 0.1 ng/g to 8.5 ng/g depending on the compounds.

<Reference>

[1] Determination of pyrrolizidine alkaloids (PA) in plant material by SPE-LC-MS/MS, BfR-PA-Tea-2.0/2014

09-SSK-015-EN First Edition: May. 2023

Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu Scientific Korea
www.shimadzu.co.kr

› Please fill out the survey

Related Products Some products may be updated to newer models.

› Nexera series
Ultra High Performance Liquid Chromatograph

› LCMS-8060
High Performance Liquid Chromatograph Triple Quadr···

Related Solutions

› Food and Beverages

› Price Inquiry

› Product Inquiry

› Technical Service / Support Inquiry

› Other Inquiry