

Determination of isoquinoline alkaloids by UPLC-ESI-Q-TOF MS: Application to *Chelidonium majus* L.

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Abstract: In this study, we set up an analytical method that can be used for rapid and accurate determination of representative isoquinoline alkaloids in medicinal plants using UPLC-ESI-Q-TOF MS (ultra pressure liquid chromatography–electrospray ionization–quadrupole–time-of-flight mass spectrometry). The compounds were eluted on a C18 column with 0.1 % formic acid and acetonitrile, and separated with good resolution within 13 min. Each of the separated components was characterized by precursor ions (generated by ESI-Q-TOF) and fragment ions (produced by collision-induced dissociation, CID), which were used as a reliable database. We also performed method validation: analytes showed excellent linearity (R^2 , 0.9971–0.9996), LOD (5–25 ng/mL), LOQ (17–82 ng/mL), accuracy (91.6–97.4 %) as well as intra- and inter-day precisions (RSD, 1.8–3.2 %). In the analysis of *Chelidonium majus* L., magnoflorine, coptisine, sanguinarine, berberine and palmatine were detected by matching retention times and characteristic fragment ion patterns of reference standards. We also confirmed that, among the quantified components, coptisine was present in the highest quantity. Furthermore, alkaloid profiling was carried out by analyzing the fragment ion patterns corresponding to peaks of unknown components. In this manner, protopine, chelidonine, stylophine, dihydroberberine, canadine, and nitidine were tentatively identified. We also proposed the molecular structure of the fragment ions that appear in the mass spectrum. Therefore, we concluded that our suggested method for the determination of major isoquinoline alkaloids by UPLC-Q-TOF can be useful not only for quality control, but also for rapid and accurate investigation of phytochemical constituents of medicinal plants.

Key words: UPLC-Q-TOF, Isoquinoline alkaloids, *Chelidonium majus* L., Chemical profiling

1. Introduction

The alkaloids are basic organic compounds containing nitrogen, occurring mainly as secondary plant metabolites.¹ Most alkaloids are said to function as a defense against herbivorous animals and pathogenic

bacteria, some of them are characterized by clear physiological action on the human body such as toxicity and arousal.² Isoquinoline alkaloids, one of the various alkaloid groups, are commonly found in *Berberidaceae*, *Ranunculaceae* and *Papaveraceae* species, and are well known for their beneficial

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pharmacological effect.³ The representative pharmacologically-active compounds are berberine, palmatine, sanguinarine, coptisine, jatrorrhizine, tetrahydropalmatine, isocorydine and magnoflorine. These are reported to exhibit anti-diabetic (berberine),⁴ liver protection (berberine and isocorydine),^{5,6} anti-inflammatory (magnoflorine),^{7,8} anti-bacterial (sanguinarine and coptisine),⁹ and anti-cancer (sanguinarine)¹⁰ effects. *Chelidonium majus* L. is a herbaceous plant belonging to the *Papaveraceae* family, which has been reported to contain 46 isoquinoline alkaloids, including some of the above-mentioned alkaloids.¹¹ It is also registered as an official medicinal plant in the Korean Pharmacopoeia, and various clinical trials have been conducted on the pharmacological effects of isoquinoline alkaloids obtained from *Chelidonium majus* L. Due to the pharmacological importance of these compounds, determination of individual components in a plant resource is important in evaluating new drug development, utilization and quality control.

Reverse phase HPLC methods combining UV-visible, fluorescence and mass spectrometry detectors are generally used to determine the alkaloid.^{12,13} However, HPLC requires a long analysis time to perform simultaneous analysis and involves inconvenient pretreatment due to matrix effects.¹² Research has continuously focused on improving these problems. A recent achievement in the development of LC technology is UPLC-Q-TOF. This technique has attracted attention as an extremely powerful tool in the investigation of complex plant extracts.¹⁴ UPLC is an upgrade on traditional HPLC that enables rapid analysis, high resolution and enhanced sensitivity using elution of mobile phase with high pressure and narrow inner diameter columns (2 μm or less).¹⁵ Q-TOF measures mass information with high accuracy and precision, and can be used to predict molecular formulae with low parts-per-million error.¹⁴ It is a hybrid technology that combines the advantages of the MS/MS technique, as well as being recorded together with the fragment ions generated by collision-induced dissociation (CID) of the precursor ion.¹⁴ Moreover, unknown components can be identified by profiling of fragment ion patterns.¹⁶

Sun et al and *Qi et al* proposed a chemical profiling method to distinguish physiologically active isoquinoline alkaloids present in *Rhizoma corydalis* and golden seal using UPLC-Q-TOF.^{17,18} *Lee et al.* have validated an analytical method for rapid screening of three aconitum alkaloids with toxicity in food. Evidently, measurement of physiologically active substances using UPLC-Q-TOF has increased gradually.¹⁹ However, the compilation of an accurate database using standard compounds and the verification of a quantitative analysis method are still incomplete. In this study, we aim to establish a rapid and accurate method for the analysis of representative pharmacologically active isoquinoline alkaloids derived from medicinal plants using UPLC-Q-TOF. We selected *Chelidonium majus* L. as the representative plant sample, since it is known to be rich in isoquinoline alkaloids, and applied the analysis method to it. We also proposed and described a method for chemical profiling of fragment ions that enables identification of unknown components.

2. Experiments

2.1. Chemicals and standard solution

Standards of berberine chloride, palmatine chloride (purity, $\geq 98\%$), tetrahydropalmatine (purity, $\geq 98\%$), coptisine chloride (purity, $\geq 98\%$), jatrorrhizine (purity, $\geq 95\%$), sanguinarine chloride hydrate (purity, $\geq 98\%$), isocorydine hydrochloride and magnoflorine (purity, $\geq 98\%$) were purchased from Sigma-Aldrich (St. Louis, USA). Methanol, acetonitrile, dichloromethane and water were purchased from Merck (Darmstadt, Germany). All solvents used for the analysis were HPLC grade. Analytical grade formic acid was used for experiments.

Stock standard solutions were prepared by dissolving approximately 1 mg of each substance in a 1 mL aliquot of methanol. The mixed standard solutions were stored in the refrigerator at 4 °C prior to use.

2.2. Instrumentation conditions

UPLC analysis was performed on a Waters ACQUITY UPLC system (Waters Co., USA) equipped

with a binary solvent delivery pump, auto-sampler and photo-diode array detector. The chromatographic separation of alkaloids was carried out on a Waters ACQUITY UPLC BEH C18 2.1 × 50 mm, 1.7 μm column. The mobile phase consisted of an aqueous phase containing 0.1 % formic acid and an organic phase composed of acetonitrile. The column temperature and flow rate were maintained at 0.4 mL/min and 35 °C, respectively. The gradient elution was performed as follows: 8–15 % B from 0 to 10 min; 15–20 % B from 10 to 13 min; 20–24 % B from 13 to 16 min; 24–90 % B from 16 to 17 min; post-run at 8 % B for 3 min. The injection volume was 2 μL.

Q-TOF MS (quadrupole time-of-flight mass spectrometer) analysis was operated on a Waters Xevo G2 (Waters Co., USA) using an electrospray ionization (ESI) source in the positive mode. Raw data acquisition and processing were executed with UNIFI Ver 1.8 software (Waters Co., USA). The source temperature and desolvation gas temperature were 120 °C and 300 °C, respectively. The flow rate of desolvation and cone gas were set at 800 L/h and 60 L/h, respectively. The voltage of capillary and cone in the positive mode were set at 3.0 kV and 30 V, respectively. Leucine enkephalin (*m/z* 556.2766 in the positive mode) was used for accurate mass calibration in real time. MS^c was applied for the MS/MS with a low collision energy of 6 eV and a high collision energy of 20–50 eV.

2.3. Method validation

The proposed method was validated for linearity, limits of detection (LOD), limits of quantification (LOQ), as well as inter- and intra-day accuracies and precisions. The LOD and LOQ were determined for analytes under the chromatographic conditions at the signal to noise (S/N) of 3 and 10, respectively. Based on the measured LOQ, the stock solution of the eight standards was prepared and diluted to six concentrations (range, 20–2000 ng/mL) for the establishment of calibration curves. The intra- and inter-day accuracies and precisions of the method were determined by analyzing nine replicates of the middle concentration

corresponding to the calibration curve, and on three consecutive days, respectively. The accuracy and precision are expressed in recovery (%) and relative standard deviation (RSD), respectively.

2.4. Application of the method

In order to apply the verified analytical method on medicinal plants, *Chelidonium majus* L. was selected as a representative sample. The *Chelidonium majus* L. sample used had been cultivated in Yeongcheon, Gyeongsangbukdo, Republic of Korea. It was ground evenly in a dry state. Ten grams of the dried powder was accurately measured and extracted with 70 % methanol by initial sonication for 1 h, followed by incubation at room temperature for 24 h. After extraction, the methanol was removed using the rotary vacuum evaporator at 40 °C and lyophilized to remove the aqueous layer. Crude extracts were obtained in a yield of 10.8 %.

Pretreatment procedures for the determination of isoquinoline alkaloids from crude extracts were performed with reference to *Sun et al.*¹⁷ One gram of crude extract was accurately measured and transferred to a falcon tube with 100 % methanol. It was re-extracted for 1 h at room temperature and centrifuged (3,500 rpm) for 30 min. The supernatant was then filtered through a 0.45 μm membrane filter into an autosampler vial prior to UPLC-Q-TOF analysis.

2.5. Matrix effect

The matrix effect means that the LC-ESI-MS analysis affects the components of the sample by suppression or enhancement in the detection of analytes or internal standard. Evaluation of the matrix effect is performed by calculating the ratio of the known standard concentration and the concentration of that component in the extracted sample; the ratio of the areas of the peaks is measured by instrumental analysis.^{20,21}

3. Result and Discussion

3.1. Optimization of chromatographic separation

The representative chromatogram of isoquinoline

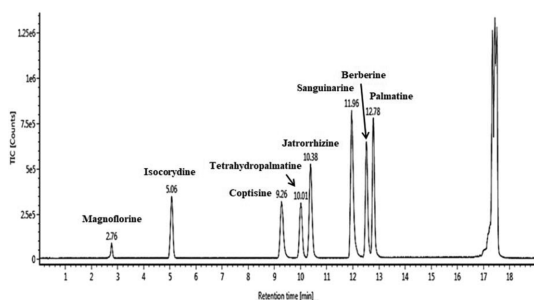


Fig. 1. Total ion current (TIC) chromatogram of the mixture of eight alkaloid standards.

alkaloids separated by UPLC is shown in Fig. 1. Analytes were separated in order of magnoflorine

(R_t , 2.76 min), isocorydine (5.06 min), coptisine (9.26 min), tetrahydropalmatine (10.01 min), jatrorrhizine (10.38 min), sanguinarine (11.96 min), berberine (12.53 min) and palmatine (12.78 min), respectively, within 13 min. The separation of alkaloids on a C18 column is affected by the pK_a value of the compounds.²² It has been reported that the elution order of berberine and palmatine changed when using an aqueous solution of pH 4.2 by adjusting pH with formic acid and ammonium acetate.¹⁶ Since most alkaloids are basic, having an unshared electron pair on nitrogen, they can be ionized on a C18 column, resulting in peak tailing or a decline in

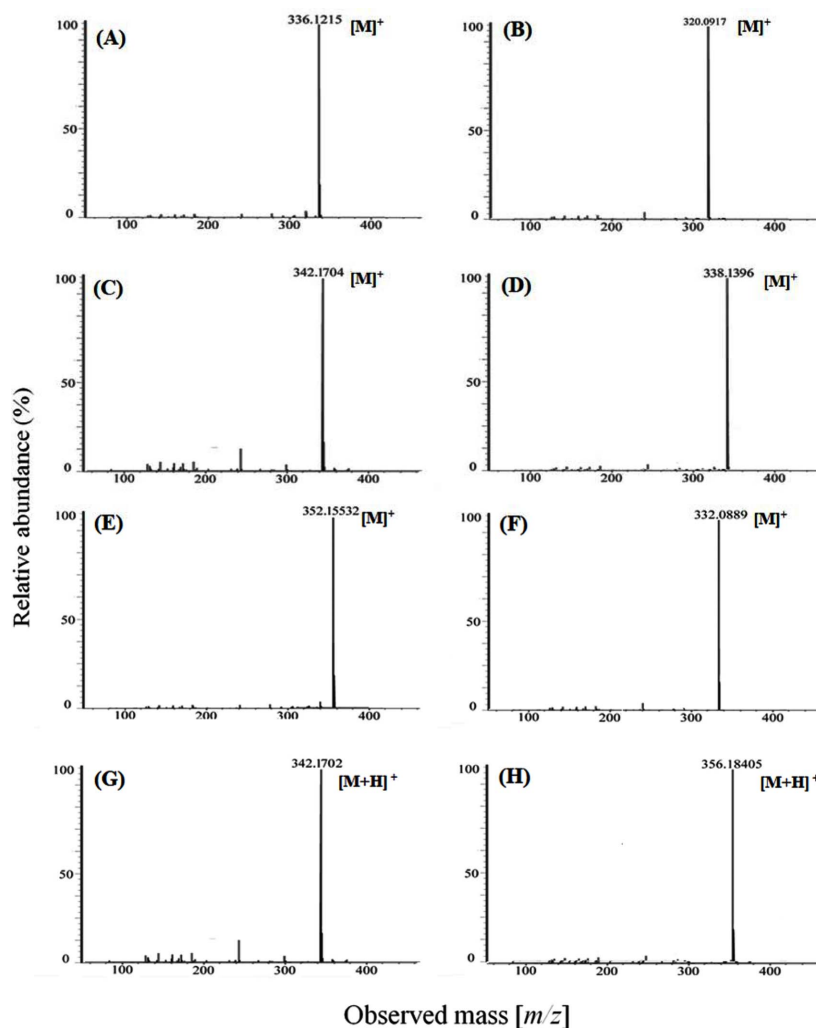


Fig. 2. MS spectrum of berberine (A), coptisine (B), magnoflorine (C), jatrorrhizine (D), palmatine (E), sanguinarine (F), isocorydine (G) and tetrahydropalmatine (H) by UPLC-Q-TOF-MS in positive mode.

separation.¹³ Also, since the commonly used HPLC column has a longer length and a larger inner diameter (3 μm or more) than UPLC column, ionization of the alkaloids can result in frequent peak tailing due to large contact area with the functional groups in the column. A buffer solution with ammonium formate or ammonium acetate was used to improve the resolution and inhibit ionization.¹⁶ However, simultaneous analysis of eight or more alkaloids requires a very long analysis time of 25–40 min or more.¹² The UPLC is capable of giving good peak resolution within a short time by passing the analytes through a column of short length and narrow inside diameter with the elution of a high-pressure mobile phase. Thus, in this study, the established separation conditions can give a good resolution in a fast time without using buffer solution. In addition, it is considered an economical method that can reduce analysis time, as well as reagent and solvent use.

3.2. MS spectra of reference standard by ESI-Q-TOF/MS

The ionized spectra of the reference standards separated in the positive mode by UPLC are shown in Fig. 2. Berberine (A), coptisine (B), magnoflorine (C), jatrorrhizine (D), palmatine (E) and sanguinarine (F) were acquired as molecular ions $[\text{M}]^+$ at m/z 336.1215, m/z 320.0917, m/z 324.1704, m/z 338.1396, m/z 352.1553 and m/z 332.0889, respectively. Isocorydine (G) and tetrahydropalmatine (H) were acquired as molecular ions $[\text{M}+\text{H}]^+$ at m/z 342.1702 and m/z 356.1840, respectively. This result shows a very accurate mass value in the range of 0–0.5 mDa

when compared with the actual mass value of each component. Since alkaloids contain nitrogen atoms, they are easily protonated in the positive mode and the method exhibits excellent sensitivity. On the other hand, in the negative mode, alkaloids cannot be detected or are detected with lower sensitivity than the positive mode.²³ The types of alkaloids are classified differently, depending on the number of hydrocarbon group substituents on the nitrogen atom. Thus, because the difference between the protonated ions of each component depends on the number of substituent hydrocarbons on the nitrogen atom, isocorydine and tetrahydropalmatine, corresponding to tertiary ammonium alkaloids, are $[\text{M}+\text{H}]^+$ ions, and berberine, palmatine, coptisine, jatrorrhizine, sanguinarine and magnoflorine, corresponding to quaternary ammonium alkaloids, are protonated to $[\text{M}]^+$ ions.²⁴

In addition, a singularity was found during database set-up with the fragment ion patterns produced by CID. In particular, the fragment ion patterns of berberine and palmatine appeared very similar. Fig. 3 shows the spectrum of fragment ions generated by CID from the molecular ions of berberine and palmatine. As shown by Fig. 3, the palmatine precursor ion $[\text{M}]^+$ was observed at m/z 352.1553, and produced fragment ions at m/z 336.1213, m/z 320.0911, m/z 292.0942 and m/z 278.0811 by CID. Similar to palmatine, berberine produced fragment ions at m/z 320.0912, m/z 292.0943 and m/z 278.0812 and the precursor was observed as precursor ion $[\text{M}]^+$ at m/z 336.1215. Also, these components were detected at similar retention time. This can cause confusion when

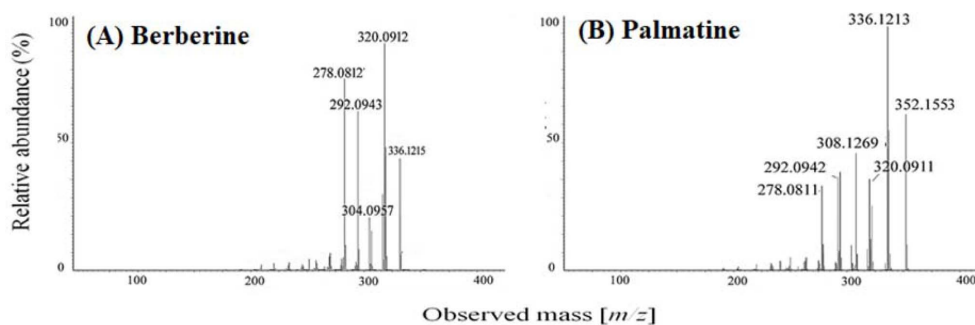


Fig. 3. MSMS spectra of berberine (A), palmatine (B) through collision energy as ramped 20–50 eV.

Table 1. Linearity, equation, LOD, LOQ, accuracy and precision of isoquinoline alkaloids

Compounds name	Regression equation	R^2	LOD (ng/ml)	LOQ (ng/ml)	Intra day		Inter day	
					Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Magnoflorine	$y = 33407x - 1238.9$	0.9984	25	82	91.6	3.2	93.5	1.8
Isocorydine	$y = 69270x - 2087.6$	0.9996	15	50	94.6	2.7	93.5	2.0
Coptisine	$y = 86051x - 3991.5$	0.9971	10	33	90.8	3.4	100.5	1.4
Tetrahydro-palmatine	$y = 100925x - 1756$	0.9995	5	17	95.1	3.8	96.1	2.1
Jatrorrhizine	$y = 96389x - 2725.5$	0.9991	10	33	96.0	3.2	98.0	3.2
Sanguinarine	$y = 170717x - 2775.2$	0.9982	15	50	96.2	3.7	98.4	2.6
Berberine	$y = 105905x - 3514.8$	0.9988	5	17	95.5	2.4	100.5	2.0
Palmatine	$y = 145472x - 7214$	0.9973	5	17	97.4	1.4	92.5	2.3

identifying the two components using fragment ion patterns. Therefore, simultaneous analysis of berberine and palmatine requires chromatographic separation of the two components.

3.3. Method validation

Table 1 shows the linearity (R^2 , correlation coefficients), LOD, LOQ, accuracies and precisions (inter- and intra-day) for the analytes. The linear regression of the peak area ratios vs concentrations (ng) was fitted over the concentration range of 10–2000 ng/mL for the analytes. The correlation coefficient was 0.9971 or more, indicating excellent linearity. The LOD range was calculated as 5–25 $\mu\text{g/mL}$, and the LOQ range was calculated as 17–70 $\mu\text{g/mL}$. The intra- and inter-day precisions and accuracies were determined by repeatedly analyzing the intermediate reference material 9 times every 3 days. The accuracy was expressed as recovery rate (%), and precision was expressed as RSD (relative standard deviation, %). The intra- and inter-day accuracies ranged from 90.8 % to 97.4 % and from 92.5 % to 100.5 %, respectively. The intra- and inter-day precisions ranged from 1.4 % to 3.8% and from 1.4 % to 3.2 %, respectively. This data showed that the established analytical method is a reliable one.

3.4. Application of the method

Fig. 4 shows the chromatogram of the extracts of *Chelidonium majus* L., analyzed by UPLC-Q-TOF.

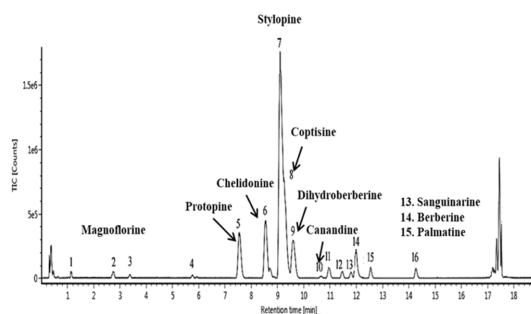


Fig. 4. Total intensity chromatogram (TIC) of *Chelidonium majus* L. extract. Refer to Table 2 for compound identification.

A total of 16 peaks were observed and five of eight isoquinoline alkaloids were detected by matching fragment ion patterns and retention times with those of the standards. We then performed chemical profiling for MS spectra and MSMS spectra of unknown peaks using Waters UNIFI software, chemical formula databases (www.chemspider.com and <http://www.massbank.jp>) and literatures. As a result, six components assumed as a type of isoquinoline alkaloid were tentatively identified. Table 2 summarizes characteristics such as mass value and formula for all components identified by UPLC-Q-TOF. The recorded mass values of all detected components ranged from 0 to -0.8 mDa and were very close to the natural mass. The content of five alkaloids in the extract was calculated from the calibration curves (Table 1) and the content of each component is shown in Table 3. Among the detected alkaloids, the coptisine content

Table 2. Compounds identified in *Chelidonium majus* L. extract by UPLC-Q-TOF-MS in positive mode

Peak number	Component	Formula	Identification status	Neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	[M] ⁺ or [M+H] ⁺	Assumed compound name
1	unknown				-					
2	Magnoflorine	C ₂₀ H ₂₄ NO ₄	Identified	342.1705	342.1695	-0.5	-1.4	2.7	[M] ⁺	
3	unknown				-					
4	unknown				-					
5	unknown	C ₂₀ H ₁₉ NO ₅	Identified	353.1263	354.1328	-0.8	-2.2	7.52	[M+H] ⁺	Protopine
6	unknown	C ₂₀ H ₁₉ NO ₅	Identified	353.1263	354.133	-0.6	-1.6	8.55	[M+H] ⁺	Chelidonine
7	unknown	C ₁₉ H ₁₇ NO ₄	Identified	323.1158	324.1229	-0.1	-0.3	9.15	[M+H] ⁺	Stylophine
8	Coptisine	C ₁₉ H ₁₄ NO ₄	Identified	320.0923	320.0917	0	-0.1	9.19	[M] ⁺	
9	unknown	C ₂₀ H ₁₉ NO ₄	Identified	337.1314	338.1385	-0.2	-0.6	9.57	[M+H] ⁺	Dihydroberberine
10	unknown	C ₂₀ H ₂₁ NO ₄	Identified	339.1471	340.1541	-0.3	-0.8	10.93	[M+H] ⁺	Canadine
11	unknown				-					
12	unknown				-					
13	Sanguinarine	C ₂₀ H ₁₄ NO ₄	Identified	332.0923	332.0915	-0.2	-0.7	12.02	[M] ⁺	
14	Berberine	C ₂₀ H ₁₈ NO ₄	Identified	336.1236	336.1225	-0.5	-1.5	12.63	[M] ⁺	
15	Palmatine	C ₂₁ H ₂₂ NO ₄	Identified	352.1549	352.1542	-0.1	-0.4	12.94	[M] ⁺	
16	unknown	C ₂₁ H ₁₈ NO ₄	Identified	348.1236	348.1224	-0.6	-1.7	14.57	[M] ⁺	Nitidine

Table 3. Alkaloid content ($n=3$) of isoquinoline alkaloid detected in *Chelidonium majus* L. extract

Compound	Amount ($\mu\text{g/g}$)	RSD (%)
Magnoflorine	0.8 \pm 0.1	1.0
Coptisine	198.3 \pm 4.8	2.4
Sanguinarine	12.6 \pm 0.8	6.3
Berberine	10.4 \pm 0.1	1.1
Palmatine	0.5 \pm 0.1	14.7

was highest, the berberine and palmatine contents were similar, and the magnoflorine and sanguinarine contents were lowest. The higher content of coptisine among the constituents of *Chelidonium majus* L. corresponds to the previously reported literature.^{12,25} However, although magnoflorine is a constituent of *Chelidonium majus* L., it is not included in the analysis studies using HPLC and UPLC-UV, because UV detection exhibits a relatively lower sensitivity for magnoflorine than for other components. Pretreatment methods, such as micro-extraction, are used to increase the sensitivity to magnoflorine,²⁶ but with a Q-TOF analyzer detection was possible without special pretreatment. Therefore, it was confirmed that the analytical method using Q-TOF is an ideal for simultaneous analysis of isoquinoline alkaloid mixtures

containing magnoflorine.

The MSMS spectra of the components identified from the chemical databases and their corresponding predicted molecular structures are shown in Fig. 5. The molecular structures were described by Waters UINIFI software only as spectra that could theoretically be fragment ions. Firstly, in the previous results (Refer to Fig. 2), alkaloids showed different ionized forms depending on the nature (tertiary or quaternary) of the ammonium salt. The alkaloids of ionized form at [M]⁺ ion were protopine, chelidonine, dihydroberberine and canadine, corresponding to tertiary ammonium salt.²⁷⁻³⁰ Since nitidine is a quaternary alkaloid, it was ionized at [M+H]⁺ ion³¹. This result suggests that the profiling of an ionized form of a molecule is very close to the actual component. Secondly, as can be seen in Fig. 5, protopine, chelidonine, stylophine, dihydroberberine and canadine were observed to have the highest spectral intensity generated by cleavage of the carbon ring of the molecular structure. Also, the protopine, chelidonine and canadine have been reported to be easily fragmented due to the Retro-Diels Alder (RDA) reaction of a ring conjugated with a heterocyclic structure containing a nitrogen atom in the molecular

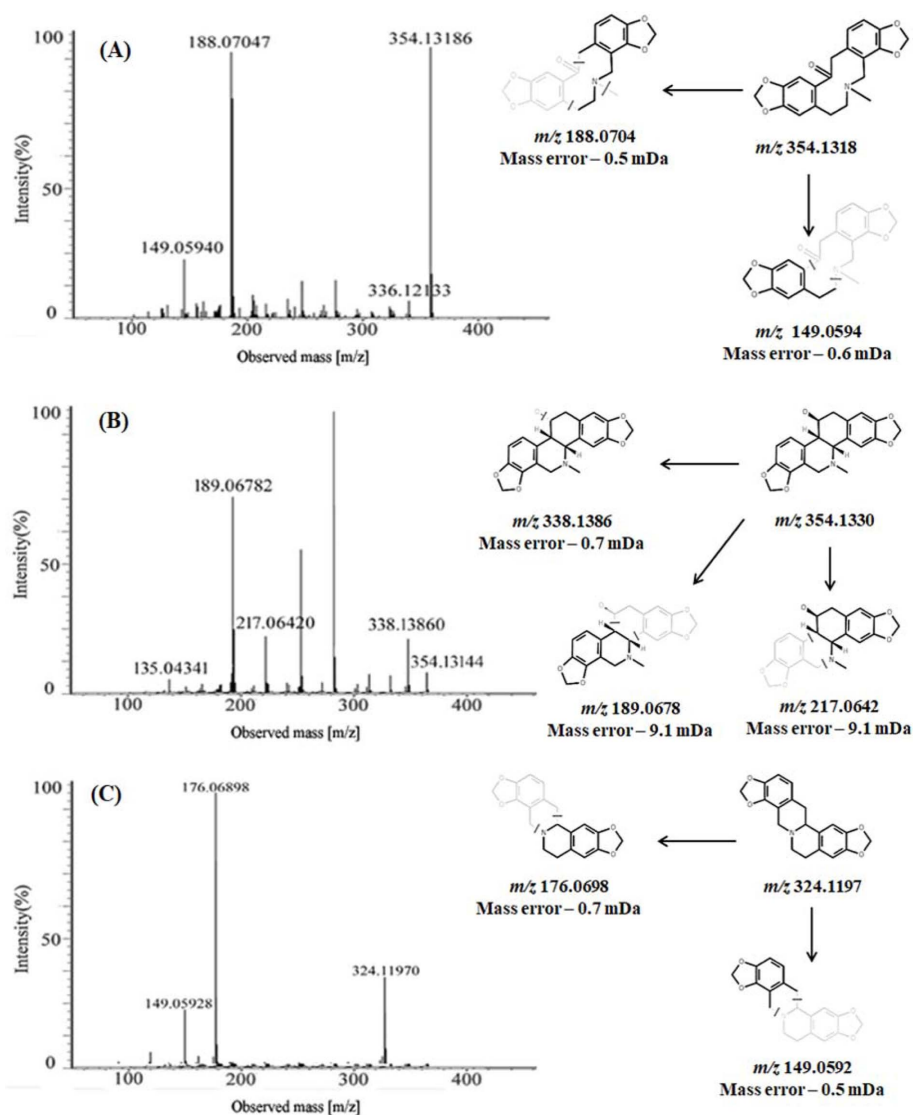


Fig. 5. MS/MS spectrum and proposed fragmentation pathways of identified compounds; protopine (A), chelidonine (B), stylophine (C), dihydroberberine (D), canadine (E) and nitidine (F).

structure.^{17,32} Unlike other alkaloids, nitidine produced characteristic fragment ions formed by loss of $-\text{CO}$ (m/z 332.0888) and $-\text{CH}_3$ (m/z 304.0960) from the precursor ion, and was reported to produce mainly m/z 332 in the range of collision energy 19–31 eV.³¹ This result can be interpreted to show that the carbon ring conjugated with a heterocyclic structure containing nitrogen atom of the nitidine species contains double bonds and is not fragmented at low collision energy. A collision energy of 40 eV or more should be used

to produce detailed fragment ions. Finally, there are no reports in the literature on the characteristic fragment ion patterns of dihydroberberine and stylophine. However, considering the mass error range of the precursor ions and the fragment ions, it was expected to be consistent with the searched components. Also, all identified components were reported to be present in *Chelidonium majus* L.¹¹ Therefore, the alkaloids identified using profiling were considered to be accurate and will be used as part of the database for

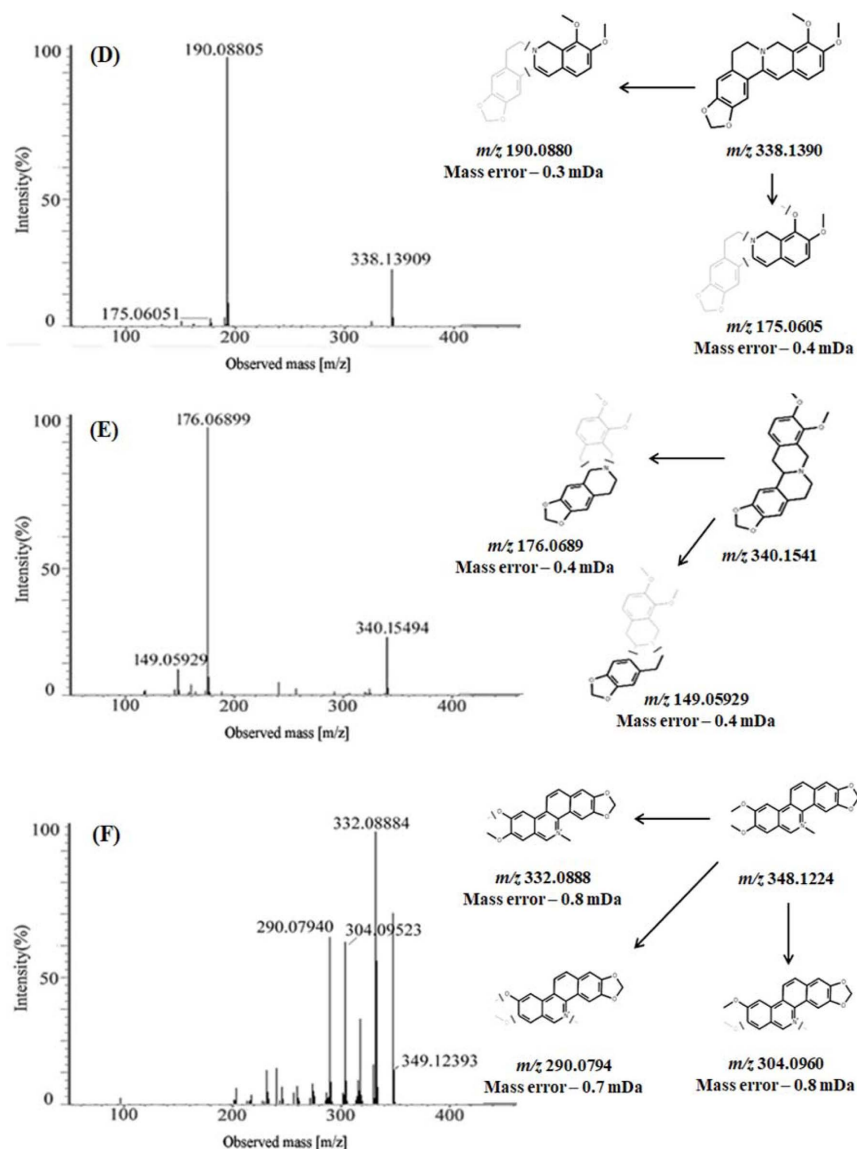


Fig. 5. Continued.

qualitative research.

3.5. Matrix effect

The matrix effect was determined by analyzing three concentrations (10, 100 and 1000 $\mu\text{g/mL}$) of isocorydine, tetrahyropalmatine and jatrorrhizine with respect to the concentration in the extracted sample. The matrix effect is generally included in the evaluation content in the method validation. However, it can be accurately evaluated when the sample such

as human urine and rat plasma does not contain any object of the analyte. Therefore, in this study, the matrix effect was evaluated by three components except for the components detected in the sample. Table 4 showed the result of the matrix effect. The range of the measured values was 90.14–102.01 %, and the overall value was slightly lower. This result can be expected to include some substances that interfere with the ionization process of the analytes during UPLC-Q-TOF component analysis.

Table 4. Matrix effect for UPLC-Q-TOF-MS of three alkaloids in *Chelidonium majus* L. extract

Compound	Spiked concentration of standard (ng/mL)		Factor of matrix effect*	RSD% (n=4)
Isocorydine	low	10	90.14 %	5.56
	middle	100	95.01 %	6.87
	high	1000	93.30 %	2.63
Tetrahydropalmatine	low	10	90.63 %	4.66
	middle	100	92.26 %	8.02
	high	1000	91.29 %	3.08
Jatrorrhizine	low	10	102.01 %	4.19
	middle	100	99.29 %	11.21
	high	1000	93.08 %	3.48

*100% : no matrix effect

<100% : ionization suppression

>100% : ionization enhancement

4. Conclusions

In this study, representative isoquinoline alkaloids with physiological activity were resolved by UPLC within 13 min and a database of ionized molecular ions generated in the positive mode and characteristic fragment ion patterns determined using reference standards was established through Q-TOF analysis. By applying the validated analytical method to *Chelidonium majus* L. extract, eleven alkaloids were detected. Among the detected alkaloids, coptisine content was confirmed to be the highest. In addition, six isoquinoline alkaloids were tentatively identified by performing profiling with molecular ions and fragment ions of unknown peaks. This data will be used as a database for qualitative research. The analysis method proposed in this study will be useful data for determining isoquinoline alkaloids in *Chelidonium majus* L. and other medicinal plants.

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