Identification and metabolite profiling of alkaloids in aerial parts of *Papaver rhoeas* by liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry

Jae-Hyeon Oh \(^1\) | In Jin Ha \(^2\) | Min Young Lee \(^2\) | Eun-Ok Kim \(^2\) | Dain Park \(^2\) | Jun-Hee Lee \(^{2,4}\) | Seok-Geun Lee \(^{2,3}\) | Do-Wan Kim \(^1\) | Tae-Ho Lee \(^1\) | Eui-Ju Lee \(^{2,4,*}\) | Chang-Kug Kim \(^1\)

\(^1\) Genomics Division, Department of Agricultural Biotechnology, National Institute of Agricultural Science (NAS), Rural Development Administration (RDA), Jeollabuk-do, Republic of Korea

\(^2\) Korean Medicine Clinical Trial Center (K-CTC), Kyung Hee University Korean Medicine Hospital, Seoul, Republic of Korea

\(^3\) KNU-KIST Department of Converging Science & Technology, Kyung Hee University, Seoul, Republic of Korea

\(^4\) Department of Sasang Constitutional Medicine, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea

Correspondence
Dr. Chang-Kug Kim, Genomics Division, Department of Agricultural Biotechnology, National Institute of Agricultural Science (NAS), Rural Development Administration (RDA), Jeollabuk-do 54874, Republic of Korea.

Email: chang@korea.kr

* Additional corresponding author

Eui-Ju Lee

Email: sasangin@hanmail.net

In Jin Ha and Jae-Hyeon Oh made an equal contribution to this work.

* * *

**Papaver** plants can produce diverse bioactive alkaloids. *Papaver rhoeas* Linnaeus (common poppy or corn poppy) is an annual flowering medicinal plant used for treating cough, sleep disorder, and as a sedative, pain reliever, and food. It contains various powerful alkaloids like rhoeadine, benzylisoquinoline, and proaporphine. To investigate and identify alkaloids in the aerial parts of *P. rhoeas*, samples were collected at different growth stages and analyzed using liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. A liquid chromatography with mass spectrometry method was developed for the identification and metabolite profiling of alkaloids for *P. rhoeas* by comparing with *Papaver somniferum*. Eighteen alkaloids involved in benzylisoquinoline alkaloid biosynthesis were used to optimize the liquid chromatography gradient and mass spectrometry conditions. Fifty-five alkaloids, including protoberberine, benzylisoquinoline, aporphine, benzophenanthridine, and rhoeadine-type alkaloids, were identified authentically or tentatively by liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry in samples taken during various growth stages. Rhoeadine alkaloids were observed only in *P. rhoeas* samples, and codeine and morphine were tentatively identified in *P. somniferum*. The liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry method can be a powerful tool for the identification of diverse metabolites in the genus *Papaver*. These results may help understand the biosynthesis of alkaloids in *P. rhoeas* and evaluate the quality of this plant for possible medicinal applications.

**KEYWORDS**

alkaloids, liquid chromatography, metabolite profiling, *Papaver rhoeas*, *Papaver somniferum*
INTRODUCTION

The annual flowering plant *Papaver rhoeas* L. (red poppy or corn poppy), is a species of the poppy family, Papaveraceae. It has been used in folk medicine for the treatment of inflammation, cough, diarrhea, respiratory problems, asthma, insomnia, and pain, and it can also be consumed as food [1–5]. Various phytochemical components can be found in corn poppy, such as alkaloids [2,6–8], anthocyanins [9], flavonoids [10], and essential oils [11]. *P. rhoeas* can be cultivated in regions with a temperate climate. In Korea, it is widely distributed and is grown as an ornamental plant. This plant is sometimes mistaken for *Papaver somniferum* L. (PS), the strictly controlled opium poppy, containing narcotic substances such as morphine, codeine, and the baine [12]. Plants in the genus *Papaver* can produce diverse alkaloids, mainly in the form of benzylisoquinoline alkaloids (BIAs) [13,14]. Alkaloids from *Papaver* species possess significant biological activity [13,15], and the alkaloid content of *Papaver* plants varies greatly depending on their growth stage, conditions, and origin [4,12,16]. Rapid and reliable analytical techniques must be developed to facilitate identification and structural determination of alkaloids in complex extracts and medicine herbs [17–23]. To discover medicinally relevant compounds and potential alternatives to plants as the commercial source of valuable BIAs, metabolomic studies of the *Papaver* species have been reported by metabolomics with chemometric approaches and transcript profiling [13,15,24–26]. However, to date, most studies have focused on the opium poppy and there have been few analytical studies on the alkaloids in *P. rhoeas* [4,12]. This is despite various reports on biological activity of its extracts based on both in vitro and in vivo experiments and clinical studies [4,5,7,27]. Many alkaloids likely present in this plant are yet to be identified. Therefore, investigation and identification of known and unknown alkaloids in *P. rhoeas* is needed for pharmacological studies, plant extract quality evaluations, and to understand species-specific biosynthetic metabolism. This study aims to develop an analytical method based on LC–QTOF-MS/MS for metabolite profiling and identification of alkaloids in *P. rhoeas* in a single LC run. Two types of *P. rhoeas* with different origins and one *P. somniferum* were cultivated and collected at different growth stages to measure the growth stage dependent variation of metabolites and to limit environmental influences. Samples collected at different time points were analyzed to achieve a more comprehensive understanding of BIA biosynthesis. The aim of the present study, in addition to developing novel LC–QTOF-MS/MS methods, was to investigate alkaloids and their changes in *P. rhoeas* at different growth stages.

MATERIALS AND METHODS

2.1 Plant material

The aerial parts of *P. rhoeas* harvested at three different growth stages were provided by the National Academy of Agricultural Science, Rural Development Administration (Korea). The specimens used in this study were deposited in the Genomics Division of the National Academy of Agricultural Science. The two types of *P. rhoeas* seeds were of different origins; one (resource name: Shiri) originated from Moscow, Russia (RS) and the other (resource name: WS2981) originated from Hoengseong in the Province of Gangwon, South Korea (RA). The seeds were used after disinfection according to a method described elsewhere [28], and preprocessing with low temperature treatment at 4°C for 90 days [29]. Two types of *P. rhoeas* (RS and RA) and one type of *P. somniferum* (PS) were sown in April. The growth conditions were 26°C and relative humidity of 45% under natural light conditions in a glass greenhouse at the NAAS (latitude [N 35° 49′ 53″; longitude [E 127° 3′ 48″]) in the Republic of Korea. For the *Papaver* genus, the floral axis is observed at 60 days, and flower closing and fruit-bearing occurs 90 days after germination. Hence, samples were collected at 30, 60, and 90 days.

2.2 Reagents and chemicals

Eighteen BIAs were purchased as authentic standards and are listed in Table 1. The reference standard of demethylcoclaurine (20 mg; purity ≥ 98%) and tetrahydropapaverine (10 mg; purity ≥ 98%) were purchased from ALB technology (Mongkok Kowloon, Hong Kong). Coclaurine (10 mg; purity ≥ 97.2%), corytuberine (10 mg; purity ≥ 98%), tetrahydroberberin (20 mg; purity ≥ 98%), berberine (20 mg; purity ≥ 98%), stylophone (10 mg; purity ≥ 98.3%), dihydrosanguinarine (10 mg; purity ≥ 98%), sanguinarine (20 mg; purity ≥ 99.8%), protopine (20 mg; purity ≥ 98.3%), and dihydroberberine (10 mg; purity ≥ 98%) were purchased from Chemface (Wuhan, China). (S)-Reticuline (10 mg; purity ≥ 98%) and L-reticuline (10 mg; purity ≥ 98%) were purchased from Carbosynth (Berkshire, UK). Tetrahydrocolumbamine (10 mg; purity ≥ 98%) and scoulerine (10 mg; purity ≥ 98%) were purchased from ChemNorm biotech (Wuhan, China). L-Tetrahydropalmatine (20 mg; purity ≥ 98%) and allocryptopine (20 mg; purity ≥ 98%) were purchased from Biopurify phytochemicals (Chengdu, China). Chelidonine (10 mg; purity ≥ 97%) was purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile (ACN) and methanol (MeOH) were purchased from Honeywell.

Reagents and chemicals

- Demethylcoclaurine (20 mg; purity ≥ 98%)
- Tetrahydropapaverine (10 mg; purity ≥ 98%)
- Coclaurine (10 mg; purity ≥ 97.2%)
- Corytuberine (10 mg; purity ≥ 98%)
- Tetrahydroberberin (20 mg; purity ≥ 98%)
- Berberine (20 mg; purity ≥ 98%)
- Stylophone (10 mg; purity ≥ 98.3%)
- Dihydrosanguinarine (10 mg; purity ≥ 98%)
- Sanguinarine (20 mg; purity ≥ 99.8%)
- Protopine (20 mg; purity ≥ 98.3%)
- Dihydroberberine (10 mg; purity ≥ 98%)
- Tetrahydrocolumbamine (10 mg; purity ≥ 98%)
- Scoulerine (10 mg; purity ≥ 98%)
- Reticuline (10 mg; purity ≥ 98%)
- L-Reticuline (10 mg; purity ≥ 98%)
- Chelidonine (10 mg; purity ≥ 97%)
- Acetonitrile (ACN)
- Methanol (MeOH)
| Peak no. | Alkaloid name       | RT (min) | Formula       | Adduct | Observed mass (Da) | Mass error (ppm) | BIA structural subgroups derived from the basic benzylisoquinoline subunit |
|---------|---------------------|----------|---------------|--------|-------------------|------------------|---------------------------------------------------------------------|
| 1       | DL-Demethyl-         | 4.98     | C₁₆H₁₇NO₃    | +H     | 272.1281          | −0.1             | Benzylisoquinoline                                                   |
| 2       | Coclaurine           | 6.01     | C₁₇H₁₉NO₃    | +H     | 286.1438          | 0.8              | Benzylisoquinoline                                                   |
| 3       | Tetrahydropapaverine | 9.10     | C₂₀H₂₂NO₄    | +H     | 344.1856          | 1.0              | Benzylisoquinoline                                                   |
| 4       | S-Reticuline         | 6.72     | C₁₉H₂₃NO₄    | +H     | 330.1700          | 0.8              | Benzylisoquinoline                                                   |
| 5       | Corytuberine         | 6.29     | C₁₉H₂₁NO₄    | +H     | 328.1543          | 0.8              | Aporphine                                                           |
| 6       | 1-Tetrahydrocolumbamine | 8.37 | C₂₀H₂₃NO₄    | +H     | 342.1700          | 0.1              | Protoberberine (tetrahydroprotoberberine)                             |
| 7       | Scoulerine           | 6.57     | C₁₉H₂₁NO₄    | +H     | 328.1543          | 0.4              | Protoberberine (tetrahydroprotoberberine)                             |
| 8       | 1-Tetrahydropalmatine| 10.51    | C₂₀H₂₃NO₄    | +H     | 356.1856          | 0.9              | Protoberberine (tetrahydroprotoberberine)                             |
| 9       | Tetrahydroberberine  | 11.64    | C₂₀H₂₃NO₄    | +H     | 340.1543          | 0.8              | Protoberberine (tetrahydroprotoberberine)                             |
| 10      | Berberine            | 13.80    | C₂₀H₁₈NO₄    |        | 336.1236          | −0.6             | Protoberberine                                                       |
| 11      | Stylopine            | 10.85    | C₂₀H₁₉NO₄    | +H     | 324.1230          | 0.9              | Protoberberine (tetrahydroprotoberberine)                             |
| 12      | Dihydrosanguinarine  | 19.10    | C₂₀H₁₅NO₄    | +H     | 334.1074          | 1.4              | Benzophenanthridine                                                  |
| 13      | Sanguinarine         | 12.75    | C₂₀H₁₄NO₄    |        | 332.0923          | −0.6             | Benzophenanthridine                                                  |
| 14      | Protopine            | 9.22     | C₂₀H₁₉NO₅    | +H     | 354.1336          | 0.8              | Protopine                                                           |
| 15      | Allocryptopine       | 10.21    | C₂₁H₂₃NO₅    | +H     | 370.1649          | 0.1              | Protopine                                                           |
| 16      | Chelidonine          | 10.18    | C₂₀H₁₉NO₅    | +H     | 354.1336          | −0.1             | Benzophenanthridine                                                  |
| 17      | Dihydroberberine     | 10.95    | C₂₀H₁₉NO₄    | +H     | 338.1387          | 0.1              | Protopine                                                           |

Burick & Jackson (Morristown, NJ) and were of HPLC grade. Analytical-grade formic acid (99% purity) and ammonium formate were obtained from Sigma–Aldrich. Deionized water (>18 mΩ) was obtained by a pure water purification system (Human, Korea).

### 2.3 Sample preparation

Aerial parts of the lyophilized *Papaver* species were first ground into a fine powder. Approximately 2 g of each sample was then ultrasonicated in 5 mL of ethanol for 30 min, and centrifuged at 13 000 rpm for 15 min at 4°C. The supernatants were filtered through a 0.2 μm polytetrafluoroethylene syringe filter (Thermo Scientific, Waltham, MA). Finally, the filtrate was diluted with ethanol to 20 mg/mL and transferred to an LC sample vial before use.

### 2.4 LC and MS analysis conditions

The LC–MS system consisted of a Thermo Scientific Vanquish UHPLC system (Thermo Fisher Scientific, Sunnyvale, CA) with an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.7 μm; Waters, Milford, MA) and a Triple TOF 5600+ mass spectrometer system (Triple TOF MS; QTOF, Sciex, Foster City, CA). The QTOF-MS, equipped with a Duospray™ ion source, was used to complete the high-resolution experiment. The LC gradient used a mobile phase A containing 0.05% formic acid and 2.5 mM ammonium formate in water and a mobile phase B of acetonitrile. The flow rate was kept constant at 0.4 mL/min and the injection volume was 1 μL. The gradient elution system began at 1% B for 2.5 min, 1 to 10% B from 2.5 to 3.0 min, 10 to 19% B from 3.0 to 6.0 min, 19 to 22% from 6.0 to 9.0 min, 22 to 25% B from 9.0 to 14.0 min, 25 to 70% B from 14.0 to 17.0 min, and then increased to 100% B at 19.0 min, held at 100% B for 3 min and then returned to the initial conditions for re-equilibration.

Mass data acquisition was performed with a Triple TOF 5600+ in positive ion mode using the following parameters: source temperature was set at 450°C with a curtain gas flow of 25 L/min (GS1 and GS2 both 50), the ion spray voltage was set at 4500 V, declustering potential was 50 V, and the collision energy was 10 V. High-purity nitrogen gas was used for the nebulizer/Duospray™ and curtain gases. The QTOF and
RESULTS AND DISCUSSION

3.1 Sample preparation

Five different extraction solvents were examined for effectiveness in metabolite extraction from the aerial parts of plants: 50 and 80% methanol in water, pure methanol, pure ethanol, and ethyl ether with 10% ammonia. Samples were extracted and analyzed by the LC–QTRAP (hybrid triple quadrupole/linear ion trap) MS system (ABSCIEX, Foster City, CA). To study the effects of the extraction process on metabolite quantitation, the peak intensities of the extracted metabolites were compared. As a first approximation, a gradient system developed in a previous study was used [30]. The protopine authentic metabolite was detected in all tested samples. When ethyl ether was used, the peak area of protopine was the highest of all the extraction solvents tested, as shown in Supporting information Table S1 and Figure S1. According to the peak areas, the efficiency of extraction solvents was as follows: ethyl ether with 10% ammonia > pure ethanol > 80% methanol in water > methanol > 50% methanol in water. However, ethyl ether is volatile, and its extracts require additional sample preparation steps including evaporation and re-dilution to obtain reproducible data. For metabolomics, sample preparation should be easy, convenient, robust, and reproducible, especially for large-scale analyses. Therefore, ethanol, the second most efficient extraction solvent was selected as the ideal extraction solvent for the aerial parts of 
P. rhoeas
and 
P. somniferum
.

3.2 LC–MS analysis

Several previous studies analyzed and separated BIAs using similar analytical techniques as presented here [17–19,30–32]. The simultaneous analysis of the alkaloids with different skeletons present in the 
P. rhoeas
and 
P. somniferum
genera was not a simple task. The chromatographic retention behavior of alkaloids on an RP column significantly decreases at low pH, and significantly increases at neutral pH [33,34]. To optimize the gradient and mass spectrometer conditions, analysis of a mixture of 18 authentic standards (Table 1) was performed with the UHPLC–QTOF system. To identify suitable chromatographic conditions, different elution systems were tested and their selectivity, sensitivity, resolution, tailing factor, and peak widths were considered as important parameters. First, a gradient system with the mobile phases of 5 mM ammonium formate in water (A) and acetonitrile (B) was optimized on C18 and pentafluorophenyl columns. Although the pentafluorophenyl column separated allocryptopine, protopine, and chelidonine well, which were not separated by the C18 column, it showed peak broadening of (dihydro)berberine and (dihydro)sanguinarine using 5 mM ammonium acetate or ammonium formate as mobile-phase adducts. In addition, the repeatability was suboptimal because of retention time (RT) drift. The aqueous C18 column, compatible with 100% aqueous mobile phase, exhibited more reproducible RTs and was selected as the ideal analytical column for the LC–QTOF-based metabolomic analysis of alkaloids in 
P. rhoeas
. Then, three elution conditions were tested on the Acquity UPLC HSS T3 column; 5 mM ammonium formate, 0.1% formic acid, and a combination of 0.05% formic acid and 2.5 mM ammonium formate as additives. Based on the optimized gradient system, elutions with different additives were performed on the authentic standards and the results are shown in Figure 1. The chromatographic separation and resolution among authentic standards were good for the ammonium formate additive, as shown in Figure 1A, but peak tailing and broadening were observed. This resulted in peak splitting and difficulties during the metabolomics data pre-processing for samples containing a complex mixture of metabolites. As shown in Figure 1B, the use of moderately acidic additives improved the chromatographic properties when compared to ammonium formate alone. The use of 0.05% formic acid together with the addition of 2.5 mM ammonium formate improved the chromatographic resolution and detection sensitivity of the alkaloids (Figure 1C), which agrees well with the literature [33].

3.3 Identification of the alkaloids

Eighteen authentic standards representing the major alkaloids in 
P. rhoeas
and 
P. somniferum
were analyzed by LC–QTOF with the column described in Section 2 and optimized LC conditions. Table 1 summarizes the chromatographic information (RTs) and MS-based information (adduct forms, observed mass, product ions). Highly abundant protonated molecule [M+H]+ ions of most alkaloids, except for the peak no. 11 (berberine) and 14 (sanguinarine), were observed in the ESI mass spectra due to the strong basicity of the secondary or tertiary amine group. Characteristically, berberine and sanguinarine showed strong intensities of intact [M]+ ion in the ESI-MS analysis because of the cation on the nitrogen atom as explained in the previous report [35]. The MS/MS spectra of the authentic standards are shown in Supporting Information Figure S2 and were used for identification of alkaloids from the 
P. rhoeas
(RS and RA)
Representative extracted ion chromatography (XIC) of 18 authentic standards with different mobile phase compositions: 5 mM ammonium formate in water/acetonitrile (A), 0.1% formic acid in water/acetonitrile (B), 2.5 mM ammonium formate and 0.05% formic acid in water/acetonitrile (C); (1) DL-demethylcoclaurine, (2) coclaurine, (3) tetrahydropapaverine, (4) S-reticuline, (5) corytuberine, (6) L-reticuline, (7) tetrahydrocolumbamine, (8) scoulerine, (9) L-tetrahydropalmatine, (10) tetrahydroberberine, (11) berberine, (12) stylopine, (13) dihydrosanguinarine, (14) sanguinarine, (15) protopine, (16) chelidonine, (17) allocryptopine, and (18) dihydroberberine and P. somniferum (PS) samples. In addition, structural information such as formula and fragment patterns of many of the BIAs have been previously reported [2,6,8,13,14,24,30,36]. This information combined with the in-house library on the Sciex instrument and online databases such as Metlin and MS bank were used for the tentative identification of various alkaloids in the RS, RA, and PS samples collected at 30, 60, and 90 days. As can be seen in Figure 2, the samples taken at 90 days show quite different chromatograms because they are composed of a larger variety of metabolites (the chromatograms of the other samples are shown in Supporting Information Figure S3). The identification of each component in the extract was first analyzed by comparison to authentic standards (Table 1) with MasterView in the PeakView software. Eight metabolites, demethylcoclaurine, coclaurine, (R/S)-reticuline, stylopine, dihydrosanguinarine, and protopine were identified in the three samples during all growth stages even though their abundance varied depending on species and growth stage. Protopine (a protopine-type alkaloid) and stylopine (a tetrahydroprotoberberine-type alkaloid) from the RA and RS samples were more abundant during early growth stage (30 days) than at 60 and 90 days and could be characteristic metabolites of P. rhoeas (Figure 3). DL-Demethylcoclaurine, coclaurine, and (R/S)-reticuline (a benzylisoquinoline-type alkaloid) and corytuberine (an aporphine-type alkaloid) were observed in low abundance in RS and RA, but in the PS samples, these alkaloids significantly increased over the course of the growth process. Tetrahydrocolumbamine, tetrahydropalmatine, and tetrahydroberberine (a tetrahydroprotoberberine-type alkaloid) and berberine (a protoberberine-type alkaloid) were found in low abundance and only in the RS samples harvested at 30 and 90 days. Sanguinarine (a benzophenanthridine-type alkaloid) was found
FIGURE 2  Representative base peak ion chromatograph (BPC) of the ethanol extracts of RS at 90 days (A), RA at 90 days (B), and PS at 90 days (C). The two types of *Papaver rhoeas* seeds have different origins; one (resource name: Shiri) originated from Moscow, Russia (RS) and the other (resource name: WS2981) originated from Hoengseong in the Province of Gangwon, South Korea (RA).

only in the RS samples at 60 days. Also, dihyrosanguinarine (a benzophenanthridine-type alkaloid) in the RS samples harvested at 60 days was significantly more abundant than the other specimens. Tetrahydropapaverine (a papaverine-type alkaloid), scoulerine (a protoberberine-type alkaloid), and allocryptopine (a protopine-type alkaloid) were not observed in either RS or RA samples. Dihydroberberine (a protoberberine-type alkaloid) and chelidonine (a benzophenanthridine-type alkaloid) were not observed in any samples. According to the results, there is no significant change of representative metabolites in BIA biosynthesis from *P. rhoeas* (RS and RA).

For further identification of the alkaloids, the LC–QTOF base peak chromatogram collected in information-dependent acquisition scan mode was processed to search and screen alkaloid metabolites by exact masses against an in-house library and databases such as Chemspider, Metlin, and MS bank. The proposed identity of the alkaloid was compared...
to the RT and characteristic product ions (fragmentation patterns) in the reference MS/MS spectra. A total of 54 alkaloids were identified or tentatively identified in the base peak chromatograms of the RA, RS, and PS samples. Table 2 lists the benzylisoquinoline alkaloids that could be authentically or tentatively identified in the samples by the developed LC–QTOF-MS/MS method. Alkaloids identified or tentatively identified in the aerial parts of the cultivated \textit{P. rhoeas} and \textit{P. somniferum} are summarized in Supporting Information Table S2. Also, metabolites (colored or bold text) from \textit{P. rhoeas} (RS and RA) identified in benzylisoquinoline alkaloid (BIA) biosynthesis pathways \cite{24} were marked as shown in Supporting Information Figure S4. Further analysis of precursors of BIA was conducted by LC–QTOF. The precursors such as tyrosine, 4-hydroxyphenyacetaldehyde, tyramine, dopamine due to polarity were effectively extracted with distilled water from aerial parts of RS and RA. Those were identified, and intensities as determined by LC–QTOF analysis for samples at different growth stages (30, 60, and 90 cultivating days) are expressed in Supporting Information Figure S4. Tyramine, dopamine, and 4-hydroxyphenyacetaldehyde increased during growth, and the contents of tyramine increased significantly. Stylopine, protopine, and rhoeadine were mainly observed as secondary metabolites over all stages from the aerial parts of \textit{P. rhoeas}.

4 | CONCLUDING REMARKS

In this study, an effective LC–QTOF-MS/MS method was developed for the simultaneous identification of alkaloids in \textit{Papaver} species. Alkaloids in \textit{P. rhoeas} were separated and analyzed by LC–QTOF in a single run. Simultaneously, narcotic substances such as morphine and codeine derived from the opium poppy could be monitored. This could be helpful in the discrimination between species in the genus \textit{Papaver}, as some are controlled substances whereas others are commonly found ornamental plants. In this first attempt to investigate the content, abundance, and identity of alkaloids present during the growth of \textit{P. rhoeas}, information related to the growth stage and origin could be useful in understanding the biosynthesis and metabolism of alkaloids in \textit{P. rhoeas}, even those in low abundance using MS-based metabolite profiling.
| Peak no. | Name                        | Formula       | Expected RT (min) | Predicted mass (Da) | Adduct form [M]+ or [M+H]+ | Observed mass (Da) | Product ions (MS/MS fragment ions, m/z) |
|---------|-----------------------------|---------------|-------------------|---------------------|-----------------------------|-------------------|----------------------------------------|
| 1       | DL-Demethylcoclaurine       | C_{18}H_{21}NO_{3} | 4.98              | 271.1208            | [M+H]+                      | 272.1281          | 107.0893, 255.1015, 161.0591, 143.0490 |
| 2       | Coclaurine                  | C_{17}H_{19}NO_{3} | 6.03              | 285.1365            | [M+H]+                      | 286.1438          | 107.0894, 269.1167, 175.0744, 237.0905 |
| 3       | Tetrahydropapaverine        | C_{20}H_{24}NO_{4} | 9.11              | 343.1784            | [M+H]+                      | 344.1856          | 192.1023, 189.0912, 151.0575, 327.1600 |
| 4 & 6   | Reticuline                  | C_{19}H_{21}NO_{4} | 6.74              | 329.1627            | [M+H]+                      | 330.1700          | 192.1017, 137.0598, 143.0490, 175.0749 |
| 5       | Coryzisterpine              | C_{18}H_{22}NO_{4} | 6.28              | 327.1471            | [M+H]+                      | 328.1543          | 265.0859, 237.0908, 297.1122, 205.0644 |
| 7       | Tetrahydrocolumbamine       | C_{20}H_{23}NO_{4} | 8.38              | 341.1627            | [M+H]+                      | 342.1700          | 178.0870, 163.0634, 176.0711          |
| 8       | Scoulerine                  | C_{19}H_{22}NO_{4} | 6.57              | 327.1471            | [M+H]+                      | 328.1543          | 237.0919, 207.0445, 211.0478, 239.0707 |
| 9       | 1-Tetrahydropalmitatine     | C_{21}H_{23}NO_{4} | 10.53             | 355.1784            | [M+H]+                      | 356.1856          | 192.1017, 165.0905, 176.0700          |
| 10      | Tetrahydroberberine (canadine) | C_{20}H_{24}NO_{4} | 11.68             | 339.1471            | [M+H]+                      | 340.1543          | 176.0707, 149.0595, 174.0548          |
| 11      | Berberine                   | C_{20}H_{24}NO_{4} | 13.83             | 336.1236            | [M+]+                        | 336.1236          | 320.0922, 292.0970, 321.1000, 306.0762, 278.0813 |
| 12      | Stylopine                   | C_{18}H_{21}NO_{3} | 10.94             | 323.1158            | [M+H]+                      | 324.1230          | 176.0705, 149.0596                     |
| 13      | Dihydrosanguinarine         | C_{20}H_{24}NO_{4} | 19.10             | 333.1001            | [M+H]+                      | 334.1074          | 318.0974, 319.0842, 304.0967, 276.1016 |
| 14      | Sanguinarine                | C_{20}H_{22}NO_{4} | 12.77             | 332.0923            | [M]+                        | 332.0923          | 317.0683, 274.0861, 304.0969          |
| 15      | Protopine                   | C_{19}H_{22}NO_{4} | 9.22              | 353.1263            | [M+H]+                      | 354.1336          | 188.0702, 189.0778, 149.0591         |
| 16      | Allocryptopine              | C_{21}H_{23}NO_{3} | 10.21             | 369.1576            | [M]+                        | 370.1649          | 188.0701, 189.0780, 290.0393         |
| 17      | Cheilodine                  | C_{20}H_{23}NO_{3} | 10.18             | 353.1263            | [M+H]+                      | 354.1336          | 275.0704, 247.852, 305.0815, 323.0917 |
| 18      | Dihydroberberine            | C_{20}H_{24}NO_{4} | 10.95             | 337.1314            | [M+H]+                      | 338.1387          | 322.1071, 294.1123, 308.0914, 279.0876 |
| 19      | Tyramine                    | C_{3}H_{11}NO    | 1.91              | 137.0841            | [M+H]+                      | 138.0913          | 77.0401, 121.0649, 103.0356, 91.0551  |
| 20      | Dopamine                    | C_{4}H_{11}NO_{2} | 0.89              | 153.0790            | [M+H]+                      | 154.0863          | 91.0599, 137.0600, 119.0496          |
| 21      | 4-Hydroxyphenylacetaldehyde | C_{6}H_{8}NO_{2} | 0.89              | 136.0524            | [M+H]+                      | 137.0597          | 91.0551, 65.0420, 119.0501, 63.0269  |
| 22      | Morphine                    | C_{2}H_{14}NO_{3} | 4.10              | 285.1365            | [M+H]+                      | 286.1438          | 201.0900, 229.0849, 185.0584, 211.0747 |
| 23      | Mecambrine                  | C_{14}H_{17}NO_{3} | 7.68              | 295.1208            | [M+H]+                      | 296.1281          | 202.0853, 171.0674, 280.0965         |
| 24      | Codeine                     | C_{14}H_{17}NO_{3} | 5.08              | 299.1521            | [M+H]+                      | 300.1594          | 215.1064, 243.1013, 225.0906, 199.0748 |
| 25      | (S)-N-Methylococlarine      | C_{14}H_{17}NO_{3} | 6.09              | 299.1521            | [M+H]+                      | 300.1594          | 269.1180, 107.0495, 271.1348         |
| 26      | Armepavine                  | C_{14}H_{17}NO_{3} | 5.64              | 313.1678            | [M+H]+                      | 314.1751          | 107.0495, 58.0675, 269.1160, 271.1328, 298.1070 |
| 27      | (S)-3″-Hydroxy-N- methylcoclarine | C_{14}H_{17}NO_{3} | 5.53              | 315.1471            | [M+H]+                      | 316.1543          | 192.1010, 123.0430, 285.1116, 300.1196 |
| 28      | (S)-Cheilanthifoline        | C_{14}H_{14}NO_{4} | 7.70              | 325.1314            | [M+H]+                      | 326.1387          | 178.0865, 190.0862, 163.0625         |

(Continues)
| Peak no. | Name                                           | Formula          | Expected RT (min) | Predicted mass (Da) | Adduct form | Observed mass (Da) | Product ions (MS/MS fragment ions, m/z) |
|---------|------------------------------------------------|------------------|-------------------|---------------------|-------------|-------------------|----------------------------------------|
| 29      | Papaverine                                     | C_{20}H_{21}N_{10}O_{4} | 10.15             | 339.1471            | [M+H]^+     | 340.1543          | 202.0867, 324.1216, 296.1288, 171.0663 |
| 30      | Cryptopine                                     | C_{21}H_{23}N_{10}O_{5} | 8.97              | 369.1576            | [M+H]^+     | 370.1649          | 352.1190, 205.1099, 165.0913, 190.0862  |
| 31      | Noscapine                                      | C_{22}H_{23}N_{10}O_{7} | 10.59             | 413.1475            | [M+H]^+     | 414.1547          | 220.0967, 353.1030, 365.1012, 179.0705  |
| 32      | 4-Hydroxyphenylpyruvate                        | C_{8}H_{8}O_{4}   | 4.86              | 180.0423            | [M+H]^+     | 181.0495          | 89.0402, 135.0438, 163.0396, 145.0291, 117.0345 |
| 33      | Codeinone                                       | C_{14}H_{12}N_{10}O_{3} | 5.64              | 297.1365            | [M+H]^+     | 298.1438          | 283.1208, 282.1125, 254.1180, 266.1213  |
| 34      | Morphine N-oxide                               | C_{14}H_{12}N_{10}O_{4} | 4.16              | 301.1314            | [M+H]^+     | 302.1387          | 284.1280, 241.0860                      |
| 35      | Flavinantine                                   | C_{14}H_{12}N_{10}O_{5} | 7.17              | 327.1471            | [M+H]^+     | 328.1543          | 178.0855, 163.0621                      |
| 36      | 8,14-dihydroflavinantine (or salutaridinol)    | C_{14}H_{12}N_{10}O_{7} | 5.17              | 329.1627            | [M+H]^+     | 330.1700          | 285.1121, 123.0345, 58.0665, 143.0482   |
| 37      | (S)-cis-N-Methylstylopine                     | C_{20}H_{20}N_{10}O_{4} | 10.20             | 338.1392            | [M+H]^+     | 339.1465          | 191.0891, 190.0857, 149.0589            |
| 38      | Isocorydine                                    | C_{20}H_{23}N_{10}O_{4} | 7.25              | 341.1627            | [M+H]^+     | 342.1700          | 297.1120, 265.0856, 237.0904            |
| 39      | Pseudoprotopine                                | C_{20}H_{21}N_{10}O_{5} | 7.69              | 353.1263            | [M+H]^+     | 354.1336          | 188.0703, 189.0775, 149.0592            |
| 40      | Amurenamine N-oxide A (or amurenamine N-oxide B) | C_{20}H_{23}N_{10}O_{4} | 7.61              | 355.1420            | [M+H]^+     | 356.1493          | 190.0567, 191.0944, 277.0864, 151.0756   |
| 41      | Rheagenine (or isorheagenine)                  | C_{20}H_{21}N_{10}O_{7} | 10.19             | 369.1212            | [M+H]^+     | 370.1285          | 352.1185, 340.1180, 324.1226            |
| 42      | Rheoadine (or isorheoadine)                    | C_{20}H_{21}N_{10}O_{8} | 7.77              | 383.1369            | [M+H]^+     | 384.1442          | 321.0763, 303.0649, 291.0653, 366.1341  |
| 43      | Glaucamine (or isoglaucamine)                  | C_{21}H_{23}N_{10}O_{6c} | 8.64              | 385.1525            | [M+H]^+     | 386.1598          | 368.1499, 338.1042                      |
| 44      | Coptisine                                      | C_{19}H_{14}N_{10}O_{4} | 7.65              | 320.0923            | [M]^+       | 320.0923          | 292.0964, 277.0728, 290.0805, 318.0755, 262.0858 |
| 45      | Unknown M1                                     | C_{14}H_{12}N_{10}O_{3} | 4.98              | 271.1208            | [M+H]^+     | 272.1281          | 227.1772, 107.0496, 161.0580, 209.1688, 235.1030 |
| 46      | Unknown M2                                     | C_{14}H_{12}N_{10}O_{4} | 4.32              | 283.1208            | [M+H]^+     | 284.1281          | 175.0568, 129.0169, 203.0549, 227.0707  |
| 47      | Unknown M3                                     | C_{14}H_{12}N_{10}O_{5} | 5.64              | 297.1365            | [M+H]^+     | 298.1438          | 283.1211, 254.1180, 177.0774, 148.0776, 107.0498  |
| 48      | Unknown M4                                     | C_{14}H_{12}N_{10}O_{4} | 7.60              | 311.1521            | [M+H]^+     | 312.1594          | 267.1008, 252.0774, 181.0599, 121.0299  |
| 49      | Unknown M5                                     | C_{14}H_{12}N_{10}O_{4} | 5.32              | 315.1471            | [M+H]^+     | 316.1543          | 298.1430, 283.1218, 254.1169, 121.0647  |
| 50      | Unknown M6                                     | C_{14}H_{12}N_{10}O_{4} | 7.18              | 327.1471            | [M+H]^+     | 328.1543          | 178.0855, 163.0614, 151.0750, 91.0572, 176.0685 |
| 51      | Unknown M7                                     | C_{20}H_{20}N_{10}O_{5} | 10.45             | 383.1369            | [M+H]^+     | 384.1442          | 352.1180, 190.0863, 188.0710, 303.0653, 334.1077 |
| 52      | Unknown M8                                     | C_{20}H_{20}N_{10}O_{4} | 9.81              | 354.1705            | [M+H]^+     | 355.1778          | 207.1204, 206.1172, 191.0913,           |

(Continues)
| Peak no. | Name            | Formula  | Expected RT (min) | Predicted mass (Da) | Adduct form [M]+ or [M+H]+ | Observed mass (Da) | Product ions (MS/MS fragment ions, m/z) |
|---------|-----------------|----------|-------------------|---------------------|--------------------------|-------------------|---------------------------------------|
| 53      | Unknown M9      | C22H19NO4 | 7.22              | 399.1682            | [M+H]+                   | 400.1755          | 337.1073, 319.0962, 204.1006, 58.0664, 382.1637 |
| 54      | Unknown M10     | C23H21NO4 | 13.81             | 337.1314            | [M+H]+                   | 338.1387          | 321.0953, 322.1021, 293.1003, 307.0820 |
| 55      | Unknown M11     | C21H19NO4 | 12.20              | 383.1369            | [M+H]+                   | 352.1380, 384.1442 | 190.0861, 320.0923, 291.0654, 263.0704 |

Peak no. 1–18 identified by direct comparison to authentic standards.
Peak no. 19 and 20 tentatively identified by comparison to the in-house MS/MS library (Sciex) and Metlin database.
Peak no. 21–29 tentatively identified by comparison to the Metlin database.
Peak no. 30 and 31 tentatively identified by comparison to the MS bank database.
Peak no. 32–44 tentatively identified by isotope MS pattern and previous reports.
Peak no. 45–55 unknown.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Institute of Agricultural Sciences, Rural Development Administration (Project title: “Omics data analysis and infrastructure construction for medical material in the Alkaloid biosynthesis crops,” Project no. PJ01184702), Republic of Korea. And it was supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant no. HB16C0010).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Jae-Hyeon Oh http://orcid.org/0000-0003-1100-6953

REFERENCES

1. Pourmotabbed, A., Rostamian, B., Manouchehri, G., Pirzadeh-Jahromi, G., Sahraei, H., Ghoshooni, H., Zardooz, H., Kamalnegad, M., Effects of Papaver rhoeas extract on the expression and development of morphine-dependence in mice. J. Ethnopharmacol. 2004, 95, 431–435.
2. Kalav, Y. N., Sariyar, G., Alkaloids from Turkish Papaver rhoeas. Planta Med. 1989, 55, 488.
3. Zargari, A., Medical Plants, Tehran University, Tehran 1995.
4. Coban, I., Toplan, G. G., Ozbek, B., Gurer, C. U., Sariyar, G., Variation of alkaloid contents and antimicrobial activities of Papaver rhoeas L. growing in Turkey and northern Cyprus. Pharm. Biol. 2017, 55, 1894–1898.
5. Günaydın, Y. K., Dündar, Z. D., Çekmen, B., Akılı, N. B., Köyüli, R., Cander, B., Intoxication due to Papaver rhoeas (corn poppy): five case reports. Case. Rep. Med. 2015, 2015, 321360.
6. El-Masry, S., El-Ghazooly, M. G., Omar, A. A., Khafagy, S. M., Phillipson, J. D., Alkaloids from Egyptian Papaver rhoeas. Planta Med. 1981, 41, 61–64.
7. Todorova, T., Pesheva, M., Gregan, F., Chankova, S., Antioxidant, antimutagenic, and anticarcinogenic effects of Papaver rhoeas L. extract on Saccharomyces cerevisiae. J. Med. Food. 2015, 18, 460–467.
8. Rey, J.-P., Levesque, J., Pousset, J.-L., Roblot, F., Analytical studies of isorhoeadine and rheogenine in petal extracts of Papaver rhoeas L. using high-performance liquid chromatography. J. Chromatogr. A 1992, 596, 276–280.
9. Matysik, G., Benesz, M., Thin-layer chromatography and densitometry of anthocyanins in the petals of red poppy during development of the flowers. Chromatographia 1991, 32, 19–22.
10. Hillenbrand, M., Zapp, J., Becker, H., Depsides from the petals of Papaver rhoeas. Planta Med. 2004, 70, 380–382.
11. Dogan, G., Bagci, E., Essential oil composition of Papaver rhoeas L. (corn poppy) (Papaveraceae) from Turkey. Hacettepe J. Biol. & Chem. 2014, 42, 545–549.
12. Choe, S., Kim, S., Lee, C., Yang, W., Park, Y., Choi, H., Chung, H., Lee, D., Hwang, B. Y., Species identification of Papaver by metabolite profiling. Forensic. Sci. Int. 2011, 211, 51–60.
13. Hagel, J. M., Facchini, P. J., Benzylisoquinoline alkaloid metabolism: a century of discovery and a brave new world. Plant. Cell. Physiol. 2013, 54, 647–672.
14. Beaudoin, G. A., Facchini, P. J., Benzylisoquinoline alkaloid biosynthesis in opium poppy. Planta 2014, 240, 19–32.
15. Farrow, S. C., Hagel, J. M., Facchini, P. J., Transcript and metabolite profiling in cell cultures of 18 plant species that produce benzylisoquinoline alkaloids. Phytochemistry 2012, 77, 79–88.
16. Bernáth, J., Dánsó, B., Veres, T., Szántó, J., Tétényi, P., Variation in alkaloid production in poppy ecotypes: Responses to different environments. Biochem. Syst. Ecol. 1988, 16, 171–178.
17. Ren, L., Xue, X., Zhang, F., Xu, Q., Liang, X., High performance liquid chromatography-mass spectrometry analysis of protoberberine alkaloids in medicine herbs. J. Sep. Sci. 2007, 30, 833–842.
18. Li, Y., Zhang, T., Zhang, X., Xu, H., Liu, C., Chemical fingerprint analysis of Phellodendron amurenensis cortex by ultra performance LC/Q-TOF-MS methods combined with chemometrics. J. Sep. Sci. 2010, 33, 3347–3353.
19. Le, P. M., McCooeye, M., Windust, A., Application of UPLC-QTOF-MS in MS(E) mode for the rapid and precise identification of alkaloids in goldenseal (Hydrastis canadensis). Anal. Bioanal. Chem. 2014, 406, 1739–1749.
20. He, J., Liu, Y., Kang, Y., Yang, P., Wang, Y., Guo, J., Huang, J., Identification of alkaloids in Stephania hainanensis by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Phytochem. Anal. 2016, 27, 206–216.
21. Li, D., Xu, G., Ren, G., Sun, Y., Huang, Y., Liu, C., The application of ultra-high-performance liquid chromatography coupled with a LTQ-Orbitrap mass technique to reveal the dynamic accumulation of secondary metabolites in licorice under ABA stress. Molecules 2017, 22, 1742.
22. Hagel, J. M., Mandal, R., Han, B., Han, J., Dinsmore, D. R., Borchers, C. H., Wishart, D. S., Facchini, P. J., Metabolome analysis of 20 taxonomically related benzylisoquinoline alkaloid-producing plants. BMC Plant. Biol. 2015, 15, 220.
23. Mao, Z., Di, X., Zhang, J., Wang, X., Liu, Y., Di, X., Rapid and cost-effective method for the simultaneous quantification of seven alkaloids in Corydalis decumbens by microwave-assisted extraction and capillary electrophoresis. J. Sep. Sci. 2017, 40, 3008–3014.
24. Ziegler, J., Facchini, P. J., Geissler, R., Schmidt, J., Ammer, C., Kramell, R., Voigtlander, S., Gesell, A., Pienkny, S., Brandt, W., Evolution of morphine biosynthesis in opium poppy. Phytochemistry 2009, 70, 1696–1707.
25. Schmidt, J., Boettcher, C., Kuhnt, C., Kuchan, T. M., Zenk, M. H., Poppy alkaloid profiling by electrospray tandem mass spectrometry and electrospray FT-ICR mass spectrometry after [ring-13C6]-tyramine feeding. Phytochemistry 2007, 68, 189–202.
26. Gurkok, T., Turktas, M., Parmaksiz, I., Unver, T., Transcriptome profiling of alkaloid biosynthesis in elicitor induced opium poppy. Plant Mol. Biol. Report. 2015, 33, 673–688.
27. Osanloo, N., Najafi-Abedi, A., Jafari, F., Javid, F., Pirpiran, M., Jafari, M. M., Khosravi, S. A. M., Behzadi, M. R., Ranjbaran, M., Sahaie, H., Papaver rhoas L. hydroalcoholic extract exacerbates forced swimming test-induced depression in mice. Basic. Clin. Neurosci. 2016, 7, 195–202.
28. Park, K. W., Lee, G. P., Park, K. W., Jeong, J. C., Seed morphology of thirty Korean wild green species and effect of seed stratification on germination. Hortic. Sci. Technol. 1998, 39, 129–134.
29. Ahn, Y. H., Son, J.-E., Lee, S. J., Jin, Y. H., Choe, C.-Y., Lee, K. M., Kim, M. Y., Lee, S. C., Germination characteristics and early growth of Papaver radicatum var. pseudoradicatum seeds. J. Environ. Sci. Int. 2011, 20, 541–549.
30. Farrow, S. C., Facchini, P. J., Dioxygenases catalyze O-demethylation and O,O-demethylation with widespread roles in benzylisoquinoline alkaloid metabolism in opium poppy. J. Biol. Chem. 2013, 288, 28997–29012.
31. Acevska, J., Stefkov, G., Petkovska, R., Kulevanova, S., Dimitrovska, A., Chemometric approach for development, optimization, and validation of different chromatographic methods for separation of opium alkaloids. Anal. Bioanal. Chem. 2012, 403, 1117–1129.
32. Wang, F., Zhang, Q., Lu, Z., Wang, Q., Yang, P., Wang, M., Liu, Y., Fu, S., Gao, X., Tang, X., Identification of chemical constituents in traditional Chinese medicine formula using HPLC coupled with linear ion trap-Orbitrap MS from high doses of medicinal materials to equivalent doses of formula: study on Xiang-Sha-Liu-Jun-Zi-Jia-Jian granules. J. Sep. Sci. 2016, 39, 1619–1627.
33. Sim, H. J., Yoon, S. H., Kim, M. S., Kim, B., Park, H. M., Hong, J., Identification of alkaloid constituents from Fangchi species using pH control-liquid-liquid extraction and liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Rapid. Commun. Mass. Spectrom. 2015, 29, 837–854.
34. Kim, J. H., Sim, H. J., Lee, K. R., Hong, J., UHPLC separation of structurally diverse markers in Fangchi species. Bull. Korean Chem. Soc. 2013, 34, 1–4.
35. Jeong, E. K., Lee, S. Y., Yu, S. M., Park, N. H., Lee, H. S., Yim, Y. H., Kwang, G. S., Cheong, C., Jung, J. H., Hong, J., Identification of structurally diverse alkaloids in Corydalis species by liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid. Commun. Mass. Spectrom. 2012, 26, 1661–1674.
36. Tani, C., Tagahara, K., Studies on the alkaloids of papeveraceous plants. XXVIII. The biosynthesis of rhoeadine (author's transl). Yakugaku Zasshi 1977, 97, 93–102.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Oh J-H, Ha IJ, Lee MY, et al. Identification and metabolite profiling of alkaloids in aerial parts of Papaver rhoas by liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. *J Sep Sci* 2018;41:2517–2527. [https://doi.org/10.1002/jssc.201701402](https://doi.org/10.1002/jssc.201701402)