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ORIGINAL RESEARCH  
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# Development of validated UHPLC–PDA with ESI–MS–MS method for concurrent estimation of magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine in *Berberis aristata*

ISHITA A. BASERA<sup>1</sup> , ABOLI GIRME<sup>2</sup> , VIJAY P. BHATT<sup>3</sup>,  
GANESH SASTE<sup>2</sup> , SANDEEP PAWAR<sup>2</sup> ,  
LAL HINGORANI<sup>2</sup> and MAMTA B. SHAH<sup>1\*</sup>

<sup>1</sup> Department of Pharmacognosy, L. M. College of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India

<sup>2</sup> Pharmanza Herbal Pvt. Ltd., Anand, 388435, Gujarat, India

<sup>3</sup> Herbal Research & Development Institute, Mandal, Gopeshwar, Chamoli, Uttrakhand, India

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## ABSTRACT

A validated UHPLC-PDA with an ESI-MS/MS method has been developed for simultaneous estimation of six bioactive alkaloids (magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine) in the different extracts of the roots of *Berberis aristata* DC (Family:Berberdiaceae). It is an important medicinal herb native to Northern Himalaya and commonly known as ‘daruharidra’, ‘daruhaldi’, ‘Indian barberry’ or ‘tree turmeric’. An insight into the research literature uncovered reports on isoquinoline alkaloids like magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine, and berberine as major bioactives in *B. aristata* roots, possessing different pharmacological and therapeutic effects. In the present study, these aforementioned alkaloids were separated on Phenomenex Luna<sup>®</sup>, 5 µm-C8 analytical column. The HPLC-MS analysis was performed at a flow rate of 0.90 mL min<sup>-1</sup>. Each alkaloid that is resolved was characterized by precursor ions and fragment ions with electrospray ionization (ESI) source in both positive and negative ionization using scan mode. The limit of detections (LODs) were 0.087, 0.727, 0.035, 0.124, 0.782 and 0.794 µg mL<sup>-1</sup> for magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine, respectively. The proposed UHPLC-PDA method was fully validated according to international (ICH) guidelines and was found to be selective, sensitive and highly accurate for the concomitant estimation of the aforementioned symbolic bio-markers of *B. aristata* roots.

## KEYWORDS

berberine, berbamine, columbamine, simultaneous UHPLC-MS/MS, jatrorrhizine, magnoflorine, palmatine

## INTRODUCTION

Each botanical species depicts a distinct chemical profile that can be generated using hyphaenated techniques like HPTLC and HPLC coupled with spectroscopy. The present analytical study is planned with an aim to develop validated ultra high-performance liquid chromatography coupled to PDA (UHPLC-PDA) with electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) method and implement it to quantify six major isoquinoline alkaloids in the roots of *Berberis aristata* DC. It is an important multipurpose shrub native to hilly parts of Northern India and Nepal and commonly known as ‘daruhaldi’

\*Corresponding author. Department of Pharmacognosy, L. M. College of Pharmacy, Navrangpura, Ahmedabad 380009, Gujarat, India.  
E-mail: mbshah2007@rediffmail.com

and ‘citra’. And as the common name suggests it has similar property like turmeric (commonly known as ‘haldi’) and is used to treat diarrhea, jaundice, menorrhagia and skin diseases [5–7]. The roots and its extract “rasot” are important ingredients of many Ayurvedic formulations since ancient times [1–4]. A wide range of pharmacological activities exhibited by roots has been attributed to its isoquinoline alkaloids like magnoflorine (1), berbamine (2), columbamine (3), jatrorrhizine (4), palmatine (5) and berberine (6) (Fig. 1) [3, 8–11].

Alkaloids, berberine, palmatine, and magnoflorine are well-noted to exhibit a multifarious of pharmacological effects, including anti-cancer, anti-diabetic, anti-oxidative, anti-inflammatory, immunomodulation, neuroprotection, anti-bacterial, anti-viral and regulating blood lipids [12–14]. Berbamine is proved to exhibit to inhibit the proliferation and induce apoptosis in a variety of cancer cell types [15, 16]. Jatrorrhizine (dihydro berberine) and columbamine are shown to be potent antifungal and anti-viral alkaloids [17, 18]. An inquest into the former studies on qualitative and quantitative analysis of bioactive principles either singly or simultaneously in group in the roots of *B. aristata* revealed reports of the application of high-performance thin-layer chromatography (HPTLC) [19, 20] and high-performance liquid chromatography (HPLC) methods [21–23]. However, no UHPLC-MS/MS method has been reported to estimate magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine, and berberine probably complementing actions of each other and representing the diverse groups of the bioactive constituents of *B. aristata* root. The proposed UHPLC-PDA and ESI-MS/MS method would serve as a useful tool for quantitative analysis for these six biomarkers in *B. aristata* species, and connotes its identification too, since, under the optimized conditions, a distinct chemical profile is generated [9]. Validation results confirmed that

specificity, sensitivity and accuracy of the developed UHPLC-PDA method.

## EXPERIMENTAL

### Plant material

The roots of *B. aristata* were collected from Garhwal, Uttarakhand, dried, powdered and passed through to 60 # size sieve and stored in an airtight container at room temperature.

### Chemicals and reagents

All reagents were of analytical HPLC grade. Acetonitrile and methanol were procured from Rankem. IN and MS grade water was purchased from JT Baker, FS, IN. Trifluoroacetic acid was purchased from the Loba Chemie Pvt. Ltd., Mumbai, India. The 0.45  $\mu\text{m}$  membrane filter was purchased from Pall Life Sciences, Mumbai, India. Reference standards, berberine, berbamine, and palmatine were bought from Natural Remedies Pvt. Ltd., Bangalore, India and ANJ Biomedicals, Mumbai, India, while jatrorrhizine, magnoflorine and columbamine were procured from Chemfaces (Hubei, PRC).

### Instrument conditions for UHPLC–PDA and mass spectrometry

The UHPLC analysis was carried out using instrument Shimadzu Nexera X2 (Shimadzu Tech., Kyoto, Japan) consisting of a quaternary pump (LC-30AD), auto-sampler (SIL-30AC), column oven (CTO-20AC) with a diode-array detector (SPD-M20A), coupled with LCMS-8045 (Shimadzu Tech., Kyoto, Japan), and triple-quadrupole mass detector supplied with a thermally aided ESI source. An outlet of the

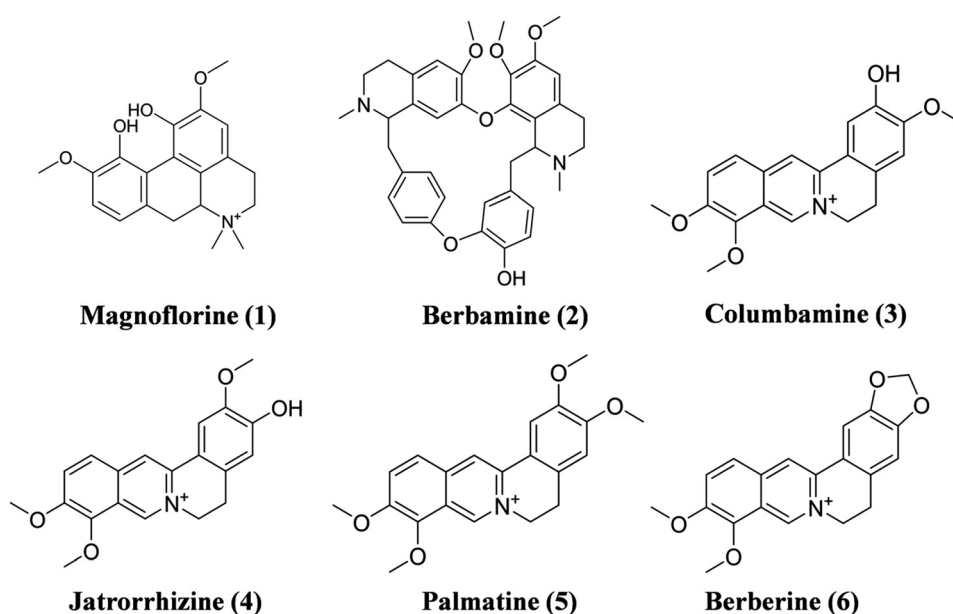


Fig. 1. Structures of identified compounds from *Berberis aristata*

PDA detector was linked to a splitter for splitting the flow (2:1 mL min<sup>-1</sup>). A Phenomenex Luna 5 µm C8 (2) 100 Å column (150 × 4.6 mm × 5 µm), column was used and the column temperature was maintained at 25 °C. The mobile phase used was 0.1% trifluoroacetic acid in water (A), 0.1% trifluoroacetic acid in acetonitrile (B) with a gradient elution mode of 0.01 min, 20% B; 0.01–17.00 min, 20–45% B; 17.00–17.50 min, 45–40% B; 17.50–18.00 min, 40–20% B; 18.00–20.00 min, 20% B. The analysis was done by keeping injection volume and the flow rate 10 µL and 0.90 mL min<sup>-1</sup> respectively. The PDA detector was set to 190–600 nm and detection was carried out at 280 nm. Mass analysis was conducted in scan and MS/MS settings in positive as well as negative ion modes and corroborating the product ion by a precursor scan. The interface for ESI was kept at 350 °C. The heat block and desolvation line temperatures were maintained to 300 and 400 °C, respectively. The other parameters that may enhance sensitivity of the mass spectrometer like flow rates of nebulizing gas, heating gas and the drying gas were set at 3.0 L min<sup>-1</sup>, 10.00 L min<sup>-1</sup> and 10.00 L min<sup>-1</sup>. The fragmentation of the aforementioned alkaloids was achieved at CE 20 eV and the analysis was performed in the Q1 scan mode, keeping the scan range 50–1,000 *m/z* and scan speed 5,000 u/sec in a positive and negative mode. The alkaloids were confirmed in the product ion scan mode for *m/z*342.40, *m/z*609.15, *m/z*338.15, *m/z*338.70, *m/z*353.60 and *m/z*337.35 for magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine, and berberine respectively as per their mass ion. The Lab Solution software (Version 6.80) was used for data analysis.

### Preparation of the standard solution

The stock solution for compounds (1–6) (500 µg mL<sup>-1</sup>) was prepared by weighing accurately 5.0 mg of each reference standard in 10 mL volumetric flask and adding 5.0 mL of methanol. Then sonicated for 10.0 min and filled volume up to the mark with methanol and were stored at 4 °C. The working standard solution was prepared daily for the stock solution with diluent; the final concentration was 500 µg mL<sup>-1</sup>.

### Sample solutions

Accurately weighed root powder (10.0 g) of *B. aristata* was extracted separately with different solvents visually, methanol, methanol:water (70:30), methanol:water (40:60) and

water. The four individual extracts were filtered and concentrated under reduced pressure to yield methanolic extract (yield, 5.3%), methanol:water (70:30) (yield, 8.0%), methanol:water (40:60) (yield, 7.0%) and water extract (yield, 6.0%). 10.0 mg of these extracts were dissolved and sonicated into 5.0 mL of methanol in 10.0 mL volumetric flasks for 10.0 min separately, and volume was made up to 10 mL in each using methanol. The working solution of samples 1,000 µg mL<sup>-1</sup> were prepared, filtered through the 0.22 µm filter, and used for injection.

### UHLC–PDA method validation

The calibration curves (CC) for each alkaloid were derived by injecting five different concentrations of each alkaloid (1–6) in triplicate and recording their peak area followed by plotting a graph of peak areas versus concentration. The linearity range for CC ranged between 0.312 and 20 µg mL<sup>-1</sup> for magnoflorine, 4.68–300 µg mL<sup>-1</sup> for berbamine, 0.16–10 µg mL<sup>-1</sup> for columbamine, 0.469–30 µg mL<sup>-1</sup> for jatrorrhizine, 2.19–140 µg mL<sup>-1</sup> for palmatine and 4.68–300 µg mL<sup>-1</sup> for berberine. The calibration curve slope (CCS) method was used to obtain regression equations and determine the LOD and LOQ (Table 1).

The intraday and interday precision and accuracy were determined by injecting same concentrations of the standard thrice and measuring the peak area. The experiment was repeated (*n* = 3) on the same day and the consecutive day (*n* = 3). The measurement precision for the amount of aforementioned alkaloids (1–6) separately was represented in the RSD. The recovery was ascertained by adding known concentration of standard alkaloids to the pre-quantified sample solution and estimating the quantity of combined content by the proposed method. Each level was prepared by three replicate samples (*n* = 3) (Table 2). The recoveries were obtained (between 80.0 and 120.0%), and the precisions were lower than 5.0% as per the ICH guidelines.

### Mass spectrometry

The combined use of ESI and collision-induced dissociation in TQ-MS/MS was applied to investigate the structural characterization of these six alkaloids (1–6) of *B. aristata* in the protonated and deprotonated forms. The mass ion ratio (*m/z*) was ascertained on the basis of molecular ions of the aforementioned six alkaloids (1–6). The scan range in the positive and negative ion modes was found to be

Table 1. Retention time, Range, Linear Regression, Peak purity, LOD and LOQ in the Developed UHPLC–PDA for magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine

Sr. No.	Analyte	Rt	Range (µg mL <sup>-1</sup> )	Linear regression equation	r <sup>2</sup>	Peak purity value	LOD (µg mL <sup>-1</sup> )	LOQ (µg mL <sup>-1</sup> )
1	Magnoflorine	4.395	0.312–20	y = 3685.5x + 1029.2	0.9998	98.00	0.087	0.264
2	Berbamine	6.837	4.68–300	y = 1883.5x + 4696.7	0.9997	92.97	0.727	2.203
3	Columbamine	8.320	0.16–10	y = 11145x + 676.16	0.9998	98.00	0.035	0.105
4	Jatrorrhizine	8.683	0.469–30	y = 21129x + 3133.2	0.9997	98.00	0.124	0.378
5	Palmatine	10.400	2.19–140	y = 21093x + 15145	0.9997	98.00	0.782	2.369
6	Berberine	11.157	4.68–300	y = 18020x + 27872	0.9996	98.00	0.794	2.405



Table 2. Precision (% RSD) ( $n = 3$ ), and Recovery (%) ( $n = 3$ ) in the Developed UHPLC–PDA for magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine, and berberine

Sr. No.	Analyte	Recovery	Precision (% RSD)			
			System precision	Repeatability	Reproducibility	Intermediate precision
1	Magnoflorine	94.70	1.295	1.246	1.445	2.822
2	Berbamine	84.74	0.312	2.371	4.487	3.816
3	Columbamine	96.35	0.344	2.968	1.503	4.671
4	Jatrorrhizine	88.41	0.469	2.134	3.906	3.220
5	Palmatine	88.09	0.450	1.707	2.588	4.111
6	Berberine	88.47	0.393	1.949	2.865	4.096

50–1,000  $m/z$ . These alkaloids (1–6) were studied in positive electron spray ionization (ESI+) and grouped in consonance with their optimal determination in each ionization mode. It was observed that alkaloids exhibited signal sensitivity in the positive ionization mode as  $[M + H]^+$  and  $[M]^+$  ions at  $m/z$  342.17 (1),  $m/z$  609.15 (2),  $m/z$  338.10 (3),  $m/z$  338.15 (4),  $m/z$  352.15 (5),  $m/z$  336.05 (6). Furthermore, data on proposed fragmentation patterns with ion structures and deprotonated forms on the reference compounds has been generated (1–6).

## RESULTS AND DISCUSSION

### UHPLC–PDA method optimization

*B. aristata* root extract was prepared using a reflux extraction process. The study is aimed to separate these alkaloidal bioactives with MS-compatible solvents for their corroboration in the root extract of *B. aristata*. To optimize the largest peak responses and check relatively high sensitivity experiments were performed at 240, 260, 280, and 350 nm wavelengths. Based on the results (peak shape and response), the optimal wavelength confirmed for the study was 280 nm for compound identification and analysis (chemical profiling) of *B. aristata* root extract. Separation of these alkaloids was achieved after repeated trials and combination of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile were ascertained as mobile phases. Furthermore, as compared to isocratic elution use of gradient elution method gave better separation of chemical constituents of the root extract. The MS optimization study for the mass ion fragmentation revealed that the temperature of 350 °C of electrospray ionization (ESI) interface resulted in better selectivity and efficiency for selective ion monitoring in both the polarities than at 250, 300, and 400 °C. Similarly, at CE 20 eV higher intensities of peaks and lower background noise were observed for the fragmentation opposed to 5, 10, 15, 25, 30, and 40 eV. The UHPLC–PDA method is validated as per International Conference on Harmonization (ICH) guidelines [24].

### Validation results of the UHPLC–PDA method

The UHPLC–PDA method is validated as per International Conference on Harmonization (ICH) guidelines

(ICHQ2R1) [24]. The alkaloidal bioactives got eluted in the order of 1–6 at mean retention times of 4.395, 6.837, 8.320, 8.683, 10.400, and 11.157 min, respectively (Fig. 2) indicating good resolution of each alkaloid peak. The system suitability was conducted on parameters such as retention time (Rt), response, number of theoretical plates ( $n$ ), tailing factor (Tf), resolution factor (Rs), and capacity factor (K).

**Selectivity, linearity, LOD, and LOQ.** The method was found to selectively differentiate the analytes from the matrix interferences as evident from comparison of the representative chromatogram of standards and spiked samples. The LOD and LOQ were determined for linearity and the CCS method. The LODs were 0.087, 0.727, 0.035, 0.124, 0.782 and 0.794  $\mu\text{g mL}^{-1}$  for magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine, respectively; and LOQs were 0.264, 2.203, 0.105, 0.378, 2.369 and 2.405  $\mu\text{g mL}^{-1}$  respectively. The calibration curve showed linearity for all compounds with  $r^2 > 0.99$ . Linear regression analyses of the calibration curves of these compounds are provided in Table 1.

**Accuracy, precision, and recovery.** The developed UHPLC–PDA method's precision was verified by repeated injections of the *B. aristata* root extract for quantification of 1–6 at six concentrations (Fig. 3a). The intraday and interday variations by the developed UHPLC method were evaluated by the relative standard deviation (RSD) values of active compounds in the *B. aristata* root extracts. The analytical recovery was performed by analyzing the analytes by spiking with the six reference standards in the root extracts. The recovery percentages was found in the range of 84.74% and 96.35% for 3 different concentrations in 3 replicates for the aforementioned six alkaloids. The developed method was found to be specific for estimating alkaloids (1–6) as evident from their peak purity values and the absence of any co-eluting peaks (Table 1).

### Characterisation by RP-UHPLC-PDA-ESI-MS and MS/MS

Confirmation of alkaloids magnoflorine (1), berbamine (2), columbamine (3), jatrorrhizine (4), palmatine (5) and berberine (6) has been done by ESI–MS/MS coupled with UHPLC–PDA in the *B. aristata* root extracts. The



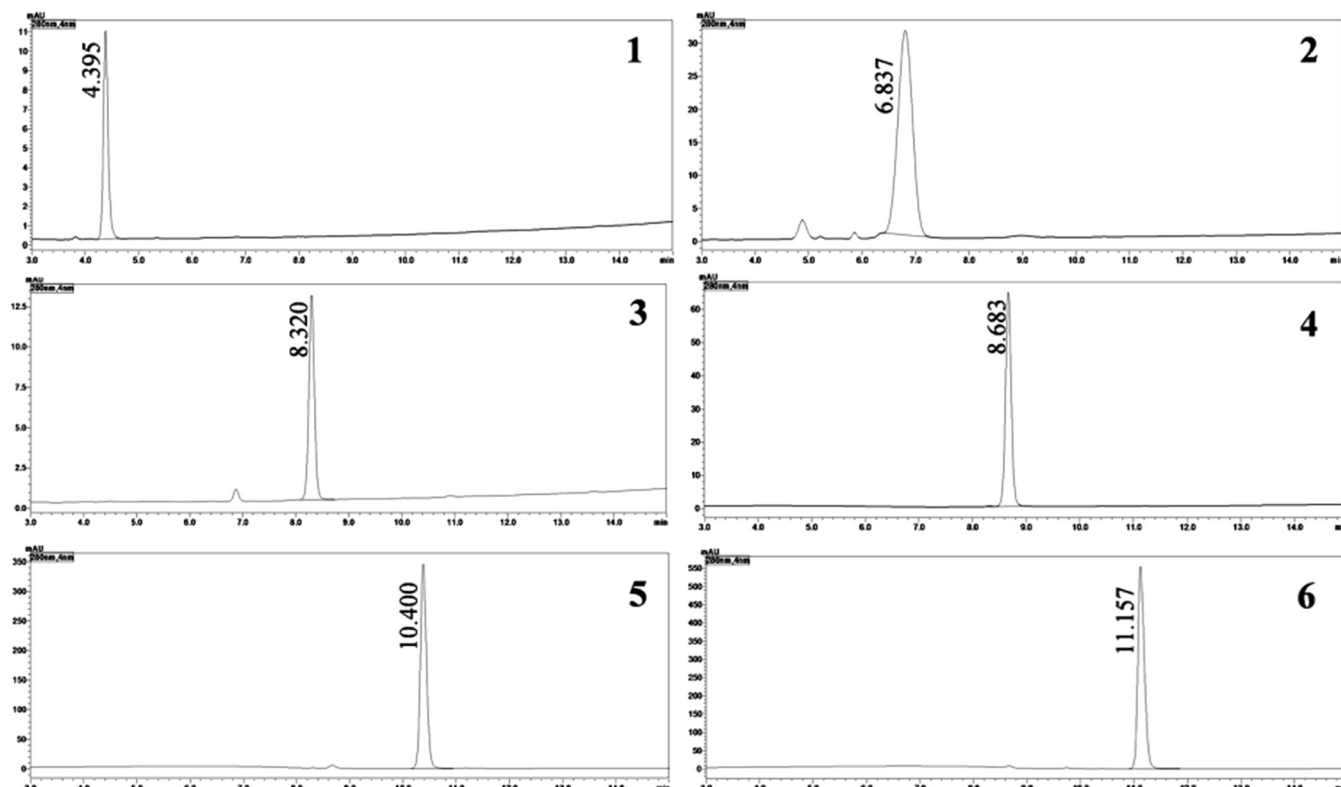


Fig. 2. UHPLC-PDA method optimization for BA compounds (1–6) at 280 nm in where 1, magnoflorine; 2, berbamine; 3, columbamine; 4, jatrorrhizine; 5, palmatine, 6, berberine

molecular weight of compounds 1–6 was calculated using chembiodraw 16.0, which are similar to the observed molecular weight (Table 3). The compounds showed  $m/z$  342.17,  $m/z$  609.15,  $m/z$  338.10,  $m/z$  338.15,  $m/z$  352.10 and  $m/z$  336.05 respectively, for in the *B. aristata* (Fig. 3b). During optimization of the ESI-MS method it was found that positive ionization sensitivity was higher and all the aforementioned alkaloids showed very strong signal responses in the positive ion mode compared to that in negative ionization and hence the method was set in positive ionization. The  $m/z$  ions were monitored in precursor and product ion scan mode to increase the specificity of the detection method. In the total ionization current (TIC) and extracted ion current chromatogram [Fig. 4]. Under the optimized UHPLC and ESI MS/MS conditions, all the six alkaloids could be explicitly identified in *B. aristata* root extracts by comparing their retention times and UV and MS data with reference standards. The identification of compounds in the extracts via collision-induced dissociation (CID) fragmentations is the strategy followed that consisted of the generation of the molecular formula of the detected ions in the chromatographic profile [Fig. 3b]. The structure was further characterized by studying the fragmentation behavior based on the literature [25]. The results provided by the ESI MS/MS analyzer are given in Table 4, which shows MS experimental data, retention time (RT), and main fragments generated by ESI from root extracts. The  $[M]^+$  ions of the quaternary alkaloids magnoflorine,

columbamine jatrorrhizine, palmatine and berberine eluting at 4.29, 8.07, 8.40, 10.09, and 10.85 min were observed.

The fragmentation patterns of magnoflorine were characterized by cleavage of the substituted groups of the alkaloid core, whereas no ring fusion was observed. In this sense,  $[M]^+$  ion at ( $m/z$  342) shows the primary loss of  $(CH_3)_2NH$  (45 Da) at  $m/z$  297.10 due to the presence of a two-methyl substituent in the amino group; this is a characteristic of this type of alkaloid [26]. Further fragments were observed at  $m/z$  265.05, 282.05, and 237.05 due to the loss of  $CH_3OH$ ,  $CH_3$ , and  $C_2H_4$  groups.

Protoberberine alkaloids, columbamine, jatrorrhizine, palmatine, and berberine showed  $[M]^+$  ions at  $m/z$  338.10, 338.15, 352.10, and 336.05, respectively. The CID spectra of the  $[M]^+$  ions were studied. Isomers columbamine and jatrorrhizine showed significant  $[M-CH_3-H]^+$  fragments with characteristic  $m/z$  values as the prominent peak at 322.15 in the product-ion spectra [27–29]. In addition,  $[M-3CH_3-H]^+$ ,  $[M-2CH_3]^+$ ,  $[M-2CH_3-2H]^+$  and  $[M-2CH_3-CO]^+$  fragments were also observed at  $m/z$  294.00, 308.05, 306.20 and 280.0 in the product-ion spectra. The MS/MS spectrum of berberine (Fig. 3b), showed the major product ions at  $m/z$  320.10, 321.00 probably due to the removal of the methyl group and  $CH_4$  respectively from the methoxy substituent. The ion at  $m/z$  306.00 arised because of continual exclusion of two methyl groups and the ion at  $m/z$  303.95 appeared by the depletion of  $CH_3OH$  from the precursor ion. The elimination of CO from the ions at  $m/z$  320.10 and  $m/z$  306.00 results in the

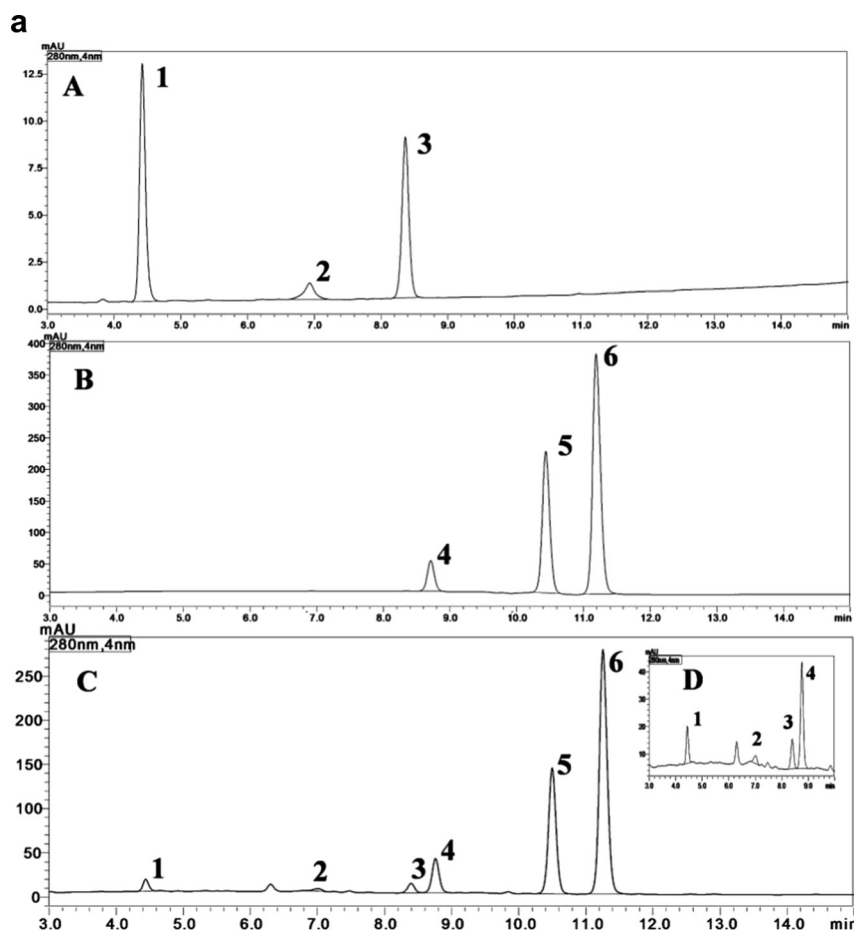


Fig. 3a. UHPLC-PDA chromatogram with detection alkaloids compounds (1–6) at 280 nm in the *Berberi aristata* root extracts as A & B-reference standards; C-sample chromatogram; D-sample chromatogram for compound. Where 1, magnoflorine; 2, berbamine; 3, columbamine; 4, jatrorrhizine; 5, palmatine, 6, berberine

Table 3. Characteristic ions observed for the MS spectrum of magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine

Entry	Ion Identified As	MF	Polarity	Calcd. MW (ChemBioDraw 16.0)	Observed ( $m/z$ )
1.	Magnoflorine	$C_{20}H_{24}NO_4$	+ve	342.17	342.40
2.	Berbamine	$C_{37}H_{42}N_2O_6$	+ve	610.30	609.15
3.	Columbamine	$C_{20}H_{20}NO_4^+$	+ve	338.38	338.15
4.	Jatrorrhizine	$C_{20}H_{20}NO_4^+$	+ve	338.38	338.70
5.	Palmatine	$C_{21}H_{22}NO_4$	+ve	353.42	353.60
6.	Berberine	$C_{20}H_{19}NO_4^+$	+ve	337.37	337.35

appearance of ions at  $m/z$  292.10 and 278.10 respectively, and the loss of a methyl radical and CO consecutively is archetypal fragmentation style followed by this protoberberine (benzyl-tetrahydroisoquinoline) alkaloids. Similar fragmentation behavior also occurs in another protoberberine alkaloid such as palmatine [27, 29]. Thus, palmatine predominantly formed  $[M]^+$  ion at  $m/z$  352.10 in the precursor ion scan and the product ion spectra showed characteristic  $m/z$  values at 336.10 and 337.05 respectively due to  $[M-CH_3-H]^+$  and  $[M-CH_3]^+$ . In addition,  $[M-3CH_3-H]^+$ ,  $[M-2CH_3]^+$  and  $[M-2CH_3-2H]^+$  fragments were also observed at  $m/z$  308.05, 322.00 and 320.05 in the product-ion spectra of palmatine.

Tertiary alkaloid berbamine/oxycanthine gave  $[M + H]^+$  ion as precursor at  $m/z$  609.25 eluting at 6.77 min that was observed to undergo further fragmentation reaction. It showed the primary loss of  $C_2H_5N$  (43 Da) at  $m/z$  566.20 due to the presence of a methyl substituent in the amino group. The fragments were observed at  $m/z$  551.75, 578.10, and 527.75 due to  $[M-C_2H_5N-CH_3-H]^+$ ,  $[M-OCH_3]^+$  and  $[M-2C_2H_6N_2]^+$  respectively. Furthermore, fragments were observed at 499.65 and 465.00 due to the loss of two and one  $CH_3$  radical. The ion at  $m/z$  188 was significant in the MS/MS spectra [30]. The loss of  $H_2O$  could be adopted to identify a carbonyl group in ortho-positions, and hence the

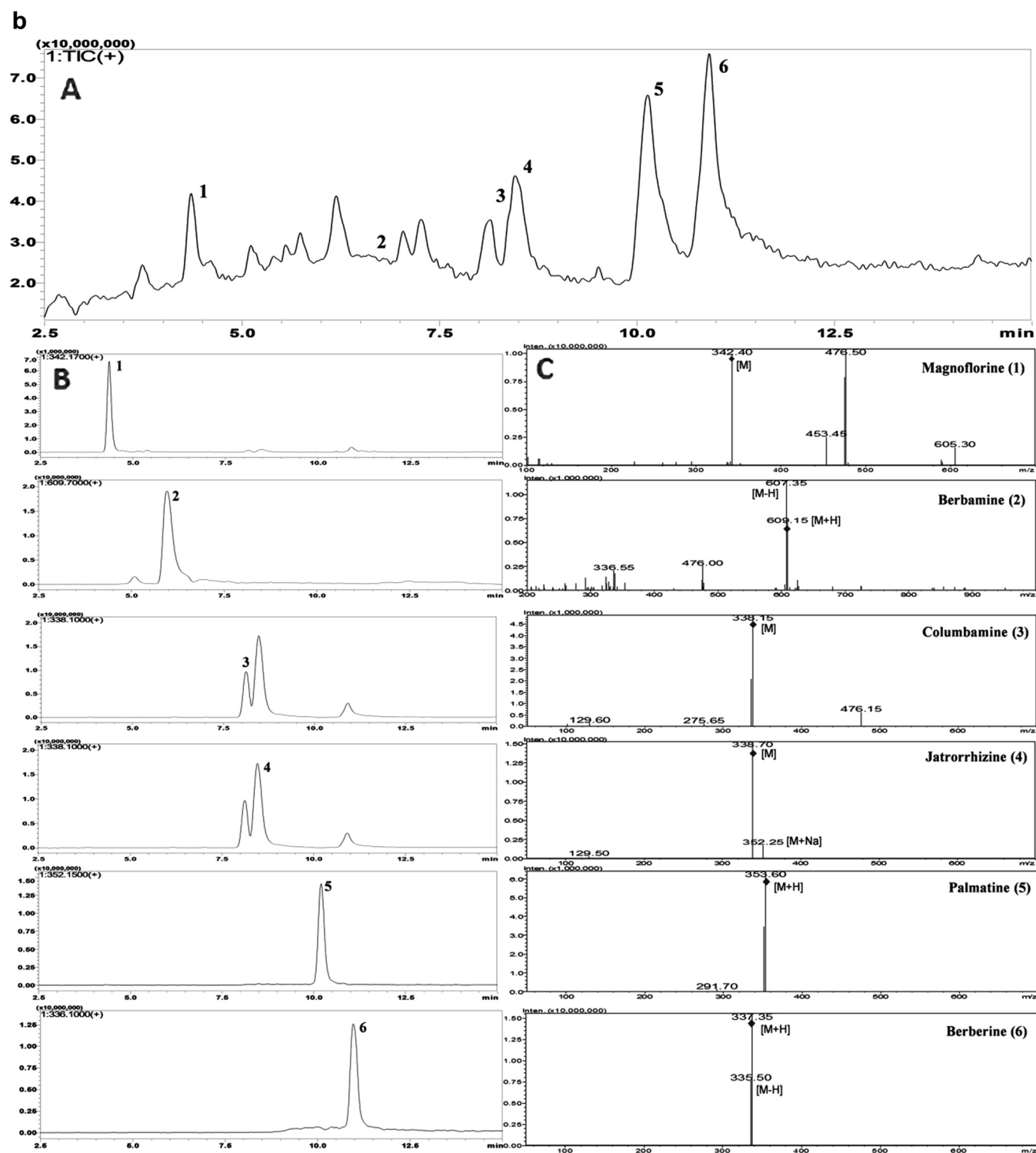


Fig. 3b. TQ-ESI-MS/MS based confirmation of 6 alkaloids in the BA with  $m/z$  values in selective ion scan mode. (A) TIC (+ve); (B) ion chromatograms extracted at  $m/z$  values with either the negative or positive ion or the fragment ion (C) molecular ion and  $m/z$  values of each of the identified compounds (1-6) in the *Berberis aristata* root extracts

$[M + H]^+$  ion was identified as protopine. To obtain the maximum sensitivity, the Interface voltage, Q1 and DL bias voltage, collision energy, and maximum and stable response nebulizing, heating, drying gas and Interface, and DL temperature were optimized.

### Quantification of analyte

Quantification data shows that water extract has highest content of magnoflorine. Berberamine and jatrorrhizine content were found to be more in methanolic extract while columbamine and palmatine have its highest content in

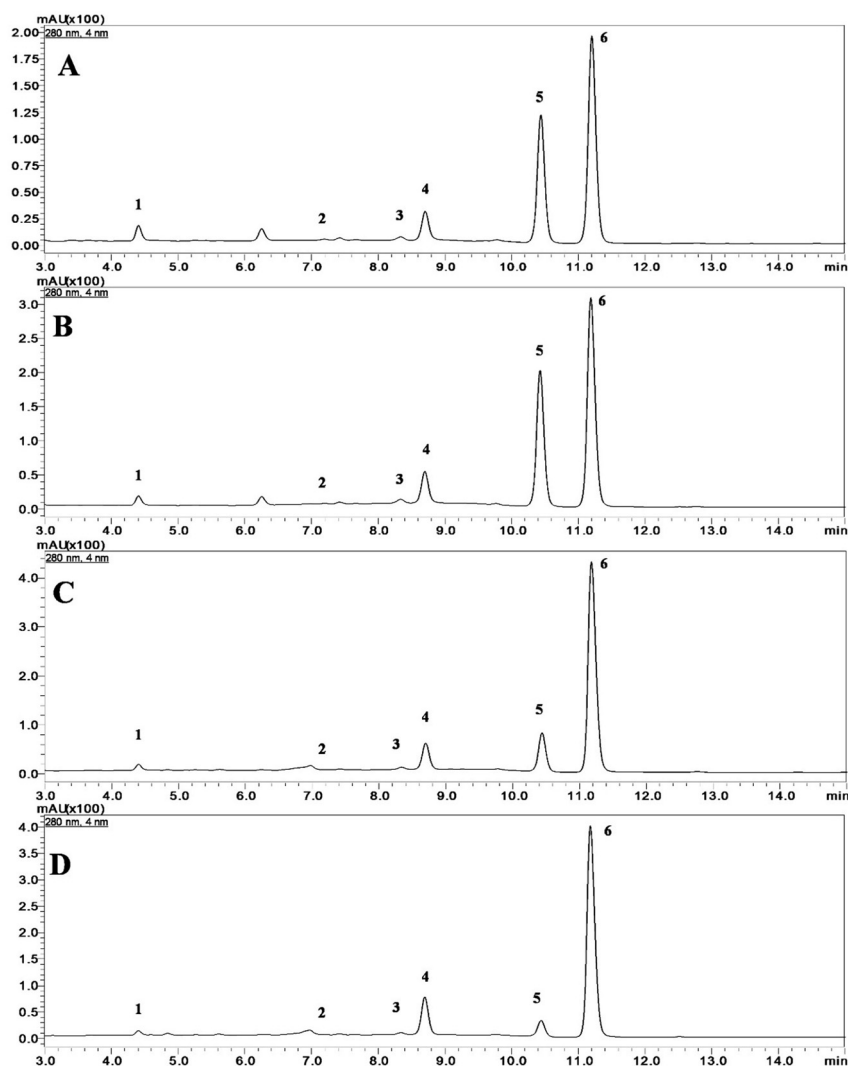


Fig. 4. UHPLC-PDA chromatogram of *Berberis aristata* extracts as A- water extract; B- hydroalcoholic extract (70:30) C- hydroalcoholic extract (40:60); D- alcoholic/methanolic extract identified as alkaloids compounds (1–6) at 280 nm

Table 4. The optimized compound dependent MRM parameters and transitions for each analyte in the UPLC–ESI–MS/MS analysis

Peak No.	RT (min)	Analyte	Precursor ion ( $m/z$ )	DP (V)	EP (V)	CE (eV)	Quantifiers	Qualifiers
1	1.49	Magnoflorine	342.1 [M] <sup>+</sup>	50	10	27	342.1→296.7 (20)	342.1→282.0 (12)
2	3.11	Berbamine	336.0 [M] <sup>+</sup>	40	10	45	336.0→320.0 (5)	336.0→292.2
3	2.50	Columbamine	352.2 [M] <sup>+</sup>	32	10	40	352.2→336.0 (15)	352.2→308.1 (16)
4	2.00	Jatrorrhizine	338.0 [M] <sup>+</sup>	50	10	55	338.0→307.3 (15)	338.0→322.1 (11)
5	2.50	Palmatine	352.2 [M] <sup>+</sup>	32	10	40	352.2→336.0 (15)	352.2→308.1 (16)
6	3.11	Berberine	336.0 [M] <sup>+</sup>	40	10	45	336.0→320.0 (5)	336.0→292.2 (8)

Table 5. Quantification ( $n = 3$ ) data for six analyte by UHPLC-PDA method of *Berberis aristata* extracts

SR. NO	ANALYTE	Water ext	HA 70:30 ext	HA 40:60 ext	Alcoholic extract
1	Magnoflorine	2.194	2.038	1.752	1.182
2	Berbamine	0.007	0.012	0.064	0.769
3	Columbamine	0.204	0.353	0.246	0.202
4	Jatrorrhizine	0.901	1.477	1.674	2.204
5	Palmatine	4.162	6.812	2.582	1.036
6	Berberine	8.08	12.771	17.633	16.415

hydroalcoholic extract (methanol:water, 70:30) and berberine content was found to be more in hydroalcoholic extract (methanol:water, 40:60) (Table 5) (Fig. 4).

## CONCLUSION

The advent of hyphenated analytical techniques like UHPLC-TQ-MS/MS has aggrandized qualitative and quantitative analysis of bioactive phytoconstituents with precision [31, 32].





In the proposed study, representative bioactive isoquinoline alkaloids, magnoflorine, berbamine, columbamine, jatrorrhizine, palmatine and berberine of root of *B. aristata* were resolved by developing validated UHPLC-PDA method that was simple and rapid giving separation within 13 min. The method was extended to MS/MS based identification of bioactive alkaloids (fingerprint profile) present in the roots by assessing the fragment ion patterns corresponding to peaks of reference alkaloids. This is the first study on based on UHPLC-PDA-MS/MS method for the analysis of these alkaloids in *B. aristata* root extract, which is commercially important drug that is very popularly used in many traditional and modern formulations. Thus, the present method for determining major isoquinoline alkaloids by UHPLC-PDA and ESI-MS/MS could be a useful gauge for rapid and precise estimation of aforementioned bioactive alkaloids of *B. aristata*.

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