Characterization of 10 Species of *Mahonia* by Capillary Electrophoresis

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Key Words

Capillary electrophoresis
Mahonia
Fingerprint, alkaloids
Berberine, palmatine and jatrorrhizine

Summary

A simple, rapid and effective capillary electrophoresis method was developed for the characterization of 10 different species of *Mahonia*. A fingerprint of the extract of each species was constructed using a mixed buffer of borate and phosphate, containing methanol, pH 8.0. The effective electrophoretic mobility and % normalized area of each peak in electrophoregrams were evaluated to characterize various species. Three alkaloids: berberine, palmatine and jatrorrhizine were found in all 10 species. The CE technique appears to provide a powerful tool for the identification and quality control of plant drugs.

Introduction

Mahonia belongs to the family of berberidaceae. There are 109 species of this genus recorded in the world and 44 species are found in China [1]. The dried stems of *Mahonia bealei* and *Mahonia fortuner* are commonly used against fever, swelling, inflammation, janundice, dysentery and constipation in Chinese traditional medicine [2]. The pharmaceutically relevant compounds in *Mahonia* are alkaloids, including berberine, berbamine, isotetrandine, oxyacanthine, palmatine, jatrorrhizhine magnoflorine and columbamine [3–5]. Methods such as TLC and HPLC have been used for the analysis and detection of alkoids in *Mahonia* [6,7].

As a modern separation method, CE has been applied to the analysis of traditional Chinese medicines [8]. Nevertheless, most reports have focused on the detection and determination of alkaloids in the plants and have paid little attention to the potential of CE in the construction of fingerprints for distinguishing different species and their identification [9–14]. At present, the identification of various species is performed by morphology and TLC. An infrared spectrometric method for comparing different components in six species of *Mahonia* has been reported recently [15]. However, none of these methods is entirely adequate for identification of various species because of the variety and complexity of *Mahonia*.

In this study, the fingerprints of the extracts of ten different species of *Mahonia* are constructed by CE. Three alkaloids: berberine, palmatine and jatrorrhizine were found in all 10 species. Their structures are shown in Figure 1. The results are of great significance in comparing the chemical constituents among various species and in identification of different species within a genus.

Experimental

Apparatus and Conditions

All separations were performed on a HP^{3D}CE system with air-cooling and a diode array detector (Hewlett-Packard, USA). An uncoated, fused-silica capillary 50 μm I.D. × 375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, P. R. China) was used. The total length of the capillary was 50 cm, 41.5 cm to the detector. The temperature of the capillary was maintained at 20 °C. Sample was injected by applying 5000 Pa for 10 s. The applied voltage was 30 kV. Detection was at 200 nm, 254 nm, 265 nm, and 320 nm.

New capillary was purged with 1.0 mol L^{-1} NaOH for 1 h, followed by water and background electrolyte for 10 min each. Between consecutive analyses, the capillary was flushed with 0.1 mol L^{-1} NaOH for 2 min, followed by water and background electrolyte each for 3 min.

The electrolyte solution was 0.05 mol L^{-1} phosphate and 0.05 mol L^{-1} borate buffer containing 50 % methanol (pH 8.0), which was degassed in an ultrasonic bath before use.

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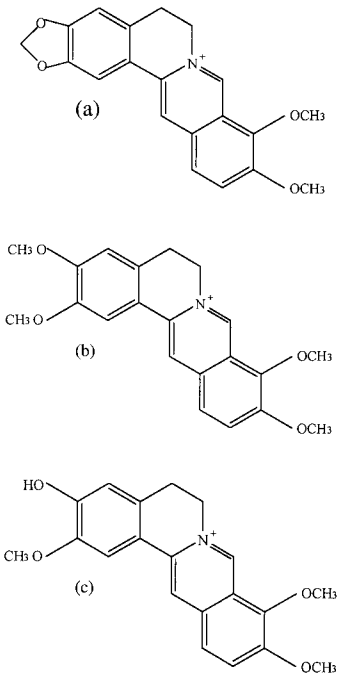

Figure 1
Structures of (a) berberine, (b) palmatine, (c) jatrorrhizine.

Reagents and Materials

Ten species of *Mahonia: M. bealei, M. fortunei, M. eurybracteata, M. japonica, M. confusa, M. bodinieri, M. shenii, M. veitchiorum, M. fargesii,* and *M. gracilipes* were collected in China and were identified by Beijing University of Chinese Medicine. Authentic samples are preserved in the unit for identification of medicinal herbs in Beijing University of Traditional Chinese Medicine. The three standard alkaloids, berberine, palmatine and jatrorrhizine were from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). All other chemicals were of analytical grade. Re-distilled water was used.

Sample Preparation

A 2.0 g sample of pulverized dried stem of each of the 10 species of *Mahonia* was extracted with 10.0 mL methanol ultrasonically for 30 min. Extraction was repeated twice with 5.0 mL methanol for 20 min. The extracts were combined and centrifuged at 4000 rpm for 15 min, then filtered through a 0.45 μm filter. Formamide (FA) was added to the sample solution as an indicator of electro-osmotic flow (EOF).

Solution for Identification of Alkaloids

Three standard alkaloids: berberine, palmatine and jatrorrhizine were dissolved in methanol at concentration of 0.1 mg mL^{-1} and filtered through a 0.45 μm filter before electrophoresis.

Result and Discussion

Optimization of Analytical Conditions

The major components in *Mahonia* are alkaloids. Most alkaloids carry a single positive charge at low pH. Their charge status depends on their pKa values and the pH of the running buffer. Our studies showed that at pH < 11.0, the alkaloids were positively charged. So, it was necessary to find a counter-ion that has different interactions with the positively charged nitrogen of the alkaloids. Phosphate, borate and acetate systems have been tried for analysis of plant extracts. The results showed that alkaloids eluted before the EOF marker were well separated from each other in phosphate buffer. Whereas the peaks after the EOF marker were separated better in borate buffer, probably because borate ions can form complexes with compounds having vicinal hydroxyl groups enhancing the selectivity of separation. Therefore, a mixed buffer of 0.05 mol L^{-1} phosphate and 0.05 mol L^{-1} borate was chosen as background electrolyte for optimum results.

The borate-phosphate buffer system was tested pH 5.0–10.0 to investigate the effect of pH on separation, and pH 8.0 was found to produce the highest resolution.

Organic modifiers in running buffer can varym the EOF greatly and improve resolution. The peaks before the EOF marker overlapped when no methanol was added, but they were made sharper after adding methanol to the buffer. After a series of experiments with concentrations of methanol varying 20 %–65 % was tested, 50 % methanol was demonstrated to be optimum, because lower concentrations of methanol gave only a slight improvement in separation, while higher concentrations prolonged analysis time.

Applied voltage and detection parameters were also optimized for this analysis. After a series of comparative voltages 20–30 kV, 30 kV proved the best. Detection was at variable wavelength 200–320 nm. Finally, 200 nm was selected because the number of peaks that could be detected was greatest at 200 nm.

Method Validation

The apparent electrophoretic mobility (μ_a) of the compound in CE tends to be poorly repeatable, mainly due to changes in the electrophoretic flow (EOF) from run to run, capillary to capillary and instrument to instrument. Therefore, we use effective electrophoretic mobility (μ_e) instead of μ_a as one parameter to assign the same compound in different electrophoregrams; μ_e values are calculated by the following equations:

$\mu_e = \mu_a - \mu_{EOF}$;

$\mu_a = lL / tV, \quad \mu_e = lL / t_0 V$

where l and L are effective and total length of the capillary, respectively; t is migration time of the compound, and t_0 is migration time of FA used as a EOF marker.

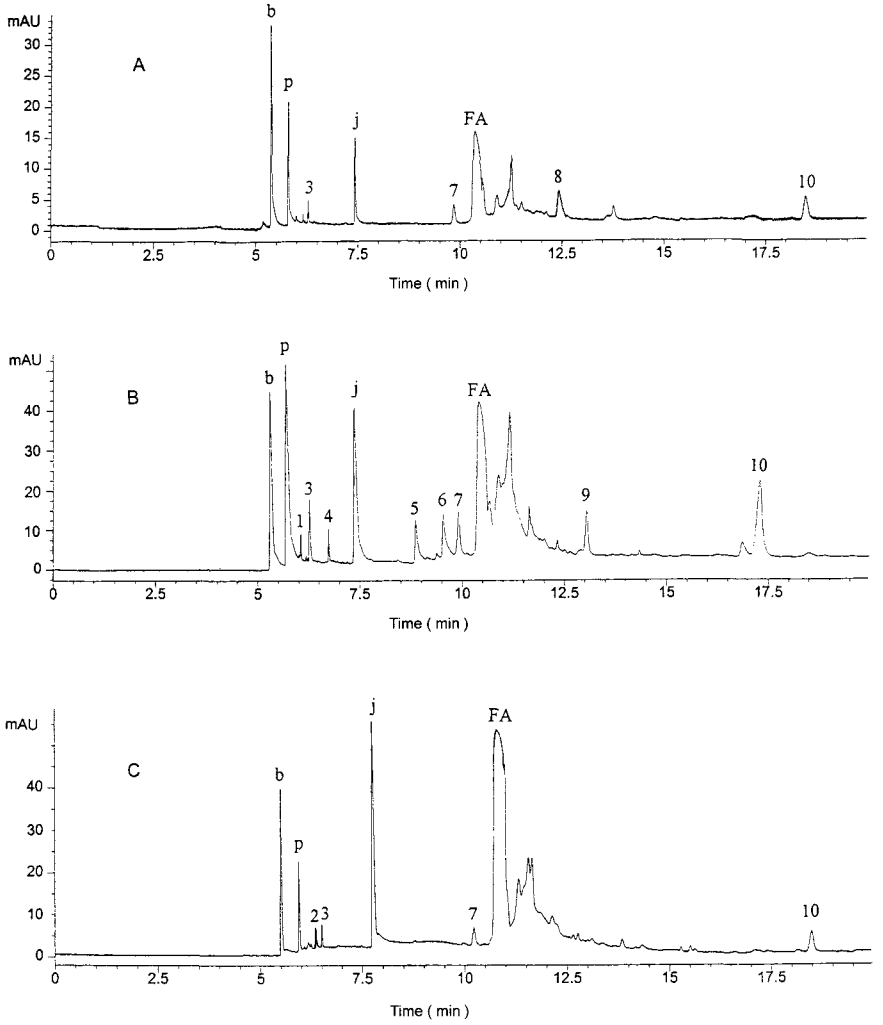

Figure 2

Electrophoregrams of three species of *Mahonia: M. bealei* (A), *M. japonica* (B), and *M. gracilipes* (C). Peaks **b** = berberine, **p** = palmatine, **j** = jatrorrhizine, **FA** = formamide. Peaks **1–10** are unknown compounds. Conditions: 0.05 mol L^{-1} borate and 0.05 mol L^{-1} phosphate buffer with 50 % methanol, pH 8.0; applied voltage: 30 kV; detection: 200 nm; injection: 5000 Pa for 10 s; 20 °C.

Since the area of a peak is proportional to both the solute concentration and peak residence time in the detector, normalization of CE peak areas by their division by migration times is necessary to decrease RSD values and comparison of peak areas of components within one separation [16]. The % normalized area of the peaks were calculated according to the following equation:

A % = normalized peak area divided by total normalized areas of all peaks marked except FA.

Four batches of samples from the same species of *M. berlie* were analyzed under the same condition. The RSD of μ_e and A % of the same peak in different samples range from 0.56 %–1.2 % and 2.5 %–4.9 %, respectively. The results demonstrate that deviations of μ_e and peak area for different samples in the same species are within the range of deviation for replicate injections of the same sample. Consequently, the difference between samples in the same species can be ignored in the identification procedure by fingerprints.

Fingerprints of 10 Species of *Mahonia*

Figure 2 shows the electrophoregrams of the extracts of three species: *M. bealei, M. japonica,* and *M. gracilipes* with peak b representing berberine, p representing palmatine, j representing jatrorrhizine and FA representing formamide which indicates the EOF. Peak purity for all peaks marked in the electrophoregrams were evaluated using the built-in purity function of HP ChemStation and a purity factor 961.4–999.5 was obtained, indicating that the separation was satisfactory-based on the purity threshold of 950. Although the peaks numbered 1–10 were unknown compounds, we can identify the same compounds in various species by comparing their UV absorption spectrum, as well as by μ_e. The results indicated that the profiles of these species were more or less similar and that such characteristic profiles could be used to distinguish this genus from other genera and to identify adulterates. Table I listes the average effective electrophoretic mobility and the normalized percent

Table I. The average effective electrophoretic mobility and normalized area percents of peaks marked in electrophoregrams of 10 species

Peaks *	*M. bealei*		*M. fortunei*		*M. eurybracteata*		*M. japonica*		*M. confusa*	
	m_e	*A* %	μ_e	*A* %	μ_e	*A* %	μ_e	*A* %	μ_e	*A* %
b	6.2	45	6.3	40	6.3	31	6.3	21	6.3	57
p	5.3	21	5.2	4.2	5.3	4.91	5.3	30	5.4	10
1	–	–	4.8	0.86	4.8	2.3	4.8	1.2	–	–
2	–	–	4.7	3.0	–	–	–	–	–	–
3	4.4	2.9	4.3	1.9	4.4	2.0	4.4	3.7	–	–
4	–	–	–	–	–	–	3.6	1.0	–	–
j	2.7	13	2.7	30	2.7	32	2.6	17	2.7	25
5	–	–	–	–			1.1	3.9	–	–
6	–	–	0.58	13	0.60	17	0.59	4.4	–	–
7	0.39	4.9	0.36	6.1	0.39	6.0	0.35	4.4	0.39	6.1
8	–0.79	8.4	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–1.4	3.2	–	–
10	–2.6	4.6	–2.7	2.2	–2.7	4.5	–2.7	9.7	–2.7	2.2

Peaks**	*M. bodinieri]*		*M. sheii*		*M. veitchiorum*		*M. fargesii*		*M. gracilipes*	
	μ_e	*A* %	μ_e	*A* %	μ_e	*A* %	μ_e	*A* %	μ_e	*A* %
b	6.3	48	6.4	75	6.2	12	6.3	12	6.2	29
p	5.3	13	5.2	15	5.3	4.7	5.3	23	5.3	12
1	–	–	–	–	–	–	4.7	2.4	–	–
2	–	–	–	–	–	–	–	–	4.6	2.6
3	–	–	4.3	1.7	–	–	4.3	2.8	4.2	2.6
4	–	–	–	–	–	–	–	–	–	–
j	2.6	25	2.6	3.3	2.6	13	2.6	26	2.6	46
5	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	0.60	7.0	0.61	22	–	–
7	0.36	11	0.34	5.2	0.33	64	0.34	7.0	0.35	4.0
8	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	–	–
10	–2.6	2.8	–	–	–	–	–2.6	4.7	–2.7	4.0

Peak codes are as those in Figure 2; μ_e expressed in $cm^2\ KV^{-1}\ min^{-1}$.

area of peaks in the electrophoregram of 10 species. The RSD of μ_e and A % varied 0.26 %–1.7 % and 0.45 %–5.2 %, respectively. It can be seen that even though the concentrations of sample solutions from different species were about the same, the total number of peaks separated was different. Furthermore, the area percentages of the same compound from different species were significantly different. These two features could support the development of a CE-based fingerprint technique for identification of different species within a genus.

Identification of Alkaloids in 10 Species

The assignment of the peaks of berberine, palmatine and jatrorrhizine in the electrophoregrams of extracts was achieved by spiking standards as well as by comparing their UV absorption spectrum with those of the standards. The μ_e of the standard peaks and their corresponding UV spectra matched well with those of the standards. The results demonstrate that the three alkaloids were present in all 10 species, but their contents varied from species to species. Since alkaloids are considered pharmaceutically active compounds all the 8 species besides *M. bealei* and *M. fortunei* may have potential as a new pharmaceutical resource.

Conclusion

A simple, rapid and effective method of capillary electrophoreisis was developed for the construction of fingerprints of 10 species of *Mahonia*. Three alkaloids were as berberine, palmatine and jatrorrhizine identified in all 10 species. The results indicate that besides the morphological method, CE could be an alternative tool in distinguishing and identification of different species of *Mahonia* by comparing the electrophoregram of unknown species with the fingerprints. It can simplify the procedure of identification and decrease the possibility of wrong conclusions. Our work also suggests that CE could be useful for the analysis of pharmaceu-

tical plant products for quality control purposes and for the search for new pharmaceutical plant resources.

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