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SEPARATION AND DETERMINATION OF MATRINE, OXYMATRINE AND SOPHORIDINE IN COMPOSITE PREPARATIONS BY NON-AQUEOUS CAPILLARY ELECTROPHORESIS

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Abstract

A new, simple, and quick method for the simultaneous determination of three alkaloids (matrine, sophoridine, and oxymatrine) in composite preparations by nonaqueous capillary electrophoresis has been developed. A running buffer composed of 60% acetonitrile + 0.75% acetic acid + 25 mM ammonium acetate in methanol was found to be the most suitable for this separation. The limits of detection for three alkaloids were over the range of 0.45–0.6 μg/ml. In the tested concentration range, good linear relationships between peak areas and concentrations of the analytes were obtained. This method has been successfully applied to simultaneous determination of the three alkaloids with recoveries over the range of 95.6 to 97.2%. This new non-aqueous capillary electrophoresis method established has been successfully used for the simultaneous separation and determination of sophoridine, matrine and oxymatrine in complex preparations in 5 minutes.

Key words: nonaqueous capillary electrophoresis, matrine, sophoridine, oxymatrine

Introduction. Sophorae Flavescentis Radix is the dried root of *Sophora flavescens*, a leguminous plant, which has the functions of clearing heat and dampness, killing insects and diuresis. It is a widely used Chinese herbal medicine and

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can be used as an insect repellent, antidote and heat-clearing agent to treat various diseases. Matrine, sophoridine and oxymatrine are the main biologically active components of *Sophora flavescens*. These alkaloids have a wide range of pharmacological effects, such as anti-tumour, antiarrhythmic, anti-inflammatory, anti-viral, analgesic, and so on. Oxymatrine has an inhibitory effect on the proliferation of vascular endothelial cells induced by lung cancer and gastric cancer cells; sophoridine also has a certain anti-tumour effect, and matrine is the strongest anti-tumour active ingredient in *Sophora flavescens*; in addition, these three alkaloids also have certain toxicity [1–4]. Therefore, it is extremely important to develop a simple, rapid, quantitative analysis method for these alkaloids.

Thin-layer chromatography [5–7], high-performance liquid chromatography [8–12], and capillary electrophoresis [13–16] have been used for the separation of alkaloids. However, thin-layer chromatography lacks quantitative accuracy and generally separates one or two components, with limited separation capabilities. Although liquid chromatography is a mature separation technology with high accuracy, it generally requires a longer analysis time, the sample and solvent consumption is large, the separation column easily contaminated. High-performance capillary electrophoresis is a new separation technology developed in the 1980s, with the advantages of high separation efficiency, short analysis time, low sample and solvent consumption, low operating costs and relatively simple instruments.

Non-aqueous capillary electrophoresis [17–21] is an important supplement to capillary electrophoresis for aqueous systems. Due to the diversity of organic solvents and their properties, non-aqueous capillary electrophoresis has many advantages compared to aqueous systems: (1) low Joule heat allows higher separation voltage, which greatly shortens the separation time; (2) reduces the adsorption of the analyte on the capillary wall, thereby increasing the reproducibility of the method. Practice has proved that non-aqueous capillary electrophoresis is a better method for the determination of drugs and their active ingredients, as well as a better method for pharmacokinetic research. Song et al. [17] used 25 mM ammonium acetate-10% tetrahydrofuran-0.5% acetic acid as electrolyte and separated matrine, sophoridine and oxymatrine by non-aqueous capillary electrophoresis, with the separation time being 18 min.

This article used a new capillary electrophoresis method to achieve complete separation of the three alkaloids within 5 min. The results have proved that the method provided in this article is simple, fast and reproducible.

Experimental. ***Materials and apparatus.*** The experiment used the P/ACE 5510 capillary electrophoresis instrument of Beckman Coulter Instrument, Fullerton, CA, USA. The system is controlled by the P/ACETM workstation, using the Beckman V 8.1 gold software; the detector is a photodiode detector with a detection window of 100 µm×200 µm. The temperature of the capillary tube was adjusted and constant by the cooling liquid; the capillary tube used is the product of Hebei Yongnian Optical Fibre Factory, with an inner diameter of 75 µm, a total

length of 47 cm, and an effective length of 40 cm. The new capillary was rinsed with 0.1 m hydrochloric acid, 0.1 m sodium hydroxide, water, methanol and acetonitrile, respectively, for 5, 10, 10, and 5 min before use; between two injections, it was rinsed with running buffer solution for 2 min; at the end of the experiment, it was rinsed with methanol and water for 5 min. The sample was injected from the anode end, the injection pressure was 0.5 psi, and the injection time was 3 seconds. It was detected from the cathode end with a detection wavelength of 206 nm.

Materials and reagents. Sophoridine (1), matrine (2), and oxymatrine (3) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products; acetonitrile, glacial acetic acid and ammonium acetate were purchased from Tianjin No. 1 Chemical Plant; methanol was purchased from Shanghai Zhenxing Chemical plant; and samples of *Sophora flavescens* tablets and antidiarrheal tablets were purchased from Zhongyou Pharmacy in Lanzhou, China.

Preparation of standard solutions and buffer solutions. The storage solution of the standard product was prepared with methanol. The concentrations of the three standard products were: 1000.0 µg/ml (sophoridine, 1), 1160.0 µg/ml (matrine, 2), and 1160.0 µg/ml (oxymatrine, 3), diluted with methanol to the required concentration when used. The buffer solution was prepared by the following steps to compound a 200 mM ammonium acetate solution with methanol, and then mix an appropriate amount of 200 mM ammonium acetate, glacial acetic acid, methanol and acetonitrile to obtain the required buffer solution. All solutions were filtered with a 0.45 µm organic filter membrane before use.

Preparation of sample solution. 1.0043 g of the ground samples of *Sophora flavescens* tablets was weighed to put into a 25 ml colourimetric tube; 20 ml of methanol was added to extract ultrasonically for 30 min and the extraction was repeated three times; the extract was combined three times and condensed by vacuum distillation, resulting in a final volume of 10 ml. The sample solution was filtered by 0.45 µm organic membrane and analyzed by capillary electrophoresis.

Results and discussion. Based on the molecular structure of the analyte, sophoridine and matrine are similar in structure, but differ in the way one hydrogen atom is connected; and there is one more oxygen atom in the structure of oxymatrine. Non-aqueous media can amplify their differences, so we chose non-aqueous capillary electrophoresis to separate them. Based on the UV absorption spectrum, 206 nm was selected as the detection wavelength. This paper systematically investigated the effects of ammonium acetate concentration, acidity, and solvent composition on separation.

The effect of ammonium acetate concentration on separation. Figure 1a shows the effect of ammonium acetate concentration on analyte migration time. It can be seen from the figure that as the concentration of ammonium acetate rose, the migration time of each analyte increased, which was due to

the decrease of electroosmotic flow; and the resolution also increased with the increase of ammonium acetate concentration; when the concentration of ammonium acetate was 25 mM, the three analytes were separated better, while at this moment the current was about 30 µA and the migration time was not long, so we chose the concentration of 25 mM ammonium acetate as the best condition.

The effect of acetonitrile content. Figure 1b shows the effect of the percentage of acetonitrile on the migration time of the analytes. It can be seen from the figure that as the percentage of acetonitrile rose, the migration time of the three analytes first decreased and then increased. When the acetonitrile content was 60 and 70%, the separation time was shorter; the resolution between two adjacent analytes also varied with the acetonitrile content. Considering the

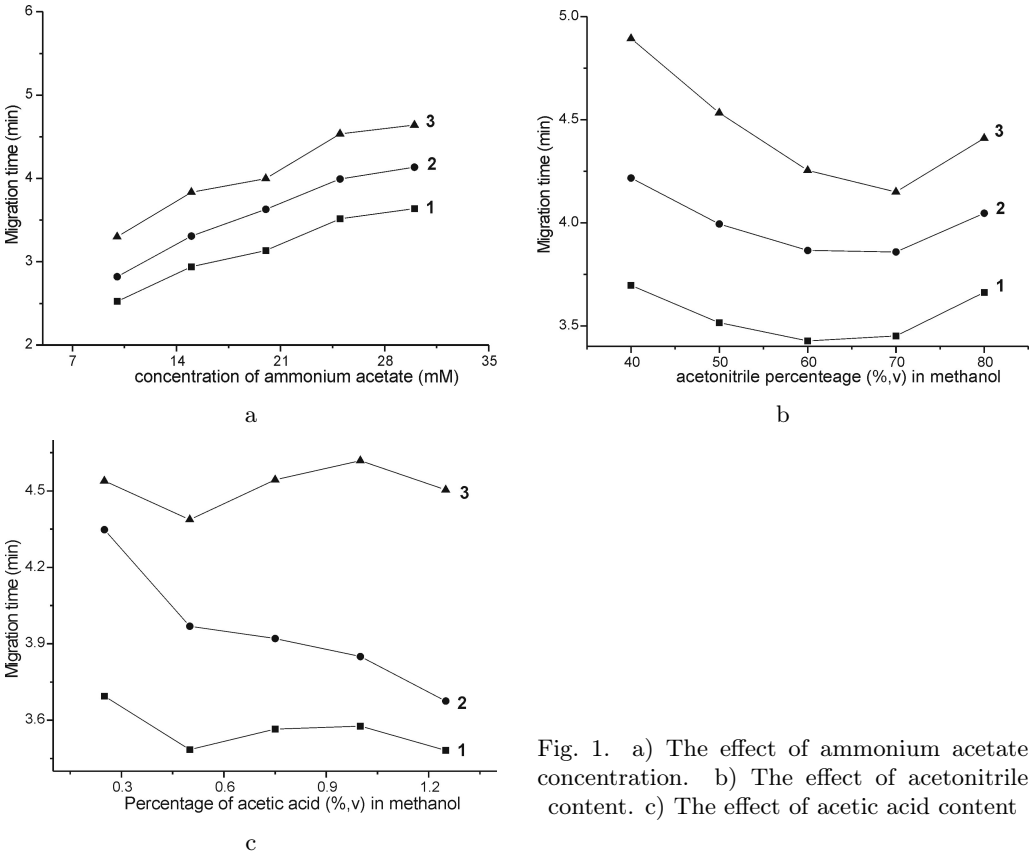

Fig. 1. a) The effect of ammonium acetate concentration. b) The effect of acetonitrile content. c) The effect of acetic acid content

Analytes: 1=sophoridine, 2=matrine and 3=oxymatrine. Buffer: 10–30 mM ammonium acetate +50% acetonitrile+0.5% acetic acid in methanol. Capillary: uncoated fused-silica capillary 47 cm (40 cm injector to detector) × 75 µm i.d. UV detection wavelength: 206 nm. Applied voltage: 20 kV. Capillary temperature: 16 °C. Analytes: 1=sophoridine, 2=matrine and 3= oxymatrine. Buffer: 40–80% acetonitrile +25 mM ammonium acetate +0.5% acetic acid in methanol. Analytes: 1=sophoridine, 2=matrine and 3= oxymatrine. Buffer: 0.25–1.25% acetic acid + 60% acetonitrile +25 mM ammonium acetate in methanol.

above two factors, 60% acetonitrile was selected as the best condition.

The effect of acetic acid content on separation. Figure 1c shows the effect of acetic acid content on separation. Acidity plays an important role in electrophoretic separation. It can be seen from the figure that the resolution between two adjacent analytes and the migration time of each analyte varied with the change of acetic acid content. Considering the resolution and migration time, 0.75% acetic acid was selected as the best separation condition.

Discussion. ***Effect of separation voltage and capillary temperature.*** In the experiment, we have also studied the effect of separation voltage (15–25 kV) and capillary temperature (16–30 °C) on separation. The separation time can be greatly reduced by increasing the voltage, but the noise will rise by increasing the voltage; it is found in the experiment that when the voltage reaches 25 kV, the flow will be cut off easily. The reason may be that the temperature of capillary rises with the increase of voltage, which causes local liquid vapourization. Considering comprehensively, 20 kV was selected as the optimized separation voltage. With the increase of capillary temperature, the separation time of the analytes was shortened, which was mainly due to the decrease of liquid viscosity. However, too high a temperature can also cause a loss of flow. Considering comprehensively, 20 °C was selected as the optimized capillary electrophoresis temperature.

According to the previous experimental results, the best separation conditions were 60% acetonitrile + 0.75% acetic acid + 25 mM ammonium acetate in methanol as the running buffer solution; the separation voltage was 20 kV, the capillary temperature 20 °C. The electropherogram of the mixed standards obtained under the best experimental conditions is shown in Fig. 2a. Baseline isolation of matrine, oxymatrine, and Sophoridine was achieved in less than 5 min.

Working curve, detection limit and reproducibility. In the optimal separation conditions, the linear range, standard curve and detection limit of each analyte are listed in Table 1. The detection limit was determined with a signal-to-noise ratio equal to 3. The relative standard deviations (RSD) of migration time and peak height obtained by running the standard solution for 5 consecutive times

T a b l e 1

The regression data and detection limits

compound	Regression equation[a)]	Correlation coefficient	Linear range (μg/ml)	LOD[b)] (μg/ml)
1 sophoridine	Y = 6.88 + 0.42X	0.984	1.73–166.6	0.56
2 matrine	Y = 9.76 + 0.35X	0.968	2.01–193.3	0.45
3 oxymatrine	Y = 6.91 + 0.30X	0.983	2.01-193.3	0.6

a) Y and X were the peak height and concentration (μg/ml) of the analytes, respectively.
b) The limit of detection (LOD) was obtained based on the signal-to-noise ratio of 3

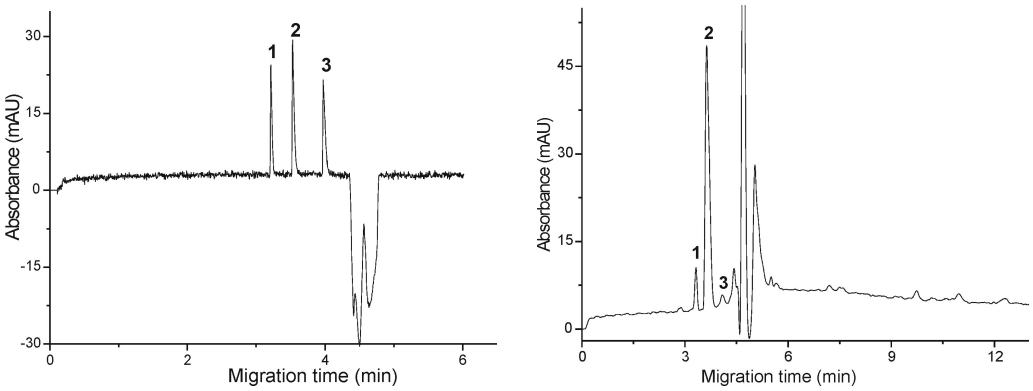

Fig. 2. a) The electropherogram of the standard mixture. b) The electropherogram of the actual samples

1=sophoridine, 2=matrine and 3= oxymatrine. Experiment conditions: 25 mM ammonium acetate, 60% acetonitrile, 0.75%acetic acid in methanol; Applied voltage: 25 kV; Capillary temperature: 20 ° C. Detection wavelength: 206 nm

were 1.45 and 2.1% (1), 1.4 and 2.3% (2), and 0.14, and 2.6% (3), respectively.

Analysis of the actual samples. In the best experimental conditions, the actual samples (*Sophora flavescens*) were analyzed, and the electrophoresis diagram is shown in Fig. 2b. By comparing the migration time and the standard addition method, the peaks of the three components in the actual sample electropherograms were identified. The content of each component in the sample is listed in Table 2. In this paper, the standard addition method was used to determine the recovery rates of matrine, oxymatrine and sophoridine. The results are listed in Table 2.

T a b l e 2

The content and recovery rate of each component in the actual sample

Compound	Content (mg/g)	Recovery (%)
1	0.249	95.6
2	1.869	97.2
3	0.325	96.3

Conclusion. The new non-aqueous capillary electrophoresis method established in this paper can be used for the simultaneous separation and determination of matrine, oxymatrine and sophoridine in compound preparations. The method provided in this article is simple, fast, and reproducible. It is expected to be used as a quality control method for compound preparations containing these three alkaloids.

REFERENCES

[1] Hu Y., S. Wang, Y. Xie, T. Kang (2003) Research on the Anti-tumor Effect of Oxymatrine, J. Liaoning Univ. Trad. Chinese Med., **5**(1), 3–5.

[2] Wang B., G. Wang, J. Xu (2000) Inhibitory Effect of Oxymatrine on Tumor-induced Vascular Endothelial Cell Proliferation, J. Pract. Oncol., **15**(5), 297–230.

[3] Zhang M., Y. Shen (2021) Research progress on the antitumor pharmacological action of sophoridine, Drug Evaluat. Res., **44**(02), 452–460.

[4] Kang J., X. Liu (2020) Progress in pharmacological action of sophoridine and sophoridine N-oxide in various inflammatory diseases, Chinese Wild Plant Resour., **39**(06), 40–47.

[5] Jiang H., Y. Chen, H. Zhang (2001) Determination of Matrine and Oxymatrine in Different Products of Radix Sophorae Flavescentis, Chinese Trad. Patent Med., **3**, 31–33.

[6] Fang Y., H. Zhu, X. Liu, L. Chen, C. Meng et al. (2020) Study on revising the quality standard of *Sophora flavescens*, China J. Chinese Mater. Med., **45**(08), 1756–1763.

[7] Li R., J. Su (2000) Quantitative Analysis of Matrine in Shikangfu Granules II, Chinese Trad. Herb. Drugs, **1**, 18–20.

[8] Cang S., R. Liu, T. Wang, X. Jiang, W. Zhang et al. (2019) Simultaneous determination of five active alkaloids from Compound Kushen Injection in rat plasma by LC–MS/MS and its application to a comparative pharmacokinetic study in normal and NSCLC nude rats, J. Chromatogr. B, **1126**, 121734.

[9] Fan R., R. Liu, R. Ma, K. Bi, Q. Li (2013) Determination of oxymatrine and its active metabolite matrine in human plasma after administration of oxymatrine oral solution by high-performance liquid chromatography coupled with mass spectrometry, Fitoterapia, **89**, 271–277.

[10] Guo P., F. Zhong, Y. Zhao, X. Xu, W. Xue et al. (2022) Thermosensitive molecularly imprinted polymer coupled with HPLC for selective enrichment and determination of matrine in traditional Chinese medicine, J. Chromatogr. B, **1191**, 123130.

[11] Zhang L., W. Liu, R. Zhang, Z. Wang, Z. Shen et al. (2008) Pharmacokinetic study of matrine, oxymatrine and oxysophocarpine in rat plasma after oral administration of *Sophora flavescens* Ait. extract by liquid chromatography tandem mass spectrometry, J. Pharm. Biomed. Anal., **47**(4–5), 892–898.

[12] Zheng H., G. Chen, L. Shi, Z. Lou, F. Chen et al. (2009) Determination of oxymatrine and its metabolite matrine in rat blood and dermal microdialysates by high throughput liquid chromatography/tandem mass spectrometry, J. Pharm. Biomed. Anal., **49**(2), 427–433.

[13] Cheng Y., H. Chen, Y. Li, X. Chen, Z. Hu (2004) Separation and determination of aloperine, sophoridine, matrine and oxymatrine by combination of flow injection with microfluidic capillary electrophoresis, Talanta, **63**(2), 491–496.

[14] Wang H., Y. Lu, J. Chen, J. Li, S. Liu (2012) Subcritical water extraction of alkaloids in *Sophora flavescens* Ait. and determination by capillary electrophoresis with field-amplified sample stacking, J. Pharm. Biomed. Anal., **58**, 146–151.

[15] Hou Z., G. Sun, Y. Guo, F. Yang, D. Gong (2019) Capillary electrophoresis fingerprints combined with Linear Quantitative Profiling Method to monitor the quality

consistency and predict the antioxidant activity of Alkaloids of *Sophora flavescens*, J. Chromatogr. B, **1133**, 121827.

[16] Yin J., Y. Xu, J. Li, E. Wang (2008) Analysis of quinolizidine alkaloids in *Sophora flavescens* Ait. by capillary electrophoresis with tris(2,2-bipyridyl) ruthenium (II)-based electrochemiluminescence detection, Talanta, **75**(1), 38–42.

[17] Song J., H. Xu, S. Tian, P. But (1999) Determination of quinolizidine alkaloids in traditional Chinese herbal drugs by nonaqueous capillary electrophoresis, J. Chromatogr. A, **857**(1–2), 303–311.

[18] Kenndler E. (2014) A critical overview of non-aqueous capillary electrophoresis. Part II: Separation efficiency and analysis time, J. Chromatogr. A, **1335**, 31–41.

[19] John A. S., M. M. Sidek, L. Y. Thang, S. Sami, H. Y. Tey et al. (2021) Online sample preconcentration techniques in nonaqueous capillary and microchip electrophoresis, J. Chromatogr. A, **1638**, 461868.

[20] Niedermeier S., G. E. Scriba (2020) Chiral separation of four phenothiazines by nonaqueous capillary electrophoresis and quality by design-based method development for quantification of dextromepromazine as chiral impurity of levomepromazine, J. Chromatogr. A, **1624**, 461232.

[21] Hou J., G. Li, Y. Wei, H. Lu, C. Jiang et al. (2014) Analysis of five alkaloids using surfactant-coated multi-walled carbon nanotubes as the pseudostationary phase in nonaqueous capillary electrophoresis, J. Chromatogr. A, **1343**, 174–181.

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