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Short communication

Simultaneous determination of atropine, anisodamine, and scopolamine in plant extract by nonaqueous capillary electrophoresis coupled with electrochemiluminescence and electrochemistry dual detection

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ABSTRACT

A rapid and simple method was demonstrated for the analysis of atropine, anisodamine, and scopolamine by nonaqueous capillary electrophoresis (NACE) coupled with electrochemiluminescence (ECL) and electrochemistry (EC) dual detection. The mixture of acetonitrile (ACN) and 2-propanol containing 1 M acetic acid (HAc), 20 mM sodium acetate (NaAc), and 2.5 mM tetrabutylammonium perchlorate (TBAP) was used as the electrophoretic buffer. Although a short capillary of 18 cm was used, the decoupler was not needed and the separation efficiency was good. The linear ranges of atropine, anisodamine, and scopolamine were 0.5–50, 5–2000, and 50–2000 μM, respectively. For six replicate measurements of 100 μM scopolamine, 15 μM atropine, and 200 μM anisodamine, the RSDs of ECL intensity, EC current, and migration time were less than 3.6%, 4.5%, and 0.3%, respectively. In addition, because the organic buffer was used, the working electrode (Pt) was not easily fouled and did not need reactivation. The method was also applied for the determination of these three alkaloids in *Flos daturae* extract.

1. Introduction

Atropine, anisodamine, and scopolamine are extensively used in clinic because of their strong parasympatolytic and anticholinergic actions [1]. These three tropane alkaloids are widely distributed in the Solanaceae plants, such as *Flos daturae*, *Atropa*, and *Hyoscyamus* [2]. So, it is very important to develop a rapid, sensitive, and accurate method for the analysis of these alkaloids in plant extracts. Thin layer chromatography (TLC) [3], gas chromatography (GC) [4,5], and high-performance liquid chromatography (HPLC) [6,7] have been developed for the determination of these three alkaloids.

Capillary electrophoresis (CE) with aqueous solution has been also applied for the analysis of these alkaloids [1,2,8–14]. However, it is very difficult to separate these three alkaloids because of their similar structure (see Fig. 1), and some surfactants (such as cyclodextrin [1] and sodium dodecyl sulfate [14]) are typically used as additives in the electrophoretic buffer. In addition, these alkaloids are unstable in aqueous solution.

Nonaqueous capillary electrophoresis (NACE), which is based on the use of the electrolyte solution prepared from pure organic solvents or their mixture, has received considerable attention [15–17]. Compared with aqueous CE, NACE has several advantages. Firstly, it can be used to separate insoluble and unstable compounds in water. Secondly, the electrophoretic current is lower in NACE. Finally, NACE is ideally suite for coupling with electrochemistry detection and electrospray ionization mass spectrometry. Lower electrophoretic current and wide electrochemical window in organic solvent is beneficial for electrochemical detection, while high volatility and low surface tension of some organic solvents is useful for electrospray ionization mass spectrometry. NACE coupled with UV [14,18,19] or MS [20] has been successfully used for the analysis of tropane alkaloids. However, there is no report about the simultaneous determination of atropine, anisodamine, and scopolamine by NACE. Recently, we presented a novel tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) electrochemiluminescence (ECL) and electrochemistry (EC) dual detection scheme for NACE (NACE-ECL/EC), and the detectors were compatible with NACE very well [21]. In this study, NACE-ECL/EC system was successfully used for the fast analysis of atropine, anisodamine, and scopolamine with good repeatability, and the effect of buffer composition on the separation was investigated in detail. This proposed method was also applied for the simultaneous determination of these three alkaloids in *F. daturae* extract, and satisfied results were obtained.

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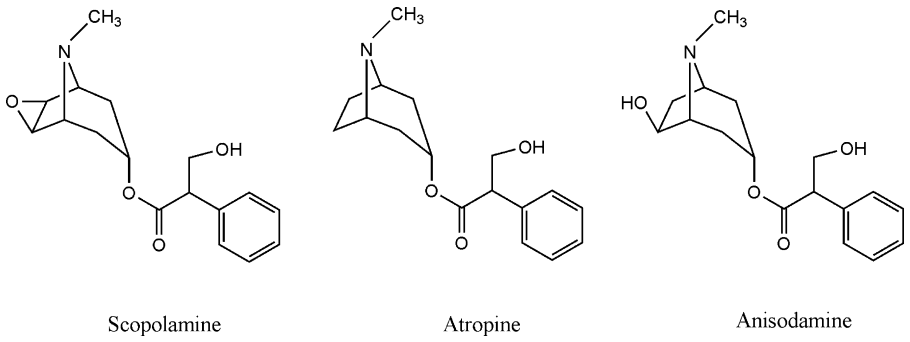

Fig. 1. Structure of scopolamine, atropine and anisodamine.

2. Materials and methods

2.1. Chemicals

Tris(2,2′-bipyridyl)ruthenium(II) dichloride hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$) was purchased from Aldrich (Milwaukee, WI, USA) and used without further purification. Tetrabutylammonium perchlorate (TBAP, electrochemical grade) was obtained from Fluka (Milwaukee, WI, USA). Acetonitrile (ACN), 2-propanol, acetic acid (HAc), and sodium acetate (NaAc) were purchased from Beijing Chemical Factory (Beijing, China). Atropine sulfate, anisodamine hydrobromide, and scopolamine hydrobromide were obtained from Tianjin Yifang Science Tech Co., Ltd. *F. daturae* was provided by Heilongjiang University. All the reagents and chemicals used were of analytical reagent grade.

2.2. Apparatus

NACE-ECL/EC dual detection was performed on a MPI-A electrophoretic instrument (Xi'an Remex Electronic Science Tech Co., Ltd., Xi'an, China) equipped with a high-voltage power supply, an EC potentiostat, a luminescence detector, and a data collection analyzer. Electrochemical measurements were performed with a CH Instrument model 800 voltammetric analyzer (Austin, TX, USA). The ECL/EC dual detection cell and the three electrodes system have been illustrated in detail in our recent work [21].

A 18 cm length of uncoated fused-silica capillary (25 μm i.d., 360 μm o.d.) was used for separation (Yongnian Optical Fiber Factory, Hebei, China). Before use, the capillary was rinsed with doubly distilled water, pure ACN, and the electrophoretic buffer for 10 min, respectively. The electrophoretic buffer consisted of 2.5 mM TBAP, 1 M HAc, and 20 mM NaAc in the mixture of ACN and 2-propanol.

2.3. Plant sample preparation

First, dried *F. daturae* was ground into powder. Next, 0.5 g powder was weighed and extracted in 6 mL ethanol with ultrasonication. After that, the solution was centrifuged at 3000 rpm for 10 min. Later on, the extraction procedure was repeated twice. Finally, the extracts were collected and filtered through a 0.22 μm cellulose acetate membrane.

3. Results and discussion

3.1. Optimization of detection conditions

Most of NACE is carried out in the mixed organic solvents since the selectivity of the analytes can be modified by varying their ratio, and the mixture of ACN and alcohol is typically used [22]. In our study, it was found that the addition of some 2-propanol to ACN was not only compatible with ECL detector but also increased the ECL intensity of these alkaloids. The concentration of 2-propanol in the detection cell was optimized, and 20% 2-propanol was selected as the optimized concentration in the detection cell (Supplementary material, Figs. 1 and 2). In order to decrease analysis time, a much shorter capillary (18 cm) and higher separation voltage (20 kV) was employed in our study. However, the high separation voltage had no negative effect on the detectors even without the decoupler, and the RSD (n = 12) of ECL intensity was 0.55% (see Supplementary

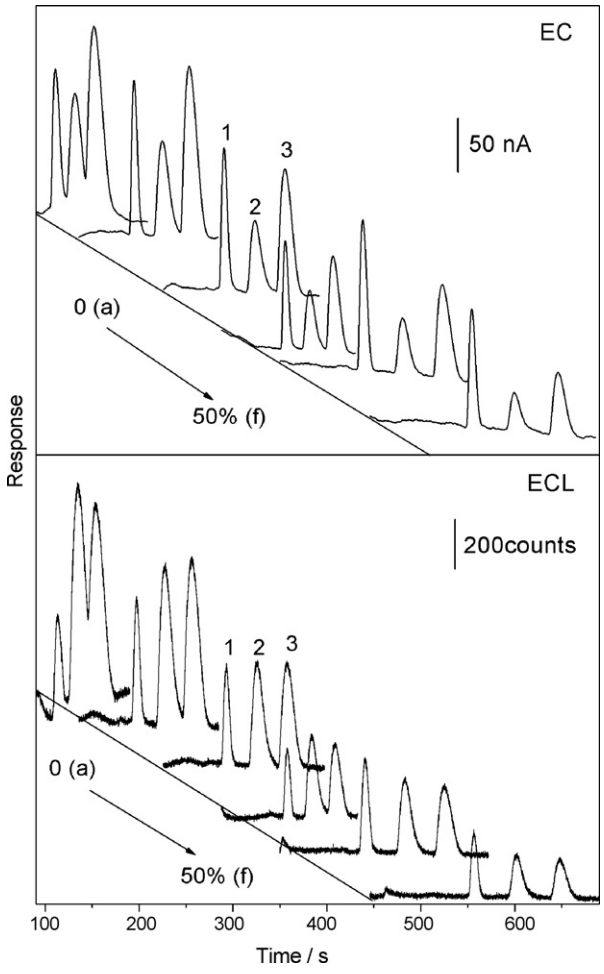

Fig. 2. Effect of the addition of 2-propanol (v/v) in buffer on the separation of 100 μM scopolamine (1), 15 μM atropine (2), and 200 μM anisodamine (3) with ECL and EC dual detection. Detection potential, 1.2 V; solution in detection cell, 2 mM $Ru(bpy)_3^{2+}$, 40 mM TBAP, 20 mM NaAc, and 1 M HAc in the mixture of 2-propanol and ACN (v/v, 1:4); capillary dimensions, 18 cm × 25 μm i.d.; separation voltage, 20 kV; electrokinetic injection, 12 s × 5 kV; electrophoretic buffer, 2.5 mM TBAP, 20 mM NaAc, and 1 M HAc in the mixture of 2-propanol and ACN (v/v).

Table 1
Detection of atropine, anisodamine, and scopolamine by different methods.

Method	Analyte	Linear range (μM)	LOD[a] (μM)	Migration time (min)	Voltag(e/kV)	Capillary dimension	RSD (%)	Ref.
CE-ECL	Atropine	0.2–100	0.016	10	16	50 cm × 25 μm i.d.	5.3	[1]
	Anisodamine	0.2–100	0.01	8	16	50 cm × 25 μm i.d.	2.8	
	Scopolamine	2–200	0.2	11	16	50 cm × 25 μm i.d.	3.4	
CE-ECL	Atropine	1–20	0.05	7.4	15	50 cm × 25 μm i.d.	5.16	[9]
	Scopolamine	10–1000	1	7.8	15	50 cm × 25 μm i.d.	5.07	
CE-UV	Atropine	5.8–51.8		11.5	20	42 cm × 50 μm i.d.	1.34	[8]
	Anisodamine	6.7–61.4		11.2	20	42 cm × 50 μm i.d.	1.27	
	Scopolamine	5.5–49.7		11.8	20	42 cm × 50 μm i.d.	1.81	
CE-UV	Atropine	35–440	4.6	6.6	15	55 cm × 50 μm i.d.	0.9	[10]
	Scopolamine	44–552	8	7.6	15	55 cm × 50 μm i.d.	1.47	
CE-UV	Atropine	14.4–180	1.4	3.47	30	56 cm × 50 μm i.d.	2.11	[11]
	Scopolamine	22.8–285.2	2.3	3.53	30	56 cm × 50 μm i.d.	2.21	
CE-UV	Atropine	7.2–201.5	1.4	5	20	60 cm × 75 μm i.d.	4.62	[12]
	Scopolamine	17.1–479.1	3.4	5.5	20	60 cm × 75 μm i.d.	5.27	
CE-MS	Scopolamine		0.0023	7.6		80 cm × 50 μm i.d.		[13]
NACE-UV	Atropine			6.5	25	65 cm × 50 μm i.d.		[14]
	Scopolamine			6.0	25	65 cm × 50 μm i.d.		
NACE-EC[b] (our work)	Atropine	0.5–50[c]	0.5	3.2	20	18 cm × 25 μm i.d.	1.6[d]	
	Anisodamine	5–1000[c]	2	4	20	18 cm × 25 μm i.d.	3.6[d]	
	Scopolamine	50–2000[c]	5	2.5	20	18 cm × 25 μm i.d.	1.6[d]	
NACE-ECL[b] (our work)	Atropine	0.5–50[c]	0.5	3.2	20	18 cm × 25 μm i.d.	2.6[d]	
	Anisodamine	5–2000[c]	5	4	20	18 cm × 25 μm i.d.	4.5[d]	
	Scopolamine	50–1000[c]	50	2.5	20	18 cm × 25 μm i.d.	4.3[d]	

[a] Based on S/N = 3.
[b] Under the optimized conditions.
[c] Correlation coefficients were 0.996, 0.995, 0.991, 0.999, 0.992, and 0.992, respectively.
[d] Based on six replicate injections of the mixture of 100 μM scopolamine, 15 μM atropine, and 200 μM anisodamine. The RSDs of the migration time were 0.31%, 0.30%, and 0.27% for atropine, anisodamine, and scopolamine, respectively.

material, Figs. 3 and 4). Effect of injection time (injection voltage, 5 kV) and detection potential on the ECL and EC responses was also investigated (Supplementary material, Figs. 5 and 6), and 12 s and 1.2 V was selected as the optimal injection time and detection potential, respectively.

3.2. Effect of 2-propanol concentration (v/v) on the separation

ACN is an aprotic solvent that can only accept proton. However, 2-propanol is an amphiprotic solvent, which can act as both proton acceptor and proton donor. 2-Propanol has been successfully used in NACE [23–25] and chiral NACE [26] to improve the selectivity. Fig. 2 illustrates the effect of the addition of 2-propanol in buffer on the separation of atropine, anisodamine, and scopolamine. We can see that these three alkaloids could not be well separated in pure ACN and the baseline noise was much larger (curve a). While 10% 2-propanol was added in buffer, the separation efficiency of these three alkaloids was improved (curve b). These three alkaloids could be baseline separated in 20% 2-propanol (curve c). The higher 2-propanol concentration was used in buffer, the better separation was achieved. However, when the percentage of 2-propanol was beyond 20%, the ECL and EC responses decreased (curve d–f). In addition, higher 2-propanol concentration resulted in longer analysis time. Therefore, 20% 2-propanol was added in buffer, which was the same as that in the detection cell.

3.3. Repeatability, linearity, and detection limit of these three alkaloids

Under the optimized conditions: detection potential at 1.2 V; electrokinetic injection for 12 s at 5 kV; separation voltage at 20 kV; buffer solution containing 2.5 mM TBAP, 1 M HAc, and 20 mM NaAc in the mixture of 2-propanol and ACN (1:4), these three alkaloids were detected by NACE-ECL/EC dual detection. The analysis results were compared with other methods (Table 1). The detection limits of atropine, anisodamine, and scopolamine were 0.5, 2, and 5 μM, which is lower than and comparable to that obtained by CE-UV [8,10–12] but higher than that obtained by CE-ECL [1,9] and CE-MS [13]. The reason of lower sensitivity than CE-ECL (50 cm × 25 μm i.d.) might be caused by the short capillary (18 cm × 25 μm i.d.) we used. By this ECL/EC detection, the linear ranges of atropine, anisodamine, and scopolamine were 0.5–50, 5–2000, and 50–2000 μM, respectively, which is wider than or comparable to that reported [1,8–12]. These three alkaloids could be baseline separated within 4.5 min without any surfactant, which is much faster than the reported literatures [1,2,8–14].

For six replicate measurements of 100 μM scopolamine, 15 μM atropine, and 200 μM anisodamine, the RSDs of ECL intensity, EC current, and migration time were less than 4.5%, 3.6%, and 0.3%, respectively (Table 1). The major advantage of this NACE-ECL/EC system is that the working electrode did not need reactivation with using organic buffer, which resulted in good repeatability. However, in aqueous CE-ECL or CE-EC, it is necessary to reactivate the electrode after each run because the adsorption of products on the electrode surface can decrease ECL or EC signal [27–31]. In addition, the electrode must be equilibrated for several minutes after the pretreatment until the baseline was stable. When water was replaced by ACN, products adsorbed on the electrode surface can be dissolved in organic phase buffer. Therefore, the fouling of electrode surface by products in NACE-ECL/EC system was suppressed.

3.4. Application

The proposed method was applied for the determination of scopolamine, atropine, and anisodamine in *F. daturae* extract. The peak identifications of these three alkaloids were assured by

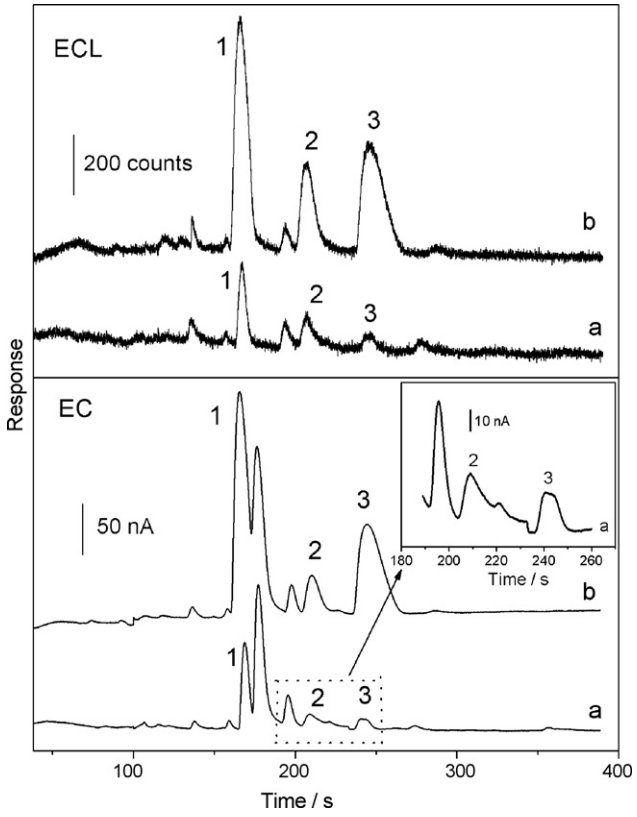

Fig. 3. Typical electropherograms of the *Flos daturae* extract (a) and the extract spiked with 100 μM scopolamine, 6 μM atropine, and 150 μM anisodamine (b) with simultaneous ECL and EC detection. Electrophoretic buffer, 2.5 mM TBAP, 20 mM NaAc, and 1 M HAc in the mixture of 2-propanol and ACN (v/v, 1:4). Other conditions are the same as in Fig. 2.

standard addition method. Fig. 3 shows the typical electropherograms of the plant extract (curve a) and the plant extract spiked with 100 μM scopolamine, 6 μM atropine, and 150 μM anisodamine standard solutions (curve b). We can see that peaks 1, 2, and 3 in curve a were obviously increased when 100 μM scopolamine, 6 μM atropine, and 150 μM anisodamine were added to a (curve b). Hence, peaks 1, 2, and 3 were deemed to the peaks of scopolamine, atropine, and anisodamine, respectively. It was noted that there was an unknown peak overlapping with peak 1 in EC detection while not in the ECL detection. In addition, the responses of peaks 2 and 3 in ECL detection were too small to determine. Therefore, scopolamine was determined according to the ECL detection while atropine and anisodamine were detected according to the EC detection, which suggested that this ECL/EC dual detection could help to determine and identify the analytes from unknown ingredients since the plant sample was very complex. According to the linear equation, the contents of scopolamine, atropine, and anisodamine in *F. daturae* were calculated to be 1.08, 0.69, and 0.20 mg/g, respectively. The recoveries of spiked scopolamine (100 μM), atropine (6 μM), and anisodamine (150 μM) were 77%, 70%, and 83%, respectively.

4. Conclusions

A rapid and simple method was presented to determine atropine, anisodamine, and scopolamine in *F. daturae* extract by NACE-ECL/EC dual detection. This ECL/EC dual detection mode could provide more information of analytes and help to determine and identify analytes from unknown gradients in the plant sample. NACE-ECL/EC could be a potential method for the quantitative and qualitative analysis of herbal medicine.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.008.

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