Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1553-1556

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Detection of Galanthamine in *Bulbus lycoridis radiatae* by Capillary Electrophoresis with an End-column Electrochemiluminescence of *Tris*(2,2'-bipyridyl)ruthenium(II)

Q. XIANG[1], H. WANG[2], Y. XU[3], L. SUN[1] and Y. GAO[1,3,*]

[1]Department of Applied Chemistry, Changchun Institute of Technology, Changchun, P.R. China
[2]College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, P.R. China
[3]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, P.R. China

*Corresponding author: Fax: +86 431 85689711; Tel: +86 431 85711660; E-mail: gaoy5680@163.com

(*Received*: 7 May 2010; *Accepted*: 4 December 2010) AJC-9355

A capillary electrophoresis with an end-column electrochemiluminescence detection method is described for the determination of galanthamine in *Bulbus lycoridis radiatae* without derivatization. To minimize the matrix effect and get rid of the interfering substances in crude extract of the herb, the effect of running buffer on separation and detection of galanthamine in real sample was investigated. Under the optimized conditions: electrochemiluminescence detection at 1.2 V, 15 mmol/L phosphate buffer at pH 8.49, 13 kV separation voltage, the calibration of galanthamine was linear (r = 0.998) with detection limit of 1.0×10^{-9} mol/L.

Key Words: Electrochemiluminescence, Capillary electrophoresis, Galanthamine.

INTRODUCTION

In recent years, attention has been focused on the quality control and quantitative analysis of active principles in Chinese traditional medicine. However, there are two obvious challenges in analysis of Chinese traditional medicine. Firstly, because Chinese traditional medicine contains many interfering components which are also detected, target analytes must be sufficiently resolved to avoid interference. Secondly, the detection sensitivity of the analytes must be improved for active component with low concentration.

Bulbus lycoridis radiatae is a widely used traditional Chinese plant medicine. Galanthamine in *Bulbus lycoridis radiatae* is a major biologically active ingredient, which is a cholinergic drug used for the treatment of Alzheimer's disease by inhibiting acetylcholinesterase and positively modulating nicotinic acetylcholine receptors[1]. Molecular structure of galanthamine is shown in Fig. 1.

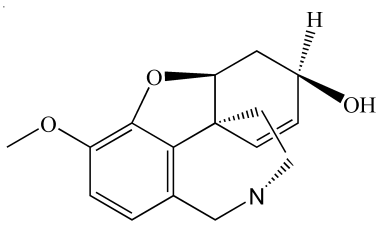

Fig. 1. Molecular structure of galanthamine

Several reports have described the analysis of galanthamine in Chinese traditional medicine, pharmaceutical products, biological fluids and tissue samples, respectively. Gotti *et al.*[2] determinated amaryllidaceae-type alkaloids in the bulbs of Narcissus species by using GC-MS and capillary electrophoresis, respectively. Berkov *et al.*[3] detected the contents of galanthamine type alkaloids in plants by capillary gas chromatography-mass spectrometry. Ingkaninan *et al.*[4] reported the separation, detection and identiflcation of acetylcholinesterase inhibitors from natural products by using HPLC with on-line coupled UV-MS-biochemical detection. In order to monitor and identify quality of pharmaceutical products, Visky *et al.*[5] analyzed impurities of galantamine hydrobromide in an extended release capsule by CE-MS. Micellar electrokinetic chromatography (MEKC) with UV detection was also used for the separation of acetylcholinesterase inhibitors including galantamine, rivastigmine and major metabolite in plasma[6]. In addition, capillary zone electrophoresis[7], LC-MS-MS method[1] and HPLC method with fluorescence detection[8] were applied to the separation and the determination of galanthamine in biological fluids and tissue samples, respectively.

Recently, there are increasing interests in coupling (CE) separation with electrochemiluminescence (ECL) detection for alkaloids analysis[9,10]. Especially, electrochemiluminescence of *tris*(2,2'-bipyridyl) ruthenium(II) is a highly sensitive and selective detection means in analytical chemistry because of

its excellent stability and high efficiency in the aqueous phase. Therefore, the combination of CE with $Ru(bpy)_3^{2+}$ ECL detection (CE-ECL) have been paid more attention to the analysis of alkaloids[11-17] and the detection of active principles in Chinese traditional medicine[18-22]. However, in our best of knowledge, such CE-ECL procedure has not been reported for the determination of galanthamine in Chinese traditional medicine.

The aim of this research is to develop a robust CE-ECL method that can be used for the sensitive analysis of galanthamine with other coexisting substances in *Bulbus lycoridis radiatae*. Optimization of separation and detection conditions for ganlanthamine in real sample was performed in order to minimize the matrix effect and get rid of the disturbing substances. Under the optimized conditions, the content of galanthamine in *Bulbus lycoridis radiatae* was successfully determined within 10 min.

EXPERIMENTAL

Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Chinese herb *Bulbus lycoridis radiatae* was obtained from Baishan Zhengmao Medicine Industry Co. Ltd. (Ji Lin, China). Galanthamine was purchased from State Narcotic Laboratory (Beijing, China). The content of alkaloid is over 99 % and it can be used directly in the experiment without further purification. Sodium hydroxide, hydrochloric acid, sodium phosphate (Na_2HPO_4 and NaH_2PO_4) and ethanol were purchased from Beijing Chemical Co. (Beijing, China). Chemicals and reagents used were all analytical reagent grade. The buffer used throughout the study was sodium phosphate system. A series of different concentration galanthamine and phosphate buffer were prepared with double-distilled water.

Capillary electrophoresis separation and ECL detection were carried out with a CE-ECL instrument (Xi'an Remax Electronics Co., Xi'an, China), consisting of a high-voltage power supplier for separation and injection, a potential control system, a chemiluminescence detection system and a data processor. An uncoated fused-silica capillary (50 μm i.d., 365 μm o.d.) with the length of 42 cm was bought from Yongnian Optical Conductive Fiber Plant (Hebei, China). The separation capillary was filled with 0.1 mol/L NaOH overnight and then flushed for 10 min with 0.1 mol/L NaOH, 10 min with water and 10 min with the running buffer prior to use. Electrokinetic injected at 10 kV for 10 s. Column-end detection of ECL was employed using a three-electrode system consisting of Ag/AgCl as the reference electrode and Pt wire as the counter electrode and Pt disk as the working electrode (500 μm diameter). 300 mL of 5 mmol/L $Ru(bpy)_3^{2+}$ solution in 50 mmol/L phosphate buffer solution was added into a detection reservoir and was replaced once for 4 h. A detection potential applied at working electrode was fixed at 1.2 V. A voltage of 800 V was supplied at photomultiplier tube for collecting the ECL signal.

Preparation of standard solutions: The stock solution of galanthamine and $Ru(bpy)_3^{2+}$ were prepared by dissolving the standard samples in water and stored at 4 ºC in a refrigerator. A series of standard solutions with suitable concentrations and various running buffers with different concentrations were prepared by diluting the solution with water for the construction of calibration curve, the study of reproducibility and recovery. The double-distilled water was used throughout experiments.

Preparation of herbal drug extract: Firstly, a 0.5 g amount of pulverized *Bulbus lycoridis radiatae* was weighted accurately, fragmented in an ultrasonic bath at 40 ºC with 25 mL of 85 % ethanol as the solvent for 10 min. Then, the sample solution consisting of pulverized *Bulbus lycoridis radiatae* was extracted in a Soxhlet extractor for 2 h. The residue of *Bulbus lycoridis radiatae* was washed with 5 mL of 85 % ethanol for three times. All the extracts and washings were combined and were counteracted with 0.2 μL hydrochloric acid and filtered through a 0.45 μm membrane before use and stored this at 4 ºC in a refrigerator.

RESULTS AND DISCUSSION

Effect of applied potential: The detection voltage applied to the working electrode provides a crucial ECL coreaction platform for the alkaloid and ruthenium species. Based on this fact, the influence of the applied potential on the ECL intensity was investigated in a potential range of 1.0-1.3 V. As can be seen from Fig. 2, the signal intensity of galanthamine exhibited dependence on the detection potential. An increase in the applied potential from 1.0-1.2 V resulted in an enhancement in the ECL intensity. However, a decrease in ECL intensity at higher potential than 1.2 V was observed. An optimum detection potential was obtained at 1.2 V after evaluating the sensitivity and baseline noise, offering the most favourable signal-to-noise (S/N) characteristics.

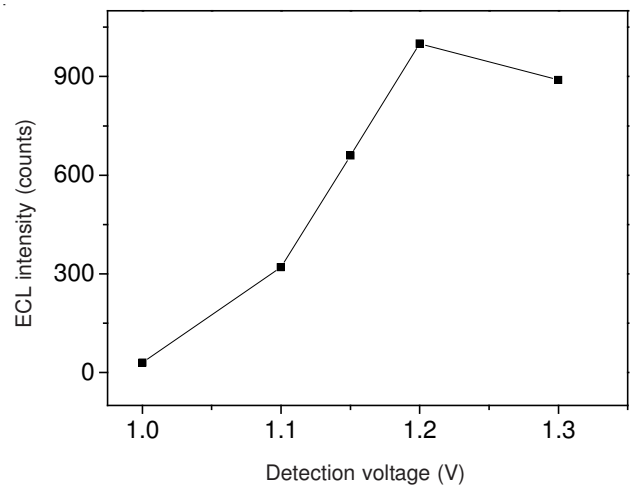

Fig. 2. Effect of detection potential on the ECL intensity: ECL intensity of 1×10^{-5} mol/L galanthamine. Conditions: running buffer, 20 mmol/L phosphate (pH 8.50); 5 mmol/L $Ru(bpy)_3^{2+}$ with 50 mmol/L phosphate buffer (pH 7.50) in the detection reservoir; electrokinetic injection, 10 s at 10 kV

Effect of running buffer concentration: Electrochemiluminescence intensity was examined when the buffer concentration changed from 5-25 mmol/L in order to investigate the effect of concentration on migration behaviour and ECL intensity of analyte. The experimental result was shown in Fig. 3, with increasing phosphate concentration, the migration time of the analyte was prolonged gradually. When buffer concentration was higher than 20 mmol/L, the negative effect

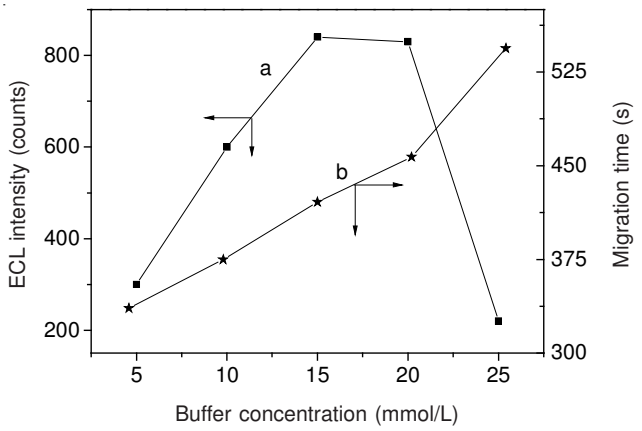

Fig. 3. Effect of running buffer concentration on the ECL intensity and migration time: (a) ECL intensity of galanthamine, (b) Migration time of galanthamine. Condition: sample, 10^{-5} mol/L galanthamine. Condition: detection voltage, 1.2 V; electrokinetic injection, 10 s at 10 kV; buffer pH, 7.01; separation voltage, 13 kV

on the baseline and ECL intensity of alalyte was observed. The highest ECL intensity of galanthamine with a suitable analysis time was obtained at a running buffer concentration of 15 mmol/L.

Effect of running buffer pH: The separation buffer pH value influences the electroosmotic flow inside the capillary, the analytes ionization and ECL reaction of analytes on the surface of the working electrode. Therefore, the effect of buffer pH on CE separation and ECL detection was studied by changing a wide range of pH from 4.51-9.93. With increasing pH of buffer, the eletroosmotic flow inside capillary increased remarkably and the migration time of galanthamine reduced gradually. On the other hand, using a running buffer at pH 6.93, ECL intensity of galanthamine reached a highest value. When exceeded pH 6.93, lower ECL intensitiy of the analyte was observed (Fig. 4).

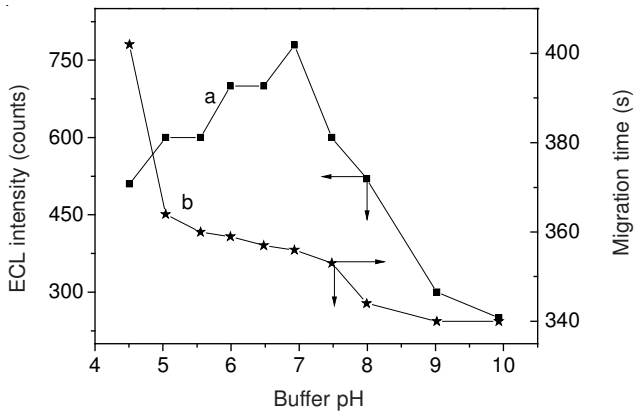

Fig. 4. Effect of running buffer pH on the ECL intensity and migration time: (a) ECL intensity of galanthamine, (b) Migration time of galanthamine. Condition: detection voltage, 1.2 V; electrokinetic injection, 10 s at 10 kV; running buffer concentration, 15 mmol/L; separation voltage, 13 kV

For practical sample analysis, a major drawback appears to be poor resolution between active ingredients, as well as active ingredient and disturbing substance in Chinese traditional medicine. When the extraction of *Bulbus lycoridis radiatae* was injected into CE-ECL analytical system, partial separation of galanthamine in traditional herbal medicine was observed using a running buffer at pH 6.93. To minimize the matrix effect and get rid of the interfering elements, buffers at pH value higher than 6.93 were used to investigate the separation of galanthamine in experimental sample. With the increase in the pH value of the running buffer, the degree of protonation of galanthamine reduced and differences of charge-mass ratio between the analyte and interfering elements increased. A baseline separation of galanthamine was obtained using a buffer at pH 8.49. Although at pH 6.93, the ECL response of galanthamine was higher than at pH 8.49, considering both the separation performance and ECL intensity of analyte, a running buffer of pH 8.49 was selected for further experiment.

Effect of separation voltage: The microenvironment of the outlet of capillary aligned with the working electrode is affected directly by the separation voltage. Then, the effect of separation voltage on CE separation and ECL intensity was investigated in the range of 10-15 kV. With the increase in separation voltage, ECL intensity of galanthamine increased and reached the maximum value at 13 kV due to electroosmosis and more analyte in the effluent arrived in the diffusion layer of working electrode within a given time[23]. When the separation voltage exceeded 13 kV, the ECL intensity decreased, at the same time the noise of the background increased due to the effect of high joule heat inside capillary. Thus, 13 kV was chosen as the separation voltage for further study.

Reproducibility, linearity and detection limit of galanthamine: Under the optimized conditions: the electrochemiluminescence detection at 1.2 V, electrokinetic injection for 10 s at 10 kV, separation voltage at 13 kV, 15 mmol/L phosphate buffer at pH 8.49, 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L phosphate buffer in the detection reservoir, the reproducibility of the ECL intensity and migration time was estimated by making repetitive injections of a standard solution containing 10^{-5} mol/L galanthamine. Relative standard derivations of the ECL intensity and the migration time were 4.27 and 0.71 % for galanthamine, respectively. To investigate the detection linearity of the galanthamine by CE-ECL system, a series of standard solutions was tested and the standard curve was linear between 2.5×10^{-7} and 5×10^{-5} mol/L for galanthamine. The calibration equations and regression coefficients were $y = 1.32 \times 10^{8}x - 39.83$ and $R = 0.998$ in terms of peak height response as a function of galanthamine concentration. The calibration curve exhibits excellent linear behaviour over the concentration range of about two orders of magnitude. Detection limit of 1.0×10^{-9} mol/L for galanthamine was obtained (S/N = 3). Comparison of the proposed method with other methods is listed in Table-1. Compared with traditional analytical method, the detection limit of galanthamine was lower than that reported in literature and could meet the analysis needs of active principles with low concentration and with other coexisting substances in real sample.

Analytical application: The developed CE-ECL method was employed for the determination of galanthamine in the extract of *Bulbus lycoridis radiatae* under the optimized conditions. Identification of the analyte in herbal extracts was confirmed by comparing the electropherograms of the extracts with that of the extracts spiked with galanthamine, where the increase of peak height at certain migration time was directly

TABLE 1
COMPARISON OF THE RESULTS OBTAINED BY THE PRESENT METHOD WITH OTHER REPORTED METHODS

Method	Detection limit (ng/mL)	Linear range (ng/mL)	Reference
LC-MS-MS	1	$1–5 \times 10^2$	[1]
GC-MS	1.8×10^3	$6.15 \times 10^3 – 6.15 \times 10^5$	[2]
NACE	2.0×10^3	$6.15 \times 10^3 – 6.15 \times 10^5$	[2]
GC-MS (in total ion current mode)	5×10^3	$5 \times 10^4 – 1 \times 10^6$	[3]
GC-MS (in selected ion monitoring mode)	1.6	$1.6 – 5 \times 10^2$	[3]
MEKC	1.0	$1.0 – 1.2 \times 10^2$	[6]
CZE	*ca.* 35	–	[7]
CE-ECL	1×10^{-9} mol/L (*ca.* 0.29 ng/mL)	$2.5 \times 10^{-7} – 5 \times 10^{-5}$ mol/L (*ca.* $7.2 \times 10 –1.4 \times 10^4$ ng/mL)	Present work

proportional to the amount spiked with galanthamine. Fig. 5 shows a typical electropherogram of the herbal extract. As shown in figure, the analyte is visible as neat electrophoretic peak without interference. The content of galanthamine found in the herb was 0.020 %.

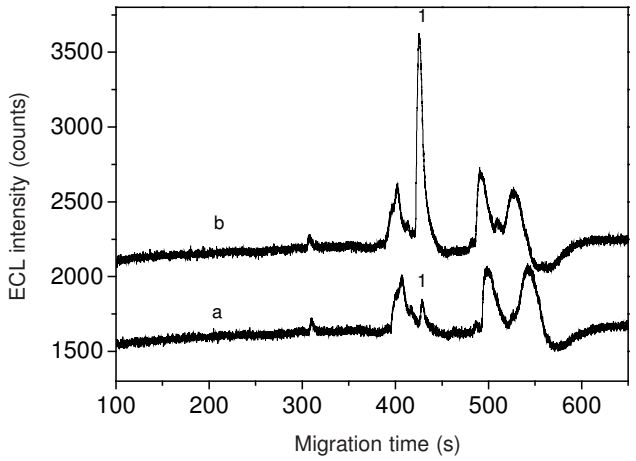

Fig. 5. Electropherograms of (a) the diluted extract and (b) the diluted extract spiked with 2×10^{-5} mol/L galanthamine; (1) ECL intensity of galanthamine. Condition: running buffer pH, 8.49; separation voltage, 13 kV; other conditions are the same as in Fig. 4.

The recoveries of the method were determined with the standard addition method in Chinese traditional medicine samples with results of 96.5-98.7 % for galanthamine. The results indicate that this method is accurate and sensitive, providing a useful quantitative method for the analyses of active ingredients in *Bulbus lycoridis radiatae*.

Conclusion

Capillary electrophoresis-electrochemiluscence was employed to analyze galanthamine in *Bulbus lycoridis radiatae* without derivatization. In comparison of the previous methods, the lower detection limit and wider linear range was obtained. It is concluded that capillary electrophoresis-electrochemiluscence is a powerful technique for study of the constituents of plant extracts and has become an alternative, competitive and supplementary method for HPLC, because of its special attributes.

ACKNOWLEDGEMENTS

This project was supported by the Foundation of State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (No. SKLEAC2010003), the Natural Science Foundation of Jilin Province, China (No.2010551) and the Foundation of Department of Education of Jilin Province (No.2006145).

REFERENCES

1. T. Verhaeghe, L. Diels, R. de Vries, M. De Meulder and J. de Jong, *J. Chromatogr. B*, **789**, 337 (2003).
2. R. Gotti, J. Fiori, M. Bartolini and V. Cavrini, *J. Pharm. Biomed. Anal*., **42**, 17 (2006).
3. S. Berkov, J. Bastida, F. Viladomat and C. Codina, *Phytochem. Anal*., **19**, 285 (2008).
4. K. Ingkaninan, C.M. de Best, R. van der Heijden, A.J.P. Hofte, B. Karabatak, H. Irth, U.R. Tjaden, J. vander Greef and R. Verpoorte, *J. Chromatogr. A*, **872**, 61 (2000).
5. D. Visky, I. Jimidar, W.V. Ael, T. Vennekens, D. Redlich and M.D. Smet, *Electrophoresis*, **26**, 1541 (2005).
6. Y.-H. Hsieh, Y.-H. Yang, H.-H. Yeh, P.-C. Lin and S.-H. Chen, *Electrophoresis*, **30**, 644 (2009).
7. L. Pokorná, A. Revilla, J. Havel and J. Patocka, *Electrophoresis*, **20**, 1993 (1999).
8. J. Maláková, M. Nobilis, Z. Svoboda, M. Lísa, M. Holcapek, J. Kvetina, J. Klimeš and V. Palicka, *J. Chromatogr. B*, **853**, 265 (2007).
9. Y. Du and E. Wang, *J. Sep. Sci*., **30**, 875 (2007).
10. X.-B. Yin and E. Wang, *Anal. Chim. Acta*, **533**, 113 (2005).
11. W. Cao, X. Yang and E. Wang, *Electroanalysis*, **16**, 169 (2004).
12. B. Deng, Y. Kang, X. Li and Q. Xu, *J. Chromatogr. B*, **857**, 136 (2007).
13. Y. Huang, W. Pan, M. Guo and S. Yao, *J. Chromatogr. A*, **1154**, 373 (2007).
14. J. Liu, W. Cao, X. Yang and E. Wang, *Talanta*, **59**, 453 (2003).
15. J. Wang, Z. Peng, J. Yang, X. Wang and N. Yang, *Talanta*, **75**, 817 (2008).
16. J. Yan, J. Liu, W. Cao, X. Sun, X. Yang and E. Wang, *Microchem. J*., **76**, 11 (2004).
17. X. Yin, J. Kang, L. Fang and E. Wang, *J. Chromatogr. A*, **1055**, 223 (2004).
18. Y. Gao, Y. Tian and E. Wang, *Anal. Chim. Acta*, **545**, 137 (2005).
19. Y. Gao, Q. Xiang, Y. Xu, Y. Tian and E. Wang, *Electrophoresis*, **27**, 4842 (2006).
20. J. Li, Y. Chun and H.J. Ju, *Electroanalysis*, **19**, 1569 (2007).
21. J. Yin, Y. Xu, J. Li and E. Wang, *Talanta*, **75**, 38 (2008).
22. M. Zhou, Y. Ma, X. Ren, X. Zhou, L. Li and H. Chen, *Anal. Chim. Acta*, **587**, 104 (2007).
23. Y. Xu, Y. Gao, H. Wei, Y. Du and E. Wang, *J. Chromatogr. A*, **1115**, 260 (2006).