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Three-channel column-switching high-performance liquid chromatography with electrochemical detection for determining bioactive redox components in *Salvia miltiorrhiza*

Xianchun Chen[a,b,c], Akira Kotani[a,*], Hideki Hakamata[a], Jie Wang[b], Shouying Du[c], Fumiyo Kusu[a]

[a] Department of Analytical Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan
[b] Guanganmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China
[c] School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China

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ABSTRACT

Three-channel column-switching high-performance liquid chromatography with electrochemical detection (3LC-3ED), which consists of three flow ways, two switching valves, four columns, and three electrochemical detectors, was developed for determining various redox components in *Salvia miltiorrhiza* with high sensitivity. In this study, the analytes were divided into three groups as follow: Group I [salvianic acid A (Danshensu) sodium salt (DSS), protocatechuic acid (PA), protocatechuic aldehyde (PAD), and caffeic acid (CA)], Group II [rosmarinic acid (RA), lithospermic acid (LA), and salvianolic acid B (SAB)], and Group III [cryptotanshinone (Cry), tanshinone I (Tan I), and tanshinone IIA (Tan IIA)]. By rotating each switching valve to change the elute flow way, the components in Groups I, II, and III were directed into the oxidative (+0.7 V), another oxidative (+0.7 V), and reductive (−0.2 V) detection channels, respectively. Chromatographic peaks of components in Groups I, II, and III appeared within 69, 53, and 60 min, respectively, in each channel. The detection limits of DSS, PA, PAD, CA, RA, LA, SAB, Cry, Tan I, and Tan IIA were 4.2, 2.8, 10, 10, 3.9, 3.8, 3.0, 132, 119, and 109 fmol, respectively, thus the sensitivity by the present 3LC-3ED is superior to those of previously reported gradient LC-ED and LC with UV detection. As an application, the phenolic acid contents and tanshinones in nine batches of *S. miltiorrhiza* were successfully determined.

1. Introduction

Traditional Chinese medicine (TCM) has been used for thousands of years in China and neighboring countries including Japan. In the last two decades, the use of TCM and its derived products has spread around the world, leading to an increase in the use of herbal medicines [1]. It is well known that there are numerous chemical components in herbal medicines, and reports have shown that the polyvalent effects of herbal medicines and their multiple components contribute to their pharmacological effects [2,3]. As such, there is a need to establish a multi-component means of analysis to evaluate the quality of herbal medicines. By comparing the present version of the Chinese Pharmacopeia from the last version, many multi-marker analysis methods have been accepted instead of single-marker analysis.

Salvia miltiorrhiza, a popular herbal medicine commonly used to cure cardiovascular disease, contains water-soluble phenolic acids and lipophilic tanshinones as its main pharmacologically active substances (Table 1). Phenolic acids such as salvianic acid A (Danshensu), protocatechuic acid (PA), protocatechuic aldehyde (PAD), caffeic acid (CA), rosmarinic acid (RA), lithospermic acid (LA), and salvianolic acid B (SAB) possess antioxidant, anti-platelet and anti-thrombotic activities, kidney function regulatory effects, liver protective effects, cardiovascular protective effects, anti-HIV activity, and so on [4–12]. In contrast, tanshinones such as cryptotanshinone (Cry), tanshinone I (Tan IA), and tanshinone IIA (Tan IIA) have many pharmacological effects, including antibacterial, antioxidant, and antineoplastic activities [13].

Owing to the fact that various compounds contribute to the pharmacological effect of *S. miltiorrhiza*, the simultaneous detection of phenolic acids and tanshinones becomes the preferable method to analyze *S. miltiorrhiza* itself and preparations containing *S. miltiorrhiza*. In order to simultaneously monitor both types of components, numerous analytical methods have been reported using chromatographic and spectrophotometric technologies coupled with various sample preparations such as thermal refluxing extraction, infrared-assisted extraction, ultrasound-assisted extraction, and microwave-assisted extraction [14–18].

* Corresponding author. Tel.: +81 42 676 4549; fax: +81 42 676 4570.
E-mail address: kotani@toyaku.ac.jp (A. Kotani).

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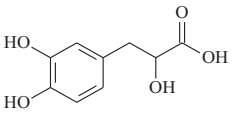
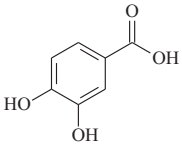
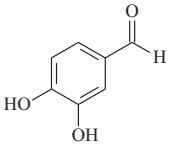
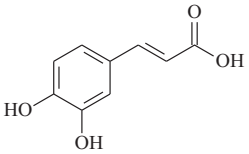
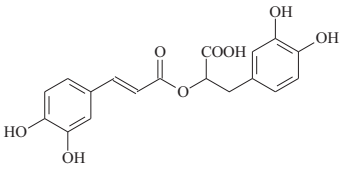
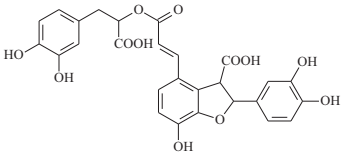
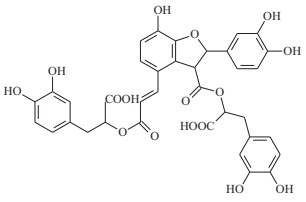
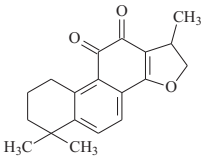
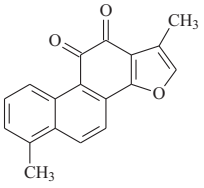
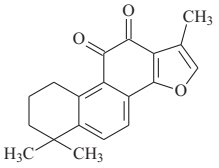
Table 1
Structure, abbreviation, and log *P* of phenolic acids and tanshinones.

Group	Analyte	Chemical structure	Abbreviation	log *P*[a]
Group I	Salvianic acid A (Danshensu)		DSS	−1.1 ± 0.4
	Protocatechuic acid		PA	1.0 ± 0.2
	Protocatechuic aldehyde		PAD	0.9 ± 0.3
	Caffeic acid		CA	0.7 ± 0.3
Group II	Rosmarinic acid		RA	0.9 ± 0.4
	Lithospermic acid		LA	−0.6 ± 0.5
	Salvianolic acid B		SAB	−0.7 ± 0.6
Group III	Cryptotanshinone		Cry	4.1 ± 0.9
	Tanshinone I		Tan I	4.3 ± 1.1
	Tanshinone IIA		Tan IIA	4.9 ± 0.8

[a] The logarithm of 1-octanol–water partition coefficient (log *P*) values were calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 for Solaris in Scifinder Web version 2012.