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Simultaneous determination of five flavonoids in licorice using pressurized liquid extraction and capillary electrochromatography coupled with peak suppression diode array detection

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ABSTRACT

Pressurized liquid extraction (PLE) and capillary electrochromatography (CEC) methods were developed for the simultaneous determination of five flavonoids, namely liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin, in licorice using baicalein as internal standard (IS). Peak suppression technique was used for the quantification of ononin because of its poor resolution with isoliquiritin. The analysis was performed on a Hypersil C_{18} capillary (3 μm, 100 μm/25 cm) with a mixture of 10 mM phosphate buffer (pH 3.0)/ACN (65:35, v/v) as mobile phase running at 25 kV and 30 °C. The detection wavelengths were set at 275 nm (without reference wavelength for liquiritin and liquiritigenin), 360 nm (without reference wavelength for isoliquiritin and isoliquiritigenin) and 254 nm (with reference wavelength of 405 nm for ononin). All calibration curves showed good linearity ($R^2 > 0.9993$) within the test ranges. The LOD and LOQ were lower than 2.1 and 8.3 μg/mL, respectively. The RSDs of intra- and interday for relative peak areas of five analytes to IS were less than 3.8 and 4.7%, respectively, and the recoveries were 98.2–103.8%. The validated method was successfully applied to the quantitative analysis of five flavonoids in licorice, which is helpful to its quality control.

1. Introduction

Licorice (or liquorice), the dried roots and rhizomes of *Glycyrrhiza* species (family Leguminosae), has been used as a natural sweetener, as well as a flavorant for a long time [1]. On the other hand, licorice is also one of the oldest and most popular herbal medicines in the world, and is recorded in the pharmacopoeias of many Asian and European countries, such as China, Japan and UK [2]. Numerous bioactive compounds, mainly including triterpene saponins and flavonoids, have been isolated from licorice. Among them, flavonoids, such as liquiritin, liquiritigenin, isoliquiritin and isoliquiritigenin, are gaining popularities because of their significant pharmacological activities including antiulcer, antioxidation, antiinflammatory, antimicrobial, antitumor effects, etc. [1–5]. Although licorice is considered as Generally Recognized as Safe (GRAS) for use in food by FDA (21 CFR 184.1408), large amount of licorice may result in severe hypertension, hypokalemia and other signs of mineralocorticoid excess [6,7]. Therefore, quality control is critical to ensure the efficacy and safety of licorice, and saponins and flavonoids are usually considered as the markers [3,8–18].

So far, a series of methods, including thin layer chromatography (TLC) [8], high performance liquid chromatography (HPLC) [9–12] and capillary electrophoresis (CE) [13–18] have been developed for the determination of saponins and/or flavonoids in licorice. However, these methods suffered from large consumption of organic solvents (HPLC), low resolution (TLC), bad repeatability (CE) and/or low sensitivity (TLC).

Capillary electrochromatography (CEC) is a hybrid technique of LC and CE, which uses an electric field to drive liquid through a packed capillary column. In this technique, the best properties of HPLC and CE operate in synergy. Due to its high selectivity and efficiency, CEC has attracted wide attention of pharmaceutical and biochemical analysts [19–21], although conventional gradient elution is difficult to perform. It has also been used for the analysis of natural products [22–24] except licorice.

On the other hand, sample preparation is crucial for quality control of herbal medicines. Traditional extraction methods such as ultrasonication, Soxhlet and reflux were usually time-consuming and solvent-intensive. As an alternative, pressurized liquid extraction (PLE), an extraction technique under elevated temperature and

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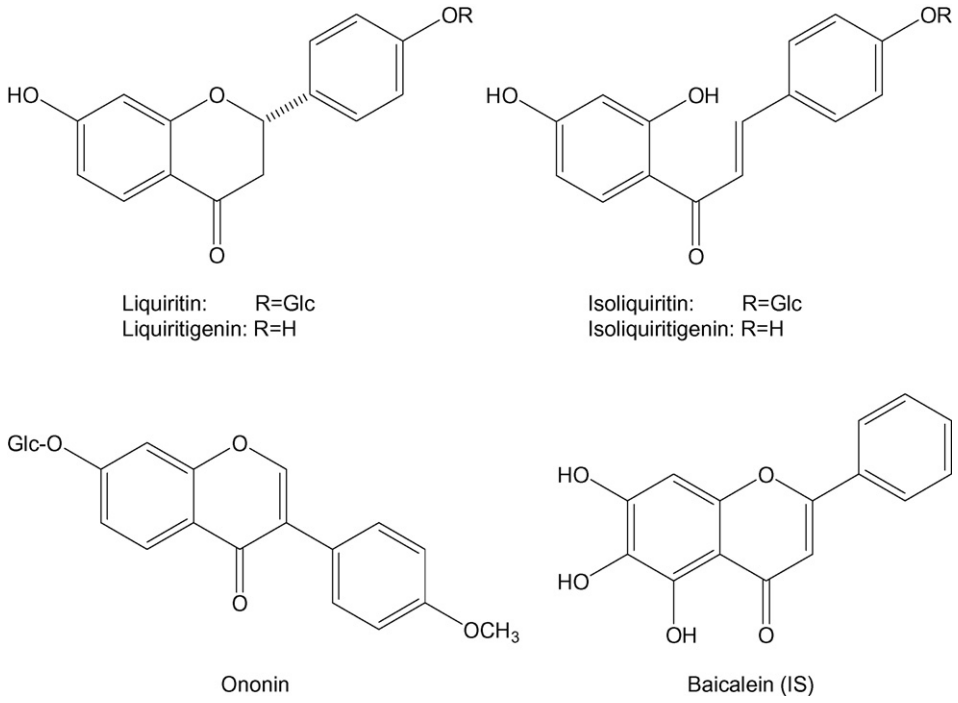

Fig. 1. Chemical structures of 5 investigated flavonoids and baicalein (IS).

pressures, could drastically improve the speed and efficiency of extraction. PLE has been applied to the extraction of glycyrrhizin in licorice using methanol [25], hot water [26] or water with surfactants [27] as solvent. However, these studies focused on glycyrrhizin, which may be unsuitable for flavonoids from licorice, although PLE has also been used for extraction of flavonoids from some other medicinal plants [28–30].

In this study, PLE and CEC coupled with peak suppression technique were developed for the simultaneous determination of five flavonoids, including liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin, in licorice. The validated method was applied to the assay of 25 samples from different species and/or parts of licorice.

2. Experimental

2.1. Chemicals and reagents and materials

Liquiritin was purchased from Mansite Pharmaceutical Co., Ltd. (Chengdu, Sichuan, China). Ononin, isoliquiritin, liquiritigenin and isoliquiritigenin were bought from Winherb Medical S&T Development Co. Ltd. (Shanghai, China). Baicalein was purchased from

Table 1
Summary for the tested samples of licorice.

Species	Sampling parts	Sources	Code
Glycyrrhiza uralensis Fisch.	Underground part	Inner Mongolia, China	GU-1
		Inner Mongolia, China	GU-2
		Inner Mongolia, China	GU-3
		Elion Resources Group Company, Inner Mongolia, China	GU-4
		Elion Resources Group Company, Inner Mongolia, China	GU-5
		Jiuquan, Gansu, China	GU-6
		Longxi, Gansu, China	GU-7
		Longxi, Gansu, China	GU-8
		Jiuquan, Gansu, China	GU-9
		Lanzhou, Gansu, China	GU-10
		Lintiao, Gansu, China	GU-11
		Jiuquan, Gansu, China	GU-12
		Yaxian, Ningxia, China	GU-13
		Ningxia, China	GU-14
		Yinchuan, Ningxia, China	GU-15
		Tongxin, Ningxia, China	GU-16
		Purchased from Heibei, China	GU-17
		Gansu, China	GU-18
	Aerial part	Gansu, China	GU-18A
		Inner Mongolia, China	GU-19A
Glycyrrhiza inflata Bat.	Underground part	Tianshan Pharmaceutical Industry Co. Ltd., Xinjiang, China	GI-1
		Tianshan Pharmaceutical Industry Co. Ltd., Xinjiang, China	GI-2
		Xinjiang, China	GI-3
		Jiuquan, Gansu, China	GI-4
Glycyrrhiza glabra L.	Underground part	Gansu, China	GG-1