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Chiral high-performance liquid chromatographic separation of evodiamine enantiomers and rutaecarpine, isolated from Evodiae fructus

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ABSTRACT

A rapid, simple and sensitive chiral HPLC method was developed and validated for quantification of biologically important alkaloids namely evodiamine enantiomers and rutaecarpine in Evodiae fructus using diphenhydramine as the internal standard (IS). Chromatographic separations were performed on a Chiralpak AD-H® column (250 mm × 4.6 mm i.d., 5 μm) with elution of *n*-hexane–2-propanol–ethanol (70:20:10, v/v/v) in a flow rate of 0.7 ml/min and at λ_{max} 225 nm. To identify the order of elution, small quantities of the each evodiamine enantiomer were isolated by semi preparative HPLC method. Extraction samples were prepared by a simple solid phase extraction (SPE) method. All calibration curves showed good linearity ($r^2 \geq 0.999$) within the test ranges. The LOD and LOQ were lower than 0.05 and 0.1 μg/ml, respectively. The RSDs of intra- and interday for relative peak areas of three analytes to IS were less than 3.2 and 2.5%, respectively, and the recoveries were 98.0–103.7%. The validated method was successfully applied to the quantitative analysis of three constituents in 13 batches of samples collected from market. The results showed that S-(+)-evodiamine was the main component while R-(−)-evodiamine was present in low concentration. This study provides a qualitative and quantitative method for analysis of evodiamine enantiomers and rutaecarpine, and should be extendable to pharmacological and toxicological studies of the individual evodiamine enantiomers.

1. Introduction

Evodiae fructus (Wuzhuyu in China) is the dried, unripe fruit of *Evodia rutaecarpa* (Juss.) Benth. or *E. rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang, and *E. rutaecarpa* (Juss.) Benth. var. *bodinieri* (Dode) Huang belonging to the family Rutaceae. It has been used as one of the traditional Chinese medicines (TCMs) for more than 2000 years and is officially listed in the Chinese Pharmacopeia [1]. Additionally, it also has traditionally been used as folk medicine in Korea for treatment of gastrointestinal disorders, postpartum hemorrhage and amenorrhea [2]. The two major bioactive alkaloids are evodiamine and rutaecarpine (Fig. 1). Modern pharmacological studies have proved their various activities, such as inhibit corticosterone production, anti-inflammation, anti-obesity, cardiotonic, center stimulative, vasodilatatory, antithrombotic and bronchoconstrictive activities [3–6].

Recent studies have shown that rutaecarpine can induce CYP1A1 expression [7], modulate drug metabolizing enzymes [8], and prevent ultraviolet A-induced reactive oxygen species generation [9]. Several studies demonstrated that evodiamine induces tumor cell death through two pathways: apoptosis and necrosis [10], became lead structure of anticancer agents [11]. Further studies demonstrate that evodiamine has anti-tumor potential by inhibiting proliferation, inducing apoptosis and reducing invasion and metastasis of a wide variety of tumor cells, including breast cancer cells [12], prostate cancer cells lines DU145 and PC3 [13], leukemic T-lymphocyte cells [14], melanoma cells [15], cervical cancer cells [16], colon cancer cells [17] and lung cancer cells [18].

Evodiamine posses one chiral center and exists two enantiomers, naturally it presents as S-(+)-form in plant [19]. S-(+)-evodiamine and its R-(−)-form can be obtained according to the chemical synthesis methods, though this alkaloid can be extracted from plants, they are industrially produced by stereoselective total synthesis [20]. Although much literature devoted to study of the pharmacology of evodiamine can be found, pharmacological studies has usually been performed on racemic evodiamine [20,21]. Significant differences between the pharmacology and toxicology of the individual enantiomers and the racemate have not been demonstrated so far. Many studies have indicated that drug enantiomers may have different pharmacodynamic and

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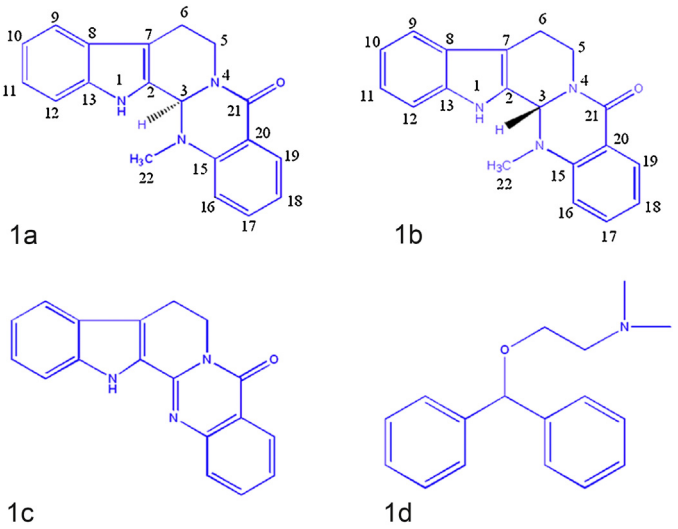

Fig. 1. Chemical structure of (a) R-(−)-evodiamine, (b) S-(+) evodiamine, (c) rutaecarpine, and (d) diphenhydramine (IS).

pharmacokinetic properties due to stereoselective interaction with optically active biological macromolecules [22]. Therefore, their pharmacological study and analysis is necessary.

Several analytical assays have been reported for determination of evodiamine and rutaecarpine which included thin-layer chromatography (TLC) [23], liquid chromatography (LC) [24], liquid chromatography–tandem mass spectrometry (LC/MS/MS) [25], and capillary electrophoresis (CE) [26]. Interestingly, all the literature has focused on the achiral analysis of evodiamine and rutecarpine in herbal extracts and biological samples.

As far as we know, no validated method for direct enantioselective analysis of S-(+)-evodiamine and R-(−)-enantiomers in Evodiae fructus has been published. Therefore, the aim of this paper is to develop a simple, rapid, sensitive, and robust analytical method for chiral separation of evodiamine enantiomers and rutaecarpine isolated from Evodiae fructus using LC with AD-H as a chiral stationary phase (CSP). Solid phase extraction was chosen to extract analytes from extract sample which resulted in good recovery, lack of endogenous interference, and moderate matrix effect. The fast, selective and sensitive chiral LC method with simple pretreatment procedures was successfully applied to assay the content of evodiamine enantiomers and rutaecarpine in commercial samples, and thus provide a basis for pharmacological and toxicological studies of the individual evodiamine enantiomers.

2. Experimental

2.1. Chemicals and solvent

(±)-Evodiamine, rutaecarpine, diphenhydramine and $CDCl_3$ were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade 2-propanol, *n*-hexane, methanol, ethanol were from Duksan Pure Chemicals Co. (Ansan, Korea). The other chemicals were of the best analytical grade available.

2.2. Plant material

Thirteen batches of samples were bought from some different herbal shops in Vietnam and Korea. A voucher specimen was deposited at Kangwon National University. Specimens were stored at desiccator cabinet before use in order to avoid moisture and chemical changes.

2.3. Apparatus

The analytical chromatography was performed on a Thermo Scientific Spectra system equipped with P 1000 pump and UV 2000 detector and 20 μl-injection loop (Waltham, MA, USA). Chromatographic data were acquired and processed by chromquest 5.0 software. The semi preparative HPLC system consisted of a Shimadzu LC-20AD pump (Kyoto, Japan) and a Water Lambda-Max 481 LC detector (Milford, MA, USA) with a Chiralcel OD-H® column (250 mm × 10 mm i.d., 5 μm).

Optical rotation of evodiamine was measured with a Jasco DIP-1000 digital Polarimeter (Tokyo, Japan).

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a Bruker Avance-600 Ultrashield NMR spectrometer (Rheinstetten, Germany) operating at probe temperature of 23 ± 1 °C and was reference to TMS taken as 0.00 ppm on the δ scale. Spectra were recorded at 600 MHz with $CDCl_3$ 99.8 atom% D contains 0.05% (v/v) TMS as an internal standard.

High-resolution and low resolution MS spectrum was acquired on a Jeol JMS-700 M station system (Tokyo, Japan). The scanned m/z range was 230–330 and 50–550, respectively. Mass spectrometer (MS) data were obtained using a quadrupole mass spectrometer equipped with electrospray ionization (ESI) source.

2.4. Preparation of evodiamine enantiomers by semipreparative chromatography

For separation of a racemate, particular attention should be paid to developing a method using a mobile phase in which the racemate is highly soluble. A variety of solvents were therefore investigated, and it was found that evodiamine was very soluble in mixture of acetonitrile and diethylamine (6:4, v/v). A concentration of 2.5 mg/ml was selected for semi preparative resolution of evodiamine using a Chiralcel OD-H® column (250 mm × 10 mm i.d., 5 μm). The mobile phase consisted of a mixture of hexane–2-propanol (85:15, v/v) at a flow rate of 2.0 ml/min. As injection volume 200 μl was used and 225 nm as UV wavelength. Two fractions, corresponding to the R-(−)- and S-(+)-enantiomers, were separately collected which R-(−)-form was eluted first, at 46 min and 55 min for S-(+)-form. The overall yield of fractions was 60% for R-(−)-evodiamine and 62.5% for S-(+)-evodiamine. 48 mg of R-(−)-evodiamine and 50 mg of S-(+)-evodiamine were obtained in this experiment.

2.5. Chromatographic conditions

Chiral chromatography was carried out on polysaccharides chiral columns Chiralcel OJ-H® (250 mm × 4.6 mm i.d., 5 μm), Chiralcel OD-H® (250 mm × 4.6 mm i.d., 5 μm), and Chiralpak AD-H® (250 mm × 4.6 mm i.d., 5 μm). All chromatographic experiments were performed in the isocratic mode. Detection wavelength was 225 nm with a 20 μl loop. The composition of the mobile phase was optimized in accordance with the guidelines suggested by the manufacturer. Initial investigations using this stationary phase focused on a mobile phase consisting of *n*-hexane and a modifier (ethanol or 2-propanol) at various percentage (10–40%). The flow rate was 0.8 ml/min.

The peak of the solvent front was considered to be equal to the dead time (t_0) and was taken from each particular run. It was about 3.5 min for the Chiralcel OJ-H®, 4.2 min for the Chiralcel OD-H®, 3.7 min for the Chiralpak AD-H®, retention times were mean values of two replicate determinations.

2.6. Calculation

The deadtime (t_0) was estimated using the peak resulting from the change in refractive index from the injection solvent on each