Journal of Chromatography A, 1219 (2012) 147–153

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Assay of caffeoylquinic acids in *Baccharis trimera* by reversed-phase liquid chromatography

Ana Lúcia Aboy[a], Miriam Anders Apel[a], Sílvia Debenedetti[b,c], Leandro Francescato[a], María Adelaida Rosella[b], Amélia Teresinha Henriques[a,*]

[a] Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[b] Cátedra de Farmacognosia Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
[c] Facultad de Ciencias Exactas y Naturales, Universidad de Belgrano, Capital Federal, Ciudad Autónoma de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 4 November 2011
Accepted 13 November 2011
Available online 28 November 2011

Keywords:
Baccharis trimera
Caffeoylquinic acids
Dicaffeoylquinic acids
Tricaffeoylquinic acids
RP-LC
Photostability

ABSTRACT

Baccharis trimera commonly named 'carqueja', is wide-spread in South America and are used as raw material for herbal medicines. A reversed-phase liquid chromatography (RP-LC) method coupled to diode array detector was developed for the analysis of caffeoylquinic acids (CQAs), the main compounds responsible for its digestive activity. The identity of the quinic acids was established by mass spectrometry and were them: 5-*O*-[*E*]-caffeoylquinic acid, 3,4-*O*-[*E*]-dicaffeoylquinic acid, 3,5-*O*-[*E*]-dicaffeoylquinic acid, 4,5-*O*-[*E*]-dicaffeoylquinic acid and a tricaffeoylquinic acid. The RP-LC method for the quantitation of the caffeoylquinic acids was validated according to ICH guidelines, based on the following parameters: linearity, selectivity, robustness, limits of detection and quantification, precision and recovery. Hydroalcoholic extracts were prepared by the maceration of the plant material with ethanol:water 1:1 (v/v) in a 0.1:25 g mL^{-1} plant:solvent ratio in a water bath at 40 °C. Validation data indicated that the HPLC method proposed is suitable for the analysis of caffeoylquinic acids in *B. trimera* raw material. The results of the LOD and LOQ analyses for the 5-CQA were 4.1 μg mL^{-1} and 12.5 μg mL^{-1}, respectively, 1.3 μg mL^{-1}, 3.9 μg mL^{-1} for 4,5-diCQA and 1.7 μg mL^{-1}, 5.1 μg mL^{-1} for triCQA. The levels of total CQAs ranged from 2.1 to 4.0 g% (w/w). The influence of season harvest and site collection was also evaluated and variations were observed in the results and can be related to phonologic phase, different locations, seasons and soil. Long term and photostability of plant material were carried out and was observed a stable behavior during the time of the experiments.

1. Introduction

In recent years despite the growth in global trade of herbal products, these are often unable to enter in international markets, due to inconsistency in quality and effectiveness. It is therefore essential the development of analytical methods that enable the standardization of extracts, as well as the stability study of herbal medicines [1]. In this context and considering the great biodiversity in South America, the countries from this region are important centers for marketing of medicinal plants, such as *Baccharis trimera*.

B. trimera (Less.) DC. (Asteraceae) is a shrub widespread in South American countries (Brazil, Argentina, Uruguay and Paraguay) that belongs to the section Caulopterae DC. [2]. This complex genus is characterized by the presence of longitudinal wings. The plant is used in folk medicine for multiple indications, mainly digestive disorders and as a diuretic [3,4]. The drug is widely used as a raw material for herbal medicines and as a food supplement. The main reported constituents for this species are flavonoids, caffeoylquinic acids and terpenoids [5–9]. Caffeoylquinic acids are considered to be the main compounds responsible for the digestive and hepatoprotective activities in some medicinal plants [10,11].

Many factors contribute with the quality of the herbal drugs, such as seasonal variation, collection period, site of collection, post-harvesting processing, procedures of extraction and storage location. Also, herbal pharmaceutical preparations are frequently dispensed as strikethrough raw material and taking in account the lability of the constituents, it is of great concern to monitor the behavior of the chemical constituents against thermal and photostability as well as the phytochemical variation related with the site of collection and seasonal variation.

Considering the wide use of *B. trimera*, appropriate quality control methods need to be developed in order to comply with regulatory requirements. Keeping these points in view, the aim of the current study was the development of a simple, effective and reliable liquid chromatography (LC) method for the quantification

* Corresponding author at: Faculdade de Farmácia, UFRGS, Av. Ipiranga, 2752, CEP 90610-000, Porto Alegre, RS, Brazil. Tel.: +55 5133085417.
E-mail address: amelia@farmacia.ufrgs.br (A.T. Henriques).

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doi:10.1016/j.chroma.2011.11.042

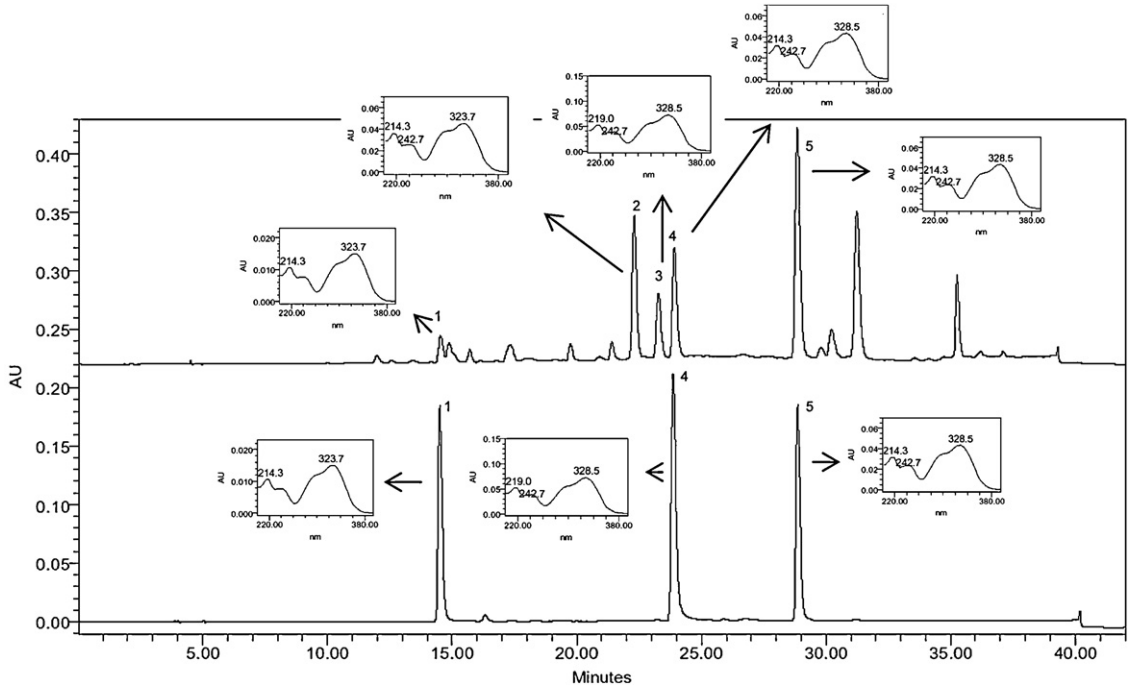

Fig. 1. Typical HPLC chromatogram for *B. trimera* ethanol extract (325 nm). The UV spectra corresponding to the main CQAs are shown above the chromatogram. Peaks: (1) 5-*O*-[*E*]-caffeoylquinic acid; (2) 3,4-dicaffeoylquinic acid; (3) 3,5-dicaffeoylquinic acid; (4) 4,5-dicaffeoylquinic acid; (5) triCQA.

of the CQAs in extracts of *B. trimera*. Optimization of the extraction conditions required to obtain the highest yield of bioactive compounds in the extracts was performed. In addition, the photostability and the influence of the phenological phase of the plant were also evaluated.

2. Experimental

2.1. Plant material

To evaluate seasonal variation, samples of the aerial parts of *B. trimera* were collected in São Jerônimo (RS-Brazil) in different phenological phases, covering the blooming and vegetative phases. A voucher specimen (ICN 128440) was deposited in the Herbarium of the UFRGS. In addition, samples of different origins were analyzed to compare their CQA contents. Samples were collected in Argentina were identified by Dr. Etile Spegazzini.

The plant material was dried at room temperature for 10 days and reduced to a fine powder (0.250 mm), using a knife mill (model A11 Basic S10, IKA® WERKE), immediately before the preparation of the extracts.

2.2. LC equipment and conditions

LC analysis were performed on a Waters Alliance 2695 chromatograph using a UV detector (UV/VIS Waters 2487) and a C18 reversed-phase column (Luna® Phenomenex; 5.0 μm, 250 mm × 4.6 mm) with a security guard column packed with Lichrosorb RP_{18} (10 × 4 mm; Merck), operated at 25 ± 2 °C. A photodiode array detector (DAD; UV/UV Waters 996) was used to check the purity and verify the specificity of the evaluated compounds. UV spectra were recorded in a range 210–400 nm. Equipment control, data acquisition and integration were performed with Waters Empower software.

The mobile phase consisted of a gradient elution of acetonitrile and water acidified with trifluoracetic acid (5:95:0.08, v/v/v) (solvent A, pH 2.1) and acetonitrile (solvent B). The gradient profile was: 0–30 min from 0 to 57% of B, 30–35 min 57–100% of B, 35–38 min 100% of B, 38–42 min from 100% to 0% of B at 0.8 mL min^{-1}. The injected volume was 10 μL and detection wavelength was 325 nm. All solutions were filtered through a 0.45 μm membrane (Millipore) prior to use.

For long term storage and photostability assays, LC analysis were performed in the same Waters HPLC apparatus, using a C18 reversed-phase column (Luna® Phenomenex; 3.5 μm, 75 mm × 4.6 mm) with the same gradient profile, but with a flow of 0.6 mL min^{-1}.

LC–MS analysis. Analyses were carried out using a Agilent (Karlsruhe, Germany) 1200 series HPLC equipped with a G1312B SL binary pump, a G1367D high-performance autosampler (HiP ALS SL+), a G1316B SL thermostated column compartment, and a Phenomenex (Torrance, CA, USA) Luna C18(2) column (250 × 4.6 mm i.d., 5 μm particle). The mobile phase consisted of 0.3% (v/v) formic acid in water (eluent A) and acetonitrile (eluent B). The gradient profile was: 5–45% B (0–30 min), 45–90% B (30–35 min), and 90% B (35–40 min). Flow rate of 0.8 mL min^{-1}, injection volume of 10 μL, and oven at 30 °C.

The mass detector was an Agilent model G6460A Triple Quadrupole fitted with an ESI source. Equipment control, data acquisition and processing were performed using MassHunter Workstation Software. Negative ion mass spectra of the column eluate were recorded in the range *m*/*z* 50–1000. The instrument was operated with the capillary voltage at 3500 V, and charging voltage at 500 V. Nitrogen was used as nebulizer gas of 45 psi, a carrier gas of 6 L min^{-1} at 350 °C, and a sheath gas of 11 L min^{-1} at 350 °C.

Semi-preparative HPLC conditions for triCQA isolation: For the semi-preparative HPLC, it was used a Waters Alliance 2695system equipped with a PrepPak 25 × 100 mm C18 6 μm column and radial compression at 700–800 lb in^{-2}, eluted at 5 ml min^{-1} from 0 to 40 min with a linear gradient solvent system from 40:60:0.1 (vol/vol/vol) methanol:water:trifluoroacetic acid to 80:20:0.1 (vol/vol/vol) methanol:water:trifluoroacetic acid (system A), or from 0 to 40 min with a linear gradient solvent system