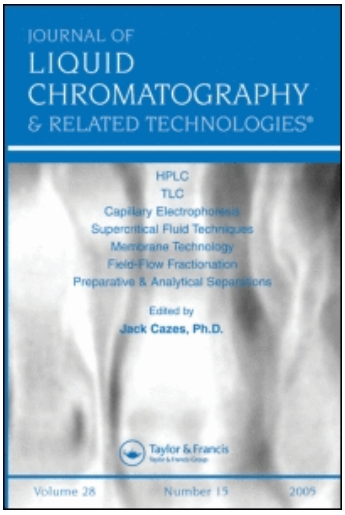

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Bicascarosides in Cascara Fluid Extract by High Pressure Liquid Chromatography

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To cite this Article De Witte, P. , Cuveele, J. and Lemli, J.(1991) 'Determination of Bicascarosides in Cascara Fluid Extract by High Pressure Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 11, 2201 — 2206
To link to this Article: DOI: 10.1080/01483919108049684
URL: http://dx.doi.org/10.1080/01483919108049684

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(11), 2201-2206 (1991)

DETERMINATION OF BICASCAROSIDES IN CASCARA FLUID EXTRACT BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

A quantitative determination of bicascarosides by HPLC in pharmaceutical preparations is described. These compounds are transformed by oxidative hydrolysis into their aglycones which can be separated on a RP-18 column. Concentrations as low as 0.05% can be determined without interference from monomeric aglycones.

INTRODUCTION

Bicascarosides (fig. 1) are dimers of cascarosides which we isolated from aromatic cascara fluid extracts described in the USP XXII AND BP 1988 (1). In the intestinal tract their aglycones (bianthraquinones) are liberated and readily absorbed. These free dimers are tightly bound to serum albumin and we were able

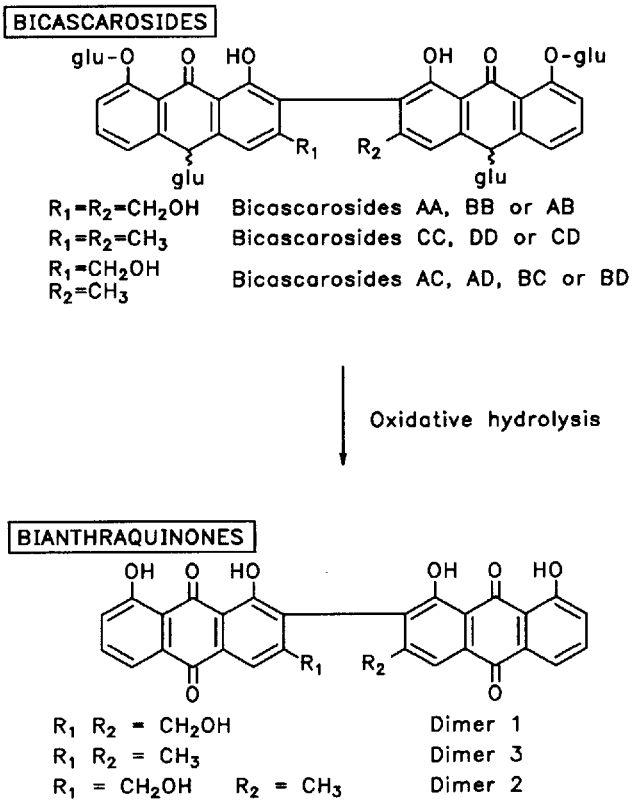

Fig. 1.

to isolate them from the plasma of a human subject (2). Therefore a quantitative estimation of these compounds in pharmaceutical preparations is of importance.

A HPLC method for the determination of the cascarosides by their aglycones (bianthraquinones) obtained after oxidative hydrolysis is presented. HPLC is the method of choice making possible the determination of the three dimers in the presence of monomeric anthraquinones.

MATERIAL AND METHODS

Instrumentation

The HPLC system consisted of a Merck-Hitachi Model L-6200 pump, Model Rheodyne 7125 injector, Model L-4000 UV detector and a Model D-2500 integrator.

The separation was performed on a Lichrocart Lichrospher (5 μm particle size) 100 RP-18 column (125 mm x 4 mm; Merck, Germany). A same precolumn but reduced to 4 mm was routinely used to protect the analytical column.

Chemicals

All chemicals (Merck, Darmstadt) were of analytical grade. Methanol, acetonitrile (Rathburn, England) and water were of HPLC grade.

Sample preparation

To 250 mg of Cascara fluid extract 60 mL water, 36 mL 36% hydrochloric acid and 4 g ferric chloride was added. The mixture was refluxed in a boiling waterbath for 4 hours. After cooling the mixture was extracted 3 times with 50 mL diethylether. The pooled ether extracts were washed twice with 30 mL water and 3 times with 5% sodium hydrogen carbonate and finally with 40 mL water. The ether extract was dried over anhydrous sodium sulphate and evaporated. The residue was dissolved in 5 mL methanol, and passed over a Bond Elut column C18 (Analytichem. Int.) and the anthraquinones were eluted with methanol untill discoloration of the elute. The methanol was evaporated and the residue dissolved in 4 mL acetonitrile. As internal standard 1 mL of a solution of 26.4 mg 1,8-dihydroxyanthraquinone in 100 mL acetonitrile was added, which was filtered through a Dynagard filter (0.45 μm). The solution was evaporated to dryness and the residue dissolved in 4.5 mL methanol and 0.5 mL 0.1% trifluoroacetic acid. An aliquot (15 μL) of this solution was injected.

Chromatography

The column was eluted at room temperature (flow rate 1 mL/min with a gradient of methanol (solvent a), water (solvent b) and acetonitrile (solvent c)). The program consisted of the following data: 0-10 min (69-74% solvent a, 30-25% solvent b, 1% solvent c), 10-15 min (74-83% solvent a, 25-15% solvent b,

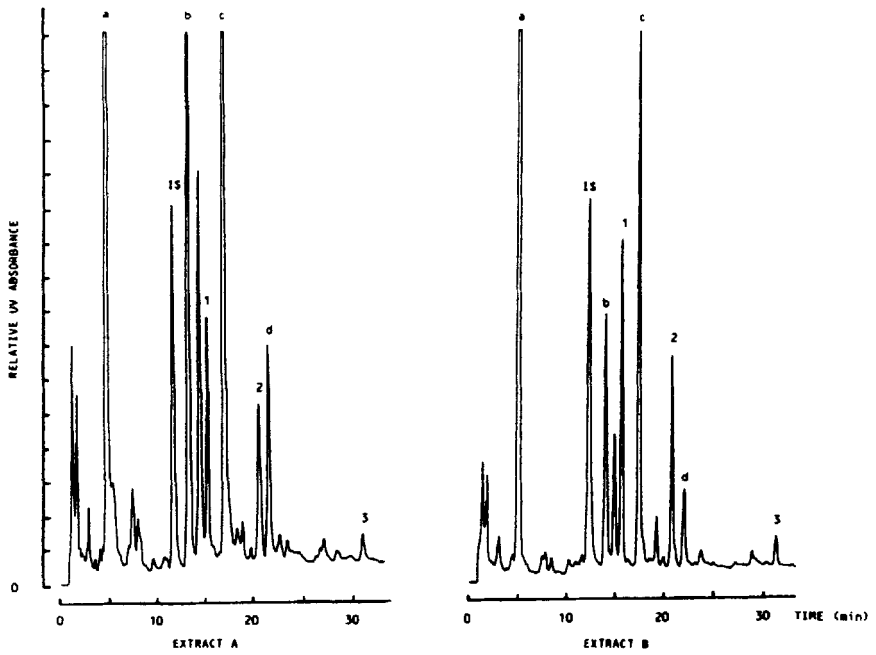

Fig. 2: Representative HPLC chromatograms of extract A with low concentration of dimers and extract B with high concentration of dimers. Monomers: aloe-emodin(a), emodin(b), chrysophanol(c), physcion(d); dimers(1), (2), (3); internal standard: IS.

1-2% solvent c), 15-50 min (83% solvent a, 15% solvent b, 2% solvent c). The anthranoids were detected at 260 nm.

Calibration graph, reproducibility and accuracy

The calibration curve was obtained by plotting the peak area agains concentration (22.7, 45.4 and 68.1 μg dimer 1/mL). Therefore, different amounts of a standard solution were processed together with the internal standard. The calibration curve was also used to calculate the concentration of dimers 2 and 3 in analyses of liquid extracts.

Assay reproducibility was assessed by repeated analyses (n=5) of dimer 1, 2 and 3 in a fluid extract with a low (ca. 50 μg/100 mg) and a high (ca. 170 μg/100 mg) content of dimers.

Table 1: Mean amounts (± RSD) expressed as μg/g fluid extract of dimer 1, 2 and 3 found by repeated analysis (n=5) of ten different extracts.

	Fluid extract A	Fluid extract B
Dimer 1	275 (4.9%)	920 (4%)
Dimer 2	178 (7.3%)	685 (0.9%)
Dimer 3	52 (5.2%)	120 (4.9%)

The accuracy of the method was evaluated by repeated analyses (n=5) of dimer 1 in a mixture of a liquid extract with a known amount of dimer 1 which was spiked with 100 μg of the same component.

RESULTS AND DISCUSSION

The method provides the separation and quantitative determination of three dimers present in cascara fluid extracts. Typical chromatograms are shown (figure 2) for an extract (A) containing a low concentration of dimers (0.5 mg/100 g extract) and an extract (B) with high concentration (1.7 mg/100 g extract). The different peaks were identified by comparison with authentic samples of the different anthranoids.

Using this system it is possible to determine separately the different dimers without any interference from the monomers or other compounds present in the extract.

The mean recovery (n=5) of 100 μg dimer 1, which was used to spike an extract with a known amount of the same component was 94.9%. The relative standard deviation was 1.4%.

The calibration curve of dimer 1 proved to be linear up to 68 μg/mL after injection of 15 μL. The correlation coefficient was 0.999. The mean amounts (± Relative Standard Deviation in %) of dimer 1, 2 and 3, expressed as μg/g fluid extract are shown in Table 1.

The proposed method allows the accurate determination of the dimers in fluid extracts. The results can be expressed in bicascarosides by applying the factor 2.14. Thus extract A contains 1.08 mg and extract B 3.69 mg bicascarosides per gram.

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