FABAD J. Pharm. Sci., 19, 139-142, 1994

RESEARCH ARTICLES / BILIMSEL ARAŞTIRMALAR

High-Performance Liquid Chromatographic Method for the Simultaneous Separation of Emodin-Type Oxidized and Reduced Anthranoids

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Summary : A new high performance liquid chromatographic method is given for the simultaneous separation of oxidized and reduced anthranoids. In the method in this study, analysis time(35 min) is shorter than the other methods. Additionally, labile anthrone glycosides can be detected at the same time with anthraquinone glycosides.

Key words : HPLC, anthrones, anthraquinones, Rhamnus frangula, Rhamnaceae

Geliş tarihi : 25.2.1994
Kabul tarihi : 5.8.1994

Emodin Tipi Okside ve Redükte Antranoidlerin Eşzamanlı Ayrımları için Yüksek Basınçlı Sıvı Kromatografi Yöntemi

Özet: Bu çalışmada okside ve redükte antranoidleri tek bir analizle ayırabilecek yeni bir yüksek basınçlı sıvı kromatografisi metodu verilmiştir. Bu metotta analiz süresi diğer metotlara göre daha kısadır(35 dak) ve buna ilaveten labil antron heterozitlerini antrakinon heterozitleri ile aynı zamanda teşhis etmek mümkün olabilmiştir.

Anahtar kelimeler : YBSK, antron, antrakinon, Rhamnus frangula, Rhamnaceae

Introduction

Anthraquinones are a pharmaceutically important group of plant constituents. Especially in western and central Europe, the bark of *Rhamnus frangula* L. has found a wide application area as a laxative. In 'Gart der Gesundheit' (Mainz 1485) frangula was not described, but it was first mentioned in Bock's herbal (Strasbourg 1539)[1]. The most active principles in the fresh bark of *R. frangula*, which is listed in various pharmacopeias because of its purgative activity[2,3], are glucofrangulin anthrones[1]. Many analytical methods have been reported of the separation of the naturally occuring anthraquinones in plant extracts. The techniques applied are paper chromatography[4,5], thin-layer chromatography[6-9], and column chromatography[10]. Gas-liquid chromatography was used for the determination of aglycones, but no progress has been made for the detection of anthracene glycosides with this technique[11]. A "prism model" was examined for the mixture of anthraquinone aglycones[12]. However, this method has not been applied to plant extracts. For the HPLC separation of the anthraquinone aglycones, such as chrysophanol, physcion, emodin, aloe-emodin and rhein, procedures based on isocratic[13] and gradient elution[14,15] have been described. The quantitative determination of emodin by HPLC, using an isocratic system has been reported previously[16]. An HPLC method for the separation of the anthraquinone glycosides, such as frangulin A, glucofrangulin A, cascaroside A and B was also reported[13,17]. Up to

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date, there is no study for the separation of labile anthrones.

This communication describes a simple and rapid gradient reversed-phase HPLC method for the simultaneous separation and identification of labile anthrone and anthraquinone glycosides in the fresh bark of *Rhamnus frangula*.

Materials and Method

Solvents

All solvents were of technical quality (Merck).

High-performance Liquid Chromatography Apparatus. The equipment consisted of a Waters 600 solvent delivery system (Waters, Milford, MA, USA) and a U6K injector. A Waters 990 photodiode-array detector coupled with a NEC APC IV personal computer (NEC Information Systems, Boxborough, MA, USA) was used for recording UV-VIS spectra(200-500 nm) of the separated compounds and standard compounds as well as for controlling time at 320 nm for anthrones and 420 nm for anthraquinones. Separations were performed on an ET 250/8/4 Nucleosil 7 C_{18} column (Macherey, Nagel, Düren, Germany) at room temperature. The mobile phase consisted of methanol:water. The flow rate was 1 mL/min. Each sample was chromatographed three times. The injection volume was 15 µL and the pressure was 1700-1800 psi.

Gradient elution:

	pump A (40 % methanol)	pump B (80 % methanol)
0 min	60	40
15 min	60	40
30 min	20	80
37 min	60	40

Extraction: Fresh barks of *Rhamnus frangula* was collected from Botanical Garden of Frankfurt/M., identified by Frangula Glycosid Konzentrat (Firma Extract Chemie). 1000,0 mg barks were preventively extracted by Ultra-Turrax with 45 mL methanol under nitrogen and in ice bath. The solution was centrifuged. The supernatant was decanted and evaporated to dryness at room temperature.

Sample Preparation: 30 mg of extract was dissolved in exactly 100 mL of methanol-water(1:1) by automatic shaking for 15 min.

Standard Samples: Glucofrangulin anthron A and B, frangulin anthron A and B, and glucofrangulin A and B were isolated by us[18].

A solution of 10 mg glucofrangulin anthrones A/B (and frangulin anthrones A/B, glucofrangulin A/B, frangulin A/B) in 10 mL of 50 % aqueous methanol was prepared as described in the sample preparation section.

Results and Discussion

A method has previously been described for the HPLC determination of glucofrangulin A and B. According to this method, retention times of glucofrangulin A/B and frangulin A/B were 41, 38 min and 68, 66 min, respectively. On the other hand, the extract was measured at 280 nm[17]; this absorption maximum was not selective for anthraquinone glycosides. There were also many peaks in this absorption maximum other than anthraquinone glycosides. Additionally, 70 min was a very long time for an analysis.

In our study, the mobil phase and flow rate were changed. At the same time, a photodiode-array detector was used, which led to gradient baseline separation of all eight substances for the first time within about 40 min, while each substance could be determined in a single HPLC run(Fig 1); that is to say, this analysis enabled simultaneous separation of both anthrone and anthraquinone glycosides within 35 min. On the other hand, this represents a significant saving of time over previous method[17]. The labile anthrone glycosides were characterized for the first time. The retention times were:

		t_R (min)
Glucofrangulin anthrone B	:	7.57
Glucofrangulin anthrone A	:	8.49
Glucofrangulin B	:	9.07
Glucofrangulin A	:	10.05
Frangulin anthrone B	:	27.15
Frangulin anthrone A	:	30.40
Frangulin B	:	30.60
Frangulin B	:	33.80

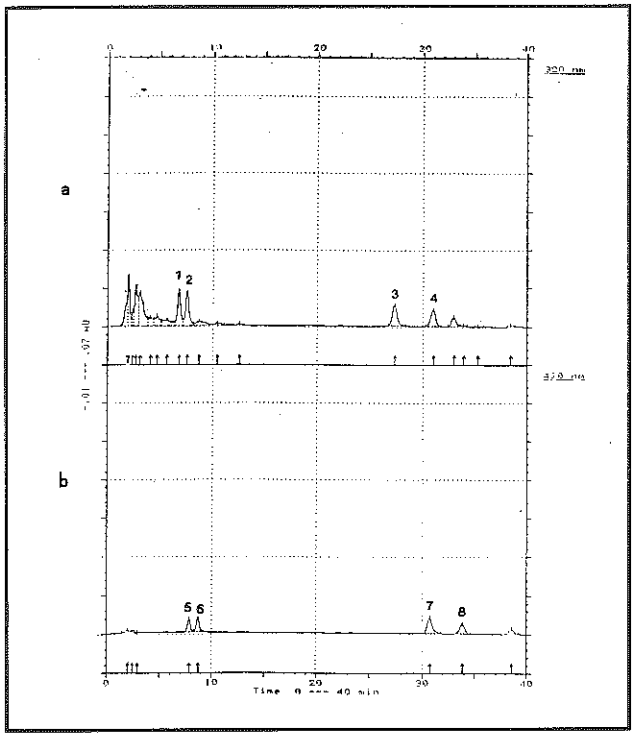

Figure 1. a. HPLC-Chromatogram of fresh bark of Rhamnus frangula at 320 nm.
1. Glucofrangulin anhtrone B, 2. Glucofrangulin anthrone A, 3. Frangulin anthrone B, 4. Frangulin anthrone A.
b. HPLC-Chromatogram of fresh bark of Rhamnus frangula at 420 nm.
5. Glucofrangulin B, 6. Glucofrangulin A., 7. Frangulin B, 8. Frangulin A.

A specific peak identification was achieved by means of the photodiode-array detection(comparison of spectra and control of retention time at 320 nm, 420 nm). In routine analysis, UV-detection for glucofrangulin and frangulin anthrones was at 320 nm, although glucofrangulins and frangulins at 420 nm was sufficient.

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