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Short communication

Determination of 1,8-dihydroxyanthranoids in senna

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Abstract

This paper describes the results of a new method for the determination of 1,8-hydroxyanthranoids in senna. This will be illustrated by examples from a study of the occurrence of 17 different 1,8-dihydroxyanthranoids (anthraquinones and their bianthranyls) in fruits and leaves of *Senna angustifolia* and *Senna acutifolia*.

The anthranoids are extracted using a mixture of acetonitrile and a solution of sodium hydrogencarbonate. The different compounds are separated and detected by HPLC using an RP-8 column and a photodiode array detector. The method was optimized by means of computer-assisted method development techniques using the DryLab software.

Keywords: Hydroxyanthranoids; Anthranoids; Anthraquinones; Bianthranyls

1. Introduction

Preparations of leaves and fruits of *Senna angustifolia* and *Senna acutifolia* are used as purgatives. Typical constituents of these medicinal herbs are sennosides (bianthranyls of several 1,8-dihydroxyanthraquinones), their aglycons, the corresponding anthraquinones and their O-glycosides. The purgative effect is based on these anthranoids.

Several studies showed that aloeemodin and emodin give positive results in in-vitro systems in terms of genotoxicity [1]. For this reason a fast and simple method was developed to determine 17 anthranoids in one HPLC run. There are several methods described in the literature to separate some of the described anthranoids, especially TLC methods using a densitometer [2], different HPLC methods [3–5], as well as comparisons between TLC and HPLC methods [6].

Up to now no analytical method could be found which is able to separate and detect most of the anthranoids in senna in one HPLC run. In Ref. [7] four HPLC methods were used to analyze 15 anthranoids. The aim of this study was to develop a method which can be carried out easily without complex sample preparation and therefore is not time-consuming.

The method development was supported by the DryLab software (Molnar Institute, Berlin, Germany). After running two or three initial linear gradients with the same starting and end point with regard to the content of organic modifier, the experimental measured retention times and peak areas of each peak are used for the simulation procedures of the software. In this way an optimum gradient time, gradient slope and pH value of the eluent could be determined. Moreover, an optimum of separation

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between some critical components was only achieved using a C_8 reversed-phase column and flow-rate programming.

2. Experimental

This method was employed as test on a variety of medicinal herbs and their extracts: senna leaves and senna fruits, rhubarb roots and cascara bark, and the corresponding pharmaceuticals (laxatives).

2.1. Reagents

All reagents used were of analytical grade purity. The extraction solvent was a mixture of acetonitrile (Baker, Cat. No. 9017-54) and a solution of sodium hydrogencarbonate (Merck, Darmstadt, Germany, Cat. No. 6329) in water (60:40, v/v). All standards were either purchased from Carl Roth (Karlsruhe, Germany) or isolated by Madaus (Köln, Germany). The identity and purity of all reference materials are documented by means of UV and IR spectroscopy and in the case of quantitative standards additionally by different NMR experiments.

The standard samples were prepared by dissolving 10 mg of each substance in 100 ml of a mixture of acetonitrile and a solution of sodium hydrogencarbonate (0.5% in water; 10:90, v/v).

The examined anthranoids are:
(1) Anthraquinones; quantitative standards: aloeemodin, emodin, rhein, physcion, chrysophanol; qualitative standard: aloeemodin-8-glucoside, rhein-8-glucoside. (2) Bianthranyls; quantitative standard: sennosid B; qualitative standard: sennosid A, A_1, C, D, D_1, their aglycons sennidin A and B and the monoglycosides sennidin-A-monoglucoside and sennidin-B-monoglucoside.

2.2. Apparatus

A sonification-bath was used for the extraction of the anthranoids. The HPLC equipment consists of two HPLC pumps (W 510 S, Waters), an injection value (Rheodyne 7125 resp. autosampler W 717, Waters), the analytical column (stainless-steel cartridge with Nucleosil 5 C_8, 5 μm, 250×4 mm I.D.) and a photodiode array detector (PDA W 996, Waters).

2.3. Extraction

A homogenous plant sample was powdered and homogenized in a laboratory mill (<0.25 mm). A 500-mg amount of the sample was weighed into a 100-ml volumetric flask (extracts 200 mg). To this sample 80 ml of a mixture of acetonitrile and a solution of sodium hydrogencarbonate (0.2% in water; 60:40, v/v) was added and extracted by sonification for 30 min. After cooling the volume was filled up to 100 ml and thoroughly shaken. The obtained solution was directly used for injection (20 μl) after membrane filtration (0.45 μm).

2.4. HPLC conditions

HPLC was carried out by gradient elution (flow-rate programming; 20°C); eluent A: 0.02 *M* potassium hydrogenphosphate in water, adjusted to pH 2 with phosphoric acid; eluent B: acetonitrile. The following gradient program was used:

Flow-rate (ml/min)	Time (min)	Eluent A (%)	Eluent B (%)
1	0	86	14
1	10	86	14
1	15	80	20
1	25	80	20
0.8	26	79	21
0.8	55	20	80
1	57	86	14
1	70	86	14

The anthraquinones are detected at 435 nm and the bianthranyls at 270 nm. The chromatograms are evaluated by a chromatography workstation (Millennium, Waters).

2.5. Calculations

The content of the anthranoids is calculated by following equation:

$$G = \frac{c}{10 \cdot m} \cdot \frac{A_2}{A_1}$$

where

G = content of the anthranoid (g/100 g)
c = concentration of the anthranoid in the standard (mg/100 ml)
m = weight of the sample (g)
A_1 = peak area of the anthranoid in the reference solution
A_2 = peak area of the anthranoid in the analyte

All bianthranyls are calculated as sennosid B. The anthraquinones aloeemodin, rhein, their 8-glucosides, physcion and chrysophanol as the corresponding anthraquinone aglyka.

3. Results and discussion

Fig. 1 shows a chromatogram overlay of a standard solution and a real sample resp. at 270 nm for the determination of the bianthranyls and Fig. 2 the corresponding chromatogram at a wavelength of 435 nm (determination of anthraquinone).

The chromatograms show a suitable separation. Satisfactory results were obtained by the present procedure.

Table 1 shows the calculated content after the analyses of six lots of senna fruits and leaves using the developed method in g/100 g.

Neither chrysophanol, nor physcion could be detected in the examined plant material. The sum of anthranoids corresponds to the content of anthranoids determined by the German Pharmacopoeia method. This was confirmed by the analysis of almost 100 lots of senna fruits leaves, extracts, tablets etc. Furthermore, some lots of senna fruits were examined, which were analyzed by W. Grimminger (see Ref. [7]) using his published methods. Almost the same results were found.

The method was validated corresponding to the analytical validation guideline of the European Community and the Tripartite ICH Text on Validation of Analytical Procedures by the CPMP working party on Quality of Medicinal Products [8]. This document is concerned with the discussion of the characteris-

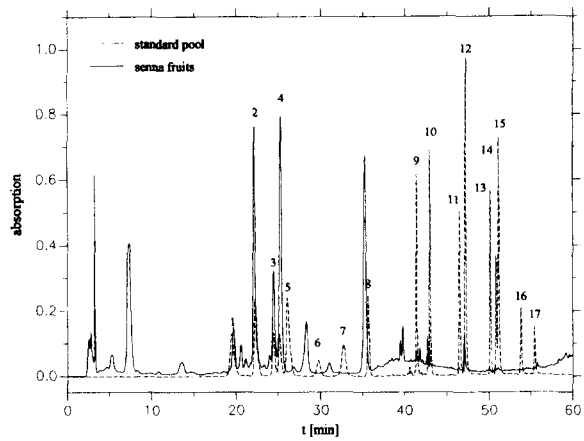


Fig. 1. (– – –) HPLC chromatogram of a standard pool containing 17 different anthranoids at a wavelength of 270 nm for the determination of the bianthranyls. 1 = aloeemodin-8-glucoside; 2 = rhein-8-glucoside; 3 = sennosid D_1; 4 = sennosid D; 5 = sennosid B; 6 = sennosid A_1; 7 = sennosid C; 8 = sennosid A; 9 = sennidin-B-monoglucoside; 10 = sennidin-A-monoglucoside; 11 = aloeemodin; 12 = rhein; 13 = sennidin A; 14 = sennidin B; 15 = emodin; 16 = chrysophanol; 17 = physcion. (———) HPLC elution profile of an extract of senna fruits at a wavelength of 270 nm for the determination of the bianthranyls.

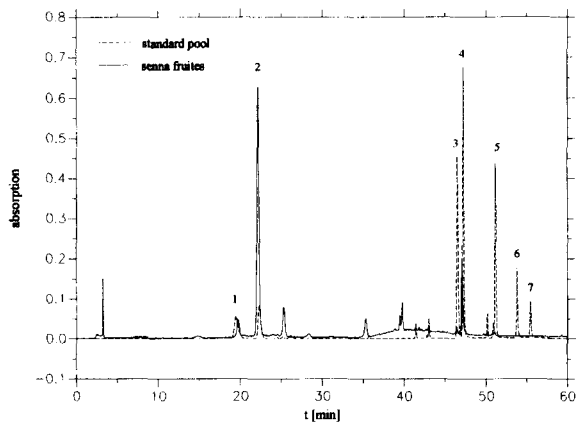


Fig. 2. (– – –) HPLC chromatogram of a standard pool containing 17 different anthranoids at a wavelength of 435 nm for the determination of the anthraquinones. 1 = aloeemodin 8-glucoside; 2 = rhein-8-glucoside; 3 = aloeemodin; 4 = rhein; 5 = emodin; 6 = chrysophanol; 7 = physcion. (———) HPLC chromatogram of an extract of senna fruits at a wavelength of 435 nm for the determination of the anthraquinones.

Table 1
Content of anthranoids in senna fruits and leaves

Anthranoid	Senna fruits, alex. %	Senna leaves, alex. %
Sennosid A	1.56 –1.99	0.65 –0.81
Sennosid A_1	0.45 –0.58	0.23 –0.27
Sennosid B	1.56 –2.14	0.85 –1.13
Sennosid C	0.11 –0.19	0.11 –0.18
Sennosid D	0.11 –0.16	0.13 –0.20
Sennosid D_1	0.19 –0.27	0.16 –0.22
Sennidin A/B	0.004–0.01	0.002–0.009
Aloeemodin	0.02 –0.15	0.01 –0.02
Aloeemodin-8-glucoside	n.d. –0.04	0.03 –0.06
Rhein	0.13 –0.54	0.03 –0.06
Rhein-8-glucoside	0.02 –0.28	0.08 –0.12
Emodin	0.003–0.007	0.001–0.002
Sennidin-A-glucoside	0.03 –0.05	0.01 –0.01
Sennidin-B-glucoside	0.004–0.01	0.002–0.007
Σ anthranoids	4.95 –5.83	2.40 –2.91

tics that must be considered during the validation of analytical procedures included as part of registration applications submitted within the EC, Japan and USA. Typical validation characteristics for assay tests are specificity, precision (repeatability), accuracy, linearity and, because of the genotoxicity risks, the detection limit of the method. The validation was carried out only for the quantitative anthranoids (sennosid B and the anthraquinone aglyka).

Table 2
Precision of the determination of anthranoids in senna fruits ($n=6$)

Anthranoid	S.D. (%)	R.S.D. (%)
Sennosid A	0.0078	0.6
Sennosid A_1	0.0039	1.0
Sennosid B	0.016	1.0
Sennosid C	0.0036	3.6
Sennosid D	0.0029	2.7
Sennosid D_1	0.0011	0.9
Sennidin A/B	0.00028	2.0
Aloeemodin	0.00025	1.8
Aloeemodin-8-glucoside	0.00090	1.9
Rhein	0.00022	0.4
Rhein-8-glucoside	0.0026	1.0
Emodin	0.00032	9.9
Sennidin-A-glucoside	0.00045	1.3
Sennidin-B-glucoside	0.00052	9.8
Chrysophanole	0.94	1.0
Physcion	4.1	4.9

3.1. Specificity

The specificity of the method is given by comparison of the retention times of the anthranoids in the standard pool and the samples. In addition to that the UV–Vis spectra of the corresponding peaks in the reference pool and the sample are measured by using a PDA detector. In this way the identity and peak purity of each compound was confirmed. The Millennium PDA software uses the Spectral Contrast technique to compare spectra. The technique converts spectra to vectors for comparison and accounts for noise and solvent effects during comparison. In this way the identity of a peak is confirmed by comparison of the peak apex spectrum with the spectrum of the standard (library). For peak purity the peak apex spectrum is compared against all the spectra from within the peak (baseline and solvent

Table 3
Recoveries of the different quantitative anthranoids in senna fruits ($n=6$)

Anthranoid	Recovery (%)
Sennosid B	99±5
Aloeemodin	108±1
Rhein	102±1
Emodin	100±0.4
Chrysophanole	95±1
Physcion	82±4

Table 4
Linearity, limit of detection, range

Anthraquinone	Regression equation	Correlation coefficient	Range (mg/100 ml)
Sennosid B	$y = 0.031 + 0.9996x$	1.000	0.006–18.0
Aloeemodin	$y = -0.0636 + 1.002x$	0.999	0.010– 9.88
Rhein	$y = 0.044 + 0.9989x$	1.000	0.010–13.3
Physcion	$y = -0.072 + 1.15x$	0.994	0.003– 4.80
Chrysophanol	$y = -0.027 + 1.003x$	1.000	0.003– 4.20
Emodin	$y = 0.031 + 0.999x$	1.000	0.010–10.2

corrected). Dependent on the width of a peak, the retention time window can be different.

3.2. Precision

The precision (repeatability) was determined by a multiple analysis of a sample of senna fruits. The precision for each compound is expressed by the standard deviation (S.D.) and the relative standard deviation (R.S.D.) ($n=6$; $P=95\%$; Table 2).

As the anthraquinone aglyka physcion and chrysophanole could not be found in the examined lots of senna fruits, the precision of the method for these compounds was determined by adding a defined amount of physcion and chrysophanole to the sample.

The high coefficients of variation for emodin and sennidin-B-glucoside can be explained by the low concentration of these compounds in the samples examined (see Table 1).

3.3. Accuracy

The accuracy of the method was examined by the determination of the recoveries of the different anthranoids (Table 3). For this purpose a standard pool with defined concentrations is added to a sample of senna fruits and analysed by the developed method ($n=6$). By comparison of the added and analysed amounts of anthranoids the recoveries could be calculated. The accuracy of the method was only determined for the quantitative anthranoids, because they are used as quantitative reference standards for the calculation of the anthranoid content. On the other hand too little of the qualitative standards were available for additional experiments.

The low recoveries for chrysophanole and physcion are not really important, because they could not be detected in the examined samples at all. But they both have to be taken into account because of legal requirements [9].

3.4. Linearity, limit of detection

The linearity of the measurements was checked for the anthranoids used as quantitative standards in a range from the limit of detection up to the specified concentration. The calibration graphs can be described as shown in Table 4.

4. Conclusions

This study describes a HPLC method which allows for the first time a simultaneous determination of 17 bianthranyls and anthraquinones in senna drugs. The analytical procedure offers a lot of advantages compared with other published methods [2–7]: easy sample preparation, complete extraction of all compounds, which is confirmed by the good recoveries (Table 3), high reproducibility (Table 2) and a short operating time.

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