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Short communication

Selective determination of aloin in different matrices by HPTLC densitometry in fluorescence mode

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ABSTRACT

A novel method based on the fluorescence excited solely on aloin by a H_3BO_3 derivatizing procedure, allowed its rapid and selective determination among the co-occurring components in a variety of complex matrices as several Aloes dried extracts and related commercial products.

HPTLC LiChrospher silica gel 60 F254S, 20 cm × 10 cm, plates with ethyl formate: CH_3OH:H_2O (100:14.5:10, v/v) as the mobile phase were used. Densitometric determinations were performed in fluorescence mode, exciting wavelength 365 nm, optical filter K540 after derivatization with H_3BO_3.

The method was validated giving rise to a dependable and high throughput procedure well suited to routine application. Aloin was quantified in the range of 110–330 ng with RSD of repeatability and intermediate precision not exceeding 2.3% and accuracy inside the acceptance limits.

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1. Introduction

Aloe is a plant genus of African origin consisting of a large number of species.

The good reputation of Aloes leaves as capable of a great deal of therapeutic activities dates back thousands of years. However, only some *Aloe* species i.e. *Aloe barbadensis* Mill. (*A. Vera* Linn.), *Aloe ferox* Mill. and its hybrids, are today of commercial interest since from their leaves dried exudates the so called "*Aloe* drug" can be obtained. These products, also employed as bittering agents in beverages, are best known for their use as components of pharmaceutical preparations with cathartic effects.

Of all the classes of compounds occurring in "*Aloe* drug", the anthrones aloin A and aloin B are by far the most important. In fact, aloin A and aloin B (collectively reported as aloin or barbaloin Fig. 1), two diastereomeric C-glucoside of the aloe-emodin aglicone differing in the configuration at C-10 aglicone moiety, are mainly responsible of the above-mentioned purgative properties. In this view, the effective value of "*Aloe* drugs", intended as ingredients of laxative pharmaceutical preparations, might be preeminently ascribed to their true content of aloin.

Up to date, a variety of analytical methods devoted to characterize Aloes dried extracts mainly based on HPLC and TLC, are reported in the literature [1–10].

In order to provide a quality control procedure for "*Aloe* drugs" and their pharmaceutical preparations relaying on a compelling quantitative approach, alternative and competitive with HPLC in term of rapidity of execution, high throughput and routine amenability, we adopted here a HPTLC-densitometric procedure in fluorescence mode, to develop and validate a novel method aimed to the selective determination of aloin in "*Aloe* drugs" and related preparations.

2. Experimental

2.1. Materials

Aloin standard was obtained from Carl Roth GmbH (Karlsruhe, Germany).

Commercial samples of *Aloe barbadensis*, *Aloe ferox* (A) and *Aloe ferox* (B) dried extracts were purchased locally.

The following pharmaceutical and health products containing aloin were analyzed: Boldina Hé (Teofarma Srl, Pavia, Italy), Sollievo tablets (Aboca SpA, Sansepolcro, Italy) and Le Dieci Erbe tablets (ESI SpA, Albissola Marina, Italy)

All solvents and chemicals were of analytical grade. Water was purified by a Milli-Q-system (Millipore Corporation, Bedford, MA, USA).

2.2. Instrumentation

A Camag (Camag, Muttenz, Switzerland) HPTLC instrumental set-up consisting of sample applicator Linomat 5, TLC Scanner 3 and DigiStore 2 Documentation System was used for the analyses under the control of the software platform winCats 1.4.2 Planar Chromatography Manager (Camag).

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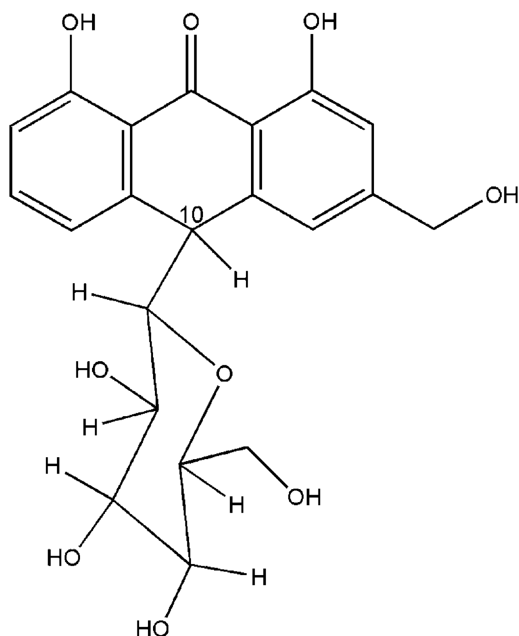

Fig. 1. Chemical structure of aloin.

HPTLC LiChrospher silica gel 60 F_{254S}, 20 cm × 10 cm, (Merck, Darmstadt, Germany) were employed and developed in a Camag Automatic Developing Chamber ADC2 with the humidity control option. Optimization of the separation conditions was carried out using a Camag HPTLC Vario System. Post-chromatographic derivatizations were performed with a Chromatogram Immersion Device III (Camag).

2.3. Chromatographic procedure

The plates were pre-washed by dipping in CH_3OH over night, dried in N_2 stream under vacuum and stored in a clean environment. Standard and sample solutions were applied to the plates bandwise (bandlength 7 mm, 100 nL/s delivery speed, track distance 9.6 mm, distance from the edge 18 mm). Plates were developed at room temperature, 37% relative humidity, in unsaturated mode with ethyl formate: CH_3OH:H_2O (100:14.5:10, v/v) as the mobile phase. After development, the plates were air-dried and scanned for qualitative documentation. For quantitative purpose the plates were subsequently derivatized by rapid immersion (1 s, speed 50 mm/s) into a solution of H_3BO_3, 10% (w/v) in CH_3OH and air-dried at room temperature for 5 min. In order to achieve the detection the plates were heated at 100 °C for 10 min.

The visual inspection and documentation of chromatograms were carried out at 254 and 365 nm. UV spectra were obtained in situ by the TLC Scanner 3. Densitometric determinations were performed in reflectance mode at 360 nm, D2 and W lamp and in fluorescence mode, exciting wavelength 365 nm, Hg lamp, optical filter K540, slit dimension 4.00 mm × 0.30 mm, scanning speed 10 nm/s, data resolution 50 μm/step. Evaluation was by peak area measurement. Curve fitting was carried out with CurveExpert 1.3 software and for the statistical analysis Excel 2003 (Microsoft Office) was used.

2.4. Sample preparation

The dried extracts of *Aloe ferox* (A) (11.06 mg), *Aloe ferox* (B) (12.50 mg) and *Aloe barbadensis* (3.00 mg) were suspended in 10 mL of CH_3OH, sonicated in an ultrasonic bath for 5 min at 20 °C and used without further manipulation.

Ten tablets of each of the following three pharmaceutical products were finely ground and the amounts indicated were suspended in 10 mL of CH_3OH and sonicated in an ultrasonic bath for 5 min at 20 °C: Sollievo (20.06 mg), Boldina Hè (18.75 mg) and Le Dieci Erbe (25.03 mg). The resulting suspensions were used as obtained.

All samples were stored under N_2 in dark vials at 4 °C and used within a week.

For the assay 3 μL of these solutions were applied.

2.5. Calibration standards

Aloin standard solution (0.11 mg/mL) was prepared by dissolving 11.0 mg of aloin in 100 mL of CH_3OH.

For the validation step three calibration CH_3OH solutions (73.3, 91.6 and 110 ng/μL) were obtained from the standard solution using the Serial Dilution option of the PerkinElmer HPLC Autosampler Series 200 (PerkinElmer, Walthman, MA, USA). For the calibration curves 3 μL of these solutions were applied.

Three validation standard solutions were prepared individually spiking 100 mL of a diluted 1:5 *Aloe ferox* (B) CH_3OH sample solution with 7.3, 8.6 and 9.2 mg of aloin.

Three microliters of these solution were applied onto the plates obtaining an aloin spiking of 219, 258 and 276 ng.

All standards were stored under N_2 in dark vials at 4 °C and used within a week.

2.6. Standard addition method

Eight depositions of 3 μL of the diluted *Aloe ferox* (B) solution were applied onto a plate. Five of these depositions were spiked with 1.0, 1.5, 2.0, 3.0, 5.0 μL of the standard solution and used to obtain the calibration line.

3. Results and discussion

3.1. Method optimization

Since it is very difficult to distinguish between aloin A and B by TLC, these epimers were collectively determined as aloin and no further effort was addressed to their chromatographic separation. Several eluent systems and chromatographic conditions, comprising those reported in the literature [9,10], were tried in order to separate aloin from the co-occurring anthrones and chromones. After thoroughly testing the most satisfying resolution was obtained with ethyl formate: CH_3OH:H_2O (100:14.5:10, v/v) as the mobile phase, in unsaturated mode with 37% controlled humidity (Fig. 2a). However, the densitogram scanned at aloin λ_{max} 360 nm evidenced a partial overlapping with the compound tentatively identified as aloeresin A (Fig. 2b).

Consequently, aloin selective detection experiments were carried out on the samples considered after chromatographic separation. Derivatization with H_3BO_3 in CH_3OH as reagent, followed by oven heating at 100 °C afforded the best results inducing a selective fluorescence of aloin without interferences from the co-occurring components (Fig. 3).

The desired selectivity of the method was so achieved. The stability of the derivatized zones was checked by scanning the layer every 2 h until 12 h. Reproducible results could be easy obtained maintaining the correct instrument setting up described in the experimental section.

3.2. Validation

The validation was carried out following the previously reported strategy based on the accuracy profiles [11].

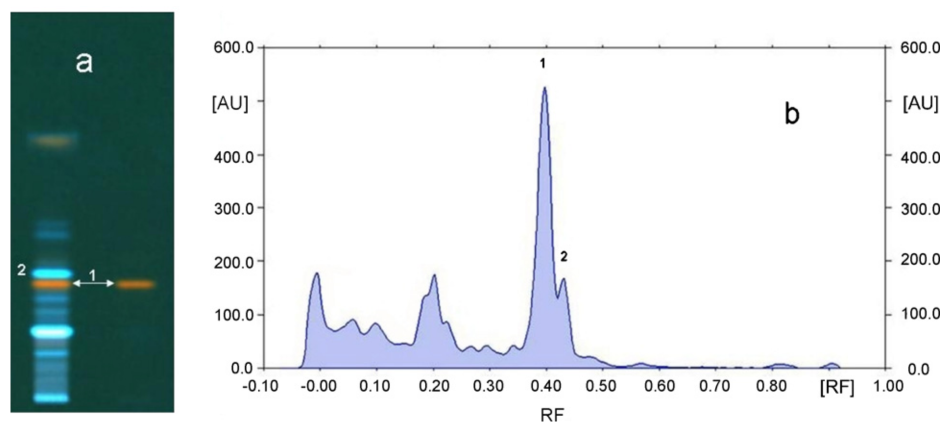

Fig. 2. Videodensitogram (a) and densitogram at 360 nm (b) of *Aloe ferox* A showing the partial overlapping of aloin (1) and aloeresin A (2).

The pre-validation step was built to identify the model to use for the calibration curves and evaluate the precision and bias of the method before designing the 'validation' phase.

Five calibration levels were obtained in quadruplicate and repeated on three different days over a range of 110–330 ng of the analyte, by applying 1.0, 1.5, 2.0, 2.5, 3.0 μL of the standard solution.

Linear model, weighed linear (1/*x*) model, quadratic model and weighed (1/*x*) quadratic model were the regression functions taken into consideration and used to back-calculate the mean bias, the repeatability and the intermediate precision for each level. The accuracy profiles (Fig. 4) obtained by using the confidence intervals allowed to select the weighed linear (1/*x*) model as the best choice, giving the smallest bias within the acceptance limit previously fixed at 15% according to the FDA guidelines concerning the complex matrices [12].

The lowest level was elected as the limit of an accurate quantitation (LOQ).

In the pre-validation step, a possible matrix effect was taken into consideration. No blank matrix being available, the method of standard addition was used. The intercept and the slope of the standard addition curve, calculated with the weighed linear (1/*x*) model ($y = 54.54x + 2513.3$) are in agreement with the calibration curve ones ($y = 48.57x + 2855.2$) obtained for the pure analyte in absence of matrix, assessing the validity of the calibration model.

As it is permitted in HPTLC, three points calibration curves were used for routine analysis. As a consequence, the amount levels 219.9, 274.8 and 330 ng were selected for the validation curves. Accordingly, three calibration points without the matrix were obtained in triplicate on three different days. As stated in the pre-validation experiments a weighed linear (1/*x*) model was used. The results for the response functions are shown in Table 1.

Table 1
Calibration standard response functions ($n = 9$).

	Day 1	Day2	Day 3
Intercept	3569.31	1388.66	−3148.79
Slope	44.21	43.08	58.85
R^2	0.9904	0.9906	0.9923

The validation standards for accuracy purpose were obtained spiking, among our samples, the less aloin containing one, i.e. *Aloe ferox* (B) dried extract. The aloin amounts suitable to obtain values inside the above-mentioned calibration range were added.

The accuracy values were obtained in triplicate on three different days at three amount levels 219, 258 and 276 ng. The bias, largely inside the acceptance limits (±15%) and the relative standard deviation of repeatability and intermediate precision between 0.8–1.9% and 1.4–2.3%, respectively, (Table 2) proved that the method fulfilled the requirements.

3.3. Assay

The method was applied to determine the aloin content in the six above-mentioned commercial samples. The results are reported in Table 3.

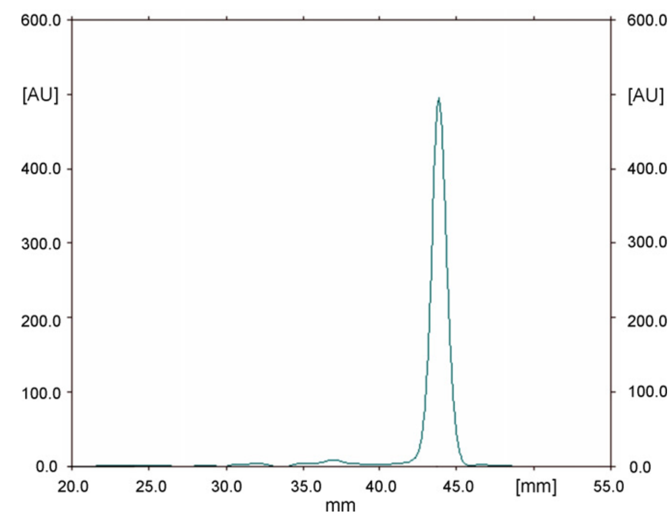

Fig. 3. Densitogram in fluorescence mode of *Aloe ferox* A after H_3BO_3 derivatization.

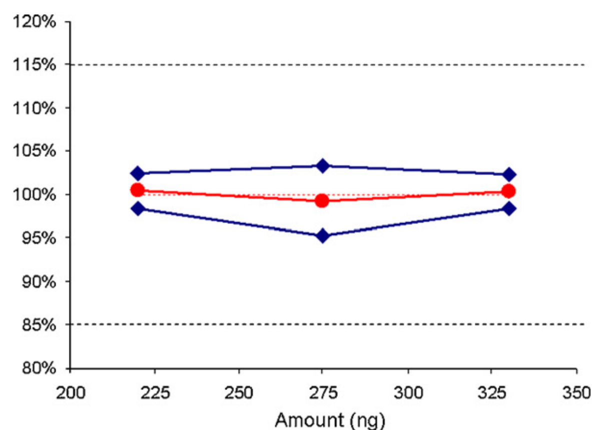

Fig. 4. Accuracy profiles of aloin obtained with weighed (1/*x*) linear model ♦ confidence intervals, ● relative trueness.

Table 2
Accuracy results.

Trueness ($n = 9$)				
Added amount (ng)	Mean result (ng)	Absolute bias (ng)	Relative bias (%)	Recovery (%)
219	226.6	7.6	3.5	103.5
258	254.1	−3.9	−1.5	98.5
276	278.2	2.2	0.8	100.8

Precision ($n = 9$)		
Added amount (ng)	Repeatability (RSD %)	Intermediate precision (RSD %)
219	1.9	2.3
258	0.8	2.5
276	0.8	2.4

Table 3
Assay for Aloin in real samples.

Sample	Found μg[a] mean ($n = 9$)	RSD (%)
Aloe barbadensis	272.73	6.1
Aloe ferox (A)	83.04	5.9
Aloe ferox (B)	68.58	1.5
Boldina Hè®	52.77	3.1
Le 10 Erbe®	30.60	1.7
Sollievo®	50.28	4.6

[a] Referred to 1 mg of sample.

4. Conclusion

The positive circumstance that, in the operative conditions adopted, the aloin enclosed in a variety of complex matrices flouresces almost exclusively among the co-occurring compounds, led to a highly selective analytical method intended for rapid and dependable determination of aloin itself. The procedure, well suited to routine analysis, was competitive with the more popular HPLC approach in terms of rapidity, simplicity of execution and throughput, allowing *inter alia* to spare any time-consuming sample preparation step.

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