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Journal of Chromatography B, 754 (2001) 121–126

JOURNAL OF CHROMATOGRAPHY B

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Determination of aloesin in rat plasma using a column-switching high-performance liquid chromatographic assay

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Received 3 July 2000; received in revised form 23 October 2000; accepted 9 November 2000

Abstract

A column-switching high-performance liquid chromatography (HPLC) method for the determination of aloesin in rat plasma using column-switching and ultraviolet (UV) absorbance detection is described. Plasma was directly injected onto the HPLC system consisting of a clean-up column, a concentrating column, and an analytical column, which were connected with a six-port switching valve. The determination of aloesin was accurate and repeatable, with a limit of quantitation of 10 ng/ml in plasma. The standard calibration curve for aloesin was linear ($r=0.998$) over the concentration range of 10–1000 ng/ml in rat plasma. The intra- and inter-day assay variabilities of aloesin ranged from 1.0 to 4.7% and 1.1 to 8.8%, respectively. This highly sensitive and simple method has been successfully applied to a pharmacokinetic study after oral administration of aloesin to rats.

Keywords: Aloesin

1. Introduction

The term aloe refers to the dried powder extracted from the leaves of several species of *Liliaceae* such as *Aloe ferox, Aloe africana*, and *Aloe spicata*. Aloe has been widely used in health foods, pharmaceuticals and cosmetics because of its aromatic properties and pharmacological activities [1–6]. The main aloe components are aloein, aloesin, aloeresin A, hydroxyaloin, aloe-emodin, and aloenoside A and B. Among them, special attention has been paid to aloesin because this compound possesses various biological activities such as wound healing, antigastric ulcer, and chemopreventive activities [7–9]. Recently, aloesin was reported to protect the kidney against cisplatin-induced toxicity [10].

Our previous studies on the pharmacokinetics of aloesin in rats showed relatively low bioavailability when this compound was orally administered. Therefore, a sensitive and simple analytical method for the determination of aloesin in biological fluids was required to study the metabolism and disposition of the compound. An analytical method for the quantitation of aloesin by using fluoresence detection has been reported. Aloesin was derivatized with 9-anthroylnitrile but this method needed a complicated

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0378-4347/01/$ – see front matter
PII: S0378-4347(00)00593-4

separation step [10]. Another HPLC method reported previously using deproteinization was not applicable in our case due to the low detection sensitivity [11].

In this paper, we report the development of a column-switching HPLC method for the detection of low levels of aloesin in rat plasma using UV detection without extraction step of aloesin from plasma. This method was consequently very simple and highly sensitive, and was successfully applied for a pharmacokinetic assay in blood.

2. Experimental

2.1. Chemicals and material

Aloesin, 8-C-β-D-glucopyranosyl-7-hydroxy-5-methyl-2-(2-oxopropyl)-4H-1-benzo-pyran-4-one (Fig. 1) isolated from the leaves of *Aloe barbadensis* was kindly supplied from Namyang Aloe (Jincheon, Korea). Acetonitrile (HPLC grade) was obtained from J.T. Baker (Mallinckrodt-Baker, Phillipsburg, NJ, USA). Sodium phosphate was purchased from Junsei chemical (Tokyo, Japan). Water was deionized and filtered through a Milli-Q water system (Millipore, Bedford, MA, USA).

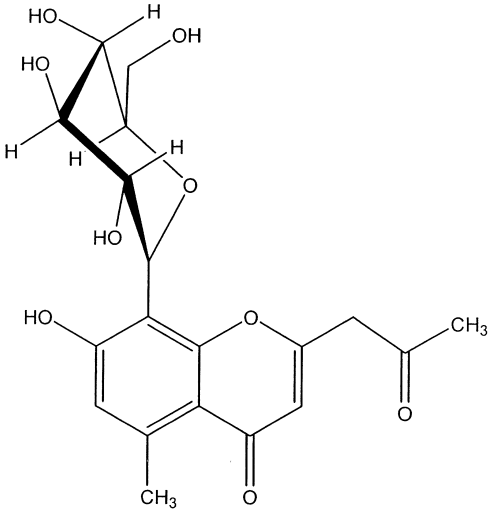

Fig. 1. Chemical structure of aloesin.

2.2. Instrumentation

The HPLC system consisted of a Shisheido HPLC system (NANOSPACE, Shisheido, Tokyo, Japan) with a SI-1/2001 pump, an automatic six-port switching valve (SI-2012), an autosampler (SI-1/2003) coupled to the degasser (SI-1/2009) and a UV–Vis detector (SI-1/2002). The columns used in this experiment were a Capcell Pak MF Ph-1 (150×4.6 mm, 5 μm, Shisheido) as column A for clean-up, a Capcell Pak C_{18} UG120 (35×2.0 mm, 5 μm, Shisheido) as column B for the concentration of the analyte, and a Capcell Pak C_{18} UG120 (250×1.5 mm, 5 μm, Shisheido) as column C for the analysis. The clean-up column was changed every 200 injections. Column temperature was maintained constant at 40°C using a thermostatically controlled column oven (SI-1/2004) except the concentration column. Quantitative evaluation of chromatograms was performed at 253 nm using a chromatogram integration software SM-C (Shisheido). Mobile phases used were 2% acetonitrile in 20 m*M* sodium phosphate buffer (pH 6.86) (buffer A) and 9% acetonitrile in 20 m*M* sodium phosphate buffer (pH 6.86) (buffer B). Plasma samples were filtered through a PVDF syringe filter (13 mm, 0.2 μm, Millipore, Bedford, MA, USA) and kept in the autosampler at 10°C until injected.

2.3. Standard solutions and spiked samples

Aloesin was dissolved in 50% acetonitrile (1.0 mg/ml) as a stock solution, then stored at −20°C. It was diluted with 20% acetonitrile for further concentration levels. Spiked plasma samples were prepared by addition of the stock solution to blank rat plasma giving final concentrations of aloesin of 10, 25, 50, 100, 250, 500 and 1000 ng/ml.

2.4. Chromatographic conditions

A schematic diagram of the HPLC system is shown in Fig. 2. Sample clean-up and chromatographic separation were performed as follows: Filtered plasma samples (40 μl) were injected onto the clean-up column. At the time of sample injection, the column-switching valve was placed in position A. Proteins and other interfering compounds were

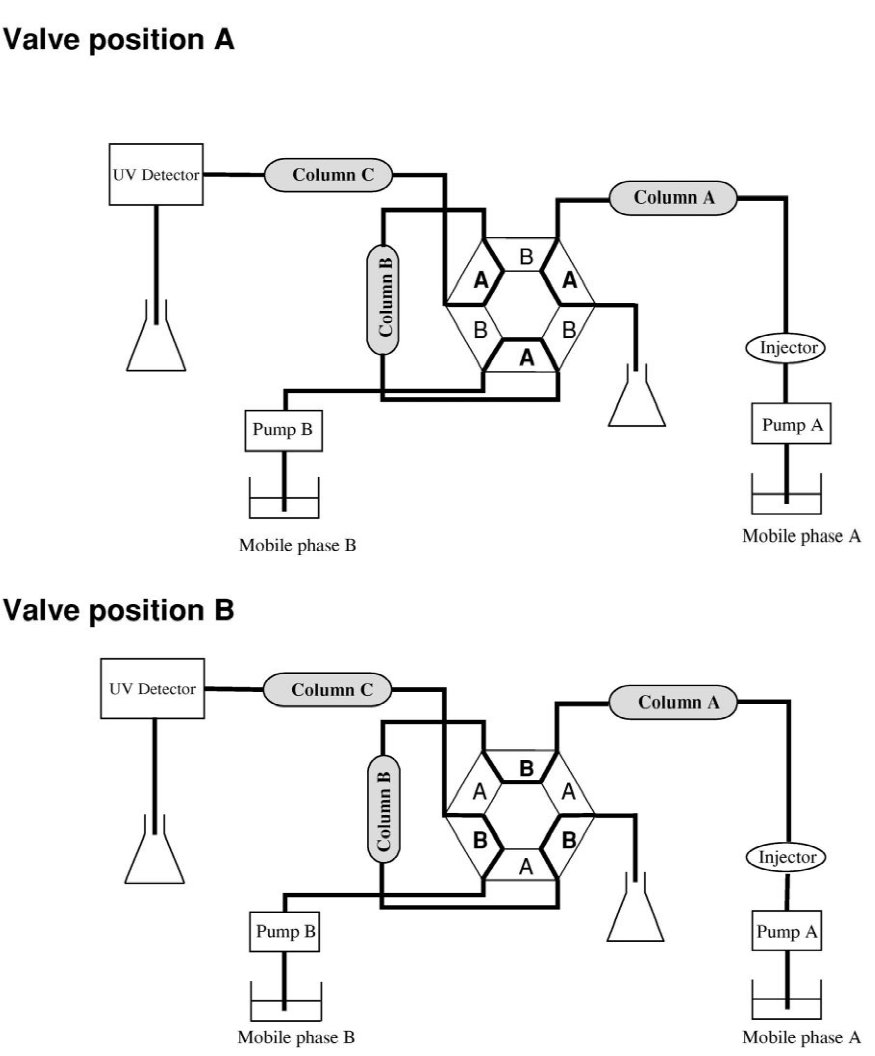

Fig. 2. Schematic diagram of the column switching HPLC system.

washed to waste by an isocratic elution with 100% of buffer A at a flow-rate of 1 ml/min. After 2 min, the switching valve was shifted to position B eluting the target compound onto the concentration column. For this step, the flow was reduced to 0.5 ml/min. After 2.8 min, the six-port valve was returned to position A and the analytical run was started. Aloesin was transferred onto the analytical column and separated by buffer B at a flow-rate of 0.1 ml/min. The eluate was monitored at 253 nm. The single analysis was completed within 20 min.

2.5. Calibration and calculations

The calibration curve for aloesin in plasma was generated by plotting the peak area versus those nominal concentrations in the standard plasma by the 1/X weighted least-square linear regression.

2.6. Coefficient of variation and accuracy

Intra-day coefficient of variation (C.V.) and accuracy of the method were evaluated by the analysis of

six plasma samples spiked at the same concentrations as the calibration standards. The C.V. and accuracy for inter-day assay were assessed at the same concentration, and repeated for five different days.

2.7. Plasma collection

Male Sprague–Dawley rats (200–220 g) were used in this experiment. For the pharmacokinetic study, the femoral artery was cannulated using PE-50 tubing (Becton Dickinson & Co., NJ, USA) 2 days before experiment. The rats were fasted overnight before use and were given a single oral dose of aloesin (100 mg/kg). Heparinized samples of blood (0.3 ml) were collected at 2, 5 10, 20, 30 min, and 1, 1.5, 2, 4, 6 and 8 h postdose. Plasma was harvested after centrifugation and stored frozen at −20°C until analyzed.

3. Results and discussion

3.1. Chromatography

Aloesin was not extracted by liquid–liquid phase extraction. The application of solid-phase extraction was not desirable because recovery of aloesin was relatively low due to its polarity. Therefore, direct injection of plasma and a column-switching method was required to obtain sufficient separation of aloesin from interfering compounds in the plasma and the required detection sensitivity. The Capcell pak MF column, used as the pre-column in this study, consists of a mixture of hydrophilic and hydrophobic phases on silicone polymer-coated silica beads [12,13]. This results in a minimized interference of the biological hydrophilic components such as proteins with the column material. By using 2% acetonitrile in 20 m*M* phosphate buffer (pH 6.86) as washing solvent, the majority of endogenous hydrophilic components were eluted out of the column before switching the valve position from A to B whereas aloesin was retained. The analyte was then transferred to the analytical column by reversing the flow and further separation was achieved. Aloesin was eluted at 11.6 min with a total run time being 18 min without carry-over. Representative chromatograms of blank rat plasma, plasma spiked with aloesin, and a plasma sample obtained 4 h after oral administration of aloesin (100 mg/kg) are shown in Fig. 3. There were no interfering peaks from endogenous compounds in plasma near the retention time of aloesin. Peak purity was further confirmed by photodiode array detection over UV wavelengths from 200 to 400 nm.

3.2. Assay validations

The standard calibration curve for spiked rat plasma containing aloesin was linear over the range of 10 to 1000 ng/ml with correlation coefficient

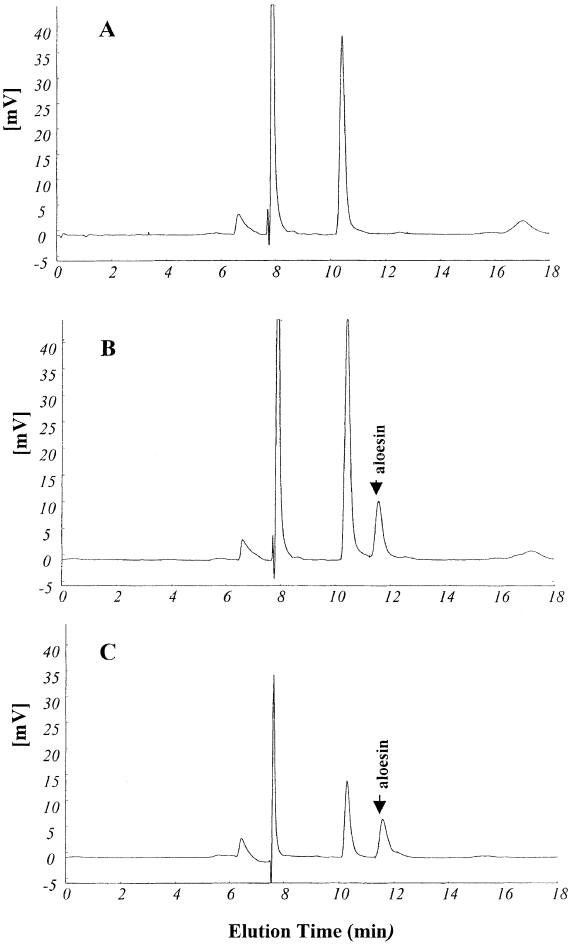

Fig. 3. Representative chromatograms of blank rat plasma (A), rat plasma spiked with aloesin (50 ng/ml) (B) and plasma obtained 4 h after a 100 mg/kg oral dose (32 ng/ml) (C). See experimental procedures for details.

greater than 0.999. The regression equation for aloesin is $y=911.9x-250$, where y indicates the peak area and x represents the concentration of aloesin in ng/ml. The intra- and inter-days variations of the aloesin determinations in plasma are summarized in Table 1. The intra-day coefficients of variation were less than 4.7% all the times, and the intra-day accuracies were between 90.4% and 99.9%. The inter-day coefficients of variation did not exceed 8.8%, and its accuracy was between 91.4% and 99.9%. The limit of quantitation for aloesin was determined to be 10 ng/ml injecting a 40 μl plasma sample volume. This is the lowest concentration of the analyte that can be measured with a coefficient of variation and an accuracy of less than 20%. The present assay utilized direct injection of plasma and column-switching technique was found to be ten times more sensitive compared to HPLC methods previously published [11].

3.3. Application

The developed method was applied for the analysis of plasma samples collected from rats dosed with aloesin. The mean plasma concentration-time curve of aloesin after oral administration of aloesin at a single dose of 100 mg/kg is shown in Fig. 4. Area under the curve (AUC), the maximum plasma concentration (C_{max}) and the time of maximum concentration (T_{max}) were determined to be 1854.6±479.2 ng·h/ml, 619.8±118.1 ng/ml, and 0.5 h, respectively. Therefore this method had adequate sensitivity for the pharmacokinetic study of aloesin after oral administration to rats.

Table 1
Recovery and intra-day and inter-day coefficient of variation and accuracy for determination of aloesin *n* rat plasma ($n=6$)[a]

Theoretical concentration (ng/ml)	Concentration found (mean±SD) (ng/ml)	C.V. (%)	Accuracy (%)
Intra-day			
10	10.44±0.30	2.9	95.5
25	27.40±0.44	1.6	90.4
50	48.27±2.25	4.7	96.5
100	95.39±3.54	3.7	95.4
250	255.19±5.90	2.3	97.9
500	502.25±7.85	1.6	99.5
1000	999.13±9.91	1.0	99.9
Intra-day			
10	10.02±0.89	8.8	99.7
25	27.14±0.44	1.6	91.4
50	50.27±3.00	6.0	99.5
100	94.83±1.62	1.7	94.8
250	260.37±2.91	1.1	95.8
500	493.49±5.74	1.2	98.7
1000	1000.74±12.22	1.2	99.9

[a] Accuracy (%)=[(*a*−B)/A]×100 where A=theoretical concentration and B=difference between theoretical and calculated concentrations.

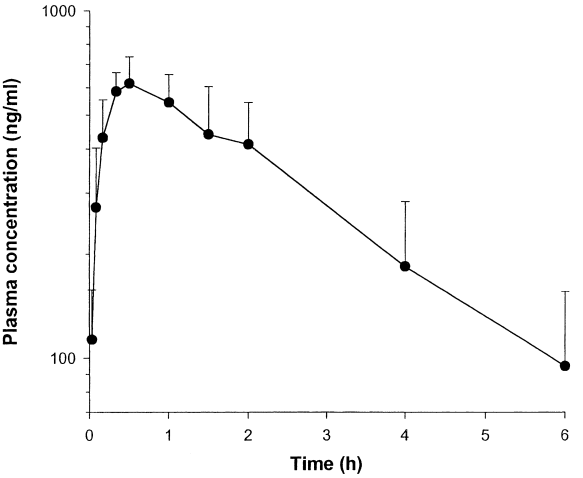

Fig. 4. Mean plasma concentrations of aloesin after single oral administration of aloesin at a dose of 100 mg/kg to rats ($n=4$).

4. Conclusions

The method described in this paper represents a specific and sensitive assay for the direct determination of aloesin in rat plasma samples within a relatively short run-time. The method seems to be advantageous over other methods reported in literature so far in terms of simplicity and sensitivity. The method was successfully applied for the determination of aloesin in rat plasma after oral administration of aloesin.

Acknowledgements

This work was supported in part by grants from the Korean Ministry of Health and Welfare and in part by grants (NRL) from Korea Ministry of Science and Technology.

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