Arch. Pharm. Res. Vol.21, No.6, pp. 651-656, 1998

# Determination of Aloesin in Plasma by High-Performance Liquid Chromatography as Fluorescent 9-Anthroyl Derivative

**Kyeong Ho Kim[1], Jin Gyun Lee[1], Jeong Hill Park[2], Young Geun Shin[2], Seung Ki Lee[2], Tae Hyung Cho[3] and Sun Tack Oh[3]**

[1]College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea, [2]College of Pharmacy, Seoul National University, Seoul 151-742, Korea and [3]Namyang Aloe Co., Jincheon 365-850, Korea

(Received February 26, 1998)

A sensitive high-performance liquid chromatographic (HPLC) method for the determination of aloesin in plasma was developed. After solid-phase extraction from plasma and derivatization of aloesin and compound AD-1, which was prepared from aloesin as a internal standard, with 9-anthroylnitrile in the presence of quinuclidine, the derivatives were separated on a Inertsil ODS-3 column using acetonitrile/methanol/water (3 : 1 : 6) as a mobile phase, and detected fluorimetrically at 460 nm with excitation at 360 nm. The detection limit of aloesin was 3.2 ng/ml in plasma (S/N=3).

**Key words :** Aloesin, 9-Anthroylnitrile, Fluorescent labelling reagent, Reversed-Phase HPLC

## INTRODUCTION

So called "Aloe" is dried powder of leaf exudates from the species, *Aloe ferox, Aloe africana,* Aloe *spicata* and so on. About 300 species of aloe were reported, for example *Aloe arborescence* MILL, *Aloe barbadensis* MILL etc, and about 130 species of aloe were distributed in South Africa (Reynolds *et al.,* 1950; 1966).

Aloe has long been used in folk medicine (Morton *et al.,* 1961) for the treatment of diarrhea, burns and dermititis. Recently, aloe has been used as cosmetics or health foods. Known activities of aloe include wound healing effects, anti-inflammatory effects, antibacterial and immuno-modulating activities, antigastric ulcer effects, hypoglycemic and antidiabetic effects (Heggers *et al.,* 1995; Hikino *et al.,* 1986; Hirata *et al.,* 1978; Obata *et al.,* 1993; Saito *et al.,* 1989; Yamamoto *et al.,* 1991).

The reported specific compounds in aloe exudates are 9,10-anthraquinones, 9-anthranol and 9-anthrones, tetrahydro-9-anthranols, anthraquinone-O-glycosides, anthranol-O-glycosides, anthrone-O-glycosides, anthrone-C-glycosides, anthraquinone diglycosides, anthrone mixed C- and O-glycosides, dimeric molecule of anthraquinones and anthranols, dimer glycosides, derivatives of 4-chromones, derivatives of 6-phenylpyran-2-one, derivatives of 2-naphthoic acid and so on (Reynolds *et al.,* 1985).

*Correspondence to:* Kyeong Ho Kim, College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea

In recent years, considerable attention has been directed to aloesin as a component used as health foods and cosmetics. Many studies on aloesin and related compounds have been reported (Conner *et al.,* 1990a, 1990b; Haynes *et al.,* 1970; Holdsworth, 1971; Mebe *et al.,* 1987; Yagi *et al.,* 1977; Yaun *et al.,* 1991). Aloesin has been reported to have various biological activities, wound healing effect, anti-gastric ulcer effect, whitening effect and reducing effect of toxicity of antineoplastics (Yagi *et al.,* 1987b). Recently we tried to investigate the reducing effect of acute toxicity of cisplatin by aloesin. And we tried the pharmacokinetic study of aloesin in rat. There was no information available on the pharmacokinetics of aloesin. Elimination of aloesin was fast and the concentration of aloesin in plasma was low. It was somewhat difficult to detect ralatively low concentration of aloesin in plasma. Therefore, the development of a sensitive method for the analysis of aloesin in plasma was needed.

Until recently, many scientists (Metori *et al.,* 1993; Chakkodabylu *et al.,* 1989; Goto *et al.,* 1983a; Goto *et al.,* 1983b; Goto *et al.,* 1983c; Wanaka *et al.,* 1992) have involved in developing new types of fluorescence labelling reagents containing a carbonylnitrile group for the sensitive HPLC methods of the hydroxyl compounds. Among the reagents, 9-anthroylnitrile was used for the derivatization of the 2'-hydroxyl group in aloesin, followed by separation and determination by HPLC with sensitive fluorescence detection. For the optimization of reaction, effects of reaction time, temperature, amount of catalyst and molar ratio on the derivatization

were examined. And solid-phase extraction method was employed using $C_{18}$ cartridge. And the assay was validated by inter-day and intra-day precision and accuracy test. Because a known compound suitable for the internal standard was not available, compound AD-1 was prepared by reduction of ketone (C-12) in the structure of aloesin.

## MATERIALS AND METHODS

### Materials and equipments

Aloesin, 8-C-β-D-glucopyranosyl-7-hydroxy-5-methyl-2-(2-oxopropyl)-4H-1-benzopyran-4-one, was isolated from the leaves of Aloe barbadensis and identified by the comparision with reported spectral data (Haynes and Holdsworth, 1970). 9-Anthroylnitrile was purchased from Wako Pure Chemical Co. (Osaka, Japan) and quinuclidine was purchased from Aldrich Chemical Co. (Milwaukee, U.S.A.). Standard solutions of aloesin, 9-anthroylnitrile and quinuclidine were prepared by dissolving in acetonitrile at concentrations of 1.5 μg/ml, 1.0 mg/ml and 1.6 mg/ml, respectively. All other reagents were of analytical reagent grade.

Silica-gel for column chromatography was Kieselgel 60 (230~400 mesh ASTM, Art. 9385, E. Merck, Darmstadt, Germany). Solid phase extraction cartridge was Sep-pak cartridge $C_{18}$ 100 mg (Part. No.20515) purchased from Waters Co. (Millford, U.S.A.) and SPE-12G vacuum manifold was purchsed from J.T.Baker Co. (Phillipsberg, U.S.A.).

High-performance liquid chromatograph was consisted of Shimadzu Model LC-9A system (Kyoto, Japan), equipped with a Rheodyne (Cotati, U.S.A.) injector and C-R4A chromatopac data processor (Kyoto, Japan). Fluorescence detector was TSP Spectra SYSTEM FL-3000 (Riviera Beach, U.S.A.) and operated at wavelength of 360 nm for excitation and 460 nm for emission. The reaction mixture was analyzed on a GL Science Inc. Inertsil ODS-3 column (4.6×150 mm with 5 μm particle size, Kyoto, Japan). For separation and determination of derivatized aloesin, acetonitrile/methanol/water (3:1:6) was used as a mobile phase which was filtered through 0.2 μm-membrane filter prior to being used. For purification of derivatized aloesin and internal standard prepared, Phenomenex ECONO-PREP C18 (10.0×300 mm with 5.0 μm particle size, Torrance, U.S.A.) column was used.

For structural elucidation of derivatized aloesin and internal standard, NMR spectra were recorded on Varian GEMINI-200 spectrometer (Palo Alto, U.S.A., 200 MHz) and Bruker AMX 500 spectrometer (Billerica, U.S.A., 500 MHz). Dimethylsulfoxide-d6 was purchased from Sigma Chemical Co. (St. Louis, U.S.A.) to be used as a solvent. The chemical shifts were recorded in δ (ppm) and coupling constant in Hz. Multiplicities of $^1$H- and $^{13}$C-NMR signals are indicated as s (singlet), d (doublet), t (triplet) and m (multiplet). IR and UV spectra were measured on Bio-Rad FTS-7 spectrophotometer (Cambridge, U.S.A.) and Shimadzu UV-1601PC spectrophotometer (Kyoto, Japan), respectively. Mass spectra were recorded on VG TRIO-II GC/MS system (Altrincham, U.K.) and Jeol AX 505 WA double focusing mass spectrometer (Akishima, Japan). Melting point was determined with Fisher Jones melting point apparatus (Pittsburgh, U.S.A.).

### General procedure for derivatization and optimization of reaction

To 200 μl of aloesin standard soln., were added 50 μl of 9-anthroylnitrile soln. and 100 μl of quinuclidine soln., and the mixture was incubated at 100°C for 5 min. After addition of 100 μl of methanol to decompose excess 9-anthroylnitrile, the mixture was evaporated to dryness under vacuum. The residue was dissolved in 250 μl of 50% acetonitrile/water and applied to a Sep-pak $C_{18}$ cartridge which was preconditioned with 5 ml of 50% acetonitrile/water for elimination of excess of the reagents. Elution was carried out under low vacuum by passing 500 μl of 50% acetonitrile/water through the cartridge. A 10 μl aliquot of the eluate was directly injected into the HPLC. Structures of aloesin and 9-anthroyl derivative of aloesin are illustrated in Fig. 1.

The optimal derivatization parameters were investigated to obtain maximum fluorescence signal. To the 200 μl of aloesin soln., various concentrations of 9-anthroylnitrile soln. and quinuclidine soln. were added and the mixtures were incubated at 100°C for 5 min and treated as described above. The effects of time and temperature on the reaction were investigated by adding 50 μl of 9-anthroylnitrile soln. and 100 μl of quinuclidine soln. to the 200 μl of aloesin soln. and the mixtures were incubated at 40, 60, 80, 100°C for 5, 15, 30, 45 or 90 min and treated as described above.

### Stability test of 9-anthroyl ester of aloesin

After derivatization, the derivatized aloesin was isolated using solid-phase extraction method as described above. A 10 μl aliquot of the eluate was injected into the chromatograph at the interval of 0, 1, 2, 4, 8, 16 and 24 hr while being kept at room temperature. The

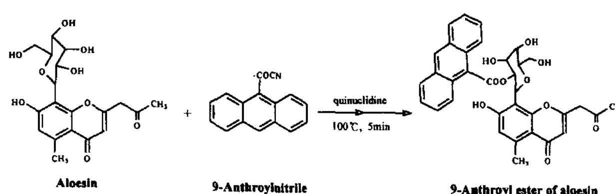


**Fig. 1.** Reaction of 9-anthroylnitrile with aloesin.

peak intensity of derivatized aloesin was monitored.

### Isolation and structural elucidation of 9-anthroyl ester of aloesin

After derivatization of aloesin with 9-anthroylnitrile, the reaction mixture was evaporated and dissolved in chloroform/methanol (9:1). And the solution was chromatographed on silica gel column chromatography using chloroform/methanol (9:1) ⟶ chloroform/methanol/water (10:3.5:1) under stepwise gradient elution.

The fraction containing derivatized aloesin was applied to semi-preparative HPLC using 40% acetonitrile in water as a mobile phase to afford 20 mg of purified 9-Anthroyl ester of aloesin (white-yellow, amorphous solid): Mp 193-8°C, UV $\lambda_{max}$; in methanol 253 nm, IR $v_{max}$ (KBr, $cm^{-1}$); 3,480 (OH), 1,625 (C=O, ester), MS (m/z); 599 $[M]^+$, $^1$H-NMR (200 MHz, DMSO-$d^6$) δ; 2.22 (s, CH3-12), 2.79 (s, CH3-5), 3.57~3.69 (m, H-3'), 3.76~3.85 (m, H-6'), 3.93 (s, H-11), 4.51~4.53 (t, OH-6'), 4.99 (d, J=10.25, H-1'), 5.28 (d, J=4.86, OH-4'), 5.76 (d, J=6.05, OH-3'), 6.09~6.19 (t, H-2'), 6.26 (s, H-3), 6.70 (s, H-6), 7.20~7.57 (m, H-2"~7"), 8.1 (d, J=8.08, H-1", -8"), 8.69 (s, H-10"), 9.27 (brs, OH-7), $^{13}$C-NMR (200 MHz, DMSO-$d_6$) δ; 202.32 (C-12), 178.50 (C-4), 167.69 (-COO-), 160.47 (C-2), 159.72 (C-7), 158.49 (C-9), 141.30 (C-5), 116.11 (C-6), 124.79~130.26 (C-1"~13"), 114.85 (C-10), 108.71 (C-8), 82.09 (C-4'), 75.93 (C-3'), 73.37 (C-2'), 71.30 (C-5'), 70.04 (C-1'), 61.59 (C-6'), 47.925 (C-11), 29.73 (C-13), 22.70 (C-14).

### Preparation of internal standard, compound AD-1

100 mg of aloesin standard was taken in 100 ml-round bottomed flask and dissolved in 30 ml of methanol. 200 mg of $NaBH_4$ was then added into aloesin solution which was precooled in ice bath to form compound AD-1 as shown in Fig. 2. The mixed solution was kept at room temperature and stirred for 12 hr.

1 N-HCl was added to adjust pH to about 2~3, and then organic solvent was removed using rotary vacuum evaporator at low temperature. The residue was dissolved in water and extracted with 30 ml of n-butanol 3 times. The solvent was evaporated to dryness and the residue was dissolved with 1 ml of methanol. And the solution was injected into semi-preparative HPLC using 20% acetonitrile in water as a mobile phase to afford 80 mg of solid which was named to compound AD-1 (White, amorphous solid): MS (m/z); 396 $[M]^+$, $^1$H-NMR (500 MHz, DMSO-$d_6$) δ; 1.12~1.15 (dd, J=6.19, 14.03, $CH_3$-12), 2.56 (brs, OH-12), 2.62 (s, H-11), 4.08 (brs, H-12), 5.95 (s, H-3), 6.68 (s, H-6), 10.56 (brs, OH-7).

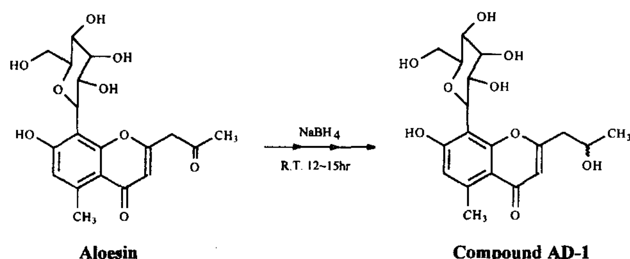


**Fig. 2.** $NaBH_4$ reduction of aloesin into compound AD-1.

### Quantitation of aloesin in human plasma and assay validation

Aloesin and internal standard were added to 1 ml of drug-free human plasma and applied to Sep-pak C 18 cartridge preconditioned with 5 ml of methanol and 5 ml of water. After elimination of co-existing substances by washing with 10 ml of water, aloesin and internal standard were eluted with 1 ml of 100% methanol. And then the solution was evaporated to dryness and the residue was dissolved in 1 ml of acetonitrile. 250 μl of this solution was reacted with 9-anthroylnitrile in the same manner described above.

The intra-day accuracy and precision of the assay were determined by analyzing five replicates from the four samples spiked with aloesin at the concentrations of 30, 67, 327, 624 ng/ml and with internal standard at 67 ng/ml to drug-free human plasma. Inter-day accuracy and precision were studied by duplicate analyses of samples at the same-four concentrations in three independent assay runs.

## RESULTS AND DISCUSSION

### Derivatization of aloesin with 9-anthroylnitrile and optimization of reaction

Many research works have been made on the reactivities of 9-anthroylnitrile and 1-anthroylnitrile toward various hydroxyl groups on the steroid nucleus. The former reagent is reported to react selectively with the primary hydroxyl groups at C-21 in various steroids in quinuclidine/acetonitrile. Therefore, 9-anthroylnitrile appeared to be also promising for derivatization of aloesin which has four free-hydroxyl groups in sugar moiety.

The optimal condition for esterification of aloesin with 9-anthroylnitrile in the presence of quinuclidine was investigated. The formation of the ester increased with concentration of quinuclidine up to 1.6 mg/ml and concentration of 9-anthroylnitrile up to 2.0 mg/ml and fluorescence response reached at maximum as illustrated in Fig. 3 and Fig. 4. The excess of the reagent which would disturb the chromatogram, could be efficiently removed by passing the reaction mixture through a Sep-pak $C_{18}$ cartridge.

The reaction rate was significantly influenced by the reaction temperature and time where the maximum

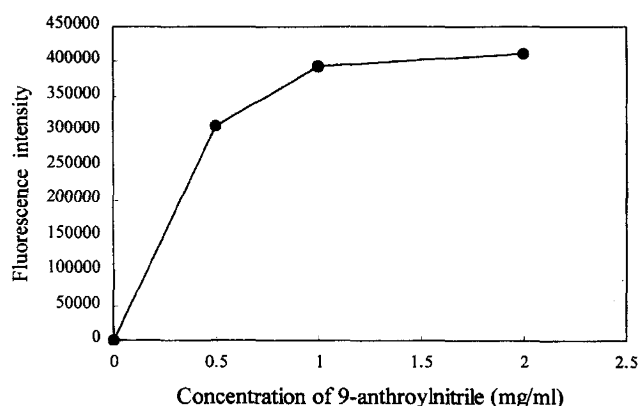


**Fig. 3.** Influence of 9-anthroylnitrile concentration on derivatization of aloesin. Concentrations of aloesin and quinuclidine were at 1.5 μg/ml and 1.6 mg/ml, respectively, with varying the concentration of 9-anthroylnitrile (0, 0.5, 1.0 and 2.0 mg/ml), and heating at 100°C for 5 min; HPLC conditions are described in Fig. 7.

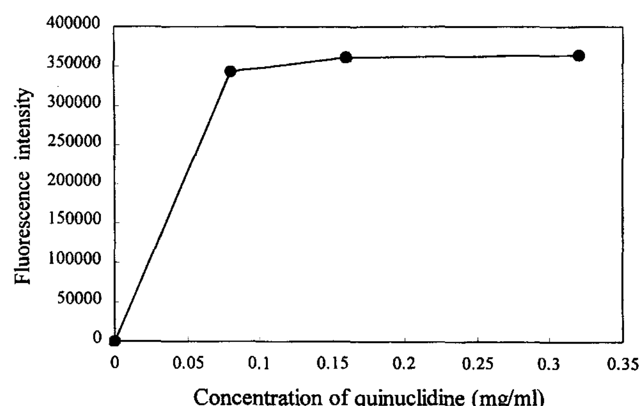


**Fig. 4.** *Influence of the quinuclidine concentration on the* fluorescence response of the 9-anthroyl derivative of aloesin. The concentration of quinuclidine was varied from 0, 0.8, 1.6 to 3.2 mg/ml in the presence of aloesin (1.5 μg/ml) and 9-anthroylnitrile (1 mg/ml). The reaction was carried out by heating at 100°C for 5 min; HPLC conditions are described in Fig. 7.

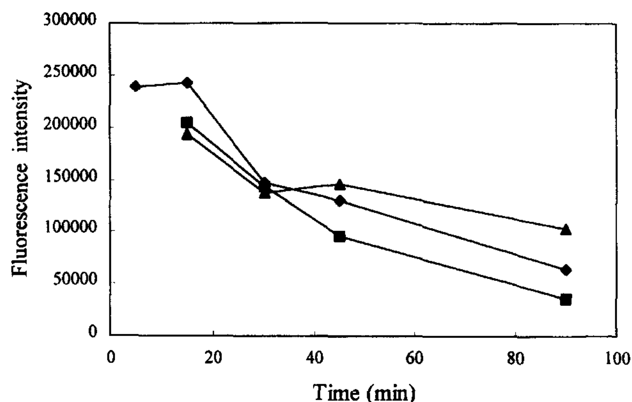


**Fig. 5.** Influence of reaction temperature and time on the derivatization of aloesin with 9-anthroylnitrile. Reaction of aloesin (1.5 μg/ml) in the presence of 9-anthroylnitrile (1.0 mg/ml) and quinuclidine (1.6 mg/ml) was carried out at three different temperatures such as ◆ (100°C), ■ (80°C) and ▲ (60°C). Aliquots of the reaction mixture were monitored for the fluorescence at regular intervals. HPLC conditions are described in Fig. 7.

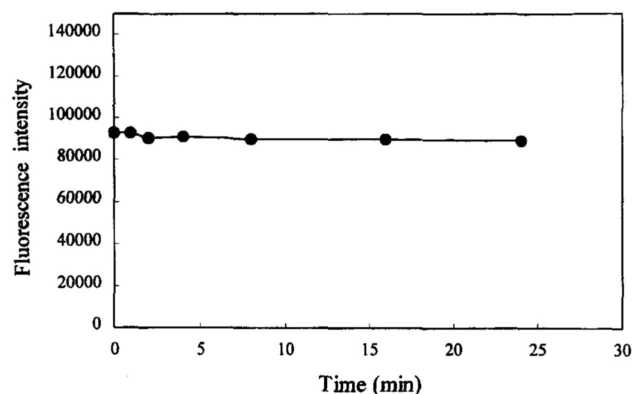


**Fig. 6.** Stability of 9-anthroyl derivatized aloesin; While the reaction mixture was stored at ambient temperature, aliquots of the mixture was analysed by HPLC at regular time intervals.

yield was obtained with incubation at 100°C for 5 min as shown in Fig. 5. The derivative, however, started to decompose when heating at this temperature longer than 15 min. The product was found to be stable for at least 24 hr during when stored at ambient temperature as shown in Fig. 6. This result indicates that fast termination of reaction appears to be important.

Based on these facts, reaction with 9-anthroylnitrile in the presence of 1.6% quinuclidine in acetonitrile at 100°C for 5 min was chosen as the optimal condition.

### Isolation and structural elucidation of 9-anthroyl ester of aloesin

In order to determine the biniding site of aloesin when derivatized with 9-anthroylnitrile, $^{1}$H- and $^{13}$C-NMR spectra were compared before and after the reaction. Both $^{1}$H-NMR spectra showed similar patterns except for the proton peaks in the sugar moiety. $^{1}$H was observed at 4.99 ppm and 3'-H, 4'-H and 6'-H were obsereved at 3~4 ppm And 3'-OH, 4'-OH and 6'-OH were founded. However, 2'-OH was not found and 2'-H was shifted 2.3 ppm downfield compared to $^{1}$H-NMR spectrum of aloesin, suggesting that 9-anthroylnitrile was esterified with 2'-OH in sugar moiety. And the $^{13}$C-NMR spectrum showed some differences compared with that of aloesin. In the sugar moiety region, only C-2 was shifted downfield. All signals were assigned using DEPT, $^{1}$H-$^{1}$H COSY and $^{1}$H-$^{13}$C COSY techniques and confirmed by comparison of chemical shifts and coupling constant value of aloesin standard.

### Preparation of internal standard and structural confirmation

Both $^1$H-NMR spectra of internal standard and aloesin were similar, except for alkyl chain moiety. 12-OH signal was observed at 2.56 ppm and 12-$CH_3$ signal which was splitted into doublet were 1.12~1.15 ppm. And all couplings in 2-hydroxylpropyl moiety were confirmed using $^1$H-$^1$H COSY technique. It was confirmed to be 8-C-β-D-glucopyranosyl-7-hydroxy-5-methyl-2-(2-hydroxypropyl)-4-H-1-benzopyran-4-one.

### Quantitation of aloesin in human plasma and assay validation

Typical chromatograms are shown in Fig. 7. Intra-, inter-day precision were determined with plasma spiked with known amounts of aloesin and internal standard. For the assay runs in one day, the differences between the added and measured mean concentrations from analysis of spiked samples in the range of 67.0~624 ng/ml were less than 8.81% (34.1% for 30 ng/ml). The coefficients of variation were in the range of 5.73%~12.84% (mean coefficient of variation, 9.54%). For the assay runs over three different days, the differences between the added and measured mean concentrations of four spiked plasma samples were less than 6.16% (36.6% for 30 ng/ml) as listed in Table I. The coefficient variation were 1.73%~4.45%.

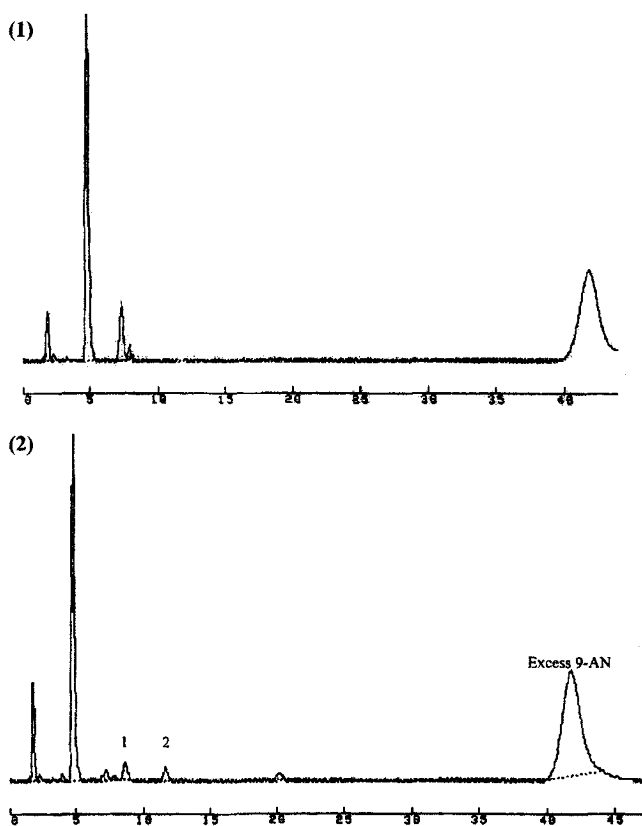


**Fig. 7.** Typical chromatograms of (1) extract of blank plasma, (2) extract of plasma spiked with aloesin at 150 ng/ml. Column; Inertsil ODS-3 (5 μm, 4.6×150 mm), mobile phase; acetonitrile/methanol/d.d.w. (3 : 1 : 6), flow rate; 1.0 ml/min, detection; fluorescence at 360 nm for excitation and 460 nm for emission. Peak 1; derivative of internal standard, peak 2; derivative of aloesin.

**Table I.** Intra-day and intra day accuracy and precision

| Added (ng/ml) | Mean observed (ng/ml)±SD (%RSD) | |
|---|---|---|
| | Intra-day (n=5) | Inter-day (n=3) |
| 30.0 | 40.3±12.8 | 41.0±1.73 |
| 67.0 | 68.5±5.73 | 71.1±3.21 |
| 327.0 | 348.3±9.69 | 335.8±3.36 |
| 624.0 | 679.7±9.89 | 646.6±4.45 |

Calibration curve was linear over the range of 10~800 ng/ml with the correlation coefficient consistently greater than 0.99. The detection limit of derivatized aloesin measured at S/N of 3 was estimated to be 3.2 ng/ml in plasma. This value was found to be 80 times lower than that of intact aloesin when monitored by UV at 253 nm.

In conclusion, the accuracy, precision and sensitivity of the present method will allow it to be used successfully for the pharmacokinetic studies.

## REFERENCES CITED

Chakkodabylu, S. R., Sensitive method for the analysis of phospholipid subclasses and molecular species as 1-anthroylnitrile derivatives of their diglycerides. *J. Chromatogr.,* 491, 37-48 (1989).

Conner, J. M., Gray, A. I., Waterman, P. G., Reynolds, T., Novel anthrone-anthraquinone dimer from *Aloe elgonica. J. Nat. Prod.,* 53(5), 1362-1364 (1990a).

Conner, J. M., Gray, A. I., Reynolds, T., Waterman, P. G., Anthrone and chromone components of *Aloe cremnophila and A. jacksonii* leaf exudates. *Phytochemistry,* 29(3), 941-944 (1990b).

Goto, J., Goto, N., Shamsa, F., Saito, M., Komatzu, S., Suzaki, K. & Nambara, T., New sensitive derivatization of hdroxysteroids for high-performance liquid chromatography with fluorescence detection. *Anal. Chim. Acta.,* 147, 397-400 (1983a).

Goto, J., Saito, M., Chikai, T., Goto, N. & Nambara, T., Determination of serum bile acids by high-performance liquid chromatography with fluorescence labelling. *J. Chromatogr.,* 276, 289-300 (1983b).

Goto, J., Shamsa, F. & Nambara, T., Determination of 6β-hydroxycortisol in urine by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.,* 6(11), 1977-1985 (1983c).

Haynes, L. J., Holdsworth, D. K., Russell, R., C-glycosyl compounds. VI. Aloesin, a C-glycosylchromone from Aloe sp. *J. Chem. Soc.,* C, (18), 2581-2586 (1970).

Heggers, John P., Kucukcelebi, A., Stabenau, Catherine

J., Ko, F., Broemeling, Lyle D., Robson, Martin C., Winters, Wendell D., Wound healing effects of Aloe gel and other topical antibacterial agents on rat skin. *Phytother. Res.*, 9(6), 455-457 (1995).

Hikino, H., Takahashi, M., Murakami, M., Konno, C., Mirin, Y., Karikura, M., Hayashi, T., Isolation and hypoglycemic activity of arborans A and B, glycans of *Aloe arborescens var. natalensis* leaves. *Int. J. Crude Drug Res.*, 24(4), 183-186 (1986).

Hirata, T., Kushi, Y., Suga, T., Christensen, A., Structural studies of aloenin; the crystal structure of its aglycone. *Chem. Lett.*, (4), 393-396 (1976).

Holdsworth, D. K., Chromones in Aloe species. Part I, Aloesin a C-glucosyl-7-hydroxychromone. *Planta Medica,* 19, 322-325 (1971).

Mebe, Paul P., 2'-p-Methoxycoumaroylaloeresin, a C-glucoside from Aloe excelsa. *Phytochemistry,* 26(9), 2646-2647 (1987).

Metori, A., Ogamo, A. & Nakagawa, Y., Quantitation of monohydroxy fatty acids by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.,* 622, 147-151 (1993).

Morton, J., Folk uses and commercial exploitation of Aloe leaf pulp. *Economic Botany,* 15, 311-319 (1061).

Obata, M., Ito, S., Beppu, H., Fujita, K., Nagatsu, T., Mechanism of anti-inflammatory and antithermal burn action of CPase from Aloe arborescens natalensis in rats and mice. *Phytother. Res.,* 7 (Spec. Issue, Proceedings of the International Congress of Phytotherapy, 1991), S30-S33 (1993).

Reynolds, G. W., The Aloes of South Africa, Johannesberg, South Africa : *The aloes of South Africa Book Fund,* (1950).

Reynolds, G. W., The Aloes of Tropical Africa and Madagascar, Mbabane, Swaziland : *The Aloes Book Fund* (1966).

Reynolds, T., The compounds in Aloe leaf exudates: a review. *Botanical Journal of Linnean Society,* 90, 157-177 (1985).

Saito, H., Imanishi, K., Okabe, S., Effects of aloe extract, aloctin A, on gastric secretion and on experimental gastric lesions in rats. *Yakugaku Zasshi,* 109(5), 335-339 (1989).

Wanaka, K. & Murui, T., Determination of sterols in edible oils and fats by high-performance liquid chromatography with fluorescence labelling. 油化學, 第41卷, 第4 (1992).

Yagi, A., Makino, K., Nishioka, I., Studies on the constituents of Aloe saponaria Haw. III. The structures of phenol glucosides. *Chem. Pharm. Bull.,* 25(7), 1771-1776 (1977)

Yagi, A., Harada, N., Shimomura, K., Nishioka, I., Bradykinin-degrading glycoprotein in *Aloe arborescens natalensis. Planta Medica.,* 53(1), 19-21 (1987).

Yamamoto, M., Masui, T., Sugiyama, K., Yokota, M., Nakagomi, K., Nakazawa, H., Anti-inflammatory active constituents of *Aloe arborescens Miller. Agri. Biol. Chem.,* 55(6), 1627-1629 (1991).

Yaun, A., Kang, S., Tan, L., Raun, B., Fan, Y., Isolation and identification of aloesin from the leaves of *Aloe vera L. var. chinensis* (*Haw.*) *Berger, Zhongguo Ihongyao Zazhi,* 16(5), 292-293 (1991).