Since as stated in Eq. 10 in the text, f_c is a constant during the β phase then

$$dX_B/dt = -\beta X_B \qquad \text{(Eq. 18}a\text{)}$$

The two-compartmental open model also indicates that the rate of loss of drug from the body (dX_B/dt) may be expressed as

$$dX_B/dt = -k_{e1}X_c \qquad \text{(Eq. 19}a\text{)}$$

or

$$dX_B/dt = -k_{e1}f_cX_B \qquad \text{(Eq. 20}a\text{)}$$

Comparing Eqs. 18*a* and 20*a*, one concludes that

$$\beta = k_{e1} \cdot f_c \qquad \text{(Eq. 21}a\text{)}$$

(Area) as a Function of Route of Administration—The total area under a plasma concentration of drug *versus* time curve upon intravenous administration is given by Eq. 14*a* for the two-compartmental open system. If a drug is given by a route other than the intravenous one, the amount of drug absorbed to time t, $(A)_t$, is given by the material balance equation

$$(A)_t = C_pV_c + X_T + \int_0^t k_{e1} \cdot V_c \cdot C_p\,dt \qquad \text{(Eq. 22}a\text{)}$$

where C_pV_c is the amount of drug in the central compartment, X_T is the amount of drug in the tissue, and the integral term is the amount of drug eliminated. At $t = \infty$ Eq. 22*a* reduces to

$$(A)_\infty = k_{e1}V_c \int_0^\infty C_p dt \qquad \text{(Eq. 23}a\text{)}$$

or, assuming absorption of a dose X° is complete,

$$X^\circ = k_{e1}V_c(\text{area}) \qquad \text{(Eq. 24}a\text{)}$$

Rearranging and substituting for X°/V_c, yields

$$(\text{area}) = C_p^\circ/k_{e1} \qquad \text{(Eq. 25}a\text{)}$$

which is identical to Eq. 14*a*. Hence, assuming the body to behave as a two-compartmental open system, the total area under the plasma level of drug *versus* time curve is independent of route of administration.

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Anthraquinone Drugs I: Thin-Layer Chromatographic Identification of Aloes, Cascara, Senna, and Certain Synthetic Laxatives in Pharmaceutical Dosage Forms

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Abstract □ A new procedure for the identification of aloes, cascara, and senna is presented. The constituent glycosides are converted to aglycones and separated into acidic and nonacidic fractions. Identification is achieved by thin-layer chromatography. The method is also applicable to the mixture of these drugs and various pharmaceutical dosage forms containing danthron, phenolphthalein, and dioctyl sodium sulfosuccinate.

Keyphrases □ Anthraquinone dosage forms—analysis □ Glucosides, anthraquinone—aglycone conversion □ TLC—identification □ UV light—TLC spot visualization

Various color reactions and microscopic methods of identification of aloes, cascara, and senna prescribed by the official compendia (*e.g.*, the British Pharmacopoeia, the NF, and the USP) are often nonspecific, unreliable, and time-consuming. Paper chromatography has been applied with limited success for the identification of certain constituents of these drugs (1). With the advent of thin-layer chromatography (TLC), various attempts have been made at characterizing anthraquinone drugs employing silica gel-coated plates (2). Hoerhammer *et al.* have made a systematic attempt at their characterization on the basis of glycosides, resins, *etc.* (3), but the chromatograms exhibit a large number of spots whose resolution is markedly affected by slight variations in chromatographic conditions. In pharmaceutical preparations containing mixtures of these drugs along

with other additives, the chromatographic patterns obtained are too complex to be of any analytical significance.

The present communication describes an identification method based on the TLC identification of the corresponding aglycones after their separation into acidic and nonacidic components. A large number of laxative preparations contain extracts of one or more anthraquinone drugs (aloes, cascara, and senna) along with synthetic laxatives, especially phenolphthalein, danthron (1,8-dihydroxyanthraquinone), and dioctyl sodium sulfosuccinate (DOSSS). The present method also permits the analysis of such pharmaceutical products.

For the preparation of aglycones, Auterhoff's method has often been employed (4), whereby extraction and simultaneous hydrolysis of the glycosides is achieved with acetic acid. It was noticed that acetic acid could readily react with aloe emodin (5), thereby interfering with the TLC analysis of aglycones. Further, the C-glycosyl compounds, *e.g.*, barbaloin, do not liberate aglycones by hydrolysis, and an oxidizing agent is necessary to rupture the C—C bond (5). Hence the preparation of the corresponding aglycones was effected with ferric chloride in presence of hydrochloric acid, as originally described by Cahn and Simonsen (6).

Of the various solvent systems suitable for the TLC of these drugs (2), two have been widely employed: benzene–ethyl formate–formic acid (75:24:1) (7) for the separation of aglycones, and ethyl acetate–methanol–water (100:16.5:13.5) (3) for the separation of glycosides. During the present study, further improvement in the resolution of the spots was achieved by modifying the solvent systems.

EXPERIMENTAL

Preparation of Test Solution—*Procedure A*—(for individual drugs or mixtures not containing senna). To about 0.2–0.3 g. of the powdered drug or extract in 2 ml. methanol and 3 ml. water, add 1 ml. 25% aqueous $FeCl_3$ solution and 2 ml. concentrated HCl. Reflux on a boiling-water bath for 30 min. Cool and shake in a separator with 5 ml. chloroform. Separate the chloroform layer and evaporate to dryness. Dissolve the residue in 1.5 ml. chloroform and 0.5 ml. methanol.

For dosage forms, take one capsule or pill or tablet or about 0.2 g. of granular preparation or about 0.3 ml. of liquid preparation.

Procedure B—(for mixtures containing senna). Dilute the chloroform extract obtained as above to 10 ml. with chloroform and shake in a separator with 10 ml. freshly prepared 10% sodium bicarbonate solution. After separation, evaporate the chloroform layer to dryness and dissolve the residue in 1 ml. chloroform and 0.5 ml. methanol (Solution 1). Acidify the bicarbonate extract with concentrated HCl (about 1.2 ml., Congo red paper) and shake immediately with 10 ml. chloroform. After separation, evaporate the chloroform layer to dryness and dissolve the residue in 1.5 ml. chloroform and 0.5 ml. methanol (Solution 2). Chromatograph Solutions 1 and 2 on the same plate.

Procedure C—(for preparations containing DOSSS). Shake one capsule (or tablet or about 0.2 ml. liquid preparation or about 0.2 g. solid preparation) with 5 ml. *n*-hexane (or petroleum ether). After the solids have settled, filter the supernatant liquid carefully and employ for the TLC analysis.

Thin-Layer Chromatography—Carry out TLC on glass plates (20 × 20 cm.) coated with Silica Gel G[1] (0.3-mm. thick layers) under the following conditions: sample size: 3 μl. of the test solution; solvent systems: I, benzene–ethyl formate–formic acid (15:5:1); II, ethyl acetate–methanol–water (10:2:1); spray reagents: I, 10% methanolic KOH solution; II, 3% solution of *N,N*-dimethyl-*p*-phenylenediamine hydrochloride in methanol–water (1:1 by vol.) (8). *Detection:* most of the spots are visible in daylight. Additional spots are detected by spraying with Reagent I and observing in UV light[2] before and after spraying. The resinous constituents are seen as characteristic fluorescent spots in UV light, but only after spraying with Reagent I.

RESULTS AND DISCUSSION

Analysis of Individual Drugs—*Aloes*—Of the various commercial varieties of aloes, some pharmacopeias (German DAB.6, Austrian OeAB.9, and Switz. Ph. Helv. V) prescribe cape aloes only, while others (BP 1963, Ph. Franz. 1965, Ph. USSR IX) have curacao aloes also as official. USP XVII, in addition, allows socotrine aloes which, however, is not readily available in North America.

The chromatogram of the oxidation products from curacao aloes (Solvent System I, Spray Reagent I) shows five main spots designated as Substances 1–2 and 4–6 (see Fig. 1). Substance 6, unlike the others, is intensely purple even before spraying. Substance 3, not seen in the visible light, appears as the most prominent spot showing blue fluorescence when examined under UV light after spraying with Reagent I. Their R_f values and colorations are represented schematically in Fig. 1*a* and Table I.

In the case of cape aloes, spots for Substances 1, 3, and 4 are clearly seen while those for 2, 5, and 6 are absent. Substances 2 and 5 of curacao aloes produce faint spots only. Hence, Substance 6 forms the basis for distinguishing between cape and curacao aloes (Figs. 1*b*, 2*a*, and 2*b*.

In contrast to the above two aloe varieties, various samples of socotrine aloes did not always yield the same results when examined under identical conditions. Substances 1, 3, and 4 were always observed in the chromatograms while Substances 2, 5, and 6 were often absent. This behavior of socotrine aloes may be expected, since the drug is derived from aloe plants of different origins (Mocha, Yemen, Aden, and various types of Zanzibar aloes, *etc.*).

On the basis of the above data, the fluorescent Spot 3 may be considered as a characteristic of aloes, and the presence or absence of Spot 6 indicative of its curacao or cape origin, respectively. The two

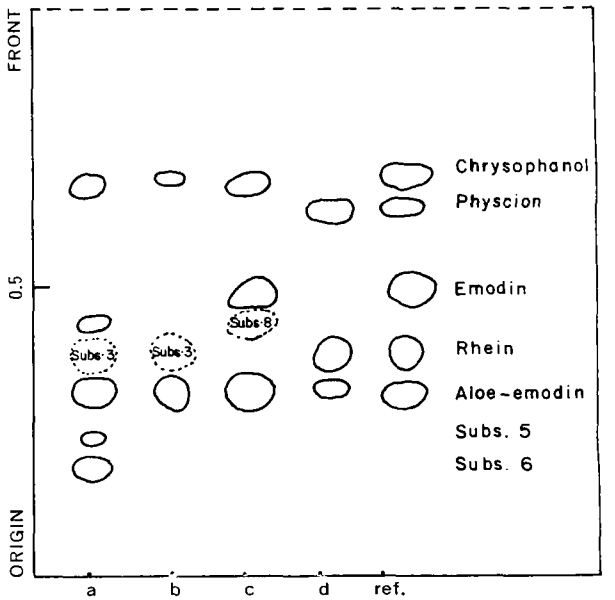

Figure 1—*Schematic chromatogram of anthraquinone drugs. Solvent system: I; spray reagent: I. Samples:* (a) *curacao aloes;* (b) *cape aloes;* (c) *cascara;* (d) *senna. Spots with dotted outline are seen in UV light only after spraying.*

[1] Merck and Co., Rahway, N. J.

[2] Black Ray UVL-22, long wave.

Table I—TLC of Aloes, Cascara, and Senna

Spot No.	$R_f \times 100$	Color of the Spot[a] — In Daylight — Before Spray	In Daylight — After Spray	In UV Light — Before Spray	In UV Light — After Spray	Drug Examined[b] — Aloes, Curacao	Aloes, Cape	Cascara[c]	Senna[d]	Identification of Spot
1	73.3	Y	P	G	G	+	+	+	−	Chrysophanol
2	46.6	YP	GP	G	G	+	−	−	−	—
3	42	—	—	—	B	(+)	(+)	−	−	Oxidn. product of *p*-coumaric acid
4	35.3	Y	P	G	G	+	+	+	+	Aloe-emodin
5	32	Y	P	R	GR	+	−	−	−	—
6	22	P	P	G	G	(+)	−	−	−	Under investigation
7	52	Y	O	G	G	−	−	(+)	−	Emodin
8	48	—	—	—	Gr	−	−	(+)	−	Resin
9	66	Y	O	G	G	−	−	−	(+)	Physcion
10	40	Y	R	G	G	−	−	−	(+)	Rhein

[a] Y = yellow; P = purple; G = gray; O = orange; R = red; B = blue; Gr = green. [b] + = present; − = absent; (+) = spot characteristic for the drug. [c] Aqueous extract of cascara, methanolic extract of cascara, and cascara powder. [d] Alexandrian senna leaf, Alexandrian senna pod, Tinnevelli senna leaf, and Tinnevelli senna pod.

types of cape aloes A and B may be readily characterized by their TLC on the basis of the presence or absence of aloinosides (9).

These criteria of identity are also applicable to samples of aloin BP and NF and may be used to determine their origin (Fig. 2*c*). Socotrine aloe is considered an adulterant in BPC. As it is not readily available on this continent and because of its batch-to-batch variation in composition it would appear desirable to exclude socotrine aloes from the USP, and thereby maintain specifications uniform with other pharmacopeias.

Cascara—Cascara is the dried bark of *Rhamnus purshiana*. Its aqueous, alcoholic, or aqueous-alcoholic extracts have often been used in laxative preparations. In the present study these extracts were found to yield the same TLC data as the powdered drug (Fig. 2*e* and *f*).

The chromatogram of cascara (Solvent System I) shows, in addition to Substances 1 and 4, an equally prominent spot of Substance 7. After spraying with Reagent I, Substance 7 turns orange; unlike Substances 1 and 4 which turn purple. In UV light an additional large spot of Substance 8 is visible immediately below that of Substance 7. Substance 8 has R_f values slightly higher than that of Substance 3 of aloes, and unlike the characteristic blue fluorescence of the latter, it shows a green fluorescence. Substances 7 and 8 were found to be characteristic of cascara (Fig. 1*c*, Table I, Fig. 2*e* and *f*).

Senna—Senna consists of the dried leaflets of *Cassia acutifolia* known commercially as Alexandrian senna, or of *Cassia angustifolia* known commercially as Tinnevelli senna. Senna pods are official in the BP and other pharmacopeias. According to the NF, they may accompany the leaves in restricted proportions. In the present study the leaves and pods of both the commercial varieties qualitatively show the same distinguishing features (Fig. 3*a–d*). The chromatogram of senna (Solvent System I, Spray Reagent I) shows three main spots corresponding to Substances 4, 9, and 10. After spraying, Substance 9 appears orange in color and Substance 10 turns red. If the test solution is prepared according to Procedure B, Substance 10 is carried over in the sodium bicarbonate extract (Solution 2) indicating its acidic nature. Substance 9, however, remains in the bicarbonate-insoluble extract (Solution 1). Substances 9 and 10 are considered to be characteristic of senna (Fig. 1*d*, Table I, Fig. 3*a–d*).

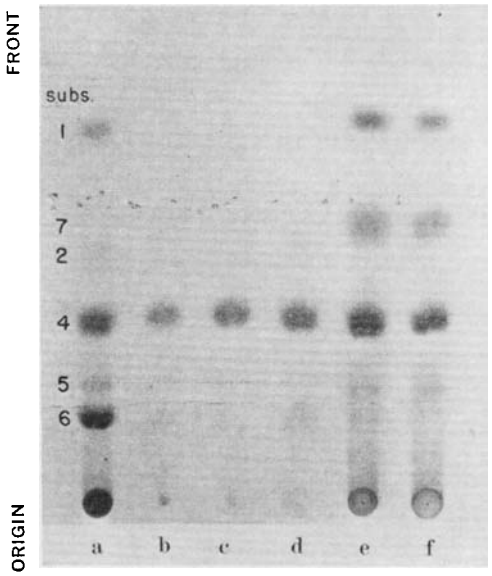

Figure 2—*TLC of the test solutions of aloes and cascara. Solvent system: I; spray reagent: I. Samples:* (a) *curacao aloes;* (b) *cape aloes;* (c) *aloin (source: cape aloes);* (d) *socotrine aloes;* (e) 70% *aqueous methanolic extract of cascara powder;* (f) *cascara powder.*

Identification of Spots—Substances 1, 4, 7, 9, and 10 have been identified as chrysophanol, aloe-emodin, emodin, physcion, and rhein, respectively, by comparison with authentic samples. The presence of these aglycones in the drugs under investigation is well established (10–13). Substance 3 of aloes is linked with *p*-coumaric acid, since the oxidation of the latter (150 mg.) according to Method A yields Substance 3 as the main product. *p*-Coumaric acid, as a component of aloe resin is already known (14). Substance 6 has aroused special interest since it has been found to be different from the aglycones reported above. Substances 2, 5, and 6 possess similar UV spectral characteristics and, therefore, appear to be closely related. The characterization of these substances is under progress. Compound 8 of cascara seems to be derived from the resinous part, though it is not identical with the $FeCl_3$ oxidation products of syringic acid, cinnamic acid, or rhamnetin, the constituents known to occur in cascara (11).

Analysis of Pharmaceutical Preparations—*Preparations Containing Combinations of Vegetable Drugs Only*—Pharmaceutical dosage forms containing combinations of aloes, cascara, and senna can be readily detected by the TLC procedure described. If senna is present along with aloes and/or cascara, the characteristic spot of rhein would partially or wholly obscure the resinous Substances 3 and 8 which are characteristic of aloes and cascara, respectively. This interference is eliminated by adopting Procedure B, in which rhein

Table II—Composition of Pharmaceutical Preparations[a]

Preparation No.	Composition
a	Aloin (source: aloes curacao), dry ext. of belladonna, ipecac, phenolphthalein
b	Aloin (source: aloes cape), dry ext. of belladonna, ipecac powder, phenolphthalein
c	Aloes, belladonna, strychnine, cascara
d	Fl. ext. cascara aromatic, psyllium husk powder, prune powder
e	Standardized concentrate of total senna-pod principles with DOSSS
f	Sennosides A and B
g	Aloes cape, methanolic ext. of cascara, Alexandrian senna pod

[a] Preparations a–f were commercial preparations; product g was a reference sample prepared in the laboratory.

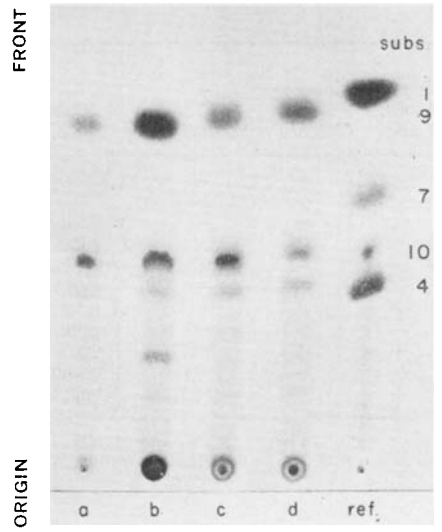

Figure 3—*TLC of the test solutions of senna. Solvent system: I; spray reagent: I. Samples:* (a) *Alexandrian senna leaf;* (b) *Alexandrian senna pod;* (s) *Tinnevelli senna leaf;* (d) *Tinnevelli senna pod.*

is separated from the test solution, by shaking with aqueous sodium bicarbonate. Substances 3 and 8 do not react with $NaHCO_3$ indicating their nonacidic nature, though Substance 3, as described previously, is derived from *p*-coumaric acid (*cf.* Fig. $4g_1$ and $4g_2$).

In addition to the above drugs some pharmaceutical specialties contain belladonna and ipecac extracts as well as strychnine. These alkaloidal extracts do not interfere with the proposed method of identification of anthraquinone drugs. Any basic constituents present in the oxidation mixture remain in the acidic-aqueous phase, while the substances extracted by chloroform stay at the starting point on the thin-layer plate (Fig. 4*a* and *b*). In certain laxative preparations, however, a red spot (orange after spraying with Reagent I) has been observed in the R_f range of chrysophanol (Solvent System I) (Fig. 4*a* and *b*). It seems to originate from some coating material or other tablet additives.

Preparations Containing Vegetable Drugs with Synthetic Laxatives—For preparations containing danthron and phenolphthalein, test solutions are prepared according to Procedure A. The former

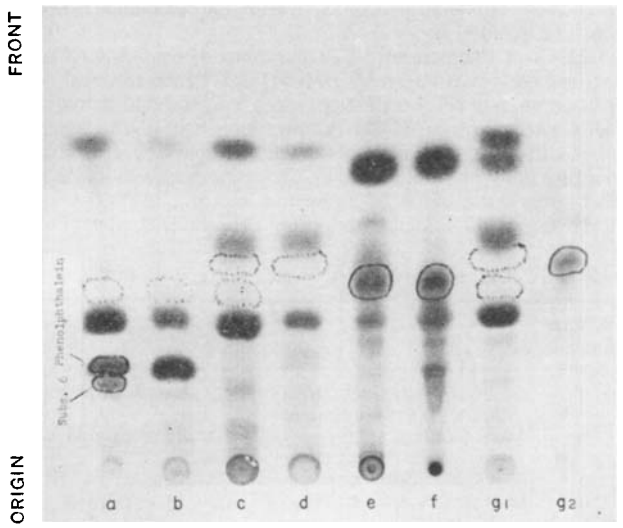

Figure 4—*TLC of test solutions of commercial laxatives. Solvent system: I; spray reagent: I. Samples:* a-f*: as described in Table II;* g_1 *and* g_2*: nonacidic and acidic fractions, respectively, derived from Sample* g *(Table II).*

Table III—TLC of Synthetic Laxatives

Substance	Prepn. Method of Test Soln.	Solvent System	Color Reagent	$R_f \times 100$	Coloration—Before Spray	Coloration—After Spray
Danthron	A	I	I	70	Yellow	Purple
Phenolphthalein	A	I	I	28	—	Red
DOSSS	C	II	II	40	—	Violet

drug can be detected on the chromatogram (Solvent System I) as a yellow spot turning purple on spraying with Reagent I, while the latter is seen as a red spot only after spraying. For dosage forms containing DOSSS, Method 3 is adopted, in which petroleum ether (or *n*-hexane) selectively removes this drug prior to $FeCl_3$ oxidation. TLC of the ether extract in Solvent System II employing Spray II may be carried out to confirm the presence of this substance. Danthron and phenolphthalein yield spots which are well separated (Table III) from the spots exhibited by the anthraquinone drugs and can, therefore, be readily detected when present along with the latter drugs.

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