THE DETERMINATION OF WATER-SOLUBLE EXTRACTIVE IN ALOES AND IN DRY EXTRACTS OF CASCARA SAGRADA AND KRAMERIA

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ALOES

THE British Pharmacopœia 1948 contains a limit for the water-soluble extractive of aloes of not less than 75 per cent., to be determined by method A of the Pharmacopœia. This method (B.P. 1948, page 771) reads as follows:—"Macerate 5 g. of the air-dried drug, coarsely powdered, with 100 ml. of chloroform water in a closed flask for twenty-four hours, shaking frequently. Filter rapidly, evaporate 25 ml. of the filtrate to dryness in a tared flat-bottomed dish, dry at 100° and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug."

The examination of numerous samples of aloes by this method has revealed none which complies with the minimum requirement of the B.P. In fact, however, the method is quite unsuitable for aloes. In the first place, the result obtained depends upon the degree of comminution of the sample tested, as is illustrated by the figures in Table I. Moreover, prolongation of the period of maceration beyond 24 hours leads to an increase in the yield of extract. Secondly, practical experience, particularly with Cape aloes, will show that on mixing with water the powdered aloes agglomerates into a sticky mass, which no amount of shaking will break up. Thirdly, the result, even when using very fine powder, is substantially lower that the total amount of cold water soluble matter actually present in the drug. There is an additional practical objection to this method, in that the 25 ml. of solution evaporated will contain something approaching 1 g. of solid matter, and to dry this to constant weight, in a dish of reasonable size, will require a very long time.

In order to obtain a fairer estimate of the amount of water-soluble extractive in the drug, a modified method was, therefore, devised. This method, which for convenience will be referred to later as method C, is as follows:—"To 1 g., finely powdered, in a stoppered flask, add 50 ml. of water at 80° C. and shake until dissolved as completely as possible. Cool, dilute to 100 ml. with water and shake. Set aside, at room temperature, overnight. Add 1 g. of kieselguhr, shake well and filter. Evaporate 25 ml. of the clear filtrate to dryness in a tared flat-bottomed dish, dry at 100°C. for 3 hours and weigh."

In the application of this procedure, the hot water takes the aloes into solution almost completely and on subsequent cooling and dilution, matter not soluble in the cold separates. This means that the difficulty of aggre-

gation mentioned above, is completely avoided and it may reasonably be assumed that all matter present, which is soluble in cold water will be taken up. The mixture is left overnight to allow adequate time for the separation of any material not soluble in the cold and kieselguhr is added to ensure that the solution obtained on filtering is perfectly clear. The total weight of extractive obtained is about 0·2 g. and experience shows that this may be dried to constant weight in 3 hours, using a dish about 6 cm. in diameter. Table I shows that this method does yield results which are considerably higher than those obtained by method A. Farther, the result is independent of the fineness of the powder used.

TABLE I

WATER-SOLUBLE EXTRACTIVES OF ALOES

Curaçao Aloes

Fineness of powder	Method A			Method C
	Macerated 24 hr. per cent.	Macerated 48 hr. per cent.	Macerated 6 days per cent.	per cent.
10/44	63·8	66·5	69·4	81·7
44/85	66·0	68·0	70·0	82·5
85/150...	67·6	68·6	70·7	82·7
150	70·0	70·5	70·6	82·6

Cape Aloes

Fineness of powder	Method A			Method C
	Macerated 24 hr. per cent.	Macerated 48 hr. per cent.	Macerated 6 days per cent.	per cent.
10/44	54·7	57·2	58·2	78·2
44/85	54·8	57·8	58·1	79·0
85/150...	55·8	57·7	57·9	78·9
150	57·3	58·3	58·4	78·6

Note.—The designations of fineness of powder in the above table follow the rules laid down in the B.P. Appendix XX (p. 833).

Having demonstrated that method of extraction C does, in fact, get significantly more water-soluble material out of aloes than does method A, the question arises whether this extra extractive is of an active nature or whether it consists merely of inert material, which would be better excluded from the assay. It may be taken that the hydroxymethyl-anthraquinone derivatives which it contains are responsible, at least in great part, for the activity of aloes. According to recent work by Brody, Voight and Maher[1] with Curaçao aloes, these derivatives are aloe-emodin, isoemodin and their anthranols, which exist in the drug both free and in the form of glycosides. From Fairbairn's work[2] on other anthraquinone-containing drugs, it may be inferred, further, that it is the glycosides which form the active fraction. It was, therefore, thought that some guidance might be obtained on this problem, by estimating the

amount of combined hydroxymethylanthraquinones present in the extractive obtained by the two methods. Such estimations were carried out on the solutions obtained by methods A and C from the aloes referred to in Table I. The method used was substantially that of Kussmaul and Becker[3], with the exception that the sodium bicarbonate extraction stage was omitted, since aloe-emodin and isoemodin are not soluble in sodium bicarbonate solution. The amount of anthraquinone derivatives present was determined by reference to a calibration curve prepared from aloe-emodin and the concentration of combined anthraquinones in the extract was calculated. The results of these experiments are given in Table II; details of the experimental procedure are recorded below.

TABLE II

AMOUNT OF COMBINED ANTHRAQUINONE (AS ALOE-EMODIN) IN EXTRACTS MADE FROM ALOES BY VARIOUS METHODS

Curaçao Aloes (150)

	Extract from 100 g. of drug g.	Combined anthraquinones, as aloe-emodin, in extract	
		per cent.	Total g.
Method C	82·6	1·44	1·190
Method A	70·0	1·27	0·890
Extra extractive obtained by method C ...	12·6	2·38	0·300

Cape Aloes (44/85)

	Extract from 100 g. of drug g.	Combined anthraquinones, as aloe-emodin, in extract	
		per cent.	Total g.
Method C	79·0	0·71	0·56
Method A	54·8	0·57	0·315
Extra extractive obtained by method C ...	24·2	1·01	0·245

The figures in Table II clearly prove that method C takes out not only more total extractive, but also a larger amount of combined anthraquinones: indeed the extra extractive obtained by the use of method C is considerably richer in combined anthraquinones than is the total extract obtained by method A.

From all these facts it seems fair to suggest that attempts to assay aloes by the present B.P. method should be abandoned and the modified method (Method C) should be used instead. In Table III are recorded the results of assay of numerous lots of Curaçao and Cape aloes carried out during the last four years by this method. They show that the present B.P. limit of not less than 75 per cent. is a fair one, provided the suggested method of assay is employed. Figures for Socotrine aloes are also included in Table III; they show the inferiority of this variety of aloes in comparison with the official varieties.

TABLE III

WATER-SOLUBLE EXTRACTIVE OF ALOES

Curaçao per cent.	Cape per cent		Socotrine per cent.
83·0	83·2	76·1	71·5
68·0	75·0	81·5	62·7
84·7	86·4	67·8	75·3
81·5	76·8	75·7	78·7
82·0	79·0	80·3	57·3
76·0	72·5	81·2	65·6
82·0	78·8	—	71·0
78·0	82·2	80·9	54·2
78·8	74·1	82·8	61·5
80·0	86·5	83·3	62·6
78·7	76·3	82·5	63·5
78·8	81·8	79·8	67·8
75·8	81·5	76·3	68·9
76·1	78·2	85·0	76·9
76·4	80·8	79·1	—
80·3	—	—	—

DRY EXTRACTS OF CASCARA SAGRADA AND KRAMERIA

In the British Pharmacopœia 1948 a special method for the determination of water-soluble extractive in dry extracts of cascara and krameria (Method B, page 771) is included which reads as follows: "Add 5 g. to 50 ml. of water at 80°C. in a stoppered flask. Shake well and allow to stand for 10 minutes; cool to 15°C. and add 2 g. of kieselguhr; filter. Transfer 5 ml. of the filtrate to a tared evaporating basin 7·5 cm. in diameter, evaporate the solvent on a water-bath, continue drying for half an hour, finally dry in a steam oven for two hours and weigh the residue. Calculate the percentage of water-soluble extractive with reference to the air-dried drug."

As has already been pointed out by Deane and Mitchell[4], this method (which is referred to below as method B) leads to fallacious results, since the total volume of a mixture of 5 g. of one of these dry extracts and 50 ml. of water is about 53 ml., an unknown fraction of which is occupied by the insoluble residue. Samples tested by this method would therefore be expected to give an apparent water-soluble extractive which is considerably lower than the true figure. Moreover, there is a practical difficulty in carrying out the B.P. procedure, because one has either to measure 50 ml. of water accurately at a temperature of 80°C. or, having measured the water in the cold, to heat it to 80°C., while avoiding loss by evaporation.

Method C is free from these objections because the mixture is diluted to a definite volume and, since only 1 g. of the drug (80 per cent. or more of which should be soluble) is dissolved in a volume of 100 ml.

the volume of the insoluble residue can safely be ignored. Farther, small variations in the volume of the hot water added to the extract make no difference to the result, and thus the difficulty of accurate measurement of the water at 80°C. is removed. In these circumstances it was thought worth while to try to apply method C to dry extracts of cascara and krameria. At the same time some interesting facts about changes in the water-soluble extractive of dry extract of cascara storage have been elicited.

Dry Extract of Cascara Sagrada B.P.

Twelve samples of dry extract of cascara B.P., prepared during the preceding 18 months, and originally examined by method B, were re-examined by this method and also by method C. The results are shown in Table IV. This table also shows the losses which occur on drying the extracts to constant weight at 100°C., both initially and after storage. In the last column, the water-soluble extractive determined by method B is shown as a percentage of that obtained by method C.

TABLE IV

DRY EXTRACT OF CASCARA SAGRADA B.P.

Sample No.	Original analysis			Second analysis				Difference in extract per cent.	Difference in loss per cent.	Method B / Method C × 100
	Date	Extract Method B per cent.	Loss at 100°C. per cent.	Date	Extract		Loss at 100°C. per cent.			
					Method B per cent.	Method C per cent.				
C1 ...	7/4/49	86·4	3·0	13/9/50	79·9	84·5	9·9	−6·5	+6·9	94·6
C2 ...	28/6/49	87·8	2·9	24/8/50	81·7	86·3	9·8	−6·1	+6·9	94·6
C3 ...	9/9/49	88·0	3·2	13/9/50	82·2	87·0	8·4	−5·8	+5·2	94·5
C4 ...	24/10/49	86·2	2·3	2/10/50	81·0	84·8	8·7	−5·2	+5·4	95·5
C5 ...	4/1/50	83·4	1·8	21/8/50	78·5	83·4	7·8	−4·9	+6·0	94·1
C6 ...	2/3/50	87·7	1·9	11/8/50	81·4	86·0	6·7	−6·3	+4·8	94·6
C7 ...	24/3/50	85·8	2·2	16/8/50	81·0	84·8	6·0	−4·8	+3·7	95·5
C8 ...	1/5/50	86·5	2·3	2/10/50	83·4	87·2	4·8	−3·1	+2·5	94·5
C9 ...	24/5/50	89·0	1·8	4/8/50	86·5	90·4	4·5	−2·5	+2·7	95·7
C10	11/7/50	84·0	1·8	21/8/50	80·5	85·0	6·4	−3·5	+4·6	94·7
C11	27/7/50	83·0	1·5	2/10/50	79·0	83·4	5·9	−4·0	+4·4	94·7
C12	11/8/50	87·4	2·2	24/8/50	85·6	89·8	4·5	−2·4	+2·3	95·3

The figures for water-soluble extractive, as determined by method C, may be taken as very close to the true amount of water-soluble material present in the drug. Table IV shows that the present B.P. method indicates only about 94 to 96 per cent. of this value. The table also shows that on storage dry extract of cascara absorbs moisture, resulting in an increase in the loss of drying at 100°C. and a roughly corresponding decrease in the water-soluble extractive. This means that a dry extract of cascara which complies with the requirements of the Pharmacopœia when prepared may fail to do so after storage.

Cascara Tablets. Dry extract of cascara sagrada is usually dispensed in the form of tablets. Such tablets are included in Part VI of the British Pharmaceutical Codex 1949, but no standard for water-soluble extractive is there mentioned. Since, however, these tablets can be prepared by direct compression of the granular dry extract with no addition, other than a small proportion of some substance to act as lubricant, it could be expected that these tablets, after removal of the coating, would comply, or at least come very close to complying, with the official standard for water-soluble extractive of dry extract of cascara. Fifteen samples of cascara tablets, of nominal size 2 or 5 grains, all sugar coated, were obtained from different manufacturers. The coatings were removed by a method similar to that described by Berry and Temple[5] and determinations of water-soluble extractive by methods B and C, and loss at 100°C. were made on the crushed tablets. The average weights of the tablets, freed from coating, were also ascertained and a search made microscopically for starch or other extraneous substances. The results are reported in Table V.

TABLE V

CASCARA TABLETS

Sample	Nominal Size grains	Average Weight g.	Maize Starch	Loss at 100°C. per cent.	Extract		Method B / Method C × 100
					Method B per cent.	Method C per cent.	
T1	2	0·131	–	9·1	75·9	79·5	95·5
T2	2	0·148	+	8·9	69·9	73·4	95·4
T3	2	0·140	–	8·2	75·2	79·8	94·1
T4	2	0·134	–	7·1	57·4	60·3	95·2
T5	2	0·165	+	7·5	50·6	54·2	93·4
T6	2	0·140	–	8·0	77·4	81·6	94·8
T7	2	0·179	+	8·7	73·8	78·8	93·7
T8	2	0·146	–	9·1	74·8	79·7	93·9
T9	2	0·141	–	7·4	70·6	74·2	95·2
T10	5	0·293	trace	9·5	78·9	84·0	93·9
T11	2	0·167	+	10·7	73·2	77·2	94·8
T12	2	0·156	+	12·1	63·4	67·5	93·9
T13	2	0·128	–	8·6	74·7	78·2	95·5
T14	2	0·129	–	8·3	75·5	80·0	94·3
T15	5	0·328	–	7·3	77·6	81·8	94·9

A number of observations may be made from the figures contained in this table. In the first place, the relationship between the two methods of determining water-soluble extractive is confirmed—method B giving results which, for these tablets, are from 93·4 to 95·5 per cent. of those obtained by method C. Secondly, not one of the tablets examined has a water-soluble extractive, as determined by the present B.P. method, which meets the standard for dry extracts of cascara. However, it is also true that all contain considerably more moisture than is usually

found in freshly prepared dry extract of cascara. If one calculates the original water-soluble extractives, on the assumption of an original water content of 2 to 2·5 per cent., which is the average for the freshly prepared extracts included in Table IV, it will be found that 9 of the 15 samples (T1, T3, T6, T8, T10, T11, T13, T14 and T15) have over 80 per cent. 5 of the tablets, T2, T5, T7,T11 and T12, contain considerable quantities of maize starch, T10 contains a small amount only. A possible explanation of the presence of this starch is that the tablets have been prepared from Dry Extract of Cascara U.S.P., which is diluted with maize starch, but a study of the average weights of the tablets suggests that it is more likely that the starch has been deliberately added as a separate ingredient. For 3 of the 5 remaining tablets, T2, T7 and T12, the presence of this starch, if so added, could account for this failure of the whole contents of the tablet to show a water-extract of over 80 per cent.; the weights of the individual tablets being sufficiently in excess of the nominal 0·130 g. to make this possible.

In an effort to find out more about the sort of analytical results which may be expected for cascara tablets, some further samples of tablets, both coated and uncoated, were examined, some of which were known to have been prepared from extracts included in Table IV. The results are in Table VI. These tablets include approximately 2·5 per cent. of lubricant. The analytical results for the extracts mentioned in the third column of this table will be found in Table IV.

TABLE VI

CASCARA TABLETS

Sample	Date Made	Extract	Uncoated			Coated		
			Extract		Loss at 100°C.	Extract		Loss at 100°C.
			Method B per cent.	Method C per cent.	per cent.	Method B per cent.	Method C per cent.	per cent.
B1 ...	7/50	C9	—	—	—	83·9	87·5	4·1
B2 ...	4/50	C6	80·0	84·5	6·1	80·1	84·5	6·0
B3 ...	1/50	C5	76·2	80·7	6·6	75·6	80·0	7·0
B4 ...	9/48	—	—	—	—	73·8	77·3	5·7

These results, in general, follow the expected lines. The figures for the last sample (B4), however, do suggest a possibility that, during the long period of storage of these tablets (about 2 years), some slight decrease in solubility of the extract has taken place, as the loss found (5·7 per cent.) is insufficient to account for the present low value of the water-soluble extractive. The original water-soluble extractive content of the extract from which these tablets were prepared is unfortunately not known exactly, although it may be taken that it was greater than 80 per cent., by method B.

DRY EXTRACT OF KRAMERIA B.P.

Five samples of dry extract of krameria, prepared by different manufacturers, were examined : determinations were made of the water-soluble

extractives by methods B and C and also of the loss on drying to constant weight at 100° C. Table VII shows the results of these experiments. In the final column of this table, the water-soluble extractive determined by method B is given as percentage of that obtained by method C.

TABLE VII

DRY EXTRACT OF KRAMERIA B.P.

Sample	Date of Analysis	Extract		Loss at 100°C. per cent.	Method B / Method C × 100
		Method B per cent.	Method C per cent.		
K1	17/7/50	73·4	82·8	6·67	88·5
K2	5/10/50	74·5	86·4	7·60	86·3
K3	5/10/50	78·6	88·4	6·70	91·0
K4	16/10/50	63·5	73·5	8·94	86·3
K5	16/10/50	73·9	86·4	3·20	85·5

It is at once apparent that, using dry extract of krameria, the water-soluble extractives, by method B, are considerably lower, relative to those by method C (only 85 to 91 per cent.) than was the case with dry extracts of cascara; considerably lower, indeed, than can be explained by the discrepancy between the assumed and true volumes of the solution in method B. This suggests that dry extract of krameria contains some substance which is only slightly soluble in water and that, in the present B.P. method, the volume of the water used is insufficient to take this ingredient entirely into solution. In an attempt to test this hypothesis, further extractions were made of the samples K1 to K5 following method C, but using 5 g. or 0·5 g. in place of 1 g. The results of these tests are reported in Table VIII, the original results by methods B and C being again included for comparison.

TABLE VIII

DRY EXTRACT OF KRAMERIA B.P. WATER-SOLUBLE EXTRACTIVES

Sample	Method B Approximately	Method C		
	1 in 10 per cent.	1 in 20 per cent.	1 in 100 per cent.	1 in 200 per cent.
K1	73·4	77·1	82·8	83·9
K2	74·5	78·9	86·4	88·0
K3	78·6	82·6	88·4	90·0
K4	63·5	67·5	73·5	74·4
K5	73·9	78·4	86·4	87·8

These figures appear to confirm the presence of a comparatively insoluble substance. Indeed, it is clear that even at a concentration of 1 in 100, which is that of the recommended method C, the whole of this constituent is not in solution. Nevertheless, it is suggested that, for practical purposes, extraction at 1 in 100 shall be adopted, since the use of still more dilute solutions would be inconvenient and liable to

lead to inaccuracies and, in any event, the difference between the results at 1 in 100 and 1 in 200 are not substantial.

Experimental

The details of the method used to determine the combined hydroxymethylanthraquinones in aloes are as follows:—To a suitable volume of the solution add a few drops of dilute hydrochloric acid and extract repeatedly with ether until the ether is no longer coloured. Mix the ether layers and wash with two quantities, each of 5 ml., of water. Mix the aqueous layer and washings and dilute to a suitable volume. To an aliquot of this solution equivalent to 0·2 g. of the original aloes add half its volume of hydrochloric acid and heat in a boiling water-bath for 15 minutes. Cool the solution, make just alkaline with solution of sodium hydroxide and wash into a separator. Make just acid with dilute hydrochloric acid and extract with ether till the ether solutions are no longer coloured. Combine the ether layers and extract with three quantities each of 5 ml. of N sodium hydroxide, collecting the aqueous layers in a boiling tube. To the contents of this tube add 0·3 ml. of solution of hydrogen peroxide (10 vol.) and heat in a boiling water-bath for exactly 4 minutes. Cool rapidly, transfer to a graduated flask and dilute to 20 ml. with N sodium hydroxide. Determine the colour of the resulting solution in a suitable colorimeter.

The calibration curve was prepared from a solution of pure aloe-emodin in N sodium hyroxide, suitable volumes of which were diluted to 15 ml. with N sodium hydroxide, oxidised as described above and finally diluted to 20 ml. The colours of the resulting solutions were read under the same conditions as those used for the aloes solutions.

Summary and Conclusions

1. It has been shown that the methods at present in use in the B.P. for the determination of water-soluble extractive in aloes and in dry extract of cascara and krameria are not satisfactory.

2. A modified method has been described which is suitable for use with all these three drugs and is free from the objections to the present methods.

3. The adoption of this modified method would not call for any alteration in the existing B.P. standards, but some allowance might need to be made for the fact that dry extract of cascara absorbs moisture during storage. The obvious way of doing this would be to calculate the water-soluble extractive with reference to the drug dried at 100°C.

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