THE COLORIMETRIC DETERMINATION OF SANTONIN IN ARTEMISIA

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A new colorimetric method for the estimation of santonin in artemisia using hydroxylamine and ferric chloride has been evolved and compared with a gravimetric and a volumetric method. Improvements in the method of extraction of santonin have also been proposed.

Several species of artemisia are grown on a commercial scale in the north-western region of West Pakistan and their unexpanded flowerheads are utilised in the manufacture of santonin which is used as an anthelmintic and is official in the British Pharmacopoeia[1].

A number of methods of assay[2–4] of this drug based on the gravimetric estimation of santonin have been described where use is made of a correction factor calculated on the basis of the solubility of pure santonin in the strength of ethanol employed for crystallisation. These corrections appear to be arbitrary and throw doubts on the accuracy of the gravimetric methods. The first method of estimation independent of a correction factor was proposed by Bohme[5] where, after extraction, santonin was determined volumetrically. This method, however, lacked specificity and could only be used for artemisia rich in santonin. Moreover, according to Bohme himself, the results were always higher than the gravimetric method.

Several methods[6–8] based on the colour reactions of santonin have been reported. In Fucci's[6] method, santonin is estimated by the colour of the sodium salt in strongly alkaline solution, but the method does not appear to possess a quantitative basis. Iwayama[7] utilises the colour reaction between an acidic solution of santonin and diazobenzene sulphonic acid, but the assay of artemisia is not described. Yamagishi and his co-workers[8] make use of the reaction with sodium methoxide for quantitative purposes but the results are imprecise because of variations in the sodium methoxide reagent; atmospheric moisture also appears to increase the experimental error.

A need clearly exists for a simple, rapid and accurate method applicable to artemisia of different santonin contents and independent of any arbitrary correction. We have evolved a new method based on Fiegl's[9] colour reaction for esters and lactones which has been applied quantitatively by Hestrin[10] and Wollish and Schmall[11]. The lactone, santonin (I) was treated with alkaline hydroxylamine and the hydroxamic acid derivative (presumably II) so formed was then treated with ferric chloride to give a purple-coloured solution of the ferric hydroxamate derivative (presumably III). The colour was found to be suitable for spectrophotometric determination. The reactions are shown in Figure 1.

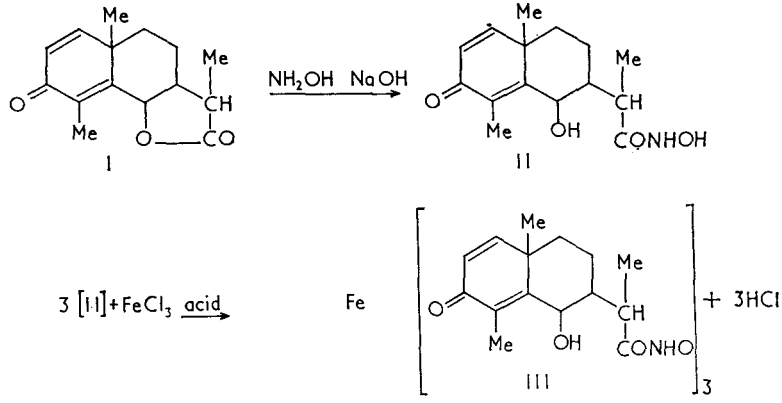

FIG. 1

We extracted santonin from the crude drug by Massagetov's method[2] and after removing the colouring matter, developed the colour in the same manner as on pure santonin. Five different samples of the drug were used for a comparison of Massagetov's gravimetric method *loc. cit.* and Bohme's[5] volumetric method with our modifications.

EXPERIMENTAL

Colorimetric Method.

Apparatus. Unicam Spectrophotometer, SP600; reflux condenser, ground-glass joints; flat bottom flasks 250 ml. ground-glass joints; steam bath; 1,000 ml. separating funnels.

Reagents. Standard solution of santonin B.P. in 50 per cent ethanol (1 mg./ml.); hydroxylamine sodium hydroxide solution: 7·5 g. in 100 ml. N sodium hydroxide freshly prepared; 2·0 per cent ferric chloride solution in water; 2,4-dinitrophenol indicator, 0·1 per cent 2,4-dinitrophenol in 90 per cent ethanol; N sodium hydroxide; N hydrochloric acid; ethanol, 95 per cent v/v 50 per cent v/v and 18 per cent v/v; calcium oxide; chloroform; hydrochloric acid, sp. gr. 1·18; sodium hydroxide solution 4 per cent w/v.

Standard Curve. Measure by pipette 1, 2, 3, 4 and 5 ml. quantities of the standard santonin solution into separate 50 ml. volumetric flasks. Add 2 ml. of hydroxylamine-sodium hydroxide solution and then 5 ml. of standard N sodium hydroxide to each flask. Allow to stand for 5 minutes, add three drops of 0·1 per cent 2,4-dinitrophenol indicator and titrate the solution carefully with standard N hydrochloric acid to a colourless end point. Make each flask up to 50 ml. with water. Place 5·0 ml. of each solution in a dry test tube, add 1 ml. of 2 per cent ferric chloride solution and mix thoroughly by shaking. Read the extinction within 3 minutes on a spectrophotometer at 500 mμ in 1 cm. cell, using water as blank, taking care that the cell is free from air bubbles. Carry out a reagent blank using 2 ml. of 50 per cent v/v ethanol in place of the santonin solution.

The standard curve is shown in Figure 2. Blank readings are usually between 0·007 and 0·010.

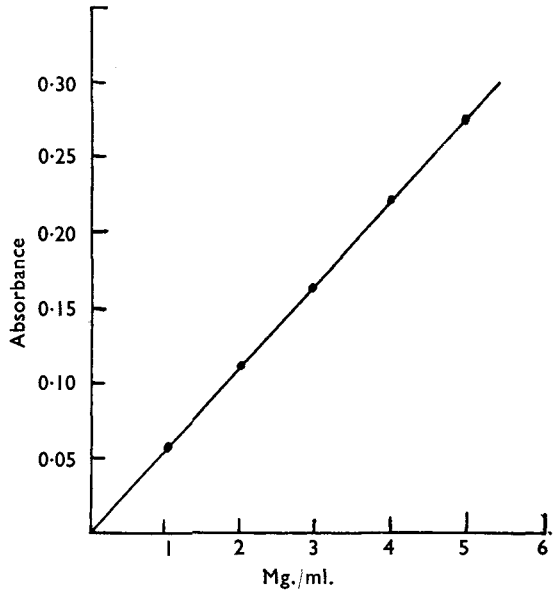

FIG. 2. Standard curve for santonin.

Preparation of sample. Weigh accurately about 2 g. of finely powdered flowerheads, grind them in a mortar with 0·5 g. of calcium oxide and then triturate with 5 ml. of hot water. Transfer the mixture to a 400 ml. beaker with 120 ml. of hot water, bring to the boil and continue boiling for 10 minutes. Filter hot and wash the residue with a further 120 ml. quantity of boiling water. Reject the residue, acidify the warm filtrate with 10 ml. of hydrochloric acid and place the beaker containing the acidified extract on the steam bath for 5 minutes. Cool and extract with 30, 20, 20 and 10 ml. portions of chloroform with vigorous agitation. Shake the combined chloroform extract twice in a separating funnel with 15 ml. of 4 per cent sodium hydroxide solution. Wash the chloroform extract with 10 ml. of water to remove the alkali, and pass through a pledget of cotton wool into a 250 ml. flask. Add 0·1 g. of animal charcoal, connect the flask to a reflux condenser on a water bath and boil gently for 10 minutes. Filter rapidly through a double filter paper (Whatman No. 41), into another 250 ml. flask and wash the first flask with two 5 ml. quantities of chloroform. Distill the chloroform and gently dry the residue. Add 2 ml. of ethanol to the residue and again evaporate to dryness to ensure complete removal of chloroform. Add 20 ml. of 18 per cent v/v ethanol and 0·1 g. animal charcoal. Connect the flask to a reflux condenser on a steam bath and boil gently for 10 minutes. Filter rapidly whilst hot through a double filter paper into a 50 ml. volumetric flask. Wash the flask twice with 5 ml. portions of hot 18 per cent v/v ethanol. Dilute to

TABLE I

COMPARISON OF THE COLORIMETRIC, VOLUMETRIC AND GRAVIMETRIC METHODS FOR THE ASSAY OF SANTONIN

Sample	S. No.	Colorimetric per cent	Melting Point °C.	Volumetric per cent	Melting Point °C.	Gravimetric per cent	Melting Point °C.	Deviation per cent: Colorimetric	Deviation per cent: Volumetric
A	1	1·82	171·0	1·88	171·0	1·82	171·0		
	2	1·85	171·0	1·88	171·0	1·82	171·0		
	3	1·83	171·5	1·88	171·5	1·81	171·0		
	Average	1·83		1·88		1·82		+0·6	+3·3
B	1	2·13	171·5	2·19	171·5	2·09	171·0		
	2	2·17	172·0	2·22	172·0	2·19	173·0		
	3	2·18	172·0	2·22	172·0	2·15	171·5		
	Average	2·16		2·21		2·14		+0·9	+3·3
C	1	2·25	171·0	2·37	171·0	2·25	172·0		
	2	2·25	172·0	2·31	172·0	2·21	173·0		
	3	2·22	171·0	2·25	171·0	2·25	172·0		
	Average	2·24		2·31		2·24		Nil	−3·1
D	1	1·95	172·0	2·00	172·0	1·93	171·0		
	2	1·90	173·0	1·97	173·0	1·90	172·0		
	3	1·95	171·0	1·97	171·0	1·97	172·0		
	Average	1·93		1·98		1·93		Nil	+2·6
E	1	2·86	172·0	2·95	172·0	2·82	172·0		
	2	2·83	172·0	2·92	172·0	2·78	172·0		
	3	2·87	171·0	2·92	171·0	2·86	173·0		
	Average	2·85		2·93		2·84		+0·4	+3·2

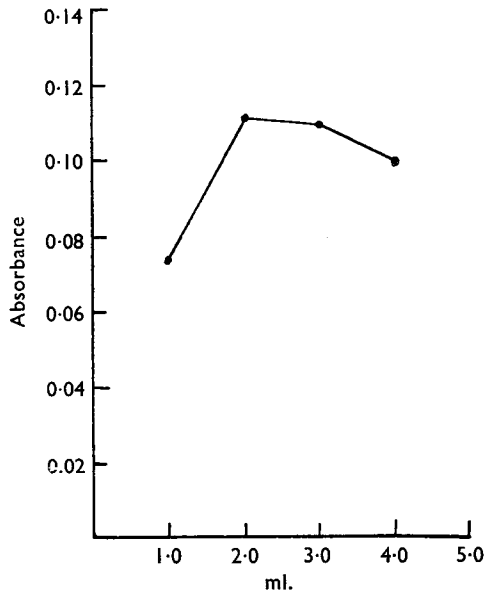

FIG. 3. Effect of varying the amounts of Hydroxylamine sodium hydroxide reagent on the development of colour.

volume with 95 per cent v/v ethanol. Pipette 3 ml. of the solution and develop the colour exactly as described under the method for the standard curve. Measure the extinction, read the concentration of santonin from the standard curve and calculate its percentage in the sample.

Volumetric Method

Weigh accurately about 5 g. of the drug and proceed for extraction as described above varying the quantities of reagents and solvent proportionately. After the complete removal of chloroform, dissolve the residue in 5 ml. of hot ethanol, add 10 ml. of 5 per cent w/v barium hydroxide solution and heat on a water bath for 15 minutes. Cool and filter through filter paper (Whatman No. 41), and wash the residue with 10 ml. of water. Add two drops of phenolphthalein indicator to the filtrate and titrate carefully with 0·5 N hydrochloric acid to a colourless end point. Add 10 ml. of 0·1 N hydrochloric acid by means of a pipette and heat on a water bath for 15 minutes. Cool and titrate with 0·1N sodium hydroxide from a fine burette until the red colour persists for 1 to 2 minutes. Carry out a blank on 10 ml. of the 0·1N hydrochloric acid and calculate the percentage of santonin in the drug as follows,

$$\frac{a \times 246{\cdot}1 \times 100}{5 \times 10{,}000}$$

where a is the difference in the titer between blank and the sample.

Gravimetric Method

Proceed for extraction as described in the volumetric method above to the complete removal of chloroform. Dissolve the residue in 2 ml. of hot ethanol, add 100 ml. of boiling water and then concentrate to 50 ml. on a water bath. Place the flask in a refrigerator (maximum temperature 10°) for 2 days. Filter the crystals of santonin which separate on a sintered glass crucible (No. 4), measure the exact volume of the filtrate, and dry at 100°, to constant weight. To the weight found add 0·0002 g. for each ml. of the filtrate and calculate the percentage of santonin in the sample.

The results on the five samples are summarised in Table I. The melting points of the crystals obtained in the colorimetric, volumetric and the gravimetric methods are also given for each determination.

Discussion

Extraction of Santonin

We chose Massagetov's method of extraction in preference to the methods of Qazilbash[3] and Kassner and others[4], because of its simplicity of operation and economy in time and solvents. The melting point determinations on the extracted santonin agreed closely with those reported by these workers on santonin extracted by their methods. We found, however, that Massagetov's method of extraction needed some improvement. The residue on being dissolved in 95 per cent v/v ethanol still contained coloured impurities which interfered in the colorimetric determination. It was therefore dissolved in hot 18 per cent v/v ethanol and

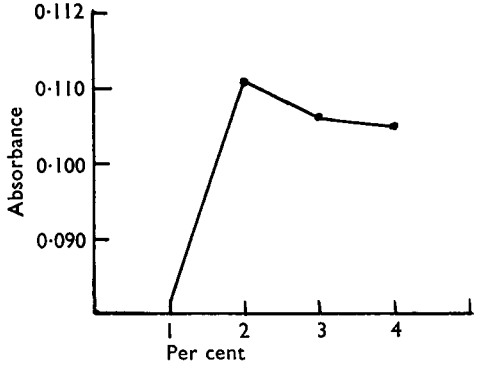

FIG. 4. Effect of varying the amount of Ferric chloride reagent on the development of colour.

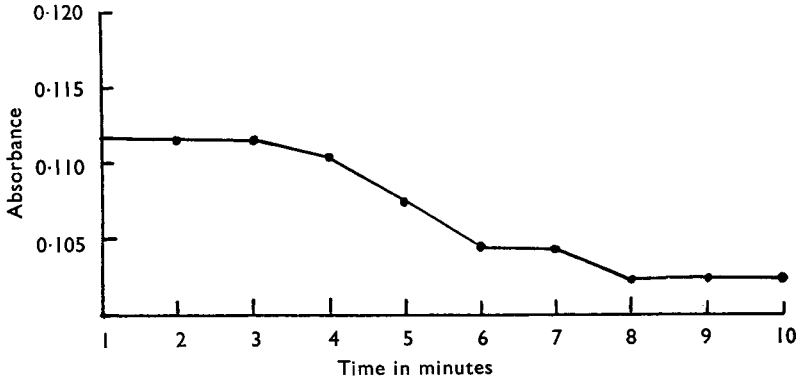

FIG. 5. Stability of the colour.

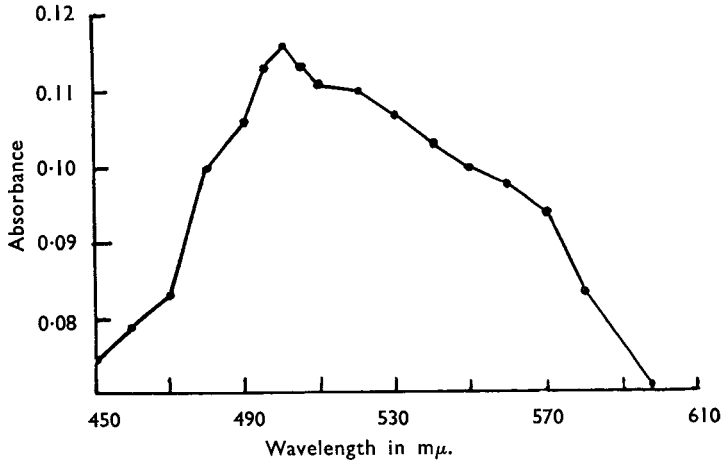

FIG. 6. Absorption curve of the developed colour.

refluxed with a further quantity of charcoal. After hot filtration, the santonin crystallised out partially but could be taken up in solution by addition of 95 per cent v/v ethanol, this time giving a clear colourless solution. On being made up to volume by water so that the final strength of ethanol was approximately 50 per cent v/v, santonin remained in solution and it was possible to develop colour on an aliquot of this solution.

Development of Colour

For the conditions affecting the development of colour from santonin we were guided by those adopted by Wollish and Schmall[8] for pantoyl lactone. The effect of varying the amounts of hydroxylamine reagent showed that 2·0 ml. of this reagent gave the maximum colour intensity (see Fig. 3). Similarly we varied the amount of ferric chloride and found that 1 ml. of 2·0 per cent ferric chloride gave the best colour as is shown in Figure 4. With the quantities of these reagents fixed, we investigated the stability of the developed colour and found that maximum intensity was reached immediately on addition of the ferric chloride and remained constant for 3 minutes. Thereafter fading took place as shown in Figure 5. The absorption curve of the developed colour was then determined and the maximum located at 500 mμ as shown in Figure 6.

The initial colorimetric determinations were made on 5 g. of the drug. After fully establishing the conditions and standardising the procedure, the quantity of the drug was reduced to 2 g. with proportionate reductions in the quantities of the reagents and solvents. The results were almost identical with those found on 5 g. of the drug.

From the standard curve it is seen that Beer's Law is obeyed with up to 5 mg. of santonin in the aliquot of the final solution.

Results and Conclusions

Table I shows that the results on the five samples by the proposed colorimetric method compare well with those given by the gravimetric method (mean deviation less than 1·0 per cent). The volumetric method gives a larger deviation, between 2·6 and 3·3 per cent. In agreement with the findings of Bohme[5] results tend to be high. Also, the low quantity of santonin in relation to the final alkali titration, would necessitate the use of a larger quantity of the drug. For artemisia deficient in santonin, the volumetric method is of little use.

Apart from the general merits of the colorimetric method over the gravimetric estimation, the former requires no arbitrary correction. It is economical in solvents since extraction can be made on smaller quantities of the drug. Also, the colorimetric method much reduces the total time for the determination from the 2 days required for complete crystallisation in the gravimetric method.

The main advantage of the colorimetric method over the existing ones lies in its ability to determine santonin in quantities as low as 1 mg. in the final aliquot and can be employed for the assay of low-grade artemisia for which, so far, no other suitable method is available.

Other lactones like artemisin, α-santonin and ψ-santonin present in artemisia are also determined by the colorimetric method. This, however, is of little consequence as these lactones are present in minute quantities and are known to have anthelmintic properties similar to santonin[12].

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