A NOTE ON THE ASSAY OF ARTEMISIA

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Although Artemisia is no longer an official drug, its assay is of some importance commercially since it is used in such large quantities for the manufacture of santonin. Numbers of samples are received each year in this laboratory, the majority coming from India; the santonin content of recent samples has varied from nil to about 3·3 per cent. The quantitative separation of santonin in a pure state presents many difficulties due to the presence of much resinous material in the drug, more especially in the poorer grades. The method in use in this laboratory until recently was similar in principle to that of the British Pharmaceutical Codex, 1934. Various modifications in technique have been introduced from time to time, resulting in a procedure which we thought eminently satisfactory from many points of view. Nevertheless, it has constantly been our interest to seek to arrive at a process free from some of the obvious difficulties.

The essentials of this method were as follows:—

(1) The use of relatively large amounts of drug to ensure that the santonin extracted shall be of a reasonable weight in relation to the correction used. For Grade A samples, 25 g. is taken, for Grade B, 35 g., in order to obtain a yield of about 450 to 500 mg.

(2) Percolation of the weighed quantity of drug to exhaustion with chloroform, a method we consider far more satisfactory than maceration and the taking of an aliquot portion.

(3) Treatment with an excess of barium hydroxide solution by boiling under a reflux condenser before removal of the chloroform by distillation, and this repeated on the cotton wool plug used for filtration, which is afterwards well washed when the chloroform has been distilled.

(4) As great an accuracy as possible in the weighing of the final solvent (ethanol 15 per cent. w/w) and very rapid filtration, during which heat is maintained throughout. The resulting filtrate contains the resin suspended in a very fine state of division and thus separation of the crystals of santonin from this is comparatively easy.

(5) Recrystallisation from the same solvent under carefully controlled conditions, guaranteeing a santonin of good colour and high melting point. If the melting point of the crystals obtained is below the limit of the British Pharmacopœia, the product is rejected and the assay is repeated, varying either the amount of drug taken or the quantity of ethanol used for the crystallisation.

The criticism which can be levelled against any assay method may be, of course, that it is only as good as the operator. This process requires a considerable experience of its operation and a high degree of skilful manipulation; under such conditions concordant results are obtained.

We tried, from time to time, other methods involving such radical changes as the use of hot extraction with chloroform or benzene, and treatment of the extracted material, after the chloroform shake-out, with ammonia, but found nothing which we considered as satisfactory as our own method.

A survey of methods in use prior to 1932 was made by Coutts[1] and his chief criticism of these was of the use of dilute ethanol for the last stage, the crystallisation of the santonin. The process he described did not involve the use of this solvent but obviously resulted in a santonin contaminated with resin since its melting point was low. Qazilbash[2], stated that he had used various assay processes and gave full details of his "new proposed method." He published a critical review of many processes[3] in 1952. His own method involves the use of ethanol (15 per cent. w/w) for the final crystallisation and his product had a melting point between 171° and 173° C. Our experience indicates to us that, without the use of this solvent, a santonin of good colour and high melting point cannot be obtained.

At our first opportunity we carried out Qazilbash's process, followed by various modifications of this. The material used for all these determinations was some Indian Grade A Artemisia of which we had a considerable quantity and which had been assayed by our own method in our laboratory by more than one worker, so that its santonin content was known with certainty. A summary of the results obtained is shown in Table I.

TABLE I

Method	Santonin per cent.	Melting point of product °C.
H. and S. (mean of various determinations)	1·80	172·8
Q	1·45*	173·2
A	1·65	Not determined
B	1·84	171·6
C	1·80	172·6
D	1·81	173·2
E	1·86	171·0

*using the correction 0·0064; if the revised correction (0·046)[5] is used, this value becomes 1·84.

By method Q, which is that of Qazilbash unaltered[2], we obtained a very white product of high melting point. We found much to criticise during the carrying out of this determination, noticing at once that his correction appeared to be wrong. We took steps to check the correction which we apply (0·05 g. for the quantity of ethanol (15 per cent. w/w) under the conditions of our assay) and confirmed our figure. We have since noted the letters on this subject by Isaacs[4] and Qazilbash.[5] If it is assumed that Qazilbash was assaying a good Grade A Artemisia containing 2·00 per cent. of santonin, the quantity of material taken for assay (10 g.) would yield a weight of 0·154 g.; in proportion to this figure the revised correction of 0·046 g. to be added is large indeed. By our method (H. and S.) the yield weighed from a similar sample would be 0·450 g. and the correction to be added 0·05 g., the proportion being only about one-third of that above. The use of only 10 g. of material and relying on maceration

for extraction of the santonin did not seem satisfactory to us, nor the mild treatment with barium hydroxide solution; moreover, we hoped that the use of kieselguhr might prove to be unnecessary.

For method A, 30 g. of sample was treated with alkali, macerated with benzene, as in method Q, and an aliquot part (= 24 g.) was taken for the assay. Not all the benzene was removed from this before adding the barium hydroxide solution and the mixture was heated on a water bath under a reflux condenser with constant swirling of the flask. The benzene was then recovered by distillation, the aqueous liquid filtered through a double plug of cotton wool, the upper layer of which was afterwards treated with benzene and barium hydroxide solution, repeating the distillation and filtration. In this way we hoped to obtain a better decomposition with barium hydroxide. Charcoal and kieselguhr were used as before and the resulting santonin was of good colour; unfortunately, by some error, the melting point was not determined.

In method B, a quantity of 25 g., after the preliminary alkaline treatment, was macerated overnight with benzene and percolated to exhaustion. The whole of the benzene was recovered, 100 g. of chloroform added and this twice treated with barium hydroxide solution as detailed above; the process was completed according to our former method. The yield was slightly higher than that obtained by method H. and S. but the product was not quite so good in colour and had a lower melting point.

Method C was carried out as for method B but the last part of the process involved the use of charcoal and kieselguhr as in method A. The result and the product were similar to those obtained by method H. and S.

Method D varied only from the foregoing in that kieselguhr was omitted. The result was close to that which we believe to be the true one for the sample, the hot filtration before the first crystallisation was carried out far more easily in the absence of kieselguhr, the product was white and the melting point slightly higher than that usually obtained by us.

Method E was carried out as for method D but involved the use of chloroform throughout instead of benzene; the result obtained was too high, the santonin being not so good in colour and having a low melting point.

The conclusions we now reached were as follows:—(1) The preliminary treatment with alkali is most valuable in removing, early in the process, a considerable quantity of the resinous matter and in changing the nature of some of the extractive so that it is more easily separable during the barium hydroxide treatment, the small amount remaining giving less trouble at the crystallisation stage.

(2) Benzene, using the method of *cold percolation*, seems a more selective solvent for Artemisia and while extracting all the santonin, removes less colour and other unwanted extractive.

(3) Heating under a reflux condenser, repeated, with barium hydroxide solution and *chloroform* ensures thorough mixing as the boiling chloroform bubbles through the aqueous layer. This eliminates the possibility of incomplete conversion of the santonin into the barium salt. Unlike

Qazilbash[3] we do not believe that any slight decomposition of chloroform, should it occur at this stage, is detrimental, since such a large excess of barium hydroxide is present. The use of benzene here is not an advantage because it forms the upper layer.

(4) Kieselguhr is unnecessary and only hinders filtration, a very fine white product being obtained by the use of charcoal only.

Method D was obviously the most satisfactory modification and has become our routine process; full details follow:—

Weigh 25 g. of bruised* drug into a 500-ml. glass-stoppered flask. Add 2·5 g. of anhydrous sodium carbonate, mix well, then add 25 ml. of 15 per cent. solution of ammonium hydroxide and shake until the drug is uniformly moistened. Add 250 ml. of benzene, shake well, set aside for 3 hours, shaking at intervals; allow to stand overnight. Shake continuously for 15 minutes and pack into a percolator. Percolate to exhaustion using approximately 1 l. of benzene over a period of about 8 hours and collecting the percolate in a tared 600-ml. Erlenmeyer flask. Recover the benzene and evaporate almost to dryness. Make the weight to 100 g. with chloroform and add 250 ml. of saturated barium hydroxide solution; place on a boiling water bath under a reflux condenser for 30 minutes, swirling frequently; distil off all chloroform. Pour through a double plug of cotton wool into a 600-ml. Erlenmeyer flask; immediately add 20 ml. hydrochloric acid to the filtrate; wash the flask and filter with small quantities of boiling water. Place the upper plug of cotton wool with the residue in the flask, add 20 ml. of chloroform and 20 ml. of saturated barium hydroxide solution, boil under a reflux condenser as before for 10 minutes, distil off all chloroform. Filter through the first cotton wool plug and wash the flask and filter with 8 portions of boiling water. Add 5 ml. of hydrochloric acid, swirl well and ascertain that excess of acid is present. Set on a boiling water bath for half an hour, cool, transfer to a separator and extract 6 times with chloroform, washing each shaking with the same water. Recover the chloroform in a 250-ml. flask, remove traces of chloroform with absolute ethanol, dry for half an hour at 100° C. and cool. Weigh the flask and contents, add 7·5 g. of absolute ethanol, warm to dissolve, then add 42·5 g. of boiling water and 0·1 g. of charcoal. Place under a reflux condenser on a water bath and boil gently for 10 minutes. Filter rapidly through a small hardened filter paper with suction, flask, funnel and filter being thoroughly pre-heated and the funnel placed in a steam-jacket. Wash the flask with 2 × 5 ml. of ethanol (15 per cent. w/w), kept hot on a water bath, then wash the filter with 2 × 5 ml. of the same solvent. Set aside overnight to crystallise at laboratory temperature. Collect the crystals on a small hardened filter paper, wash the flask with 2 × 5 ml. of ethanol (15 per cent. w/w). Dissolve from the flask and filter with hot ethanol, collecting in a 100-ml. beaker. Evaporate the ethanol on a water bath, taking care to avoid spurting towards the end.

* It is our experience that thorough bruising of Artemisia is all that is required. Extraction is not accelerated by reducing the drug to fine powder, as recommended by Qazilbash[2] whose method involves maceration only, nor is the necessary volume of solvent lessened.

Dry at 100° C. for half an hour, dissolve in 3·75 g. of absolute ethanol, add 21·25 g. of boiling water, swirl and set aside overnight. Filter off the crystals on a small hardened filter paper, washing the beaker and filter with 3 × 2·5 ml. of ethanol (15 per cent. w/w). Dry in the funnel, brush off the crystals into a small tared glass dish, dry at 100° C. and weigh. Determine the melting point of the crystals. The correction for solubility of santonin in ethanol (15 per cent. w/w), at laboratory temperature, is 0·05 g.

Experience of this method with Grade A samples shows that the same amount of santonin is obtained as by our previous method but that the product is rather better in colour and has a slightly higher melting point. When process D is used for Grade B material (taking 35 g.) in parallel with process H. and S., the figure for santonin content is somewhat higher owing to a better separation from the resins which are present in greater proportion in these samples. Some typical results are shown in Table II.

TABLE II

Sample	Santonin, per cent., and melting point	
	Method H. and S.	Method D
X	0·94 171·4° C.	1·08 171·0° C.
Y	1·01 173·2° C.	1·20 173·0° C.
Z	1·08 173·5° C.	1·19 173·2° C.

Qazilbash[3] states that his method is not suitable for Artemisias of low santonin content and that manufacturers, in any case, are not interested in such material. Our experience is that samples of this type are frequently sent to us for assay, either because new sources of santonin are being sought, or because experimental planting and/or collection is being carried out. Process H. and S. could always be adapted for dealing with such material and we have found that the method here described can be similarly modified.

For such samples as contain only small quantities of santonin, as indicated by a preliminary assay using 35 g., or for samples of which only 5 to 10 g. is available for assay purposes, we have found it best to mix these with 15 to 20 g. of a good Grade A sample of known santonin content (about 2 per cent.). There is then an assured yield of santonin of good colour and high melting point and the content of the unknown sample can be calculated.

Qazilbash criticises some methods on the grounds that large quantities of solvents are required for extraction and that the process is time-consuming; such criticisms may be levelled at Process D, but since it is our concern to extract the santonin to the last milligram, we are not able to minimise these factors with the possible sacrifice of accuracy.

Summary

1. Some difficulties in the assay of Artemisia are discussed.
2. A routine method, based on that described by Qazilbash and that formerly in use in this laboratory, is described.

References

1. Coutts, *Quart. J. Pharm. Pharmacol.*, 1932, **5**, 375.
2. Qazilbash, *J. Pharm. Pharmacol.*, 1951, **3**, 105.
3. Qazilbash, *ibid.*, 1952, **4**, 103.
4. Isaacs, *ibid.*, 1952, **4**, 423.
5. Qazilbash, *ibid.*, 1952, **4**, 511.