ASCARIDOLE STUDIES

PART II*. AN EXAMINATION OF THE IODIMETRIC AND POLAROGRAPHIC METHODS OF DETERMINATION OF ASCARIDOLE

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THE iodimetric method for the determination of ascaridole described in the B.P. 1953 and U.S.P. (XII) is based on the work of Cocking and Hymas[1]. We found that the results obtained by the official process in the assay of both commercial samples of "ascaridole" and oils of chenopodium differed greatly from the results obtained by the polarographic method[2,3] (see Table I). The two methods have therefore been investigated in some detail in an attempt to account for the observed discrepancies.

In the official method it is necessary to adhere rigidly to the conditions laid down if reproducible results are to be obtained. Furthermore, the factor given for the calculation of results is an empirical one based on the titration of a certain sample of ascaridole stated to contain 96 per cent. of ascaridole. However, this figure had been obtained by Paget's method of assay[4], using titanous chloride, where again an empirical factor based upon the reaction of titanous chloride with pure ascaridole was used. The physical constants quoted for this sample of "pure" ascaridole do not agree with the values published in more recent work. The factor adopted by the B.P. therefore seems to require further investigation. Since the factor was obtained on the basis of the reaction of a definite weight (0·25 g.) of ascaridole with acidified potassium iodide solution under rigid conditions, the use of the same factor (as in B.P.) is questionable when 0·25 g. of oil of chenopodium is used, because of the change in the ratio of ascaridole to the acidified potassium iodide solution.

In another communication[3] we have described the preparation of a product which we consider to be 100 per cent. ascaridole. The analysis of the sample by the B.P. method and using the B.P. factor gave results equivalent to 110·4 per cent. w/w of ascaridole. These results support those of Böhme and van Emster[5] who recently obtained results above 100 per cent. using the B.P. method with purified ascaridole. This incorrect factor, however, does not account for the whole of the difference in the results obtained by the polarographic and B.P. methods of assay of various oils of chenopodium (see Table I). In order to investigate the dependence of the observed percentage of ascaridole on the weight of sample used in the iodimetric method of determination, various oils of chenopodium and ascaridole were examined under experimental conditions which were constant except for the weight of sample used. These results (Table II and Fig. 1) indicate that the amount of iodine liberated is not

*The paper in *J. Pharm. Pharmacol.*, 1952, **4**, 738, is regarded as Part I.

directly proportional to the amount of sample present in the reaction mixture, and consequently the use of a constant factor for oils of differing ascaridole content must yield incorrect results.

TABLE I

Sample	Percentage w/w of ascaridole			
	B.P. method and factor	Polarographic method	B.P. method (adjusted)	B.P. method (quadratic expression)
Pure ascaridole	110·4	100·0	100·0	100·0
"Ascaridole" 1	95·1	84·4	83·9	83·6
"Ascaridole" 2	105·5	95·0	94·7	94·9
*Oil of chenopodium 1	79·8	64·0	68·0	68·3
" " 2	65·1	54·3	54·6	54·3
" " 3	68·1	57·3	58·3	57·6
" " 4	68·7	57·4	—	57·8
" " 5	77·8	64·2	—	66·7
" " 6	79·5	68·3	69·2	68·2
" " 7	74·2	63·4	—	63·1
" " 8	75·3	64·3	65·0	64·1

* This sample gives an anomalous result. We presume that it contains another substance, apart from ascaridole, which liberates iodine from acidified potassium iodide solution but does not give a reduction step in the polarographic method.

To eliminate the effect of the change in the ratio between ascaridole and acidified potassium iodide solution on observed percentages, when constant amounts of various oils of chenopodium were used, the results were corrected in the following manner. The weights of the various oils containing 0·25 g. of ascaridole were calculated from the polarographic results. The percentages of ascaridole corresponding to the use of these weights of the various oils in the iodimetric method were read off from the graphs of weight of sample against observed percentage. A further correction was applied because the B.P. factor is approximately 10 per cent. high. The resultant values for ascaridole content of the various oils (Table I, B.P. adjusted method) then corresponded with the values obtained by the polarographic method.

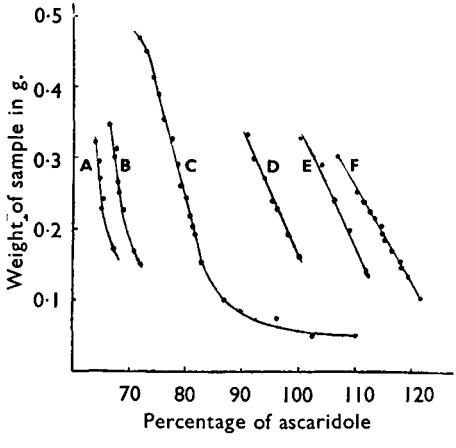

FIG. 1. The effect of the variation of weight of sample upon the observed ascaridole percentage determined by the B.P. procedure.

A. Oil of chenopodium 2.
B. Oil of chenopodium 3.
C. Oil of chenopodium 1.
D. "Ascaridole" 1.
E. "Ascaridole" 2.
F. Pure ascaridole.

It was apparent that the iodimetric method could therefore still constitute a convenient and accurate method for the analysis of oils of chenopodium if the B.P. factor were replaced by a conversion factor which varied with the amount of ascaridole coming into contact with the

TABLE II

Sample	Weight of sample g.	0·1N $Na_2S_2O_3$ (n) ml.	Percentage w/w of ascaridole	
			B.P. method and factor	B.P. method and quadratic expression
Pure ascaridole	0·3071	49·38	106·9	100·7
	0·3006	47·38	106·4	97·8
	0·2510	41·60	110·2	100·0
	0·2383	39·84	111·2	100·1
	0·2227	37·69	112·6	100·4
	0·2196	37·15	112·5	100·0
	0·2142	36·55	113·4	100·6
	0·2123	36·28	113·6	100·5
	0·2040	35·14	114·5	100·8
	0·1941	33·47	114·7	100·2
	0·1842	31·81	114·9	99·5
	0·1705	29·85	116·4	99·9
	0·1551	27·48	117·8	100·0
	0·1465	25·94	117·8	99·2
	0·1319	23·68	119·4	99·5
	0·1027	18·78	121·6	98·5
"Ascaridole" 1	0·3323	45·27	90·6	83·5
	0·2992	41·44	92·1	83·5
	0·2728	38·54	93·8	84·1
	0·2375	34·02	95·3	83·4
	0·2304	33·28	96·0	83·7
	0·1934	28·48	98·1	83·4
	0·1611	24·23	100·1	83·6
"Ascaridole" 2	0·3281	49·71	100·8	94·8
	0·2876	44·90	103·8	95·6
	0·2415	38·50	106·0	94·8
	0·1974	31·29	108·7	91·1
	0·1408	23·75	112·2	93·4
Oil of chenopodium 1	0·4661	50·14	71·5	67·6
	0·4478	48·93	72·6	67·1
	0·4123	45·82	73·9	68·3
	0·3918	44·22	75·0	68·7
	0·3548	40·60	76·1	68·8
	0·3273	38·08	77·4	69·0
	0·2901	34·28	78·6	68·9
	0·2615	31·12	79·1	68·3
	0·2471	29·67	79·9	68·7
	0·2449	29·41	79·8	68·4
	0·2187	26·54	80·6	68·2
	0·2034	24·78	81·0	67·9
	0·1926	23·60	81·5	67·9
	0·1530	19·04	82·8	67·4
	0·1015	13·26	86·9	68·6
	0·0758	10·98	96·2	75·3
	0·0496	8·21	110·0	84·5
	0·0490	7·56	102·5	78·5
Oil of chenopodium 2	0·6670	53·62	53·4	51·1
	0·6372	51·32	53·5	50·6
	0·3220	30·80	63·6	54·9
	0·2947	28·54	64·4	54·9
	0·2708	26·29	64·5	54·4
	0·2399	23·48	65·0	54·2
	0·2295	22·39	64·9	53·7
	0·1710	17·26	67·1	54·1
	0·0634	8·64	90·6	69·9
Oil of chenopodium 3	0·6132	51·05	55·4	52·2
	0·3467	34·48	66·1	58·0
	0·3110	31·51	67·4	58·3
	0·2987	30·11	67·0	57·6
	0·2641	26·92	67·8	57·4
	0·2486	25·50	68·2	57·3
	0·2276	23·53	68·8	57·2
	0·1680	17·86	70·7	57·2
	0·1498	16·25	72·1	57·8
	0·0578	8·11	93·2	71·7

acidified potassium iodide solution. A suitable expression was derived as follows:—

Let m = g. of ascaridole reacting with the acidified potassium iodide solution.

Let n = ml. of 0·1N sodium thiosulphate solution required (less blank titration).

Let x = variable conversion factor.

Then $m = xn$ .. .. (1)

$\therefore \quad x = m/n$

Furthermore $x = f(n)$ or (m) .. .. (2)

When m/n was plotted against n for pure ascaridole a straight line was obtained (Fig. 2) for values of n between 20 and 40 (corresponding to a weight of 0·11 to 0·24 g. of ascaridole).

$\therefore$ (2) becomes $x = Bn + A$ .. .. (3)

Where B and A are constants.

(The linear relationship between m and n holds beyond the values of n = 20 to 40 when pure ascaridole is used, but these seem to be about the safe limits for samples of oil of chenopodium. See Fig. 2).

Substitute for x in (1)

$\therefore \quad m = An + Bn^2$ .. .. (4)

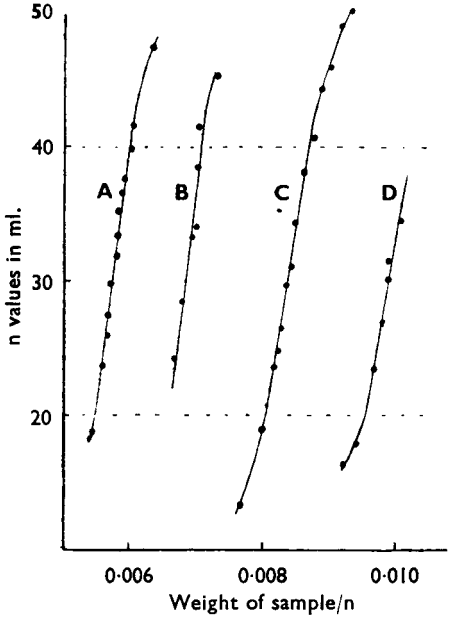

FIG. 2.

A. Pure ascaridole.
B. "Ascaridole" 1.
C. Oil of chenopodium 1.
D. Oil of chenopodium 3.

The values for A (0·00489) and B (0·0000275) for pure ascaridole were obtained graphically (Fig. 2). When the weights of commercial samples of ascaridole and oils of chenopodium were divided by their corresponding 0·1 N sodium thiosulphate figures (n) and plotted against n (Fig. 2) straight lines were also obtained for values of n between 20 and 40. The gradient of these lines increased with decreasing sodium thiosulphate titration per g. of sample as expected from the work already described, but it was necessary to establish that the constants A = 0·00489 and B = 0·0000275 obtained for pure ascaridole applied equally to the ascaridole content of oils of chenopodium before the method could be considered to be generally applicable. As a

first approximation, we applied equation (4) where A = 0·00489 and B × 0·0000275 to obtain a figure for the ascaridole content of the various oils and the weights of the various samples in Table I corresponding to titration figures (n) between 20 and 40 were then converted into weights of ascaridole. These values were divided by their corresponding titration figures (n) and plotted against n values to give straight lines which were found to be superimposable upon the m/n against n line for pure ascaridole. Thus the formula

$$m = 0{\cdot}00489\,n + 0{\cdot}0000275\,n^2 \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

is applicable to the calculation of the weight of ascaridole from the titration figure obtained in the B.P. assay process of oil of chenopodium provided the number of ml. of 0·1N sodium thiosulphate required (minus blank) is within the limits of 20 to 40. Böhme and van Emster[5] have also shown recently that, for pure ascaridole, the relation between weight of ascaridole and number of ml. of sodium thiosulphate solution can be expressed with satisfactory accuracy by means of a similar quadratic expression, but they do not indicate that the expression can only give the correct results between certain values of thiosulphate readings. The values obtained by the use of the formula (5) for the calculation of percentages [Table II—B.P. method (quadratic expression)] from the experimental results were constant (for a given oil) even if varying weights of sample were used, and these percentages agreed with the values obtained by the polarographic method. For titration figures below 20 ml. the values obtained for the percentage of ascaridole were slightly too high and above 40 ml. the values were too low. The use of the B.P. factor for the calculation of ascaridole percentages from the same experimental results gave values which varied greatly with the weight of sample used [Table II—B.P. method and factor]. A sample of oil of chenopodium which analysed at 65 per cent. w/w of ascaridole by the present B.P. method would only contain, in fact, 54 per cent. w/w (a 20 per cent. error) whereas an oil giving a result of 80 per cent. w/w would really contain 68·5 per cent. w/w (a 14·5 per cent. error).

Although it is unlikely that any of the constituents of oil of chenopodium, other than ascaridole, will give a reduction step in the polarographic method, the application of this assay process to the determination of ascaridole in oil of chenopodium has now been studied in more detail. When various mixtures of pure ascaridole with *p*-cymene (the chief constituent of oil of chenopodium other than ascaridole) were determined polarographically, the results corresponded with the amount of ascaridole added (Table III). A good correspondence of polarographic results with the theoretical values was also obtained with mixtures of pure ascaridole with oils of chenopodium of low ascaridole content, and with oil of chenopodium heated to decompose the ascaridole. The polarographic method will therefore give correct values for the ascaridole content of oil of chenopodium if the calibration curve is based upon 100 per cent. ascaridole.

The very good agreement between the results obtained by the polaro-

graphic method and the iodimetric procedure adopting the quadratic expression for the calculation of results ensures that the latter method can be regarded as suitable for simple routine analysis. We have therefore outlined the suggested iodimetric method below. It is recognised that,

TABLE III

Pure ascaridole mg.	Diluent added mg.	Total ascaridole present mg.	Ascaridole found by polarographic method mg.
11·0	A 9·6	11·0	10·8
10·2	A 5·3	10·2	10·0
15·3	A 20·0	15·3	15·3
11·3	B 30·6	11·6	11·8
16·2	B 48·8	16·7	16·4
13·3	C 9·4	18·3	18·5
15·2	C 8·4	19·7	19·7

A = *p*-cymene.
B = Oil of Chenopodium [54·2 per cent.]. heated at 130° C. for 8 hours. It was then found to contain about 1 per cent. of ascaridole [determined polarographically].
C = Low grade Oil of Chenopodium containing 54·2 per cent. of ascaridole [determined polarographically].

in presuming the two methods give correct figures for ascaridole content, we are assuming that the sample of ascaridole upon which this work is based is 100 per cent. pure. We have taken many precautions to ensure the purity of our sample, and the details of this work are published elsewhere[3].

Proposed Iodimetric Method

Perform the determination as described under chenopodium oil B.P. If the number of ml. (n) of 0·1N sodium thiosulphate required (after deduction of the blank titration) is within the limits of 20 to 40, then calculate the weight of ascaridole (m) from the formula

$$m = 0{\cdot}00489n + 0{\cdot}0000275n^2$$

If the titration is outside the stated limits, repeat the determination using more or less than the stated 5 ml. of the acetic acid solution of the oil in order to give a titration of between 20 and 40 ml. of 0·1N sodium thiosulphate, and apply the above equation.

Experimental

Pure ascaridole. Samples were prepared according to the methods to be described[3].

Polarographic method of determination of ascaridole. The details of the method have already been published[2].

Iodimetric method of determination of ascaridole. The B.P. method was used with the following minor modifications which led to an improved reproducibility of results:—(*a*) A w/w solution of ascaridole (or oil of chenopodium) was made in acetic acid (90 per cent.) and weighed amounts of this solution added to the acidified potassium iodide solution.

(*b*) After the rapid addition of the ascaridole solution and immediate thorough mixing, the mixture was set aside for 5 minutes in a cold water-bath at 5° C.

SUMMARY

1. The discrepancies between the percentage values of ascaridole in oils of chenopodium obtained by the B.P. and polarographic methods of analysis have been shown to arise because (*a*) the factor used in the B.P. is incorrect and (*b*) the amount of iodine in the B.P. method is not directly proportional to the weight of ascaridole added to the acidified potassium iodide solution.

2. A suitable quadratic expression is derived for the conversion of the titration figures obtained by the B.P. procedure into correct figures of ascaridole percentages.

REFERENCES

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2. Beckett and Dombrow, *J. Pharm. Pharmacol.*, 1952, **4**, 738.
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4. Paget, *Analyst*, 1926, **51**, 170.
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DISCUSSION

DR. A. H. BECKETT, in presenting the paper, explained that it should be considered in conjunction with a complementary paper, the publication of which had been delayed.

THE CHAIRMAN asked whether in view of the dosage range the differences in the results obtained by the different methods were of practical significance, although of course, from the analytical point of view they were very important.

DR. G. E. FOSTER (Dartford) said that it appeared that the B.P. method gave values which varied with the weight of sample taken. However, Dr. Beckett's method was still empirical, as it was based upon a sample of ascaridole which had not been proved 100 per cent. pure. He considered that the B.P. method was satisfactory as a comparative method and pointed out that infra-red absorption analysis results reported from Japan gave comparable results.

DR. A. H. BECKETT, in reply, said that veterinary surgeons desired accurate figures for the ascaridole content of oils of chenopodium and mixtures of oils of chenopodium with castor oil. He considered that the B.P. method gave results which were 20 per cent. too high in a reputed 65 per cent. oil and 14·5 per cent. too high in an 80 per cent. oil. The observations were therefore important from a dosage point of view as well as from the analytical standpoint. The sample of ascaridole they had prepared assayed 110·4 per cent. by the B.P. method. Furthermore, because results varied with the weight of ascaridole used in the test, B.P. values for oils of differing ascaridole content were not comparable. The proposed quadratic function eliminated the effect of variations in the weight of ascaridole used. Infra-red details would be published in the near future. Here, too, the result depended upon the purity of the standard and the same was true of all methods in the absence of any stoichiometric reaction of ascaridole.