THE DETERMINATION OF ASCARIDOLE IN OIL OF CHENOPODIUM AND IN SOLUTION OF OIL OF CHENOPODIUM IN CASTOR OIL

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A SOLUTION of oil of chenopodium in castor oil is used in veterinary practice as an anthelmintic and an assay of the ascaridole content was required for the British Veterinary Codex. A reliable assay is especially important because the anthelmintic effective dose is stated to be near to the toxic dose for certain animals.

The B.P. 1948 and U.S.P. (XII) method for the determination of ascaridole in oil of chenopodium, depending upon the liberation of iodine from potassium iodide solution, is obviously inapplicable in the presence of castor oil. Although the oil of chenopodium could probably be separated from the castor oil by distillation, the application of any prolonged heat treatment in an assay process would be unwise because of the ease of rearrangement or decomposition of the ascaridole molecule.

A number of methods have been used previously for the assay of ascaridole, of which the method of Cocking and Hymas,[1] upon which the B.P. method is based, has found widest acceptance, despite the fact that an empirical rather than a stoichiometric factor has to be used. Furthermore, the conditions must be controlled accurately if reproducible results are to be obtained. Halpern[2] has studied the iodination of terpenes and has shown that the non-stoichiometric behaviour of ascaridole towards the iodide ion cannot be explained by the simple iodination of olefines present in the oil of chenopodium. Do Vale[3] has modified the above method slightly by determining the liberated iodine colorimetrically. Nelson's method[4] is based on the solubility of ascaridole in 60 per cent. acetic acid, but other substances present in oil of chenopodium are also soluble in this solvent. Paget[5] used the reduction with titanous chloride as a method of assay, but here too, an empirical factor had to be applied. A colorimetric method based on the brown colour arising from the action of concentrated hydrochloric acid on a 1 per cent. ethanolic solution of ascaridole has been described.[6] Reports have appeared[7,8,9,10,11] dealing with the above methods of assay of ascaridole, and these reports indicate the limitations of these methods. The method for the assay of chenopodium involving the use of bisulphate solution and the measurement of the undissolved oil[12] is obviously not suitable for precise work. A number of colorimetric assays have been described based on the colour produced with ascaridole and strong sulphuric acid,[13] and 2:6-dichlorophenolindophenol.[14] Recently a gravimetric assay[15] has been used which involves the addition of mercuric chloride in ethanol (90 per cent.) and filtration after 48 hours in the dark.

It is apparent from a detailed consideration of the above briefly mentioned methods that a precise, reliable and relatively straightforward assay process for ascaridole has not yet been devised. Consequently we have investigated the application of polarography to the determination of ascaridole in oil of chenopodium because this approach would probably be applicable in the presence of castor oil. Since this work was commenced, the paper by Bitter[16] dealing with the polarography of some essential oils, including oil of chenopodium, came to our attention. He showed that ascaridole gave a reduction step in absolute ethanol and that polarography was a suitable method for its assay, but few details were given and the effect of varying conditions upon the results was not investigated. We now report the results of our investigations and suggest a routine method suitable for the analysis of ascaridole in oil of chenopodium and in solutions of oil of chenopodium in castor oil.

Experimental

Materials

Reagent chemical grade—lithium chloride dihydrate, lithium hydroxide monohydrate.

Analar grade—mercury, calomel, and potassium chloride.

B.P. grade—acetic acid, agar, castor oil and oil of chenopodium.

Ethanol, redistilled from commercial absolute ethanol.

Ascaridole. A pure sample was obtained in 2 ways from ascaridole kindly supplied by Dr. Foster of the Wellcome Chemical Works.

(*a*) Fractional distillation under reduced pressure. The fraction b.pt. 84° C./3 mm., $n_D^{22°\,C}$ 1·4725 gave the highest polarographic wave.

(*b*) Chromatography using alumina and benzene/light petroleum.

Polarography indicated that the sample obtained by chromatography had a purity about 1 per cent. greater than the sample obtained by fractional distillation. This work will be reported in detail in a subsequent paper dealing with the chemistry and stability of ascaridole.

Apparatus

Current-voltage curves were recorded using a Tinsley Polarograph, Mark 12. Capillary constant, $m = 1{\cdot}94$ mg. per second, $t = 3{\cdot}54$ seconds, on open circuit, in ethanol solution of the electrolyte. The temperature was controlled at $20° \pm 0{\cdot}5°$ C. The solutions were deoxygenated with oxygen-free nitrogen which had previously been passed through 97 per cent. ethanol. The recorder pen was set at zero with the electrodes disconnected.

Results and Discussion

Choice of solvent. Owing to the low water solubility of castor oil and oil of chenopodium, a solvent of low water content was required. This limited the choice of electrolyte and lithium chloride was used. Of the various solvents tried, well formed waves were given by oil of chenopodium only in methanol, industrial methylated spirit and ethanol, the latter being chosen owing to minor waves occurring at 1·7 volts in the first 2 solvents.

Buffering. The most satisfactory buffer was found to be a mixture of lithium acetate and acetic acid, each 0·01N. Neither the half wave potential nor the diffusion current of ascaridole was affected by changes of acidity or alkalinity within the range of this buffer or an ammonium chloride-ammonium hydroxide buffer of the same strength but, with the latter, maxima occurred at 1·65 volts. A second wave, due to reduction of the electrolyte, began at 2·1 volts (approx.) in these buffered solutions but was displaced to 1·8 volts in 0·15N acetic acid, thereby seriously curtailing the limiting current plateau of the first wave. Hydrochloric acid 0·01N, unbuffered, gave an interfering hydrogen wave.

Rancidity of the castor oil may give rise to a small quantity of weak acid. A sample just within the B.P. acid value limit of 4 would give a solution 0·004N in acid at the castor oil strength used in the suggested method (see below). Acetic acid 0·03N and ricinoleic acid 0·1N were found to be without effect on the diffusion current. The lithium acetate buffer was therefore used primarily as a precaution against contamination with strong acids.

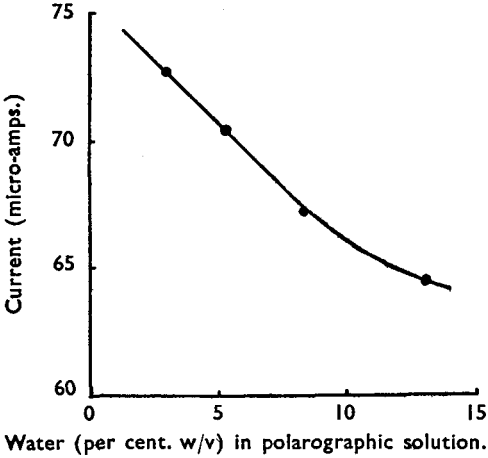


FIG. 1. Effect of water on diffusion current of ascaridole.

Water content of solvent. The effect of water on the diffusion current of ascaridole in ethanol containing the above buffer and 0·2N lithium chloride is shown in Figure 1.

The diffusion current fell by about 1·5 per cent. for a 1 per cent. increase in water content. Castor oil had little effect upon the slope. The water content was most conveniently controlled by the use of constant boiling ethanol (see section on reagents).

Electrolyte concentration and cell resistance. The half wave potential, corrected for *iR* drop, occurred at about 1·04 volts (against S.C.E.) in 0·2N lithium chloride and fell by about 50 millivolts as the electrolyte concentration was reduced to 0·05N. The main objection to low concentrations of electrolyte is the high cell resistance occurring with ethanol solutions. With the particular cell used in this work, 0·1N electrolyte was found satisfactory, but with large cells, a stronger solution would be desirable.

Temperature effect. By measurement of the diffusion current over a range from 12° to 28° C., the temperature coefficient was found to be about 1 per cent. per degree C.

Use of pool anode. Some difficulties were experienced with the agar solidified aqueous salt bridge as a result of precipitation effects at the

junction, particularly if the bridge was left in the solution during bubbling. This resulted in a fall of current due to displacement of the cathode potential to less negative values by the greatly increased *iR* drop. The trouble was eliminated by the use of a pool anode, which had the further advantage of reducing the cell resistance to 3200 ohms at an interval of about 2 cm. between the electrodes. The potential of the pool, measured against the saturated calomel electrode, varied slightly with applied voltage and current but did not exceed ± 30 millivolts (against S.C.E.) with currents up to 80μA and was generally much less.

Effect of castor oil. A method of assay was required by the British Veterinary Codex for a castor oil solution of oil of chenopodium containing 3·25 per cent. of ascaridole. Polarograms obtained with a fixed concentration of ascaridole and varying quantities of castor oil showed that an increase of 1 per cent. in the castor oil concentration depressed the diffusion current by 2 per cent. (Fig. 2).

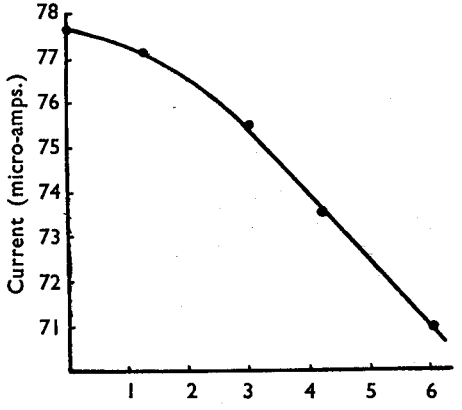


FIG. 2. Effect of castor oil on diffusion current of ascaridole.

A precision of $\pm 0{\cdot}5$ per cent. will hence be achieved, in respect of the effect of castor oil concentration, in the proposed method (below) by controlling the sample weight to within ± 5 per cent.

Calibration curves. Full polarograms were recorded for ascaridole concentrations from zero to 0·2 per cent. (*a*) in the absence, (*b*) in the presence of 6 per cent. of castor oil and the diffusion currents at various voltages plotted against the concentration (Fig. 3) without correction for the residual current. On first short-circuiting the cell at zero applied voltage, a small negative current flowed, which fell to zero at 0·05 volts, after which a normal residual current curve was obtained, rising to 0·5μA at 0·5 volts and 1·5μA at 1·65 volts in a solvent blank. Incomplete deoxygenation could therefore be detected by the presence of an oxygen wave below 0·5 volts. The current commenced to rise rapidly at 1·7 volts as decomposition of the solvent electrolyte began. This solvent wave occurred at the same voltage with low ascaridole concentrations, and was displaced progressively to above 2 volts at medium and high concentrations. The uncorrected half wave potential of ascaridole itself rose from 1·03 to 1·13 volts over the same concentration range, but on correction for the *iR* drop, a value of $1{\cdot}01 \pm 0{\cdot}02$ volts (against S.C.E.) was obtained.

The plots were very nearly linear whether the current was read at potentials of 1·35 and 1·50 volts (corrected for *iR* drop) or applied voltages

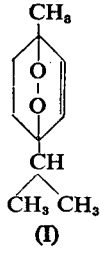
of 1·60 and 1·70. The latter plots showed slight curvature at low concentrations (under 0·1 per cent. ascaridole) with better precision on the linear portion. The former plots were more precise at the lower concentrations but showed less overall precision than the uncorrected curves. There was little to choose between results obtained at 1·6 and 1·7 volts. The values of the average current, obtained with damping, were of slightly lower precision than the instantaneous maximum currents, recorded with no damping and corresponding to maximum drop size. They were 80 per cent. of the maximum currents, slightly less than the values predicted by the Ilkovic equation.

It is clear that a calibration curve, plotted at voltages of 1·6 or 1·7, uncorrected for *iR* drop, using a cell of resistance 3200 ohms, may be used as the basis of a polarographic method for the determination of ascaridole. There is a danger with polarographic cells of high resistance, and at high values of current, that the readings may be taken slightly below the plateau of the wave, which will reduce precision, although not necessarily accuracy, in a relative method. For this reason, the higher voltage was chosen, the electrolyte concentration increased, and the residual current read on a solvent blank at 1·5 volts to allow for the slight *iR* drop in the stronger solutions.

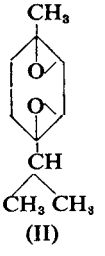
Diffusion current constants (i_dC) at 1·7 volts were calculated from the data used in plotting the calibration curves (Fig. 3) after subtracting the residual current of 1·5μA. The values for ascaridole showed an average deviation from the mean of $\pm$0·8 per cent., with no damping. With damping, the average deviation was $\pm$1 per cent. upon excluding the last 3 points obtained at very low ascaridole concentrations. Further work is being carried out to see whether the Ilkovic equation may be applied, and a comparison solution eliminated, by the use of a diffusion current coefficient for ascaridole.

Linear calibration curves of excellent precision were also obtained in the presence of a constant amount (6 per cent.) of castor oil (Fig. 3) without correction for the *iR* drop.

Ascaridole in oil of chenopodium. Oil of chenopodium contains, in addition to ascaridole (I), other constituents including *p*-cymene, *l*-limonene, *d*-camphor and cineole, as well as ascaridole glycol anhydride (II) and ascaridole glycol arising from the rearrangement of ascaridole during distillation or steam distillation.



(I)



(II)

It appears unlikely that any of these constituents will give a reduction step in the polarographic method of assay. However, this point was investigated further by heating a sample of oil of chenopodium containing 60 per cent. of ascaridole (determined polarographically) at 150° C. under reflux. Heating is known to cause rearrangement of ascaridole to ascaridole glycol anhydride (II).[17] After 2½ hours the oil was found to possess a constituent (presumed to be ascaridole)

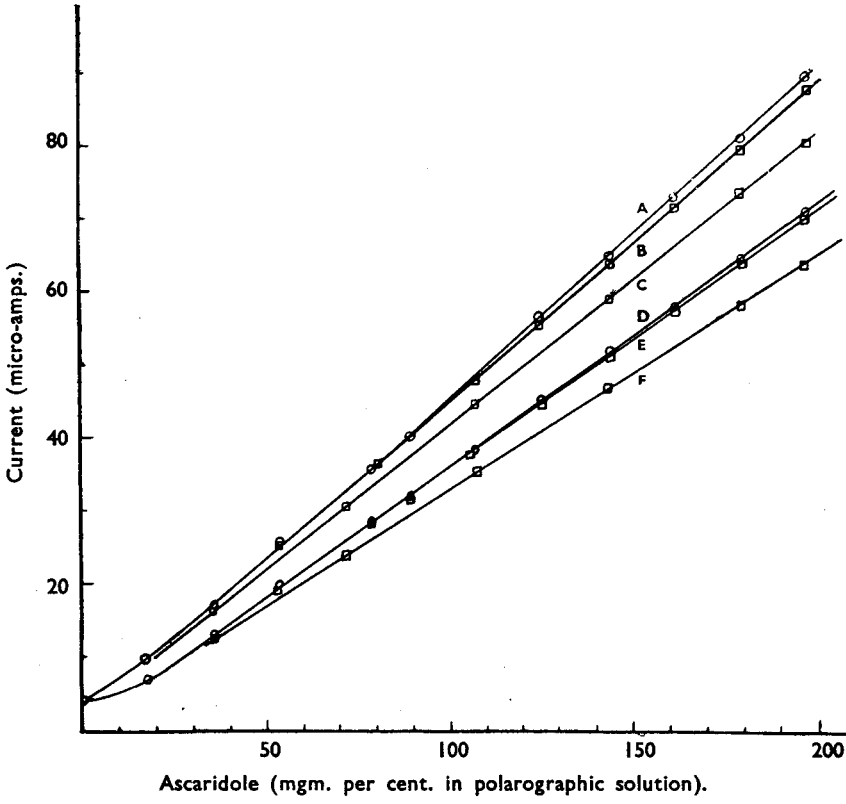


FIG. 3. Calibration curves for ascaridole in ethanol (97 per cent.) with lithium chloride 0·1N, lithium acetate 0·01N, acetic acid 0·01N.

Current at maximum drop size, undamped. A. 1·7 volts. No castor oil. B. 1·6 volts. No castor oil. C. 1·7 volts. 6 per cent. of castor oil.

Arithmetical mean current, damped. D. 1·7 volts. No castor oil. E. 1·6 volts. No castor oil. F. 1·7 volts. 6 per cent. of castor oil.

(All voltages uncorrected for iR drop and pool anode potential. m = 1·94 mgm. per second. t = 3·0 seconds at 1·6 volts, 2·9 seconds at 1·7 volts. Temperature = 20° C. ± 0·5.)

which gave a polarographic wave identical with ascaridole and the results indicated a content of 10 per cent., which fell to 4 per cent. after 4 hours heating and to about 1 per cent. after 8 hours heating. Although it is possible that this heat treatment may have caused changes in other constituents as well as in ascaridole, it is presumed that this polarographic method does in fact measure only the ascaridole content of oil of chenopodium. In connection with the possible presence of substances in oil of chenopodium or castor oil which might interfere with the polarographic determination of ascaridole, the recent publication by Willits *et al.*[18] is of interest. These authors established that a number of substances related chemically to rancidity products of fats and oils and including cumene, unsaturated esters, and epoxy and hydroxy

derivatives of fatty acids and alcohols are not reduced polarographically in a lithium chloride-methanol-benzene solvent.

Stability of ascaridole solution. Experiments are under way to test the stability of ascaridole under varying conditions. In view of the suggested use of a standard ascaridole solution, it is of interest that a 1·0 per cent. solution of ascaridole in ethanol (97 per cent.), which was prepared 2 months ago and has been exposed to sunlight and to the atmosphere frequently, still gives the same diffusion current as when it was first prepared.

Proposed Method for the Determination of Ascaridole

Solutions

Lithium acetate buffer solution (lithium chloride 0·5N, lithium acetate 0·025N, acetic acid 0·025N)

Lithium chloride dihydrate, 39·5 g.
Glacial acetic acid, B.P., 3·0 g.
Lithium hydroxide monohydrate, 1·1 g.
Ethanol (97 per cent. v/v) to 1 l.

Standard ascaridole solution

Ascaridole (freshly fractionated), 0·5 per cent. w/v in ethanol (97 per cent. v/v).

Castor oil solution

Castor oil, B.P., 30 per cent. w/v in ethanol (97 per cent. v/v).

Ethanol (97 per cent. v/v)

Prepared by dilution from absolute ethanol and subsequent distillation at atmospheric pressure, rejecting first and last portions.

Determination of ascaridole in oil of chenopodium

Weigh accurately 0·13 to 0·15 g. of oil of chenopodium into a 50 ml. graduated flask, add 20 ml. of *Lithium Acetate Buffer Solution* and dilute to 50 ml. with *Ethanol* (97 per cent. v/v). Transfer a portion to a polarographic cell, deoxygenate by bubbling with oxygen-free nitrogen which has previously been passed through a wash bottle containing *Ethanol* (97 per cent. v/v). Measure the diffusion current at 20° C. ($\pm$0·5°) and an applied voltage of 1·7 using a dropping mercury cathode and large pool anode, about 2 cm. apart or less. If the diffusion current is less than 30μA, repeat the determination using a larger weight of sample. Deduct the residual current given at 1·5 applied volts by a solvent blank, prepared and treated exactly as the above solution except that the sample is omitted. From the net diffusion current, calculate the weight of ascaridole present, either by using a calibration curve or a standard comparison solution.

Standard comparison solution. Determine the net diffusion current exactly as described above using, in place of the sample, 20 ml. of *Standard Ascaridole Solution* or an equivalent quantity of pure ascaridole. The net diffusion currents given by the sample and standard will be in the same ratio as the weights of ascaridole present in the respective solutions.

Calibration curve method. A calibration curve showing the relationship between "net current" and "weight of ascaridole in 50 ml. of prepared solution" may be prepared by carrying out the determination exactly as described above using suitable quantities of *Standard Ascaridole Solution* in place of the sample. Volumes between 12 and 20 ml. should cover normal variations in sample strength. The curve will be valid only for the particular electrode capillary used and the polarographic cell resistance should not vary appreciably.

Determination of ascaridole in castor oil solution

Carry out the determination exactly as described above using 2·6 to 2·8 g. of sample (castor oil containing 3·25 per cent. of ascaridole) in place of the oil of chenopodium.

Standard comparison solution and calibration curve. In preparing the standard comparison solution, or the solutions used in plotting a calibration curve, add 9 ml. of *Castor Oil Solution* or an equivalent weight of castor oil in addition to the other reagents, before adjusting to 50 ml.

Solutions of ascaridole in castor oil stronger than 4 per cent. may be determined by using smaller weights of sample and making a corresponding reduction in the quantity of castor oil used in any standard comparison solutions. (A correction based on the data plotted in Figure 2 may otherwise be applied.)

SUMMARY

1. The methods which are available for the determination of ascaridole are briefly reviewed.

2. The possible application of a polarographic assay of ascaridole in oil of chenopodium and in solutions of oil of chenopodium in castor oil is investigated and the effects of various conditions upon this assay are examined.

3. A method suitable for the routine determination of ascaridole in the above solutions is proposed.

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DISCUSSION

The paper was presented by DR. A. H. BECKETT.

DR. D. C. GARRATT (Nottingham) said that, apart from the desirability of standardising ascaridole analytically, the work which Dr. Beckett had undertaken arose from a specific request from the appropriate committee of the Veterinary Codex for a close assessment of the ascaridole content of oil of chenopodium in castor oil as distinct from the chenopodium oil content. That was because of the assertion that deaths had occurred as a result of the varying ascaridole content of the chenopodium oil used. In fact the ascaridole content of B.P. oils varied only within a few units per cent. It appeared that the therapeutic dose of ascaridole must be so close to the toxic dose that the conclusion might be reached that it was undesirable to use it in veterinary practice at all. Polarographic methods were not specific, and he felt that the point had been reached at which it was desirable to pause in their use. Although in the routine determination a standard ascaridole solution was desirable, who was to keep the standard, and would it deteriorate over a long period? Further, what proof had the authors that the ascaridole which they purified was in fact pure ascaridole and how did they prove that the ascaridole was still as such and was not an isomer or polymer giving the same deflection? Lastly, he asked how the results of their method compared with those obtained by chemical methods over a range of B.P. oils.

DR. G. E. FOSTER (Dartford) said that he had tried Dr. Beckett's method of assay and confirmed that a straight line calibration curve was obtained, but when the estimations were repeated it was found that the calibration curve was not altogether reproducible. After completing a number of determinations on one series of solutions one day, the next day the actual calibration was slightly different, and far from being able to estimate the ascaridole content to $\pm 0{\cdot}5$ per cent., he was of the opinion that it was not possible to get results nearer than ± 10 per cent.

DR. J. M. ROWSON (London) asked the authors whether they had any comparative figures either of the oils or anthelmintic preparations from them analysed by the polarographic method and by the pharmacopœial method? Had the pharmacologist or veterinary worker attempted to evaluate with reasonable probability the anthelmintic action of the oil and compared it with the iodimetric and polarographic methods?

MR. W. H. STEPHENSON (Nottingham) asked the authors whether they had done any work on old samples of oil and different grades of castor oil, with a view to ascertaining the interference of any peroxides which might be present.

DR. A. H. BECKETT, in reply, said that when using the B.P. method of determination of ascaridole it was possible to obtain any result, depending upon the weight of the sample taken. For a comparison between the polarographic and B.P. methods much closer standardisation of the conditions of the B.P. assay was required. It was considered

that the B.P. method gave results about 15 per cent. too high for oils containing 60 to 70 per cent. of ascaridole and 12 per cent. too high for oils containing 80 per cent. of ascaridole. Furthermore, when ascaridole was dissolved in 90 per cent. acetic acid, it decomposed very rapidly. Therefore, any comparison between the polarographic and B.P. methods was difficult. He was not certain that ascaridole had ever been obtained 100 per cent. pure, but he was continuing his work on the purification. He was investigating old samples of oils.

MR. M. DOMBROW (London), in reply, disagreed with Dr. Garratt's statement that polarographic methods were not specific. The development of polarography was more recent than that of spectrophotometry, but a great deal of information was being accumulated to suggest that polarography was quite specific under certain conditions and for certain groupings. Polarography of an oil in anhydrous ethanol was less straightforward than in aqueous solvents.