

Determination of Prophylactics in Medicated Feeding Stuff

by R. S. HATFULL

(Public Analyst's Laboratory, County Hall, Maidstone, Kent)

Analytical methods for estimation of nearly 30 prophylactic substances used as additives to animal feeding stuffs are reviewed.

The inclusion of prophylactic substances in animal feeds has been recognised for a number of years as an economical and practical way of preventing or controlling infection in large units of livestock and has, for example, proved to be particularly effective in the protection of flocks of poultry against Coccidiosis and Enterohepatitis (Blackhead).

Substances that have found favour are, in the main, fairly complex organic chemicals, but while the assay of the pure substances does not present any special difficulties, their determination in admixture with feeding stuffs has provided many problems. Most of the difficulties arise from the lack of recognised methods of analysis and to overcome this the Association of Official Agricultural Analysts appointed a number of Associate Referees for the development of methods. More recently, the Society for Analytical Chemistry has set up a Prophylactics in Feeds Sub-Committee of the Analytical Methods Committee of the Society.

Prophylactics are, in general, used at levels of 0.05 per cent. or less—the most usual concentration being in the region of 0.01–0.02 per cent., but one, Ethopabate, is normally incorporated at a level of only 0.001 per cent. As the synergistic properties of certain of these substances become better understood, the level of medication may become even lower. This also brings in the additional complication of determining the separate constituents of a mixed medication—an increasing problem as more manufacturers adopt the idea of 'polystat' addition.

Methods of analysis consist essentially of three stages:

- (a) The extraction of the substance from the feed, usually by a suitable choice of solvents and conditions.
- (b) Purification of the extract to eliminate interfering substances, e.g., by selective extraction and chromatography.
- (c) Determination of the active substance. Invariably this is done by choice of a suitable instrumental technique such as ultra-violet or visible spectrophotometry. The polarograph has proved to be of value in the determination of those substances capable of reduction, as when nitro groups are present, but such procedures will not be suitable for general adoption until more laboratories have the necessary equipment.

At these low levels of medication, sampling is of even greater importance than usual as in addition to extreme care in mixing, precautions must be taken to avoid losses due to such factors as undue exposure to heat and light.

Samples should be examined as soon as possible since it has been observed that an apparent loss of medication occurs upon ageing of a feed, due, possibly, to a binding effect of some constituent of the feed, oxidation either direct or in association with peroxidised fats and breakdown due to growth of micro-organisms.

A reduction in the amount of medicament recoverable has also been observed after pelleting and milling and for this reason it is inadvisable to regrind the sample any more than is absolutely necessary prior to analysis. Apparent losses of this nature can, however, usually be avoided by a suitable modification of the extraction procedure for example, by extending the time of the extraction or by using a hot instead of a cold extraction process. The problem of incorporating a medicament at a level of the order of 0.01 per cent. uniformly through a mass of feed on a commercial scale is very considerable as separation may occur during incorporation, bagging and even while sampling, due to differences in particle size or density. Some substances are prone to develop electrostatic changes so that they become concentrated on objects that have acquired a charge of the opposite sign such as those of glass or plastic. Even the metal parts of mixing machinery and laboratory mills may become charged unless effectively earthed. It has been reported by Woolford¹ that an apparent loss of Acinitrazole was traced to the development of static charges causing the medicament to migrate and become attached to the polythene bags used to contain the samples.

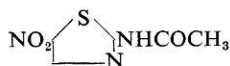
Because of these circumstances during manufacture, somewhat wider variations than those normally experienced may occur in commercial feeds and Merwin² has quoted a tolerance of ± 20 per cent. of the declared figure.

References

1. Woolford, M. H., *J. Ass. Off. Agric. Chem.*, 1961, **44**, 26.
2. Merwin, R. T., "Symposium on Medicated Feeds", Medical Encyclopedia Inc., New York, 1956, page 88.

Acinitrazole (2-acetylamino-5-nitrothiazole)

Also known as "Enheptin A" or "Cyzine", (American Cyanamid Co.), the acetyl derivative of the earlier "Enheptin".



Use

In Poultry feeds as a prophylactic against enterohepatitis (Blackhead).

Determination

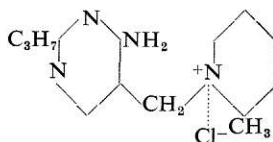
Senn³ extracted Acinitrazole with dimethyl-formamide followed by chromatography on alumina and measurement at 410 m μ of the yellow colour given by the action of NaOH.

Woolford⁴ modified this method by specifying the characteristics of the alumina more closely and while this modification is an improvement and the method was adopted and then made official by the A.O.A.C.^{5,6,7}, it is time consuming. Brown⁸ has suggested a simplified chromatographic procedure for the purification of the extract followed by determination by measuring the change in absorption at 340 m μ brought about by reduction of the nitro-group with a Zn/Cu couple. This method is under examination by the Analytical Methods Committee of the Society for Analytical Chemistry.

References

3. Senn, S. W., *J. Ass. Off. Agric. Chem.*, 1958, **41**, 329.
4. Woolford, M. H., *loc. cit.*
5. *J. Ass. Off. Agric. Chem.*, 1963, **46**, 151.
6. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 123.
7. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 195.
8. Brown, N. C., *Private Communication*.

Amprolium (1-(4-amino-2-n-propyl-5-pyrimidinyl-methyl)-2-picolinium chloride hydrochloride)



Use

In poultry feeds as a prophylactic against coccidiosis, usually in conjunction with Ethopabate as "Amprol Plus".

Determination

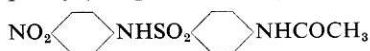
The Analytical Methods Committee of the Society for Analytical Chemistry has examined the method, adopted and made official by the A.O.A.C.^{9,10,11}, of Szalkowski & Schulz¹² and published their findings and recommended method in a Report¹³.

In this method the Amprolium is extracted with cold ethanol and interfering material is removed by chromatography on alumina. The determination is completed by reaction with a colour developing reagent and measurement at 530 m μ .

Kanora and Szalkowski¹⁴ have described a fluorimetric method which is claimed to be quicker, based upon extraction with a solution of trichloroacetic acid and formation of a fluorescent substance by a treatment very similar to the well-known procedure for the determination of aneurine.

References

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10. *J. Ass. Off. Agric. Chem.*, 1961, **44**, 154.
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12. Szalkowski, C. R. and Schulz, E. P., *J. Ass. Off. Agric. Chem.*, 1961, **44**, 154.
13. *Analyst*, 1964, **89**, 505.
14. Kanora, J. and Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 209.

APNPS (*Acetyl-(p-nitrophenyl)-sulphanilamide*)*Use*

In poultry feeds as a prophylactic against coccidiosis. Used in conjunction with DNBA under the names of "Nitrosal", "Polystat", "Tristat" and "Unistat". ("Polystat" also contains dibutyl tin dilaurate).

Determination

The Bratton-Marshall method¹⁵ was adopted by Cavett¹⁶ for the determination of APNPS in feeds.

The feed is extracted with methanol and zinc sulphate added to precipitate interfering matter from the extract. Deacetylation was then effected by boiling with HCl and the colour developed with the Bratton-Marshall reagents, followed by measurement at 540 mμ.

A collaborative study and the detailed procedure has been reported by Cavett¹⁷ and was adopted by the A.O.A.C.^{18,19}.

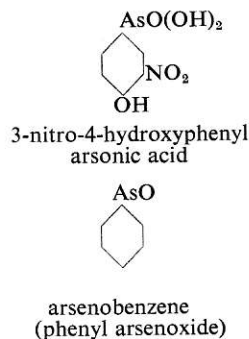
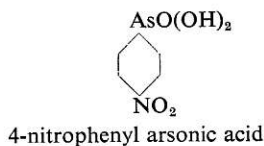
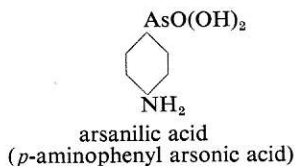
Daftsios and Schall have described the simultaneous polarographic determination of APNPS and DNBA²⁰.

References

15. Bratton, A. C. and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.
16. Cavett, J. W., *J. Ass. Off. Agric. Chem.*, 1956, **39**, 964.
17. Cavett, J. W., *J. Ass. Off. Agric. Chem.*, 1963, **46**, 422.
18. *J. Ass. Off. Agric. Chem.*, 1963, **46**, 99.
19. *J. Ass. Off. Agric. Chem.*, 1963, **46**, 153.
20. Daftsios, A. C. and Schall, E. D., *J. Ass. Off. Agric. Chem.*, 1962, **45**, 291.

Arsenic Compounds

A number of compounds of arsenic have been used in feeds, formerly as growth promoters but it is now considered that certain of these have coccidiostatic and anti-blackhead properties.

*Determination***(a) VIA TOTAL ARSENIC**

Hoffman²¹ reported on a collaborative study using a method that followed a conventional ashing procedure with MgO and Mg(NO₃)₂ by isolation as arsine

and conversion to a molybdenum blue complex. This method was adopted and finally made official by the A.O.A.C.^{22,23,24}. An alternative colorimetric procedure based on the silver diethylcarbamate colour development was studied by Hoffman^{25,26}, but was rejected by the A.O.A.C.²⁷.

(b) ARSANILIC ACID

Merwin²⁸ reported on a collaborative study using a method based upon extraction of the feed with citric acid solution followed by diazotisation and coupling, reading the colour at 545 m μ . This method was adopted and then made official by the A.O.A.C.^{29,30,31}, although it was not applicable in the presence of sulphonamides.

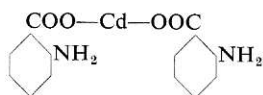
A further report by Merwin³² described a method of separation of arsanilic acid and sulphaquinoxaline based on the measurement of the total colour produced by diazotisation and coupling and after removal of the sulphaquinoxaline by extraction with ether. This method was adopted by the A.O.A.C.^{33,34}.

Senn³⁵ reported on a further collaborative study of a modified method devised to overcome the difficulty that had been encountered with superfatted feeds. This method was adopted and then made official by the A.O.A.C.^{22,24,36}, in place of that formerly adopted³⁴.

References

21. Hoffman, H. H., *J. Ass. Off. Agric. Chem.*, 1957, **40**, 455.
22. *J. Ass. Off. Agric. Chem.*, 1957, **40**, 26.
23. *J. Ass. Off. Agric. Chem.*, 1957, **40**, 65.
24. *J. Ass. Off. Agric. Chem.*, 1960, **43**, 127.
25. Hoffman, H. H., *J. Ass. Off. Agric. Chem.*, 1963, **46**, 456.
26. Hoffman, H. H., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 218.
27. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 52.
28. Merwin, R. T., *J. Ass. Off. Agric. Chem.*, 1954, **37**, 257.
29. *J. Ass. Off. Agric. Chem.*, 1954, **37**, 61.
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31. *J. Ass. Off. Agric. Chem.*, 1955, **38**, 234.
32. Merwin, R. T., *J. Ass. Off. Agric. Chem.*, 1956, **39**, 307.
33. *J. Ass. Off. Agric. Chem.*, 1956, **39**, 69.
34. *J. Ass. Off. Agric. Chem.*, 1956, **39**, 108.
35. Senn, S. W., *J. Ass. Off. Agric. Chem.*, 1957, **40**, 452.
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Cadmium Anthranilate



Use

As an anthelmintic against the larger round worm in pigs. Since the cadmium is chelated to the anthranilate moiety the compound is relatively non-toxic.

Determination

Beckman³⁷ has described a procedure based on the determination of the anthranilate part but the A.O.A.C.³⁸ has favoured the determination of the

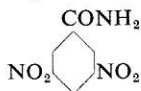
cadmium as this has the advantage of being rapid, precise and specific. A collaborative study by Stone³⁸, using the cadmium procedure, was based upon extraction of the sample with 10 per cent. trichloroacetic acid and determination of the cadmium as the dithizone complex, reading the pink colour at 518 mμ.

This method was adopted and then made official by the A.O.A.C.^{39,40,41}.

References

37. Beckman, H. F. and Feldman, L. S., *J. Agr. Food Chem.*, 1959, **7**, 350.
38. Stone, L. R., *J. Ass. Off. Agric. Chem.*, 1963, **46**, 467.
39. *J. Ass. Off. Agric. Chem.*, 1963, **46**, 99.
40. *J. Ass. Off. Agric. Chem.*, 1963, **46**, 153.
41. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 123.

3,5-DNBA (3,5-di-nitro-benzamide)



Use

In poultry feeds as a prophylactic against salmonella infections.

Used in conjunction with APNPS under the name of "Nitrosal", "Polystat", "Tristat" and "Unistat". ("Polystat" also contains dibutyl tin dilaurate).

Determination

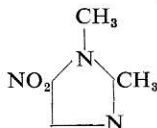
Cavett *et al.*^{42,43}, reported upon a collaborative study of a method in which the dinitrobenzamide was extracted with methanol and then reacted in dimethyl sulphoxide solution with an aged diethylamine reagent to give a colour which was measured at 560 mμ. The method was adopted and finally made official by the A.O.A.C.^{44,45}.

Daftsios and Schall⁴⁶ have described the simultaneous polarographic determination of DNBA and APNPS.

References

42. Cavett, J. W., and Heotis, J. P., *J. Ass. Off. Agric. Chem.*, 1959, **42**, 238.
43. *J. Ass. Off. Agric. Chem.*, 1959, **42**, 59.
44. *J. Ass. Off. Agric. Chem.*, 1959, **42**, 19.
45. *J. Ass. Off. Agric. Chem.*, 1961, **44**, 65.
46. Daftsios, A. C. and Schall, E. D., *J. Ass. Off. Agric. Chem.*, 1962, **45**, 291.

Dimetridazole (1,2-dimethyl-5-nitro-imidazole) ("Emtryl")



Use

In poultry feeds as a prophylactic against enterohepatitis (blackhead).

Determination

Polarographic methods have been described by Kane⁴⁷ and Daftsios⁴⁸ and while these have been satisfactory they are not suitable as general methods because of the special equipment necessary.

Stubbles⁴⁹ has suggested a process based upon extraction by chloroform of the feed made alkaline by addition of borax. Interfering substances are removed by extracting the Dimetridazole from the chloroform into dilute HCl and then back into chloroform on making alkaline, finally isolating by Thin Layer Chromatography. The Dimetridazole zones were located under U.V. light, isolated, extracted with chloroform and measured at 315–320 m μ .

Hartley⁵⁰ has modified the Stubbles⁴⁹ method by passing the chloroform extract through an alumina column, extracting into methanol/HCl and reading at 317 m μ . This method is under investigation⁵¹.

References

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48. Daftsios, A. C., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 231.
49. Stubbles, J. A., *Private communication*.
50. Hartley, A. W., *Private communication*.
51. Analytical Methods Committee, Society for Analytical Chemistry, London.

Ethopabate (Methyl 4-acetamido-2-ethoxy-benzoate)



Use

In poultry feeds as a prophylactic against coccidiosis—usually in conjunction with Amprolium as “Amprol Plus”.

Determination

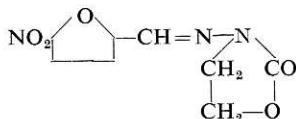
Szalkowski⁵² has described a procedure in which the Ethopabate is extracted with chloroform after a preliminary extraction with petroleum ether. Other interfering substances are then eliminated by washing the extract with dilute HCl, followed by hydrolysis of the Ethopabate to the free base. The determination is then completed by diazotisation and coupling and measuring the colour at 555 m μ . The method is claimed to be suitable for the determination of Ethopabate down to 2 p.p.m., but recoveries at the lower levels were erratic.

This method was studied further by Szalkowski⁵³ and the modified method adopted by the A.O.A.C.^{54,55}.

References

52. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1963, **46**, 464.
53. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 221.
54. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 123.
55. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 196.

Furazolidone (*N*-(5-nitro-2-furfurylidine)-3-amino-2-oxazolidine) also known as Furoxone.



Use

Control of a number of salmonella and other infections in poultry as well as blackhead. Also often compounded with the coccidiostat, Nitrofurazone, under the name of Bifuran.

Determination

Luhman⁵⁶ reporting on methods based upon those of Ells, McKay and Paul⁵⁷ carried out a preliminary extraction with petroleum ether and then extracted the Furazolidone with hot ethanol or *isopropanol*. The Furazolidone was then determined by measuring the absorption at 370 m μ against a blank produced by treating another part of the extract with sodium hydrosulphite. The method is subject to errors due to the difficulty of extraction of the furazolidone because of its low solubility and interference by pigments from feeds containing grass meals which are also effected by the sodium hydrosulphite treatment.

Buzard, Ells and Paul⁵⁸ extracted feeds with ethanol and concentrates with dimethylformamide. Conversion to the phenylhydrazone gave a red colour which was measured at 440 m μ . A modification of the procedure which re-introduced the preliminary extraction with petroleum ether before extracting with dimethylformamide was adopted by the A.O.A.C.⁵⁹

The above methods do not distinguish between Furazolidone and Nitrofurazone but Luhman⁶⁰ has described a method of separation based upon chromatography on alumina. The two eluates are treated with KOH and the blue colour given by Furazolidone read at 600 m μ and the pink-orange colour given by Nitrofurazone read at 490 m μ . A disadvantage is that both colours are unstable.

Luhman⁶¹ modified the procedure that had been adopted by the A.O.A.C.⁵⁹ by omitting the preliminary extraction with petroleum ether and extracting with hot dimethylformamide. Interfering substances were then removed by passing the extract through an alumina/magnesium hydroxide column. This method was adopted and then made official by the A.O.A.C.^{62,63}, but it does not distinguish between Furazolidone and Nitrofurazone.

Stone⁶⁴ has described recently a simplified procedure which is claimed to be specific for Furazolidone. The feed is extracted with hot dimethylformamide, diluted with carbon tetrachloride and the solution passed through an alumina column. The Furazolidone is then eluted with a mixture of carbon tetrachloride and *isopropanol*, extracted from the eluate with water and determined by measuring the absorption at 365 m μ .

A further paper by Stone⁶⁵ achieves separation of Furazolidone and Nitrofurazone by chromatography on alumina.

A polarographic method has been described by Fricke, Keppel and Hart⁶⁶.

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57. Ells, V. R., McKay, E. S. and Paul, H. N., *J. Ass. Off. Agric. Chem.*, 1953, **36**, 417.
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61. Luhman, C. A., *J. Ass. Off. Agric. Chem.*, 1960, **43**, 310.
62. *J. Ass. Off. Agric. Chem.*, 1960, **43**, 127.
63. *J. Ass. Off. Agric. Chem.*, 1961, **44**, 65.
64. Stone, L. R., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 562.
65. Stone, L. R., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 565.
66. Fricke, F. L., Keppel, G. E. and Hart, S. M., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 788.

Glycarbylamide (4,5-imidazolecarboxamide) (Merck)



Use

In poultry feeds as a prophylactic against coccidiosis.

Determination

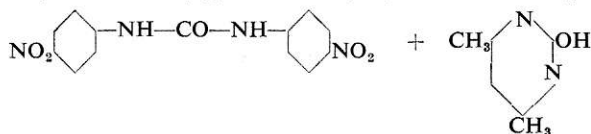
Szalkowski⁶⁷ has described a method in which the sample was extracted with hot *isopropanol*, followed by chromatography on a Dowex Resin column. The determination was completed by measuring the absorption at 288 $m\mu$ after treatment with NaOH.

A collaborative study reported by Szalkowski⁶⁸ used an improved procedure, with dimethylformamide as the extractant, followed by chromatography on alumina and an Amberlite resin, oxidation of residual impurities with bromine and differential measurement in alkaline solution at 283 $m\mu$. This method was adopted and then made official by the A.O.A.C.^{69,70}.

References

67. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1959, **42**, 241.
68. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1960, **43**, 284.
69. *J. Ass. Off. Agric. Chem.*, 1960, **43**, 127.
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Nicarbazin (Merck) (*An equimolecular mixture of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine* ("Pyrimethamine"))



Use

In poultry feeds as a prophylactic against coccidiosis.

Determination

Szalkowski *et al.*,⁷¹ have described a method based upon extraction with hot dimethylformamide followed by chromatography on alumina. 4,4'-dinitro-carbanilide was determined by measuring the yellow colour, produced by addition of NaOH, at 430 m μ and the 2-hydroxy-4,6-dimethylpyrimidine by coupling with diazotised sulphanilic acid and measuring the colour at 540 m μ . The method has the disadvantage that elution from alumina is not complete and it is necessary to compensate for the losses.

Mader⁷² reported the results of a collaborative study using a simplified version of Szalkowski's method⁷¹ and this was adopted by the A.O.A.C.⁷³.

Porter⁷⁴ avoided the use of alumina by using a series of selective solvent extraction procedures but it was then necessary to compensate for losses in the solvents.

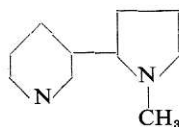
Szalkowski⁷⁵ reported on an attempt to improve and shorten the method adopted by the A.O.A.C.⁷³ by using dimethylformamide/ethanol for the extraction and the removal of interfering substances by activated charcoal. The determination was completed by coupling with diazotised *p*-amino-benzoic acid, but the results of a collaborative investigation were disappointing.

Szalkowski⁷⁶ reported on a further collaborative trial of a modified version of the earlier procedure using a more closely specified alumina and determining the 4,4'-dinitrocarbanilide moiety. This method was adopted and then made official by the A.O.A.C.^{77,78,79} in place of the earlier procedure.

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71. Szalkowski, C. R., O'Brien, M. G., Stuart, C. W. and Mader, W. J., *J. Ass. Off. Agric. Chem.*, 1955, **38**, 140.
72. Mader, W. J., *J. Ass. Off. Agric. Chem.*, 1956, **39**, 321.
73. *J. Ass. Off. Agric. Chem.*, 1956, **39**, 69.
74. Porter, C. C., *J. Ass. Off. Agric. Chem.*, 1956, **39**, 860.
75. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1957, **40**, 469.
76. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1958, **41**, 326.
77. *J. Ass. Off. Agric. Chem.*, 1958, **41**, 22.
78. *J. Ass. Off. Agric. Chem.*, 1958, **41**, 52.
79. *J. Ass. Off. Agric. Chem.*, 1959, **42**, 19.

Nicotine



Use

As an anthelmintic for poultry—but its value as a prophylactic has been questioned⁸⁰. Usually used in combination with Phenothiazine, dibutyl tin laurate and 2,2'-dihydroxy-5,5'-dichlorophenyl methane.

Determination

Root⁸¹ has reported on a collaborative study of a method adapted from a procedure described by Schuartzmar⁸² which separated the nicotine by steam

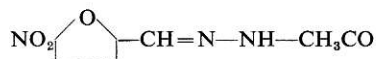
distillation and extraction from the distillate with chloroform. Interference by Phenothiazine was avoided by extracting the nicotine from the feed with NaOH and then subjecting this extract to steam distillation. The determination is completed by calculation from the U.V. absorption characteristics.

This method was adopted by the A.O.A.C.^{83,84}.

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81. Root, D. E., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 226.
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83. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 123.
84. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 198.

Nihydrazone (5-Nitro-2-furfuraldehyde acetylhydrazone)



Use

Nihydrazone is chemically similar to the other Nitrofuran prophylactics and its use in poultry feeds has been suggested⁸⁵.

Determination

Stone⁸⁵ has stated that Nihydrazone can be determined by the same method as for the chemically similar Nitrofurazone and Furazolidone but could be distinguished from the latter medicaments by the greater ease with which it formed a coloured phenylhydrazone.

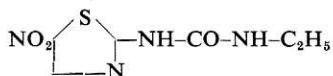
The feed is extracted with dimethylformamide and interfering material removed by chromatography on alumina/magnesium hydroxide. Determination is completed by conversion to the phenylhydrazone and measurement at 440 mμ.

The method was adopted and then made official by the A.O.A.C.^{86,87,88}, but, subsequently, slightly modified^{89,90}.

References

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87. *J. Ass. Off. Agric. Chem.*, 1961, **44**, 155.
88. *J. Ass. Off. Agric. Chem.*, 1962, **45**, 215.
89. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 53.
90. *J. Ass. Off. Agric. Chem.*, 1964, **47**, 196.

Nithiazide (1-ethyl-3(5-nitro-2-thiazolyl) urea)



Use

In poultry feeds as a prophylactic against enterohepatitis (Blackhead).

Determination

Szalkowski⁹¹ reported on collaborative trials of three methods of which one was selected for further investigation. The Nithiazide was extracted with a dimethylformamide/benzene/trichloroacetic acid mixture followed by chromatography on alumina and elution with dilute HCl. Alkaline degradation resulted in the formation of nitrite and the determination was completed by using this nitrite to diazotise procaine, followed by coupling and measuring at 540 m μ .

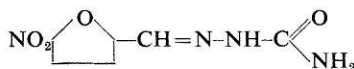
A further study reported by Szalkowski⁹² modified the extraction by using dimethylformamide only and a different preparation of alumina. This method was adopted and then made official by the A.O.A.C.^{93,94}.

References

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92. Szalkowski, C. R., *J. Ass. Off. Agric. Chem.*, 1960, **43**, 295.
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Nitrofurazone (5-Nitro-2-furfurylidene semicarbazone)

Also known as "Furacin".



Use

In poultry feeds as a prophylactic against coccidiosis. Also with Furazolidone as "Bifuran".

Determination

Nitrofurazone is closely related to Furazolidone and most of the available methods for the latter do not distinguish between these two substances so that certain of the procedures listed under Furazolidone have also been applied to Nitrofurazone^{95,96,97,98,99}.

Other methods proposed specifically for the determination of Nitrofurazone may suffer to varying degrees from interference by Furazolidone.

Dixon¹⁰⁰ carried out a preliminary extraction of the feed with petroleum ether and then extracted the Nitrofurazone with ethanol. Nitrofurazone was then separated from interfering material by paper chromatography and measured at 365 m μ .

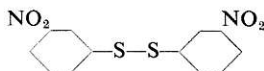
Cross, Hendey and Stevens¹⁰¹ examined the earlier procedures and the observations by van Zijl and Goosens¹⁰², that interfering substances in the extract could be destroyed by permanganate and the use, by Porter¹⁰³, of dimethylformamide. The colour produced by the addition of NaOH to nitrofurazone was found to be stable in the presence of phenol and was measured at 530 m μ . The method of Cross, Hendey and Stevens has been examined by the Analytical Methods Committee of the Society for Analytical Chemistry and published with certain modifications as a Report¹⁰⁴.

Stone¹⁰⁵, in a recent paper, determined Nitrofurazone in the presence of Furazolidone by chromatography of the extract on alumina. Furazolidone is eluted with carbon tetrachloride/isopropanol and Nitrofurazone by chloroform/acetic acid/ethanol. A polarographic method has been described by Fricke, Keppel and Hart¹⁰⁶.

References

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96. *J. Ass. Off. Agric. Chem.*, 1960, **43**, 127.
97. Puglisis, E., *J. Ass. Off. Agric. Chem.*, 1961, **44**, 30.
98. *J. Ass. Off. Agric. Chem.*, 1961, **44**, 155.
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101. Cross, A. H. J., Hendey, R. A. and Stevens, S. G. E., *Analyst*, 1960, **85**, 355.
102. van Zijl, H. J. M. and Goosens, N., *Chem. Weekbl.*, 1956, **52**, 624.
103. Porter, C. C., *Anal. Chem.*, 1955, **27**, 805.
104. *Analyst*, 1963, **88**, 935.
105. Stone, L. R., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 565.
106. Fricke, F. L., Keppel, G. E. and Hart, S. M., *J. Ass. Off. Agric. Chem.*, 1964, **47**, 788.

Nitrophenide (3,3'-dinitro-diphenyl-disulphide) ("Megasul")



Use

In poultry feeds as a prophylactic against coccidiosis.

Determination

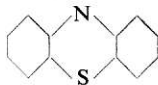
Merwin¹⁰⁷ has reported on a collaborative study in which the feed was extracted with a phosphate buffer at pH 6.6 followed by reduction to the corresponding amine. Determination was then completed by diazotisation and coupling and measuring at 545 mμ.

A further collaborative study of a slightly modified procedure was subsequently reported by Merwin¹⁰⁸ and this was adopted and then made official by the A.O.A.C.^{109,110,111}.

References

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109. *J. Ass. Off. Agric. Chem.*, 1956, **39**, 69.
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Phenothiazine



Use

As an anthelmintic, usually in conjunction with nicotine, for poultry.

Determination

Brooke¹¹² reported the results of a preliminary study in which the Phenothiazine was extracted from the feed with ethanol and the colour developed by bromination measured at 510 m μ . An alternative procedure measured the green colour produced by coupling with diazotised *p*-amino-benzoic acid, at 600 m μ .

A further report by Brooke¹¹³ in a collaborative trial recommended the alternative procedure and this was adopted and then made official by the A.O.A.C.^{114,115,116}.

References

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Piperazine



Use

As an anthelmintic in both poultry and animal feeds.

Determination

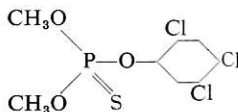
A colorimetric procedure based on Faucry's reaction¹¹⁷ was studied collaboratively by Cavett and Heotis¹¹⁸. The piperazine was extracted from the feed with water, the extract made alkaline and steam distilled. Reaction of the distillate with quinone then gave a colour that was measured at 490 m μ .

A further study of this method has been reported by Payfer¹¹⁹.

References

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118. Cavett, J. W. and Heotis, J. P., *J. Ass. Off. Agric. Chem.*, 1958, **41**, 323.
119. Payfer, R., *J. Ass. Off. Agric. Chem.*, 1946, **47**, 240.

Ronnel (O,O-dimethyl-O-(2,4,5-trichlorophenyl) phosphorothioate)



Use

For the prophylactic control of cattle grubs.

Determination

A method for the determination of Ronnel in mineral based concentrate was reported by Gehrt and Sims¹²⁰, but while this was adopted and made

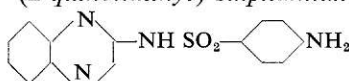
official by the A.O.A.C.^{121,122,123}, for the purpose, it was not suitable for use at a prophylactic level on ordinary feeds.

Gehrt¹²⁴ subsequently reported on a collaborative study, using a modified procedure for feeds, in which the Ronnel was extracted with methanol and hydrolysed to 2,4,5-trichlorophenate. Final separation was achieved by steam distillation and determination of the U.V. absorption characteristics.

References

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121. *J. Ass. Off. Agric. Chem.*, 1962, **45**, 121.
122. *J. Ass. Off. Agric. Chem.*, 1962, **45**, 215.
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Sulphaquinoxaline (*N*¹-(2-*quinoxaliny*l) *sulphanilamide*)



Use

In poultry feeds as a prophylactic against coccidiosis.

Determination

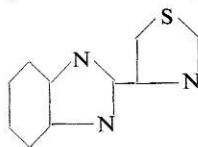
Merwin¹²⁵ has described a method in which the sulphaquinoxaline is extracted by aqueous NaOH and the determination completed by conventional diazotisation and coupling to give a colour read at 545 mμ. This method, however, had the disadvantage that other materials were also extracted, particularly amines capable of diazotisation, but was adopted by the A.O.A.C.¹²⁶.

Arsanilic acid also caused interference, but a modified procedure reported by Merwin¹²⁷, enabled both sulphaquinoxaline and arsanilic acid to be determined by measuring the total colour given by diazotisation and coupling and that given after the removal of the sulphaquinoxaline by extraction from the solution with ether. This method of separation was adopted and then made official by the A.O.A.C.^{128,129,130}. The complete separation and extraction of both constituents was finally made official¹³¹.

An investigation¹³² has been directed towards the development of a more satisfactory extraction procedure based upon the use of dimethylformamide and chloroform followed by extraction into aqueous NaOH. The determination is then completed by diazotisation and coupling similar to the original procedure¹²⁵.

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129. *J. Ass. Off. Agric. Chem.*, 1956, **39**, 108.
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132. Analytical Methods Committee, Society for Analytical Chemistry, London.

Thiabendazole (2-(4'-thiazolyl)-benzimidazole)*Use*

As an anthelmintic in pig feeds.

Determination

A collaborative study reported by Szalkowski¹³³ used a method of extraction by dilute HCl followed by extraction into chloroform and then back into acid to remove interfering substances. Reduction with zinc in the presence of *p*-phenylenediamine, followed by oxidation with ferric sulphate led to the formation of a blue complex that was measured at 600 m μ .

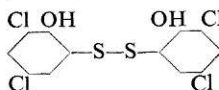
The method was adopted by the A.O.A.C.^{134,135}.

References

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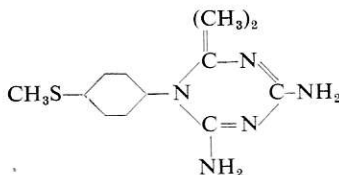
Trithiadol A mixture of:—

Bithionol, (2,2'-dihydroxy-3,3',5,5'-tetrachlorodiphenyldisulphide)



with

Methiotriazamine, (4,6-diamino-1-(4-methylmercaptophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride).

*Use*

In poultry feeds as a prophylactic against coccidiosis.

Determination

Auerbach¹³⁶ reported on a collaborative trial based upon the determination of the Bithionol component. The feed was extracted with a mixture of methanol/*n*-hexane, acidified with HCl and the determination completed by utilising the well known colour reaction of phenols with 4-amino-antipyrine^{137,138}. The results, however, were disappointing.

Auerbach *et al.*¹³⁹, re-examined the method, and a modified procedure proved to be satisfactory when subjected to a collaborative trial.

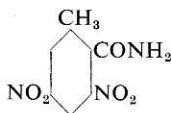
The method was adopted and then made official by the A.O.A.C.^{140,141}.

McChesney *et al.*¹⁴² have described a method for the determination of the Methiotriazamine constituent by extracting with acidified methanol and then extracting as a complex with bromothymol blue. Hydrolysis then breaks the triazine ring to give a diazotisable amine.

References

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137. Emerson, E., *J. Org. Chem.*, 1943, **8**, 417.
138. Gottlieb, S. and March, P. B., *Ind. Eng. Chem. (Anal. Ed.)*, 1946, **18**, 16.
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Zoalene (3,5-dinitro-o-toluamide)



Use

In poultry feeds as a prophylactic against coccidiosis.

Determination

Getzendaner¹⁴³ reported a procedure in which the Zoalene was extracted by hot acetonitrile. Addition of ethylenediamine to the extract gave a red-brown colour which was measured at 560 m μ . The colour was stated to be unstable.

Getzendaner & Garner¹⁴⁴ described an improved method in which interfering material was removed by adding alumina to the extract and this method was adopted by the A.O.A.C.¹⁴⁵ although a serious disadvantage of the method is that the specification of the alumina is very critical.

Further work by Getzendaner¹⁴⁶ extended the availability of this method and it was made an official method by the A.O.A.C.^{147,148}.

In a simpler procedure, at present under investigation¹⁴⁹, the Zoalene is extracted with dimethylformamide and the determination is completed by measurement of the green colour at 640 m μ given by the action of sodium methylate.

References

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147. *J. Ass. Off. Agric. Chem.*, 1962, **45**, 121.
148. *J. Ass. Off. Agric. Chem.*, 1962, **45**, 216.
149. Analytical Methods Committee, Society for Analytical Chemistry, London.