Volume 23 Part 4 December 1985

JOURNAL
OF THE
ASSOCIATION OF PUBLIC ANALYSTS

Determination of Fat in Dried Milk: Collaborative Trial

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Eight laboratories participated in a collaborative study of the official EEC method for the determination of fat in dried milk. The repeatability and reproducibility derived from this trial were 0·17 and 0·54 per cent. respectively at the 1 per cent. fat level; 0·32 and 0·73 per cent. respectively at the 13 per cent. fat level and 0·32 and 1·09 per cent. respectively at the 26 per cent fat level.

The Condensed Milk and Dried Milk (Amendment) Regulations 1982 implement Commission Directive No. 79/1067/EEC[1] which lays down *inter alia* Community methods of analysis for testing dried milk products. The method for the determination of fat is prescribed in Method 4 of Annex II to the Directive and consists of a Rose–Gottlieb extraction. The method specifies the following analytical criteria. "The difference between results of two determinations carried out simultaneously or in rapid succession on the same sample by the same analyst, under the same conditions shall not exceed 0·2 g of fat per 100 g of product with the exception of skimmed milk powder for which the difference must not exceed 0.1 g of fat per 100 g of product."

No analytical data are available to justify these acceptance requirements. The purpose of this trial was to obtain sufficient data to assess the validity of the EEC acceptance limits.

Samples and Organisation

Eight public analysts' laboratories took part in the trial. Each laboratory received three samples labelled A to C, each of which was to be analysed in duplicate for fat using the Community method. It was considered that all laboratories should be familiar with the Rose–Gottlieb method making a preliminary trial of competence in the method unnecessary. The three samples consisted of the following:

(A) Dried whole milk, approximately 26 per cent. of fat
(B) Dried partly skimmed milk, approximately 13 per cent. of fat
(C) Dried skimmed milk, approximately 1 per cent. of fat

The materials were homogenised in the co-ordinating laboratory and 10 g subsamples taken from the bulk for distribution to the participating laboratories.

0004–5780/85/040103+03 $02.00/0

Instructions for the conduct of the trial were as follows:

1. Do not deviate from the specified method in any way.
2. Make all six determinations on the same day using the same analyst.
3. Take normal precautions against uptake of moisture by the sample and report the results on the sample as received, giving the answers to two decimal places.

The results of the trial are given in Table I.

TABLE I
PERCENTAGE OF FAT IN DRIED MILK SAMPLES A, B AND C

Laboratory	Sample A		Sample B		Sample C	
1	26·61	26·46	13·67	13·88	0·72	0·66
2	26·62	26·63	13·81	13·89	0·87	0·93
3	26·99	27·01	13·94	14·19	1·02	1·00
4	26·05	26·05	13·32	13·33	0·60	0·73
5	26·78	25·60	13·85	13·69	1·01	1·15
6	26·21	26·44	13·34	13·42	0·69	0·60
7	26·55	26·24	13·74	13·63	0·84	0·78
8	25·89	25·83	13·46	13·68	0·51	0·50

Statistical Analysis of the Results

Statistical analysis of the results was made in accordance with procedures given by the British Standards Institution[2].

SAMPLE A

The results from laboratory 5 were classified as outliers by Cochran's Test and rejected after enquiries showed no reason for the poor repeatability.

SAMPLE B

The results from laboratory 4 were classified as stragglers by Dixon's Test but they showed satisfactory repeatability by Cochran's Test and were not rejected.

SAMPLE C

No results were rejected.

TABLE II
SUMMARY OF TRIAL DATA

	Sample A	Sample B	Sample C
Number of laboratories (n) after elimination of outlier	7	8	8
Mean ($\bar{x}$)	26·40	13·68	0·79
Standard deviation of repeatability (S_r)	0·11	0·11	0·06
Repeatability ($2{\cdot}83 \times S_r$)	0·32	0·32	0·17
Standard deviation of reproducibility (S_R)	0·38	0·26	0·20
Reproducibility ($2{\cdot}83 \times S_R$)	1·09	0·73	0·57
"Acceptance" repeatability	0·2	0·2	0·1

Repeatability (r) and Reproducibility (R)

The estimated precision values for seven laboratories on Sample A and eight laboratories on Samples B and C are given in Table II.

Conclusion

The data obtained in this trial show that the method is capable of a satisfactory degree of precision. It also indicated that the EEC acceptance criteria may be set too low, resulting in a lower proportion of acceptances and necessitating repeat analyses before reporting a mean figure.

References

1. Commission Directive No. 79/1067/EEC O.J. No. L327, 24.12.79 p. 29
2. British Standards Institution "Precision of test methods" BS 5497 Part 1: 1979.

J. Assoc. Publ. Analysts, 1985, **23**, 107–117

Determination of Soluble Solids in Vinegar: Collaborative Trial

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A collaborative trial by 18 UK laboratories to assess two alternative methods of analysis for the determination of the soluble solids content of vinegar has been carried out. An AOAC method and a method proposed by the UK representatives to the Codex Co-ordinating Committee for Europe were tested. It was found that the AOAC method gave better repeatability and reproducibility values for some samples but that the method proposed by the UK representatives gave more accurate results and is therefore recommended for adoption by the Codex Co-ordinating Committee.

The Codex Alimentarius Commission Co-ordinating Committee for Europe is developing a European Regional Standard for Vinegar. The Draft Standard is now at Step 8 of the Codex procedure[1]. Included in the Draft Standard are limits for the soluble solids content. These limits are:

(a) Wine vinegar: the soluble solids content, exclusive of added sugars or salt, shall be not less than 1·3 g per 1000 ml per 1 per cent. of acetic acid.
(b) Fruit (wine) vinegar, Berry (wine) vinegar or cider vinegar: the soluble solids content, exclusive of added sugars or salt, shall be not less than 2·0 g per 1000 ml per 1 per cent. of acetic acid.

The soluble solids content will be determined by a specified method of analysis. The method initially suggested for inclusion in the Draft Standard was an AOAC procedure[2]. However, at the 13th Session of the Co-ordinating Committee for Europe, the representative of the United Kingdom stated that "the method proposed by Spain (the AOAC Method) gave rise to difficulties in that some of the acetic acid remained in the residue after drying. It was common practice in the United Kingdom to wash the residue several times with distilled water (drying in between each washing), so as to allow complete volatilization of the acetic acid". The representative of the United Kingdom was asked to provide a detailed text of the method suitable for collaborative study for transmission to the Codex Committee on Methods of Analysis and Sampling[3]. This was prepared by the United Kingdom and is reproduced as Appendix I to this Report.

Nevertheless the Co-ordinating Committee for Europe, at its Fourteenth Session recommended that the AOAC method[2] be given in the Draft Standard, on the recommendation of an *ad hoc* Working Group on methods of analysis and sampling of vinegar which was convened at that Session. Members of that *ad*

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hoc Working Group "expressed their gratitude to the delegation of Spain for undertaking to test collaboratively these methods of analysis and noted the satisfactory findings on the repeatability and reproducibility of the methods"[1]. These results were given in a Report prepared for that Session by the delegation of Spain[4].

The recommendation from the Co-ordinating Committee for Europe regarding the adoption of the method for determining soluble solids content in vinegar was sent to the Codex Committee on Methods of Analysis and Sampling. That Committee, at its Fourteenth Session held November 1984, postponed endorsement of the method, stating that "it was impossible to achieve the precision reported and that it would like to have further information on conduct and calculation of the results"[5].

The method proposed by the UK has, in the meantime, been formally collaboratively studied and the results of that study are given in this Report. The study was organised by the Ministry of Agriculture, Fisheries and Food (MAFF) as one in a series of collaborative trials sponsored by MAFF to assess methods of analysis for foodstuffs. The results of such trials are normally published as Reports; the aims and objectives of the trials have been given in a previous Report[6].

Collaborative Trial Organisation, Samples, Methods and Results

There were 18 participating analysts in the trial (16 UK Public Analysts, the Laboratory of the Government Chemist and the Government Laboratory from the Isle of Man).

SAMPLES

The following samples were prepared:

(a) Vinegar, untreated.
(b) Vinegar (a) containing, in addition, 2 g/100 ml of glacial acetic acid.
(c) Vinegar (a) containing, in addition, 0·5 g/100 ml of sodium chloride.
(d) Vinegar (a) containing, in addition, 0·5 g/100 ml of citric acid.
(e) Vinegar (a) containing, in addition, 2 g/100 ml of glacial acetic acid and 0·5 g/100 ml of sodium chloride.
(f) Vinegar (a) containing, in addition, 2 g/100 ml of glacial acetic acid and 0·5 g/100 ml of citric acid.
(g) Vinegar (a) containing, in addition, 0·5 g/100 ml of sodium chloride and 0·5 g/100 ml of citric acid.
(h) Vinegar (a) containing, in addition, 2 g/100 ml of glacial acetic acid, 0·5 g/100 ml of sodium chloride and 0·5 g/100 ml of citric acid.

The samples were prepared in two litre batches. Two aliquots of 50 ml in screw-capped polyethene bottles were sent to each analyst coded as blind duplicates, i.e. each analyst received 16 samples and was asked to analyse each of these samples once only by the two methods under test. The sample codes used are given in Tables I and II.

METHODS

The methods used in the trial were as follows:

Method I: Proposed Codex method (Appendix I)

In this method the residual mass of a test portion (10 ml) was determined after evaporation on a water bath, followed by drying at atmospheric pressure in an oven at 103°C (± 2°C). To ensure the total volatilisation of the acetic acid, the evaporation step was repeated three times after restoration of the original volume with distilled water. Evaporating dishes of 75 mm diameter with close-fitting lids were recommended.

Method II: AOAC method (2) (and reproduced in Appendix II)

In this method the residual mass of a test portion (10 ml) was determined after evaporation on a water bath, followed by drying at atmospheric pressure in an oven at the temperature of boiling water. Flat-bottomed platinum evaporating dishes of 50 mm diameter were specified.

Analysts were asked to carry out each determination once only by each method and to report the total soluble solids of the samples as percentage mass to volume, i.e. x g of solids per 100 ml of sample. Analysts were also asked to describe the evaporating dish used in each method.

RESULTS

The results of the determinations for total soluble solids in vinegar by methods (I) and (II) are shown in Tables I and II respectively.

Statistical Analysis of the Results

The results reported in Tables I and II were examined for evidence of individual systematic error ($P \leqslant 0{\cdot}05$) using Cochran's and Dixon's tests progressively, by procedures described by the British Standards Institution[7].

REPEATABILITY AND REPRODUCIBILITY

Calculations for repeatability (r) and reproducibility (R), as defined by procedures given by the British Standards Institution[7], were carried out on those results remaining after removal of outliers for each method/sample calculation. These values were calculated by the procedures given by the British Standards Institution[7].

The values obtained are given in Tables I and II, and summarised in Table III. Most of the values of repeatability and reproducibility are satisfactory and are of the order of magnitude normally expected for this type of analytical procedure. The values for the proposed method for repeatability in samples (f) and (h) and of reproducibility in samples (d) and (f) are, however, greater than expected.

COMPARISON OF THE REPEATABILITY AND REPRODUCIBILITY VALUES FOR THE TWO METHODS

The values of the within and between variance ratios (defined as $F_r = r_1^2/r_2^2$ and $F_R = (2R_1^2 - r_1^2)/(2R_2^2 - r_2^2)$ were calculated.

TABLE I
SOLUBLE SOLIDS IN VINEGAR (COLLABORATIVE TRIAL)
BY PROPOSED CODEX METHOD (g/100 ml)

Laboratory	Sample Composition							
	Vinegar	Vinegar + 2 g/100 ml acetic acid	Vinegar + 0·5 g/100 ml sodium chloride	Vinegar + 0·5 g/100 ml citric acid	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml sodium chloride	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml citric acid	Vinegar + 0·5 g/100 ml citric acid + 0·5 g/100 ml sodium chloride	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml sodium chloride 0·5 g/100 ml citric acid
	2 , 10	4 , 6	8 , 11	1 , 16	3 , 15	5 , 13	7 , 9	12 , 14
1	0·83 , 0·78	0·78 , 0·82	1·28 , 1·30	1·26 , 1·20	1·30 , 1·26	1·23 , 1·19	1·71 , 1·70	1·69 , 1·69
2	0·75 , 0·73	0·72 , 0·76	1·26 , 1·23	1·21 , 1·20	1·24 , 1·23	1·18 , 1·16	1·71 , 1·68	1·69 , 1·68
3	0·81 , 0·75	0·76 , 0·77	1·27 , 1·23	1·25 , 1·17	1·29[b] , 1·20[b]	1·20 , 1·16	1·74 , 1·70	1·68 , 1·66
4	0·77[a] , 0·79[a]	0·78[a] , 078[a]	1·32[a] , 1·30[a]	1·24[a] , 1·25[a]	1·27[a] , 1·32[a]	1·21[a] , 1·23[a]	1·76[a] , 1·75[a]	1·72[a] , 1·74[a]
5	0·72 , 0·71	0·71 , 0·69	1·20 , 1·24	1·19 , 1·17	1·22 , 1·22	1·13 , 1·19	1·68 , 1·67	1·65 , 1·72
6	0·79[a] , 0·71[a]	0·75[a] , 0·77[a]	1·26[a] , 1·23[a]	1·25[a] , 1·21[a]	1·25[a] , 1·22[a]	1·24[a] , 1·18[a]	1·79[a] , 1·74[a]	1·75[a] , 1·75[a]
7	0·70[a] , 0·71[a]	0·71[a] , 0·70[a]	1·22[a] , 1·22[a]	1·17[a] , 1·18[a]	1·23[ab] , 1·15[ab]	1·15[a] , 1·22[a]	1·70[a] , 1·68[a]	1·68[a] , 1·67[a]
8	0·91 , 0·84	0·85 , 0·84	1·36 , 1·37	1·39 , 1·30	1·35 , 1·30	1·32 , 1·22	1·80 , 1·80	1·72 , 1·80
9	0·81 , 0·78	0·79 , 0·74	1·30 , 1·27	1·25 , 1·32	1·28[b] , 1·44[b]	1·26 , 1·22	1·79 , 1·73	1·73 , 1·71
10	0·80 , 1·80	0·83 , 0·78	1·29 , 1·30	1·22 , 1·23	1·29 , 1·30	1·21 , 1·23	1·72 , 1·75	1·74 , 1·76
11	0·79 , 0·85	0·80 , 0·79	1·34 , 1·33	1·20 , 1·19	1·26 , 1·25	1·20 , 1·18	1·78 , 1·76	1·81 , 1·67
12	0·73 , 0·80	0·71 , 0·70	1·24 , 1·21	1·15 , 1·15	1·21 , 1·19	1·16 , 1·15	1·70 , 1·67	1·66 , 1·69
13	0·74 , 0·73	0·74 , 0·70	1·23 , 1·25	1·23 , 1·21	1·18 , 1·23	1·20 , 1·19	1·72 , 1·71	1·71 , 1·71
14	0·69 , 0·73	0·69 , 0·68	1·20 , 1·21	1·14 , 1·17	1·17 , 1·22	1·15 , 1·15	1·66 , 1·70	1·70 , 1·67
15	0·80 , 0·73	0·73 , 0·74	1·25 , 1·25	1·25 , 1·21	1·19 , 1·23	1·20 , 1·17	1·70 , 1·68	1·68 , 1·66
16	0·86 , 0·85	0·87 , 0·87	1·36 , 1·33	1·30 , 1·28	1·35 , 1·34	1·31 , 1·34	1·83 , 1·82	1·84 , 1·78
17	0·71 , 0·73	0·71 , 0·71	1·20 , 1·22	1·16 , 1·20	1·21 , 1·21	1·12 , 1·19	1·65 , 1·71	1·78 , 1·68
18	0·67 , 0·65	0·59 , 0·64	1·17[b] , 1·06[b]	1·13 , 1·14	1·18 , 1·17	1·07 , 1·08	1·64 , 1·59	1·47 , 1·56
mean (X)	0·77	0·75	1·27	1·22	1·25	1·20	1·72	1·70
Repeatability (r)	0·09	0·06	0·05	0·08	0·06	0·16	0·07	0·11
Reproducibility (R)	0·17	0·18	0·14	0·16	0·15	0·22	0·15	0·19
Standard deviation[d]	0·059	0·062	0·050	0·056	0·053	0·078	0·054	0·066

TABLE II
SOLUBLE SOLIDS IN VINEGAR (COLLABORATIVE TRIAL)
BY AOAC METHOD

Laboratory	Samples Composition							
	Vinegar	Vinegar + 2 g/100 ml acetic acid	Vinegar + 0·5 g/100 ml sodium chloride	Vinegar + 0·5 g/100 ml citric acid	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml sodium chloride	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml citric acid	Vinegar + 0·5 g/100 ml citric acid + 0·5 g/100 ml sodium chloride	Vinegar + 2 g/100 ml acetic acid + 0·5 g/100 ml sodium chloride 0·5 g/100 ml citric acid
	2 , 10	4 , 6	8 , 11	1 , 16	3 , 15	5 , 13	7 , 9	12 , 14
1	0·91 , 0·90	0·85 , 0·91	1·31 , 1·30	1·22 , 1·21	1·29 , 1·28	1·22 , 1·20	1·80 , 1·76	1·74 , 1·75
2	0·80 . 0·80	0·88 , 0·83	1·28 , 1·30	1·28 , 1·27	1·29 , 1·29	1·27 , 1·28	1·78 , 1·79	1·80 , 1·80
3	0·87 , 0·88	0·74 , 0·82	1·30 , 1·31	1·29 , 1·25	1·34 , 1·28	1·27 , 1·24	1·79 , 1·78	1·76 , 1·77
4	0·74[a] , 0·76[a]	0·76[a] , 0·74[a]	1·28[a], 1·25[a]	1·23[a] , 1·22[a]	1·23[a] , 1·23[a]	1·21[a] , 1·20[a]	1·73[a] , 172[a]	1·69[a] , 1·70[a]
5	0·78 , 0·80	0·77 , 0·82	1·27 , 1·31	1·28 , 1·27	1·33 , 1·29	1·32 , 1·31	1·79 , 1·81	1·81 , 1·82
6	0·82[a] , 0·85[a]	0·84[a] , 0·77[a]	1·30[a] , 1·29[a]	1·34[a] , 1·30[a]	1·32[a] , 1·32[a]	1·24[a] , 1·25[a]	1·79[ab] , 1·88[ab]	1·78[a], 1·77[a]
7	0·78[a] , 0·80[a]	0 91[a] , 0·88[a]	1·29[ab] , 1·09[ab]	1·180[ab] , 1·26[ab]	1·28[a] , 1·38[a]	1·27[a] , 1·24[a]	1·78[a] , 1·76[a]	1·73[a] , 1·80[a]
8	0·91 , 0·87	0·86 , 0·81	1·35 , 1·32	1·32 , 1·35	1·31[c] , 1·49[c]	1·27 , 1·35	1·86 , 1·81	1·80[b] , 1·90[b]
9	0·85 , 0·80	0·88 , 0·96	1·39 , 1·33	1·22[b] , 1·35[b]	1·35 . 1·33	1·29 , 1·28	1·85 , 1·85	1·84 . 1·84
10	0·86 , 0·91	0·96 , 0·82	1·34 , 1·34	1·34 , 1·36	1·32 , 1·33	1·28 , 1·34	1·85 , 1·83	1·82 , 1·84
11	0·80 , 0·81	0·78 , 0·78	1·33 , 1·33	1·26 , 1·25	1·29 , 1·32	1·27 , 1·27	1·82 , 1·81	1·81 , 1·80
12	0·80 , 0·91	0·80 , 0·79	1·29 , 1·29	1·27 , 1·26	1·30 , 1·30	1·27 , 1·28	1·81 , 1·81	1·81 , 1·82
13	0·87 , 0·86	0·87 , 0·78	1·30 , 1·35	1·30 . 1·31	1·30 , 1·31	1·31 , 1·27	1·82 , 1·84	1·83 , 1·79
14	0·79 , 0·82	0·82 , 0·81	1·30 , 1·27	1·24 , 1·25	1·27 , 1·29	1·25 , 1·25	1·75 , 1·77	1·79 , 1·79
15	0·82 , 0·72	0·77 , 0·78	1·29 , 1·29	1·30 , 1·23	1·21 , 1·26	1·24 , 1·20	1·75[b] , 1·96[b]	1·69 , 1·70
16	0·79 , 0·81	0·79 , 0·79	1·32 , 1·31	1·29 , 1·24	1·31 , 1·29	1·26 , 1·30	1·76 , 1·80	1·81 , 1·78
17	0·62 , 0·77	0·78 , 0·59	1·25[b] , 1·33[b]	1·29 , 1·28	1·01[b] , 1·29[b]	1·15[b] , 1·45[b]	1·80 , 1·83	1·89 , 1·83
18	0·84 , 0·79	0·77 , 0·83	1·34 , 1·40	1·27 , 1·25	1·27 , 1·39	1·38 , 1.27	1·88 , 1·85	1·88 , 1·86
Mean (X)	0·82	0·815	1·28	1·31	1·30	1·27	1·80	1·79
Repeatability (r)	0·11	0·15	0·06	0·06	0·09	0·08	0·05	0·05
Reproducibility (R)	0·17	0·19	0·11	0·09	0·11	0·12	0·11	0·14
Standard Deviation[d]	0·060	0·068	0·040	0·033	0·039	0·041	0·038	0·050

KEY TO TABLES I AND II

a. Results reported to three decimal place but corrected to two places. All other results reported to two decimal places.
b. Results rejected by Cochran's Test $P \leqslant 0{\cdot}05$. Values not used in calculation of mean, repeatability or reproducibility.
c. Results rejected by Dixon's Test, $P \leqslant 0{\cdot}05$. Values not used in calculation of mean, repeatability or reproducibility.
d. Standard deviation calculated from all individual results but not including those rejected by Cochran's or Dixon's tests.

TABLE III
SUMMARY OF STATISTICAL RESULTS FOR THE DETERMINATION OF THE TOTAL SOLUBLE SOLIDS CONTENT IN VINEGAR

Sample	Method	No. of data pairs	Mean value (*g/100 ml*)	Repeatability (*r*)	Reproducibility (*R*)	Standard deviation
a	Proposed	18	0·77	0·09	0·17	0·059
	AOAC	18	0·82	0·11	0·17	0·060
b	Proposed	18	0·75	0·06	0·18	0·062
	AOAC	18	0·815	0·15	0·19	0·068
c	Proposed	17	1·27	0·05	0·14	0·050
	AOAC	16	1·28	0·06	0·11	0·040
d	Proposed	18	1·22	0·08	0·16	0·056
	AOAC	16	1·31	0·06	0·09	0·033
e	Proposed	15	1·25	0·06	0·151	0·053
	AOAC	16	1·30	0·09	0·11	0·039
f	Proposed	18	1·20	0·16	0·22	0·078
	AOAC	17	1·27	0·08	0·12	0·041
g	Proposed	18	1·72	0·07	0·15	0·054
	AOAC	16	1·80	0·05	0·11	0·038
h	Proposed	18	1·70	0·11	0·19	0·066
	AOAC	17	1·79	0·05	0·14	0·050

There were no significant differences between the methods (at the $P = 0{\cdot}05$ level except for the repeatabilities for samples (f) and (h) and the reproducibilities of samples (d) and (f). In these four cases, the values of r and R are better for the AOAC method than for the proposed method.

COMPARISON OF THE MEAN VALUES ATTACHED BY THE TWO METHODS

The mean values obtained by each method/sample combination are given in Tables I and II and summarised in Table III. The mean values for each sample obtained by the two methods were compared using a null-hypothesis t-test.

Only in the case of sample (c) are the two means not significantly different ($P \leqslant 0{\cdot}01$). It may therefore be concluded that the two methods give significantly different results when the analysis of the same sample is carried out.

RECOVERIES

The mean values of the different samples can be compared with the sample composition. These are given in Tables IV and V. Comments on the results obtained are given in these two tables.

For both methods it may be assumed that the addition of acetic acid, citric acid of sodium chloride, separately or in combination, does not affect their basic performance.

Conclusions

Both methods performed well when used by the participants. The presence of additional substances in the stock vinegar used in the trial did not affect the performance of either method.

The precision of the AOAC method, as assessed by the repeatability and reproducibility values obtained, is shown to be better for some samples than the proposed method. However, for most samples there was no significant difference between the two methods.

The samples analysed by the proposed method gave consistently lower mean values than when analysed by the AOAC method. These differences were shown to be statistically significant for most of the samples used in this trial.

TABLE IV
SOLUBLE SOLIDS OF VINEGAR
COMPARISON OF MEANS DETERMINED WITH DIFFERENT SAMPLE COMPOSITIONS FOR THE PROPOSED CODEX METHOD

Sample composition	Mean value (*g/100 ml*)	Comment
Vinegar	0·77	Vinegar used to prepare all samples.
Vinegar + 2 g/100 ml of acetic acid	0·75	The added acetic acid is removed quantitatively in the drying process.
Vinegar + 0·5 g/100 ml of Sodium chloride	1·27	All of the added sodium chloride is recovered.
Vinegar + 0·5 g/100 ml of citric acid	1·22	The added citric acid remains through the drying process.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of sodium chloride	1·25	The added sodium chloride is recovered quantitatively and is not affected by the presence of additional acetic acid.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of citric acid	1·20	The citric acid remains through the drying process and is not affected by the presence of additional acetic acid.
Vinegar + 0·5 g/100 ml of citric acid + 0·5 g/100 ml of sodium chloride	1·72	The added sodium chloride is recovered quantatively and the added citric acid remains through the drying process.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of sodium chloride + 0·5 g/100 ml of citric acid	1·70	The added sodium chloride is recovered quantitatively; the added citric acid remains through the drying process and neither are affected by the presence of additional acetic acid.

TABLE V
SOLUBLE SOLIDS OF VINEGAR
COMPARISON OF MEANS DETERMINED WITH DIFFERENT SAMPLE COMPOSITIONS USING THE AOAC METHOD

Sample composition	Mean value (*g/100 ml*)	Comment
Vinegar	0·82	Vinegar used to prepare all samples.
Vinegar + 2 g/100 ml of acetic acid	0·815	The added acetic acid is removed quantitatively in the process.
Vinegar + 0·5 g/100 ml of sodium chloride	1·28	All of the added sodium chloride is recovered
Vinegar + 0·5 g/100 ml of citric acid	1·31	The citric acid remains through the drying process.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of sodium chloride	1·30	The added sodium chloride is recovered quantitatively and is not affected by the presence of additional acetic acid.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of citric acid	1·27	The citric acid remains through the drying process and is not affected by the presence of additional acetic acid.
Vinegar + 0·5 g/100 ml of citric acid + 0·5 g/100 ml of sodium chloride	1·80	The added sodium chloride is recovered quantitatively and the added citric acid remains through the drying process.
Vinegar + 2 g/100 ml of acetic acid + 0·5 g/100 ml of sodium chloride + 0·5 g/100 ml of citric acid	1·79	The added sodium chloride is recovered quantitatively; the added citric acid remains through the drying process and neither is affected by the presence of additional acetic acid.

The main point of issue was whether the AOAC method, using only a single drying step, results in occlusion of acetic acid in the soluble solids residue obtained after drying. It would appear that as the proposed method, which incorporates a three-stage drying process, gives significantly and consistently lower values than the AOAC method for the determination of soluble solids in vinegar, this is the case, and the adoption of the proposed method by Codex is, therefore, recommended.

The authors thank the following analysts and their staffs who agreed to participate in the collaborative trial: R. Arnot (Government Analyst's Laboratory, Isle of Man), M. Barnett (Dr A Voelcker & Sons Ltd, London), M. J. Billington (West Midlands County Laboratory, Birmingham), J. Day (Laboratory of the Government Chemist, London), R. Fawcett (County Laboratory, Maidstone, Kent), M. W. Fogden (Fogden & Son, Nottingham), C. N. Grange (Bostock Hill & Rigby Ltd, Birmingham), A. J. Harrison (Avon County Scientific Services, Bristol), G. F. Hooke (Ruddock & Sherratt, Chester), V. Husbands (Strathclyde Regional Laboratory, Glasgow), G. V. James (Cook and Barke, Winterbourne, Avon), T. E. Johnson (Herbert J. Evans & Partners, Carmarthen), G. Keen (County Laboratory, Worcester), D. Lord (County Laboratory, Preston), R. G. Martin (Lyne, Martin & Radford, Reading), L. A. Perkin (County Analyst's Laboratory, Leeds), T. E. Rymer

(Analytical Laboratory, Lewes, Sussex), J. H. Shelton (Leo Taylor & Lucke, London).

The authors also thank Mr. E. Newman of British Vinegars Ltd, London, for the provision of the vinegar used to prepare the samples for this trial.

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Appendix I

TOTAL SOLUBLE SOLIDS IN VINEGAR

SUGGESTED CODEX METHOD (DRAFT EUROPEAN REGIONAL STANDARD FOR VINEGAR)

1. SCOPE AND FIELD OF APPLICATION

This method determines the loss of mass on drying of vinegar.

2. DEFINITION

Total Soluble Solids—the weight of matter remaining after drying by the method specified.

3. PRINCIPLE

The residual mass of a test portion is determined after evaporation on a water bath followed by drying at atmospheric pressure in an oven at 103 ± 2°C. To ensure the total volatilisation of the acetic acid the evaporation step is repeated three times after restoration to at least the original volume with distilled water.

4. REAGENTS

4.1 Distilled water.

5. APPARATUS

5.1 10 ml pipettes

5.2 Filter and filter papers (slow filtering paper)

5.3 Dishes, preferably of platinum, nickel, stainless steel or glass. The dishes must have lids which fit very well but which can be readily removed. The dishes should be 75 mm in diameter.

5.4 Water bath

5.5 Atmospheric pressure drying oven, well ventilated and thermostatically controlled with temperature regulation at 103 ± 2°C. The temperature should be uniform throughout the oven.

5.6 Desiccator containing freshly activated silica gel with a water content indicator or an equivalent desiccant.

5.7 Analytical balance capable of weighing to at least 0.1 mg.

5.8 Glass stirring rod.

6. PROCEDURE

6·1 Uncover the dish and place it and its lid in the oven at 103°C for 1 h.

6.2 Place the lid on the dish and transfer the covered dish to the desiccator.

6.3 Allow the dish to cool to room temperature and accurately weigh to the nearest 0·1 mg (M_1).

6.4 Shake the sample and filter through the filter paper.

6.5 Pipette 10 ml of sample into the dish.

6.6 Place the dish on a boiling water bath and evaporate almost to dryness.

6.7 Add 15 ml of distilled water to the dish and stir.

6.8 Wash the stirring rod with a small quantity of distilled water.

6.9 Evaporate almost to dryness on a boiling water bath.

6.10 Repeat processes 6.7 to 6.9 a further two times.

6.11 Place uncovered dish and its lid in the oven at 103°C for 3 h.

6.12 Cover the dish with the lid and transfer the covered dish to the desiccator.

6.13 Allow the dish to cool to room temperature and accurately weigh to the nearest 0·1 mg as quickly as possible (M_2).

7. EXPRESSION OF RESULTS

7.1 *Method of calculation*

Calculate the total soluble solids of the sample, expressed as a percentage mass to volume, by the formula:

$$(M_2 - M_1) \times 10$$

where;

M_1 = Weight, in grams, of empty dish and lid after process 6.3
M_2 = Weight, in grams, of the dish, its lid and final sample after process 6.13

Appendix II

TOTAL SOLUBLE SOLIDS IN VINEGAR

AOAC METHOD (30.064) (13TH EDITION OF OFFICIAL METHODS OF THE AOAC)

PROCEDURE

Measure 10 ml of sample into weighed 50 mm diameter, flat-bottom Pt dish, evaporate on boiling water bath for 30 min and dry for exactly 2·5 h in an oven at

temperature of boiling water. Cool in a desiccator and weigh. (To obtain concordant results it is necessary to use a dish of the size and shape stated and to dry for exactly the time specified.)

J. Assoc. Publ. Analysts, 1985, **23**, 119–131

Mass Spectrometry and Trace Analysis of Food Contaminants*

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The aim of this paper is to provide a critical appraisal of mass spectrometry from the viewpoint of the potential user of this analytical tool in the area of trace analysis. Technicalities associated with instrumental aspects have been deliberately avoided and a somewhat simplistic "customer-orientated" approach adopted, aimed at informing those not directly involved in the field of its potential applications. Practical examples are utilised to illustrate both the unique advantages as well as the problems associated with mass spectrometry.

Within the Food Science Division of the Ministry, mass spectrometry is used for the identification of previously unknown compounds in foods, as a detector for confirmation of data obtained by preliminary screening by other techniques and as a quantitative technique in its own right. These aspects are illustrated by recent examples drawn from the areas of nitrosamines, mycotoxins, pesticides, and veterinary drug residues in foods, which also enable the systematic approach to developing a mass spectrometric analytical procedure to be demonstrated.

Advantages of Mass Spectrometry

For identification purposes mass spectrometry has the undoubted advantage that if a spectrum can be matched between an unknown and a known compound then identification is almost unequivocal. This is illustrated in Figure 1 which shows the electron impact spectrum of the mycotoxin 4-deoxynivalenol (vomitoxin) analysed by GC/MS as its trimethylsilyl (TMS) derivative, for both the authentic compound and a component detected in a maize product[1]. For identification purposes agreement is sought both between the presence and absence of ions in the two spectra and in the relative abundances of the ions. Some variation may occur between the relative abundances of ions in spectra obtained on different occasions for the same compound, and in establishing identification, experience is important in knowing the limits within which variation is acceptable. This variation is normally attributable to instrumental factors, but data processing (particularly the background subtraction) can have an important influence on spectral quality, and again operator experience is of

* Based on the paper presented at the Association of Public Analysts Annual Conference held at Cambridge on March 29th, 1985.

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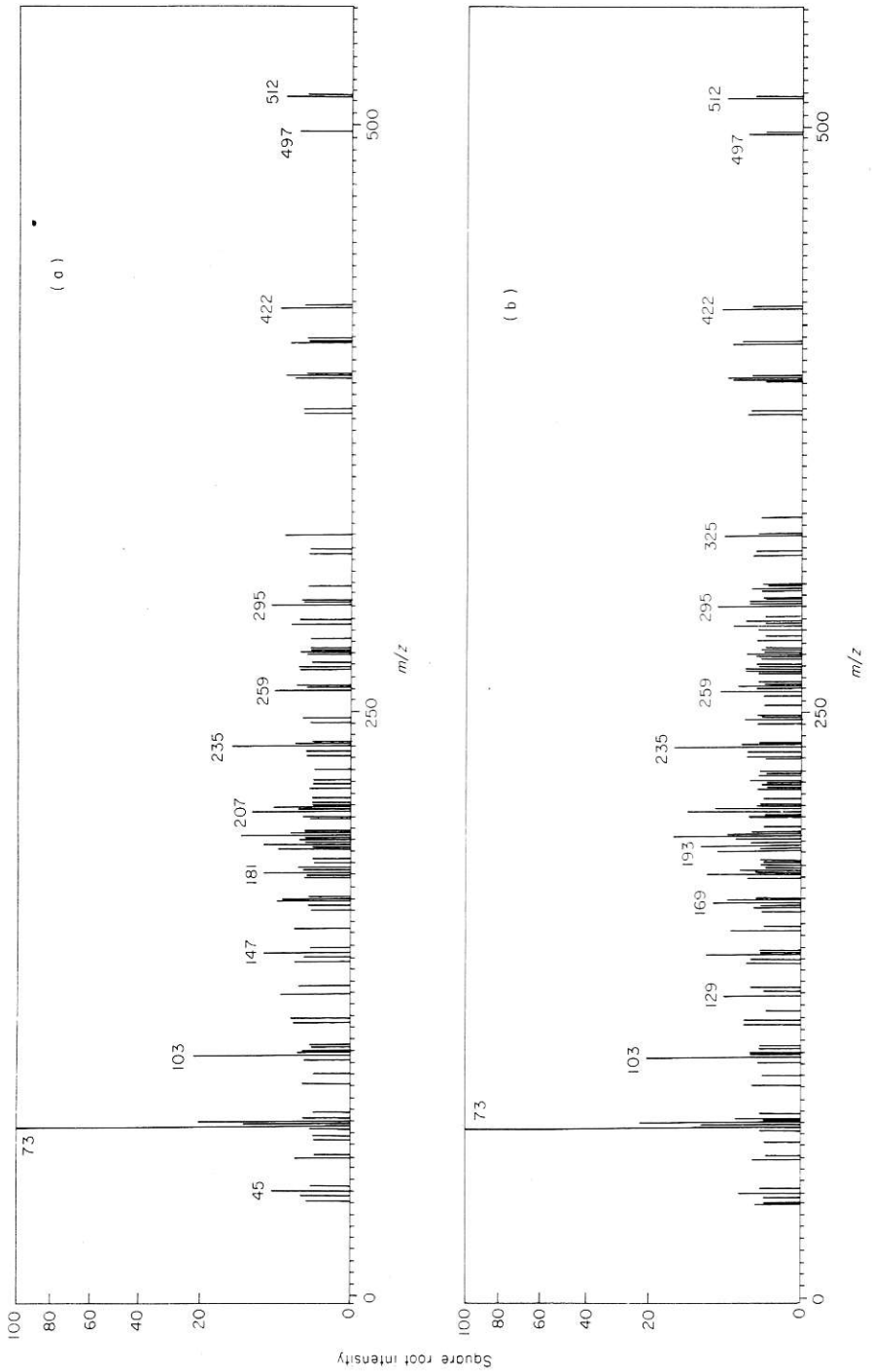

Fig. 1. Identification of the mycotoxin 4-deoxynivalenol (vomitoxin). (a) Reference spectrum of TMS-deoxynivalenol (b) Spectrum obtained from peak in extract from flaked maize. Spectra obtained by capillary column GC/MS at 70 eV using a double focusing mass spectrometer, and displayed as square root intensities for ease of comparison of high mass ions.

prime importance. In most situations some background knowledge of a particular problem area leads to possible suggestions for identification, but in the case of a complete unknown the libraries of reference spectra[2], although not often able to provide a perfect spectral match, can be of assistance in suggesting possible structural types.

Sensitivity of mass spectrometry is supreme amongst other instrumental techniques. For full scan spectra by GC/MS, identification can normally be achieved on one nanogram or less of compound on-column, which is comparable in sensitivity with a flame ionisation detector (FID). In the selected ion monitoring mode (i.e. operated as a detector rather than for identification) limits of detection in the femtogram to picogram range can be achieved, which for example for halogenated compounds is comparable to electron capture sensitivity. A more detailed comparison of sensitivities of mass spectrometry with other techniques has been published elsewhere[3].

Another important advantage of a mass spectrometer over other detectors is that in terms of selectivity it is extremely versatile, being tunable to any organic compound, but still retaining high individual compound specificity. Monitoring is normally of a molecular ion or characteristic fragment ion (or ions) chosen to be selective for a particular compound, and this can be applied to any organic compound, unlike, say, an electron capture or flame photometric detector, which depends for achieving specificity on characteristic structural features of the molecule in question. Mass spectrometry can thus be regarded as having the applicability of an FID but a unique selectivity for each and every chosen organic compound. The only limitation to this selectivity is that structurally similar isomers often give almost identical spectra so in these instances chromatographic separation may be necessary.

Although the applications of mass spectrometry discussed in the paper are mostly for compounds sufficiently volatile to be handled by GC, mass spectrometry is in fact considerably more versatile and the molecular weight range can be extended by combined LC/MS, or for molecular weights of up to several thousand by such techniques as fast atom bombardment (FAB) [4,5].

Shortcomings of Mass Spectrometry

The most obvious disadvantage of mass spectrometry is the high capital cost of instrumentation. The most basic system is four to five times more expensive than a typical GC, whereas a double focusing instrument with data handling facilities (a typical choice as a robust analytical service instrument) would cost at least twenty times that of a GC. Added to the high initial capital investment, running costs are also relatively high, and for "down-time" to be minimised, the provision of a stock of a range of expensive spares has to be taken into consideration. The other important factor is that to make effective use of this sophisticated instrumentation a skilled operator is essential. Not only is an analytical chemist required who can appreciate the background to the problems he is asked to undertake, but also someone not daunted by the complexities of ion physics, vacuum technology and electronic problems. Obviously versatility is a prime requirement for an operator and this need is further emphasised by

the increasing demand for skills in computing to handle and process the formidable amount of data that can be produced by a modern instrument.

In addition to these practical disadvantages of mass spectrometry, there are some technical problems which are not always appreciated. Firstly, the identification of complete unknowns is very difficult, and mass spectrometry really needs to be used in conjunction with other spectroscopic techniques. Secondly, as a quantitative detector, mass spectrometry is less reproducible than other commonly used detectors, and to achieve acceptable precision the use of internal standards, preferably stable isotope analogues, is desirable. These aspects are illustrated in this paper by the practical examples of identification of an unknown *N*-nitrosamine, and the quantitative determination of a veterinary drug residue (sulphamethazine) in animal tissue samples.

Identification of a Previously Unknown *N*-nitrosamine

The thermal energy analyser (TEA) is a widely used chemiluminescence detector which, coupled to GC or HPLC, is employed for specific screening of *N*-nitrosamines in foods and biological materials[6]. By coupled HPLC-TEA a number of *N*-nitroso-amino acids, *N*-nitrosothiazolidine-4-carboxylic acid and *N*-nitroso-oxazolidine-4-carboxylic acids have been identified in a range of cured meat products[7]. Additionally a compound which in retention time did not match any of the known standard *N*-nitrosamines was detected in a number of these products at estimated levels from 40 to 2100 μg/kg[7]. This nitrosamine has now been identified[8] and provides a good example of the amount of effort required using mass spectrometry to completely characterise a previously unknown compound.

Although LC/MS has been attempted for the analysis of non-volatile *N*-nitrosamines[9], for this particular problem it was regarded as unlikely to provide adequate sensitivity. The approach was adopted of trapping the "unknown" from the liquid chromatograph, reacting to form a TMS derivative, and then re-analysing by GC. Preliminary work using the TEA detector validated this approach in terms of obtaining a TEA-positive component by GC from the trapped HPLC fraction. However, on analysing by GC/MS, unlike the TEA the mass spectrometer responded to all organic compounds present and a highly complex capillary column chromatogram resulted. It was obviously difficult to pinpoint the "unknown" nitrosamine in view of the presence of the many other components not previously apparent. Only by incorporating standard *N*-nitrosamines in the extract and establishing the relative retention time of the "unknown" using the TEA and then repeating by GC/MS could a relatively minor component in the spectrum be recognised as the compound of interest. From its electron impact spectrum little structural information could be deduced at first sight other than confirming the TMS derivatization. For example, major ions at *m/z* 73 confirmed the TMS group, at *m/z* 103 indicated the presence of CH_2OTMS whilst *m/z* 147 indicated a di-TMS derivative. The molecular weight was suggested to be either 306 on the basis of the highest ion being the molecular ion, or 336 on the basis of the highest ion being M-30 a characteristic *N*-nitrosamine elimination. Experience suggested to those involved[7] that the "unknown" might be a thiazolidine derived compound, and on this

basis a number of possible nitrosamines were synthesised[8]. Of these a good agreement was obtained both in GC retention time and mass spectral characteristics between the "unknown" and synthesised 2-hydroxymethyl 3-nitrosothiazolidine-4-carboxylic acid.

The point of this example is to illustrate that, even with sophisticated instrumentation like mass spectrometry, complete interpretation of an "unknown" from first principles is rarely possible, and in practice postulated structures have to be tested by synthesis and spectral agreement demonstrated. Where amounts of material are sufficient, complementary techniques like NMR can be employed, but, for microgram amounts and less, microchemical transformations like ozonolysis and hydrogenation[10] in combination with mass spectrometry which yield spectra of derivatives or degradation products might be used to assist in an ultimate structural elucidation.

Libraries of mass spectra are available in book form[2] or as computer data bases, e.g., the EPA/NBS library of 39,000 spectra, but even this size of library is relatively small in relation to the potential number of possible organic compounds likely to be encountered. Mass spectrometry groups normally build up their own libraries based on a particular specialist area, e.g., mycotoxins or pesticides and this provides a starting point in assigning unknowns to structurally similar analogues.

Confirmation of Aflatoxin B1 in Peanut Butter

When samples for routine aflatoxin analysis have been extracted and cleaned-up by recognised methods[11] and the extracts are analysed by TLC or HPLC[12], there is rarely any need for additional confirmation other than perhaps derivative formation and re-chromatography. However in some situations, for example, if aflatoxins were found in an unexpected commodity or if the sample was particularly critical (perhaps a sample involving legal proceedings) then additional confirmatory evidence by mass spectrometry might be necessary.

Although aflatoxins have been analysed by GC/MS[13] the low volatility and high polarity of these compounds make this procedure vulnerable to adsorptive losses, and direct insertion probe analysis is preferable for isolated cases of confirmation where time is not available for optimisation of GC/MS conditions.

An approach utilising negative ion chemical ionisation mass spectrometry was proposed[14] for confirmation of aflatoxins, and data utilising this methodology were obtained by the participation of this Laboratory in a collaborative trial organised by the United States Food and Drug Administration. Dry film extracts of roasted peanuts, cottonseed and ginger were provided both artificially and naturally contaminated with aflatoxin B_1 at levels ranging from not-detectable to 40 ng/g. After a clean-up involving preparative TLC, the extracts were analysed by direct insertion mass spectrometry using iso-butane reagent gas in the negative ion chemical ionisation (NICI) mode. In Figure 2(a) the spectrum is shown of 25 ng of aflatoxin B_1 standard and in Figure 2(b) that of a sample extract of peanut butter. Under these conditions NICI response depends on an electron capturing ability, which for aflatoxins involves the conjugated carbonyl moiety. The carbonyl groups of aflatoxin B_1 conjugated through the double bond to the aromatic ring provide resonance electron

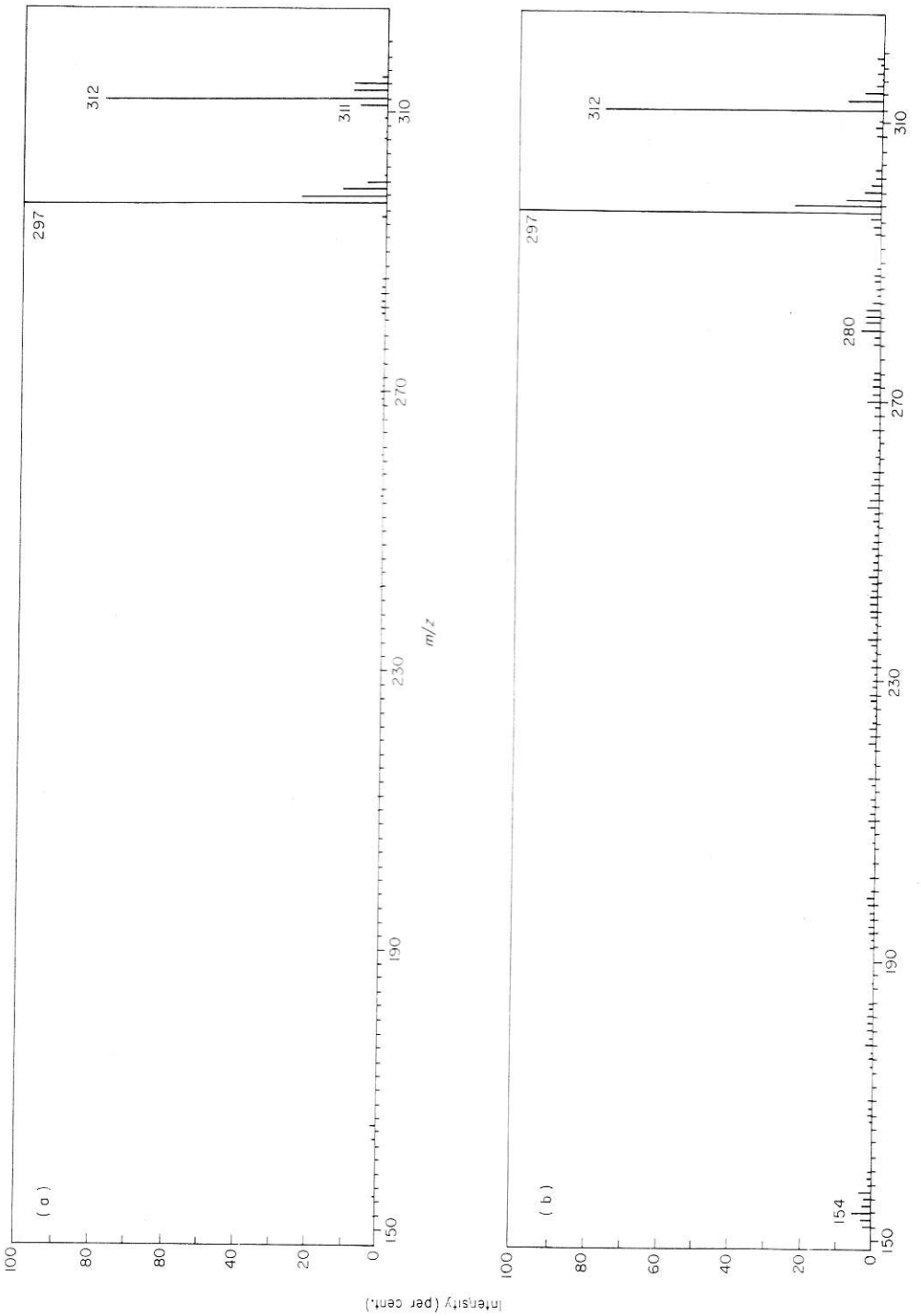

Fig. 2. Negative ion chemical ionisation spectra of aflatoxin B_1. (a) Reference spectrum of 25 ng of aflatoxin B_1. (b) Spectrum of aflatoxin B_1 in peanut butter extract. Probe analysis on a quadrupole mass spectrometer using iso-butane reagent gas operated in a NICI mode.

capturing properties, and thus in NICI the intense molecular anion is observed in the spectrum. This mode of operation provides high specificity, and thus despite other components in the extract only electron capturing compounds are evident, in this instance only those attributable to aflatoxin B_1. This spectrum provides good evidence of confirmation of aflatoxin B_1, but little quantitative information can be deduced other than establishing the absence or presence of aflatoxin at the limit of detection of the procedure. The direct insertion procedure does have the advantage of speed and relative simplicity, but ultimate sensitivity is limited by the total amount of material it is practical to analyse in the glass probe tube. In the example shown in Figure 2 the data are probably considerably simpler than would be seen in practice, where normally the four aflatoxins are found together, in which case the spectrum would contain molecular ions M^- at *m/z* 312, 314, 328, and 330 for aflatoxins B_1, B_2, G_1, and G_2 respectively with an additional contribution from their respective $(M\text{-}CH_3)^-$ ions.

Confirmation of Pesticides by GC/MS-Multiple Ion Monitoring

In complex extracts where preliminary screening utilises, say, electron capture detection, samples found to be free of contamination can be safely eliminated from further consideration, but confidence in identification for any positive samples is significantly improved by the use of mass spectrometry. For confirmation of samples screened for organochlorine pesticides in eggs a GC/MS procedure was developed for 14 compounds to be monitored in a single GC run. The requirement was for a limit of detection of 5 pg per compound on column equating to about 30 ng/g in the original foodstuff.

Initially a capillary column GC separation was established for the standard compounds as shown in Figure 3, and reference electron impact spectra were obtained for each pesticide. For a selected ion monitoring procedure a number of ions characteristic of each compound have to be chosen, choice being influenced by the fact that a high relative intensity of ion in the spectrum would favour sensitivity and a high mass in the spectrum would favour specificity. For example, in Figure 4 the mass spectra of two isomers of hexachlorohexane (HCH) are shown. For either isomer, ions at *m/z* 181, 183, 217, and 219 would appear to be suitable choices for monitoring based on the above criteria. Clearly however the two isomers are virtually indistinguishable on mass spectral characteristics alone and for differentiation, reliance must be placed on chromatographic resolution as shown in Figure 3.

After establishing chromatography and reference spectra, the confirmation procedure was then progressively developed by analysing increasingly complex standard mixtures of pesticides, ensuring that the chosen ions provided adequate sensitivity and that the chromatographic conditions allowed for separation of all the chosen components. It may be necessary in developing a multiple ion monitoring procedure such as this for a large number of compounds to switch between groups of ions for different chromatographic windows to allow for inclusion of a minimum of three ions per component. As the complexity of requirements increases, computer control in storage of the programmes, execution and processing of data becomes essential.

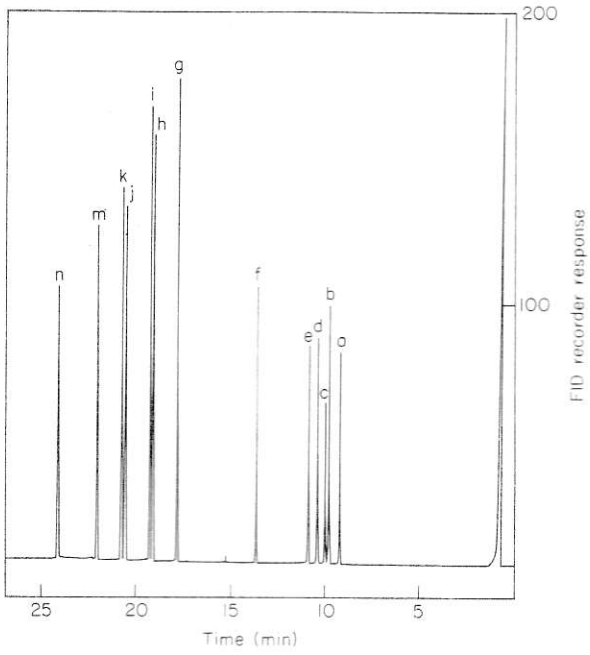

Fig. 3. FID chromatogram of organochlorine pesticides. Chromatography on a 25 m × 0·22 mm fused silica column coated with CP SIL 5CB with hydrogen carrier gas at a linear flowrate of 50 cm/s. Injection in a split mode (20 : 1) with temperature programming from 120°C (isothermal for 1 min) at 5°C per min to 250°C. Peak identification: (a) alpha-hexachlorohexane (HCH), (b) hexachlorobenzene, (c) beta-HCH, (d) gamma-HCH, (e) delta-HCH, (f) heptachlor, (g) o,p-dichlorobis(chlorophenyl)ethylene (DDE), (h) p, p-DDE, (i) o,p-dichlorobis(chlorophenyl)ethane (DDD), (j) p,p-DDD, (k) o,p-trichlorobis(chlorophenyl)ethane, (DDT), (m) p,p-DDT, (n) trichlorobis(chlorophenyl)ethanol (dicofol).

By analysing the standard mixture of pesticides a number of times successively, data can be accumulated to give some experience in the extent of variation that might be expected in ratios between ions, and this is important later in determining whether or not a particular result can be regarded as confirmation, or whether interference is being experienced. The final stage in developing the assay was to test whether the chosen ions provided adequate specificity for the pesticides in an extract, and this was achieved by analysis of a blank sample expected to be free of the compound of interest. Absence of responses at the chosen ion masses validated the procedure (and indeed the blank), whilst a positive interference would have necessitated switching choice of an ion to another suitable one in the spectrum. During actual analysis of positive samples the checking of ratios of responses for ions for each pesticide provides a further guard against interferences not identified in the development stages of the assay. In extreme cases of interferences, often the case when low mass ions are of necessity being monitored, the use of chemical ionisation or of increased mass spectrometric resolution may be needed to improve the overall specificity[15].

In Figure 5, a portion of a multiple ion chromatogram is illustrated demonstrating the presence of γ-HCH in a sample of eggs. Although a number

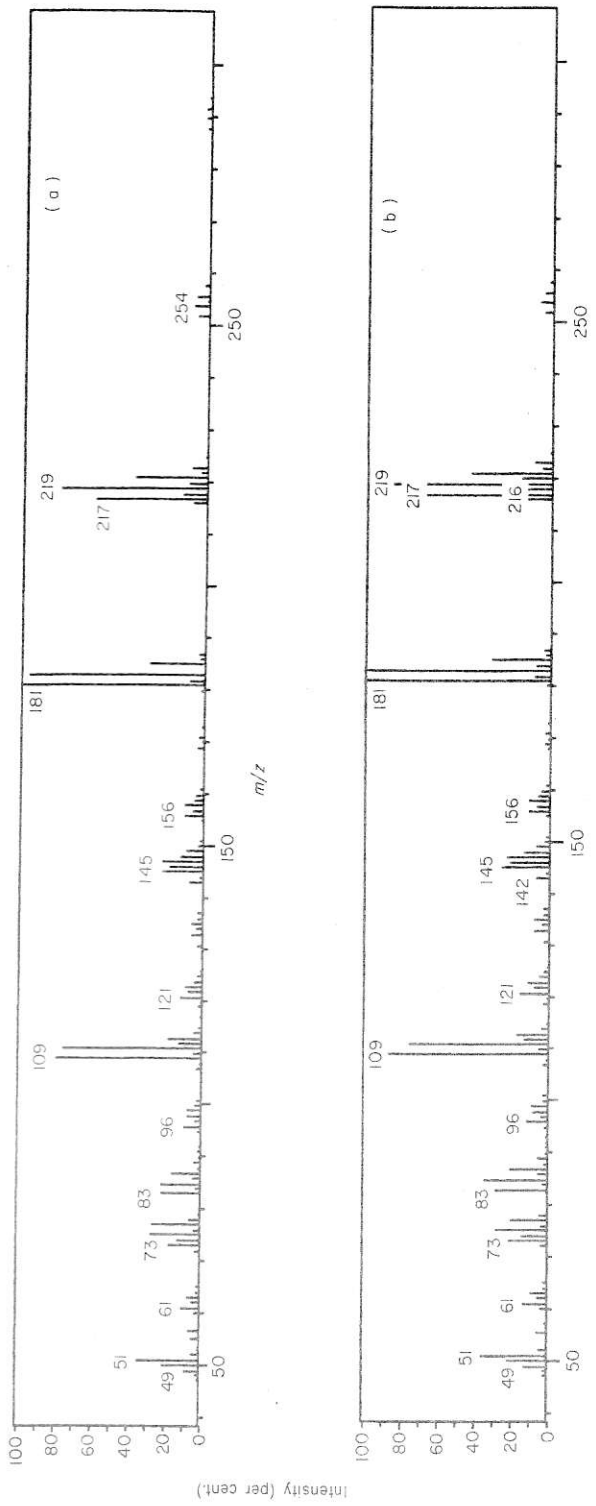

Fig. 4. Electron impact spectra of hexachlorohexane isomers. (a) γ-HCH, (b) δ-HCH. Spectra obtained on a quadrupole mass spectrometer by GC/MS in an electron impact mode.

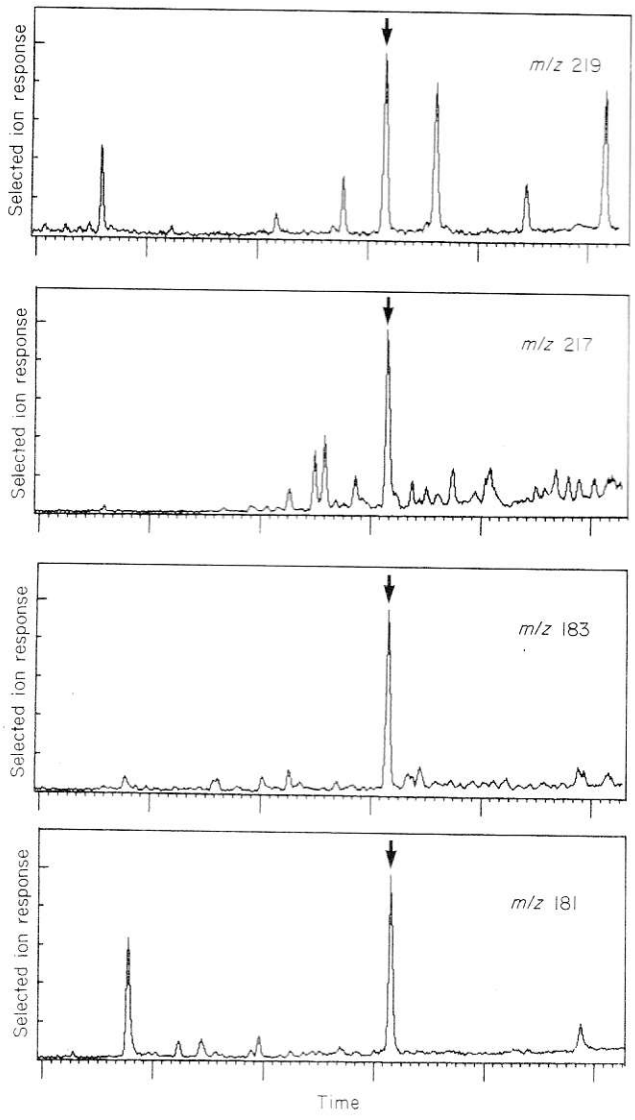

Fig. 5. Multiple ion chromatograms for the confirmation of γ-HCH in eggs. Section of chromatogram showing simultaneous responses for *m/z* 219, 217, 183, and 181, at the correct retention time for γ-HCH.

of responses are evident for each of the four ions at different retention times, only at the correct retention time of γ-HCH do all four ions respond simultaneously and a check of the relative abundances confirms the correct ratios with that obtained for the standards. This therefore constitutes an unequivocal confirmation of γ-HCH in the eggs and from the same chromatographic run data would be obtained concerning the presence or absence of any of the other pesticides being monitored by this confirmation procedure.

Quantitation of Sulphamethazine in Animal Tissue Samples

Although in the example of pesticides it was possible to obtain some quantitative information by calibration using standards of various concentrations, the reproducibility of the mass spectrometer as a detector is often poorer than other widely used GC detectors. Internal standards can significantly improve precision and where possible one would endeavour to employ a stable isotope analogue as an internal standard. The use of stable isotopes in this context has been discussed elsewhere[16]; to summarise, they offer the advantages of being chemically similar to the compound of interest and of having identical physical properties, virtually co-chromatographing, and possibly reducing adsorptive losses during chromatography by exhibiting the so called "carrier effect". The principal disadvantage of using stable isotopes is the relatively high cost which can be involved in custom synthesis of deuterium or ^{13}C-labelled materials and the requirement of high enrichment (99 per cent. +) of the final product.

For the quantitation of residues of the veterinary drug sulphamethazine in animal tissue samples, the tetradeuterated analogue (Figure 6) was synthesised for use as an internal standard. After preliminary screening by electron capture GC, the extraction and clean-up was repeated for positive samples, adding the internal standard at the outset to the macerated tissue at an accurately known concentration roughly equal to the anticipated level of contamination. A lengthy sample clean-up followed involving methylation of the sulphamethazine and preparative size-exclusion chromatography[17]. The internal standard, being present throughout all stages of this assay, provided both an overall measure of recovery of the procedure and an automatic compensation in correcting for losses of the unlabelled material during the assay.

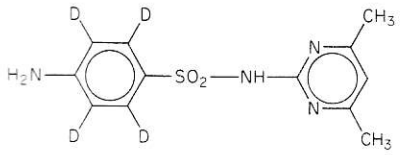

Fig. 6. Structure of tetradeuterated sulphamethazine internal standard.

The electron impact mass spectrum for methylated sulphamethazine is shown in Figure 7, and although no molecular ion is evident, characteristic ions at *m/z* 227 ($M\text{-}SO_2$) and *m/z* 228 ($M\text{-}HSO_2$) are obvious suitable choices for monitoring. The mass spectrum of the internal standard is identical to unlabelled sulphamethazine but with all ions shifted upwards by four mass units, and thus ions at *m/z* 231 and 232 would be the corresponding choice of ions to monitor. Once the necessary development has been carried out to validate the procedure in terms of establishing adequate specificity as outlined for the pesticides, quantitative aspects have to be tested and this is carried out by demonstrating good linearity of calibration. To a number of blank samples a fixed amount of internal standard is added, plus varying amounts of unlabelled material to cover the desired concentration range. After taking the samples

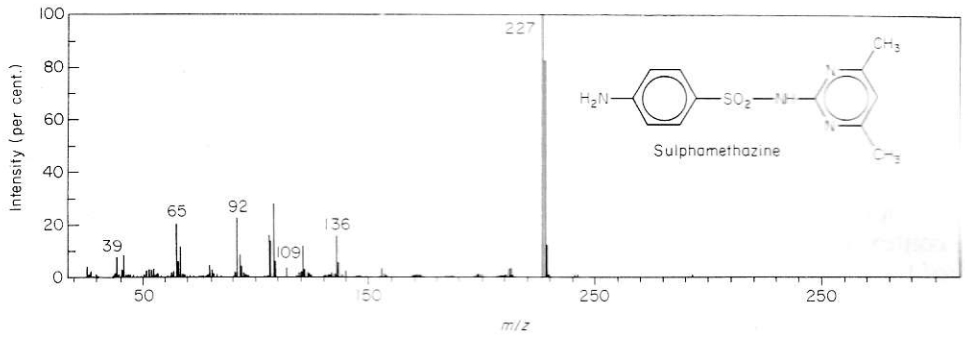

Fig. 7. Electron impact spectrum of methylated sulphamethazine. Spectrum obtained by GC/MS on a 25 m × 0·23 mm fused silica CP SIL7 column operated isothermally at 280°C using a quadrupole mass spectrometer.

through the full extraction and clean-up procedure the peak areas of the ions 227, 228, 231, and 232 at the retention time of sulphamethazine are measured. Plots of the ratio of 227/231 against the amount of unlabelled sulphamethazine produced a good straight line calibration graph as shown in Figure 8. Quantitation of the amount of unknown sulphamethazine in the positive tissue extract can be read directly from the graph, after the sample has been taken through the clean-up procedure again with the addition of the fixed amount of stable isotope internal standard. It can be seen that for this procedure all measurements are made relative to the amounts of co-eluting ions (from the internal standard) and thus there is an automatic compensation for any fluctuations in response in the mass spectrometer. Typically for amounts of sulphamethazine on-column ranging from 3 to 18 ng (equivalent to 0·2 to

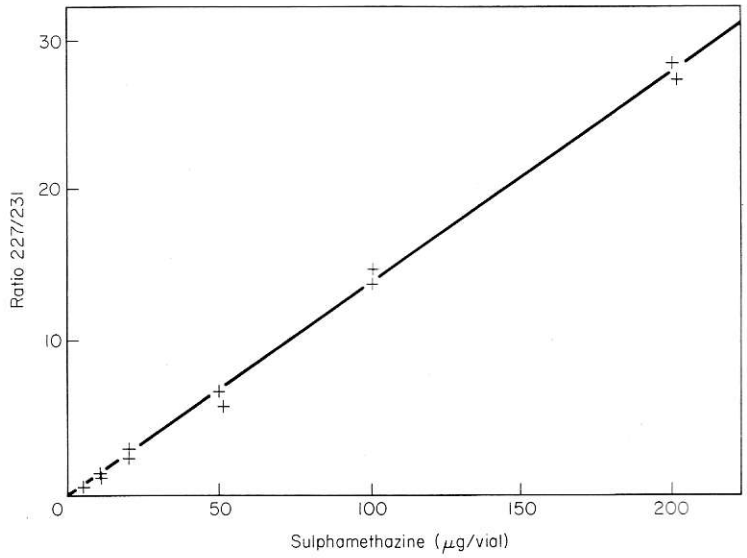

Fig. 8. Calibration graph for quantitation of sulphamethazine in animal tissue samples. Plot of ratio of ions *m/z* 227 to 231 from peak areas against the total amount of sulphamethazine spiked into blank tissue and taken through the complete extraction procedure.

1·2 μg/kg in the tissue) coefficients of variation between 4 and 6 per cent. were achieved using the procedure with a deuterated internal standard. In the absence of an internal standard variations are very compound-dependent, but precision of the order of 20–40 per cent. would not be unexpected, and thus a significant improvement is demonstrated by this use of a stable isotope analogue.

Conclusions

Although the capital costs and the demands of operation of a mass spectrometer may appear to be very high, monitoring trace contamination of foods, if it can be justified, is only worth carrying out if one has a high degree of confidence in the data ultimately generated. This paper shows some of the ways in which mass spectrometry is used for confirmation in the area of food safety, and hopefully will stimulate in the reader an increased critical awareness of reports in the literature, which are not always substantiated by rigorous confirmatory procedures.

Acknowledgement

The authors gratefully acknowledge the assistance of Dr. R. C. Massey, Mr. S. L. Reynolds, and Dr. G. Shearer of the MAFF Food Science Laboratory and Dr. D. L. Park of the U.S. Food and Drug Administration for the provision of samples for analysis and for their helpful discussion.

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Letter to the Editor

Sir,

I read with great interest the account of the collaborative trials, between twenty-two participating laboratories, of various methods for the determination of Dietary Fibre, especially since in the same issue of your journal (**23**, part 1) your reviewer of the book *Statistics for Analytical Chemistry* emphasises the need in public analytical laboratories for the monitoring of analytical methods, to ensure the output of reports of necessary precision without their involving any excesses which might produce unnecessary cost.

This almost seems to me to be a case of trying to back things both ways. The collaborative trials described in the first pages of the journal provide the means of monitoring the methods which is called for in the latter pages, but such monitoring involves considerable cost, which must be found from somewhere.

Sir Robert Mark's criticism of the legal profession has since been endorsed by the late Professor Keith Simpson, both in his autobiography and with support from other critics on radio. The message has even been taken up in that potent vehicle for bending opinion, namely fiction. In the novel *The Second Deadly Sin* by Lawrence Saunders (Panther), on page 358, the great detective is saying "look, everyone thinks a 'Not Guilty' verdict means innocence. Not necessarily. Sometimes it means the prosecutor hasn't proved his case beyond a reasonable doubt. Cases like that shouldn't go to trial. Prosecuting a weak case is a waste of time for the prosecutor, the taxpayer and everyone".

So in addition to reporting facts, a public analyst needs to arm himself with a number of opportunities to say "Yes, I *have* investigated alternative possibilities", when a Defence Lawyer (whose only consideration is to find loopholes for his client) asks questions that he knows, as well as the witness knows, are included only to confuse a jury or magistrate. To be able to do this, the Analyst often needs to extend an analysis considerably beyond what he reports.

Under such circumstances, the lowest possible charge for an analysis becomes a meaningless objective to pursue, and forensic science laboratories might actually provide a better service by coming together more, as they began to do in the investigation of methods for dietary fibre, rather than having each strive to cut a little off the cost of each analysis. Economy makes the profession unattractive (it was not heartening to read in the *Observer* Magazine for 7th April, 1985, that scientists in the Metropolitan Police Forensic Science Lab. earn less than a constable) and in some of the recent scandals, such as the Spanish Olive Oil scandal and the Ethylene Glycol in Wine scandal, one suspects that it was not a forensic science laboratory that uncovered the abuse in the first place.

A. C. BUSHNELL

J. Assoc. Publ. Analysts, 1985, **23**, 135-136

Book Reviews

BOVINE RESPIRATORY DISEASE, REGIONAL SEMINARS, October 1984, Proceedings. Smith Kline Animal Health Limited, Stevenage, Hertfordshire, 1984. Price £10.00. 52 pp.

This rather short book is a well produced account of a seminar which was addressed by eight veterinary surgeons who had specialised in the investigation of bovine respiratory diseases. Each lecture is extended by references, and often well illustrated in full colour.

Reference is made to ELISA techniques in the control of these diseases. Whilst treatment is mainly by viruses, some antibiotics are mentioned, and it is in this context that the book is brought to the notice of Public Analysts who may be concerned with the examination of milk for antibiotics.

G. V. JAMES

IMMUNOASSAYS IN FOOD ANALYSIS. Edited by B. A. Morris and M. N. Clifford. Elsevier Applied Science Publishers Ltd, 1985. Price £25.00.

This book is essentially an expanded and edited compilation of scientific papers presented at the First Symposium on "Immunoassays in Food Analysis" held at the University of Surrey on 15 and 16 September 1983.

Over the last forty to fifty years there has been a progressive and accelerating requirement for more sensitive and more specific methods suited to the routine analysis of foods. This requirement has led the food analyst to adopt and adapt techniques from diverse disciplines. One such technique is that of immunoassay, which utilises the elegantly specific and sensitive antigen-antibody reaction.

The first section of the book deals with the history and general principles of immunoassays, including ELISA, and the latest developments in the fields of fluorescent and luminescent immunoassays. It also includes a very useful and extensive glossary and bibliography.

The second section describes some quantitative analyses for macromolecules (milk protein denaturation, amyloglucosidase in beer and staphylococcal enterotoxins in foods). There are two papers on meat speciation which include enough experimental detail to make them of use to practising analysts, although the two short communications on the determination of soya protein in meat products serve to point the reader in the direction of references to more detailed published papers on the topic.

The third section is applicable to small molecules such as the mycotoxin Ochratoxin A, growth promoting hormones, and potato glycoalkaloids, and

includes a valuable general paper on the use of specific and non-specific antisera.

The book as a whole is essentially something of a research review of the food immunoassay field, and should not be regarded as an analytical method manual for use in a busy food analyst's laboratory. Nevertheless, it represents excellent background and peripheral reading for practising analysts interested in food immunoassays, and I would regard it as a very valuable acquisition for the laboratories and libraries of Public Analysts.

M. J. BILLINGTON

J. Assoc. Publ. Analysts, 1985, **23**, 137–138

James Frederick Clark, M.Sc., M.Chem.A., D.I.C., A.R.C.S., F.R.S.H., C.Chem., F.R.S.C.

James Frederick Clark, a founder member of the Association of Public Analysts, died on 13 April 1985 following a short illness, aged 77 years.

After leaving school he was employed briefly as an articled pupil to the Public Analysts of Burnley whence he won scholarships to the Royal College of Science, Imperial College, London. After two years he graduated with First Class Honours in Chemistry and a year later after further study involving the analysis of Food, Drugs and Water he was awarded the M.Sc. degree and Diploma of Imperial College.

Then followed three years as works chemist at Fry's, the cocoa and chocolate manufacturers, during which time he studied for and passed the Royal Institute of Chemistry (Branch E) examination for Fellowship in 1930.

In 1931 he was appointed Deputy Municipal Analyst, Singapore, a position he held until the city fell into the hands of the Japanese in February 1942. His wife and two children fortunately managed to escape by ship to Melbourne, Australia a few days before the city surrendered. Along with other technical officers in municipal employ he continued with some of his duties under Japanese surveillance for about a year.

The bombing of Singapore had left the water supply to the city almost non-existent and an early task of Clark's was to devise a means for the chemical treatment of the repaired supply. This he did at three points using any equipment he could lay his hands on to produce chlorination units based on the electrolysis of brine. He was, justly, proud of the fact that Singapore was free from water-borne disease during the whole of the occupation.

After a year in such a position he found it a relief to spend the remaining two and a half years anonymously as an internee.

Following a brief reunion with his wife and family in Australia after liberation he applied for the post of Liverpool City Analyst; he was successful and appointed in August 1946, a position he held for 26 years. He was only the third occupant of the post which was one of the earliest outside London, having been created in 1872. At the time he was also Public Analyst for Liverpool, Bootle, Birkenhead, Southport, Widnes, Crosby, Blackburn and Barrow-in-Furness and was Agricultural Analyst for Liverpool, Bootle and Northern Ireland. He was also the official Liverpool Port Analyst.

Fred Clark's major interests lay in fertilisers and feeding stuffs and water analysis, the latter originating, perhaps, from when he lived in his early years near the attractive Lancashire reservoirs at Rivington, a Liverpool water supply he was to monitor for many years.

Throughout his career, he was active in various regional scientific groups, being Council member and Chairman of the Liverpool and North Western

Section of the Royal Institute of Chemistry and of the Northern Section of the Society for Analytical Chemistry (formerly the Society of Public Analysts).

On 11 November 1953 he was present with J. H. Hamence, T. McLachlan, H. E. Monk, G. Taylor, R. G. Thin, E. Voelcker and E. C. Wood when the Association of Public Analysts was founded.

Following retirement in 1972, he and his wife took up residence in Surrey to be near their daughter and son. Mrs. Clark died in 1983.

Fred Clark will be remembered by his colleagues as a cheerful person with a well-developed sense of humour. He was an able and very practical analyst who did a great deal for the standing of public analysts in the North of England. Our deepest sympathy is extended to his daughter, Dr D. Burd and his son, Dr M. F. Clark.

M. McDonnell