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Determination of Tetracyclines in Milk

Collaborative Trial

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The results of a collaborative trial involving 7 laboratories on the trace residue determination of oxytetracycline, chlortetracycline and tetracycline in milk are reported. The method tested was based on metal chelate affinity chromatography (MCAC) using HPLC with UV detection. The precision parameters calculated from the trial for the determination of oxytetracycline were satisfactory for all the samples analysed. The precision for chlortetracycline although slightly worse than that for oxytetracycline, was still within expected levels for all the samples analysed. The precision of the method for the determination of tetracycline was variable, the results for three levels being acceptable, and the results for two levels being unacceptable.

Introduction

This report describes the results obtained for a collaborative trial, organised by the MAFF Food Science Laboratory Norwich, of a method for the trace residue determination of tetracycline antibiotics in milk.

Tetracyclines are a group of broad spectrum antibiotics commonly used therapeutically and prophylactically in animal husbandry. The three tetracyclines most commonly used are oxytetracycline (OTC), chlortetracycline (CTC) and tetracycline (TC). Concern about the possible occurrence of residues of these drugs in human food has produced a need for analytical methods to monitor these agents at trace residue levels. The European Community has adopted Maximum Residue Limits (MRL) for tetracycline residues of 100 µg/L for each tetracycline in milk; these MRLs have now been incorporated into UK legislation in the Animals, Meat and Meat Products (Examination for Residues and Maximum Residue Limits Regulations (1991)[(1)(2)]. There is a clear need for a precise and accurate method that can determine trace levels at the MRL, and below, of these antibiotics in food.

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The Food Science Laboratory Norwich has developed a High Performance Liquid Chromatographic (HPLC) method for the determination of trace residues of these three compounds. This procedure is based on the use of metal chelate affinity chromatography (MCAC). MCAC overcomes many of the difficulties reported in earlier procedures in that it is applicable to a wide range of tissues including milk and is capable of lower limits of determination than previously obtained[3].

The Ministry of Agriculture, Fisheries and Food, is now collaboratively testing the above method as part of its collaborative trial program. Successful validation by collaborative trial would allow the method to gain common acceptance amongst the monitoring authorities. The method would then be published as one of the series of MAFF Bulletins "Collaboratively Tested Non-Statutory Methods" [4].

Method of Analysis to be Collaboratively Tested

The method tested was developed at the Food Science Laboratory Norwich. It comprised extraction into succinate buffer, the tetracyclines were then retained on a chelating sepharose column, loaded with copper ions, residual copper and organic contaminants were removed by the use of a XAD-2 resin column, the tetracyclines being eluted with methanol[3]. The tetracyclines were separated using HPLC with UV detection at 350 nm [See appendix II for full method].

Collaborative Trial Organisation, Samples and Results

Pre-trial

Five Public Analyst laboratories together with the Laboratory of the Government Chemist (LGC) and the Food Science Laboratory (FScL) agreed to take part in the trial. Participants were sent preliminary practice samples to familiarise themselves with the method prior to the trial proper. Several comments were received and changes to the written method were made at this stage.

Trial Proper

Six samples were prepared containing combinations of chlortetracycline, oxytetracycline and tetracycline at varying concentrations from 0 to 300 ug/L (*SEE SAMPLE PREPARATION*). A blank sample, containing no added tetracyclines was also included. Each sample was sent out as a blind duplicate, i.e. 14 samples per participant.

Sample Preparation

Sufficient whole milk was purchased from a local retail outlet. The sample was homogenised and sub samples taken for analysis to verify it

as free of detectable tetracyclines. Sub samples (490 mL) for analysis were spiked with tetracyclines (in methanol) at the following concentrations.

Sample	Laboratory code (of replicates)		Concentration of Tetracycline µg/L (ppb)		
			CTC	OTC	TC
1	47	53	50	50	-
2	58	88	100	-	125
3	18	32	150	125	150
4	19	86	200	55	75
5	60	74	90	300	300
6	27	92	-	100	115
7	4	22	-	-	-

Spiking procedure

Samples (490 mL) were spiked with tetracyclines from working standards made up to 100 µg/mL in methanol with each analyte and added to samples according to the following protocol.

Sample	Volume of Added Working Standard (µL)		
	CTC	OTC	TC
1	250	250	-
2	500	-	625
3	750	625	750
4	1000	275	375
5	450	1500	1500
6	-	500	575
7	-	-	-

After spiking all samples were made up to 500 mL and re-homogenised. The homogeniser head was thoroughly washed between samples to minimise cross contamination. Samples were divided into 25 mL batches and stored at -20°C until dispatch to the participants. Before dispatch a sample from each batch was re-analysed to check the stability of samples.

Results

The results obtained by the participants are given in Tables I to X.

Statistical analysis of results

The trial results were examined for evidence of individual systematic error ($p<0.01$) using Cochran's and Grubbs' test progressively, by procedures described in the internationally agreed Protocol for the Design, Conduct and Interpretation of Collaborative Studies[5].

Horwitz Predicted Precision Parameters

There is often no validated reference or statutory method with which to compare precision criteria when assessing a new method. In such cases it is useful to compare the precision data obtained from a collaborative trial with predicted acceptable levels of precision. These levels, as predicted by the Horwitz equation, give an indication as to whether the method is sufficiently precise for the level of analyte being measured[6].

The Horwitz predicted value is calculated from the Horwitz equation[6]:

$$RSD_R = 2^{(1-0.5\log C)}$$

C = measured concentration of analyte expressed as a decimal.

e.g. 1 g/100g = 0.01

Horrat Values (Ho)

The Horrat[7] values give a comparison of the actual precision measured with the precision predicted by the Horwitz equation for a method measuring at that particular concentration of analyte. It is calculated as follows:

$$Ho_R = RSD_R(\text{measured})/RSD_R(\text{Horwitz})$$

A Ho_R value of 1 usually indicates satisfactory interlaboratory precision, while a value of >2 indicates unsatisfactory precision i.e. one that is too variable for most analytical purposes or where the variation obtained is greater than that expected for the type of method employed. Similarly Ho_r is calculated, and used to assess intralaboratory precision, using the approximation $RSD_r(\text{Horwitz}) = 0.66RSD_R(\text{Horwitz})$. (This assumes the approximation $r = 0.66R$) The Horwitz values calculated from the results of this trial are given in tables IX-X.

Repeatability and Reproducibility

Calculations for repeatability(r) and reproducibility(R) were carried out on those results remaining after removal of outliers using the procedures given in the Protocol for the Design, Conduct and Interpretation of Collaborative Studies[5]. These are given in Tables I to VII. The relatively large precision values obtained in this collaborative trial are typical of

methods measuring analyte at µg/L levels and demonstrate the problems with obtaining suitably reproducible results at trace levels.

Oxytetracycline

The precision values obtained for oxytetracycline were satisfactory for all the samples analysed, with reproducibility (R) ranging from 15 µg/L (at 40 µg/L) to 140 µg/L (at 220 µg/L). This precision corresponded to Horrat values of 0.5-1.3, which are well within the range predicted by the Horwitz equation[6] for a method determining analyte at these concentrations.

Recommended precision levels to be assigned to the method on the basis of this collaborative trial are:

(at 40 µg/L) repeatability, r = 10 µg/L

(>80 µg/L) repeatability, r = 40 µg/L

The relationship between reproducibility and concentration is approximately linear and is given by:

$$\text{reproducibility, } R = 7.1 + 0.61\,C \ \mu g/L$$

where C = concentration of oxytetracycline in µg/L

Chlortetracycline

The precision obtained for determination of chlortetracycline, while worse than that obtained for oxytetracycline, was still of the order of what would be expected when determining analyte at these concentrations. There was no clear relationship between precision and concentration, with reproducibility ranging from 35 µg/L to 78 µg/L over the sample range.

Tetracycline

The results for tetracycline were inconclusive. The precision for three levels, samples containing tetracycline at observed concentrations in the range 40-60 µg/L, was within the range predicted by the Horwitz equation. The precision values obtained for the two levels having the highest concentrations of tetracycline (67.8 and 141.8 µg/L) was unsatisfactory as demonstrated by HORRAT values of 2.3 and 2.2.

Discussion

The statistical results of this trial reveal that the method showed good precision

characteristics for the measurement of oxytetracycline in milk, satisfactory precision for higher concentrations of chlortetracycline and variable precision for tetracycline.

Most participants had initial problems with the method and required several analyses on "practice samples" prior to analysing the collaborative

trial samples. Several of the participants were unfamiliar with the determination of tetracycline residues, a technique which has traditionally been a specialised area. Initial problems centred mainly on the inability to obtain a clean system and sudden poisoning of the Lichrosorb RP8 column. 5 laboratories initially reported similar observations resulting in poor separation of the tetracyclines. Separation generally improved with, a clean system and/or a new or regenerated column. Laboratories 2 and 6 also used modified mobile phases to aid their separation.

Laboratory 1 reported some interesting findings resulting from their analysis of the samples. They found the TC and CTC peaks were each preceded by another peak, possibly a breakdown product. Assuming the peaks were breakdown products the effect of breakdown was to reduce the reported concentration by a factor of 0.53 (TC) and 0.56 (CTC). Laboratory 5 reported an impurity peak originating from the CTC standard which could not be resolved from the tetracycline peak.

Chlortetracycline elutes some time after the other two tetracyclines and in some participants' chromatograms had a much poorer peak shape, this could have an adverse effect on precision. Other workers have reported problems with epimerization of chlortetracycline-HCL to 4 epi-chlortetracycline and epimerised-dehydrated forms of TC [8][9][10][11][12][13]. Clearly, breakdown of CTC plus co-elution of CTC breakdown products with TC, could explain in some part, the poorer performance of the method for the determination of these antibiotics.

In house work carried out by the Food Science Laboratory (FScL) reported mean recoveries for: OTC of 80% and 82%; TC of 59% and 58%; CTC of 59% and 69% respectively at concentrations of 50 and 100 µg/L respectively [3]. The results of the collaborative trial were reported as uncorrected for recovery, results from initial recovery experiments carried out by participants gave the highest recovery for OTC (74-90%) with TC much lower (44-60%) with CTC somewhere in between. Comparison of observed values obtained from the collaborative trial with the concentration of OTC, CTC and TC added at the sample preparation stage, suggest that participants were recovering on average 72 %, 48 % and 48 % of OTC, CTC and TC respectively; these results are of similar order to the FScL "in house" validation work. Further work is clearly required to improve the analytical recoveries for TC and CTC.

Conclusion

The collaborative trial has demonstrated that the method is sufficiently precise for the determination of oxytetracycline and chlortetracycline in milk. The results from this collaborative trial for the determination of tetracycline, are inconclusive with the precision for three out of the five levels tested being satisfactory.

References

1. Council Regulation (EEC) No 675/92 of 18 March 1992. Amending Annexes I & III of Council Regulation (EEC) No 2377/90 of 26 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, OJ No L 73/8, 1992
2. The Animals, Meat and Meat Products (Examination of Residues and Maximum Residue Limits) Regulations, 1991, Statutory Instrument No 2843, Food, 1991, HMSO.
3. Farrington W H H, Tarbin J, Bygrave J and Shearer G, "Analysis of traceresidues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC", Food Additives and Contaminants, 1991, **8** (1), 55-64.
4. MAFF, Food Safety Directorate News Release, FSD 32/92, 9 June 1992
5. "Protocol for the Design, Conduct and Interpretation of Collaborative Studies" Ed W Horwitz, Pure and Applied Chemistry, 1988, **60**(6), 855-864
6. Horwitz W, Anal. Chem., 1982, **54**, 67A-76A
7. Peeler J T, Horwitz W and Albert R, "Precision Parameters of Standard Methods of Analysis of Dairy Products", JAOAC, 1989, **72**(5), 784-806.
8. Martinez E E and Shimoda W, JAOAC, 1989, **72**(5), 848-850.
9. McCormick J R D et al, J. Am. Chem. Soc., 1957, **79**, 2849-2858
10. Howell H R, Rhodig L L and Sigler A D, JAOAC, 1984, **67**, 572-575
11. Katz S E and Fassbender C A, JAOAC, 1967, **50**, 821-827.
12. Katz S E and Fassbender C A, JAOAC, 1968, **51**, 557-562.
13. Hermansson J and Anderson M, J Pharm. Sci., 1982, **71**, 222-229.

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APPENDIX I
Results

TABLE I
Sample A (47 & 53)

Analyte	OTC			TC			CTC
Spike Level	50			0			50
Laboratory							
1	51	37	5	ND	16	19	
2	35[a]	70[a]	ND	33	23	50	
3	25	23	5	ND	10	ND	
4	44	41	ND	ND	30	24	
5	36	35	ND	ND	22	18	
6	41	45	ND	ND	33	35	
7	44	41	ND	ND	35	26	
Statistic							
No. of Laboratories	7			-			7
No. of outliers	1			-			0
Mean $\bar{x}$	38.6			-			24.4
S_r	4.43			-			8.35
RSD_r	11.5			-			34.3
Repeatability (r)	12.4			-			23.4
S_R	8.37			-			12.48
RSD_R	21.7			-			51.2
Reproducibility	23.4			-			34.9

For Key see Table XI

TABLE II
Sample 2 (58 & 88)

Analyte	OTC			TC		CTC
Spike Level	0			125		100
Laboratory						
1	17	5	29	24	24	25
2	ND	ND	63	47	55	45
3	ND	ND	36	48	29	39
4	ND	ND	52	72	50	46
5	ND	23	61	82	32	49
6	9	13	68	80	60	62
7	7	ND	86	69	70	60
Statistic						
No. of Laboratories	-			7		7
No. of outliers	-			0		0
Mean $\bar{x}$	-			58.4		46.1
S_r	-			11.02		6.60
RSD_r	-			18.9		14.3
Repeatability (r)	-			30.8		18.5
S_R	-			20.23		15.13
RSD_R	-			34.7		32.8
Reproducibility	-			56.6		42.4

For Key see Table XI

TABLE III
Sample 3 (18 & 32)

Analyte	OTC			TC			CTC
Spike Level	125			150			150
Laboratory							
1	81	64	40	40	47	43	
2	35	80	ND	55	35	62	
3	52	67	31	40	55	33	
4	109	106	70	67	69	73	
5	86	64	100	66	72	45	
6	94	101	100	124	96	117	
7	112	115	95	121	86	112	
Statistic							
No. of Laboratories	7			7			7
No. of outliers	0			0			0
Mean $\bar{x}$	83.3			67.8			67.5
S_r	14.86			19.86			14.86
RSD_r	17.8			29.3			22.0
Repeatability (r)	41.6			55.6			41.6
S_R	25.10			37.47			27.95
RSD_R	30.1			55.3			41.4
Reproducibility	70.3			104.9			78.3

For Key see Table XI

TABLE IV
Sample 4 (19 & 86)

Analyte	OTC		TC		CTC	
Spike Level	55		75		200	
Laboratory						
1	36	42	25	29	64	66
2	27	33	25	29	76	110
3	28	36	18	34	46	84
4	51	52	65	76	86	117
5	36	1	41	5	83	89
6	41	37	48	40	108	90
7	53	55	56	59	126	135

Statistic			
No. of Laboratories	7	7	7
No. of outliers	0	0	0
Mean $\bar{x}$	40.6	42.8	91.4
S_r	3.61	6.82	16.92
RSD_r	8.9	15.9	18.5
Repeatability (r)	10.1	19.1	47.4
S_R	9.43	17.93	25.73
RSD_R	23.2	41.9	28.1
Reproducibility	26.4	50.2	72.0

For Key see Table XI

TABLE V
Sample 5 (60 & 74)

Analyte	OTC		TC		CTC	
Spike Level	300		300		90	
Laboratory						
1	179	177	84	82	28	33
2	ND[a]	204[a]	ND	100	ND[a]	65[a]
3	197	183	118	105	34	29
4	283	285	155	161	50	50
5	201	157	182	136	46	27
6	222	239	254	215	79	67
7	279	280	195	198	61	67
Statistic						
No. of Laboratories	7		7		7	
No. of outliers	1		0		1	
Mean $\bar{x}$	223.5		141.8		47.6	
S_r	14.23		31.46		7.02	
RSD_r	6.4		22.2		14.7	
Repeatability (r)	39.8		88.1		19.6	
S_R	50.05		68.21		18.54	
RSD_R	22.4		8.1		39.0	
Reproducibility	140		191		51.9	

For Key see Table XI

TABLE VI
Sample 6 (27 & 92)

Analyte	OTC		TC		CTC	
Spike Level	100		115		0	
Laboratory						
1	70	33	30	16	ND	ND
2	53	37	35	24	ND	ND
3	66	62	48	45	ND	ND
4	73	69	54	63	ND	ND
5	66	74	74	69	ND	ND
6	70	58	69	59	ND	ND
7	92	100	67	81	ND	ND

Statistic			
No. of Laboratories	7	7	7
No. of outliers	0	0	-
Mean $\bar{x}$	65.9	52.4	-
S_r	11.7	7.21	-
RSD_r	17.8	13.8	-
Repeatability (r)	32.9	20.2	-
S_R	18.32	20.75	-
RSD_R	27.8	39.6	-
Reproducibility	51.3	58.1	-

For Key see Table XI

TABLE VII
Sample Blank (4 & 22)

Analyte	OTC		TC		CTC
Spike Level	Blank		Blank		Blank
Laboratory					
1	10	7	1	2	2	2
2	ND	42	ND	ND	ND	ND
3	ND	ND	ND	ND	11	ND
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
6	10	ND	ND	ND	ND	ND
7	5	7	ND	5	ND	ND
Statistic						
No. of Laboratories	7		7		7	
No. of outliers	-		-		-	
Mean $\bar{x}$	-		-		-	
S_r	-		-		-	
RSD_r	-		-		-	
Repeatability (r)	-		-		-	
S_R	-		-		-	
RSD_R	-		-		-	
Reproducibility	-		-		-	

For Key see Table XI

TABLE VIII

Summary of Calculated Statistical Parameters for Oxytetracycline

Spike Level	obs	n	r	Sr	RSDr	Hor	R	SR	RSDR	HoR
50	38.6	6	12.4	4.43	11.5	0.7	23.4	8.37	21.7	0.8
55	40.6	7	10.1	3.61	8.9	0.5	26.4	9.43	23.2	0.9
100	65.9	7	32.9	11.74	17.8	1.1	51.3	18.3	27.8	1.2
125	83.3	7	41.6	14.86	17.8	1.2	70.3	25.1	30.1	1.3
300	223.5	6	39.8	14.23	6.4	0.5	140	50	22.4	1.1

TABLE IX

Summary of Calculated Statistical Parameters for Tetracycline

Spike Level	obs	n	r	Sr	RSDr	Hor	R	SR	RSDR	HoR
75	42.8	7	19.1	6.82	15.9	0.9	50.2	17.93	41.9	1.6
115	52.4	7	20.2	7.21	13.8	0.8	58.1	20.75	39.6	1.6
125	58.4	7	30.8	11.02	18.9	1.2	56.6	20.23	34.7	1.4
150	67.8	7	55.6	19.86	29.3	1.9	105	37.47	55.3	2.3
300	141.8	7	88.1	31.46	22.2	1.6	191	68.21	48.1	2.2

TABLE X

Summary of Calculated Statistical Parameters for Chlorotetracycline

Spike Level	obs	n	r	Sr	RSDr	Hor	R	SR	RSDR	HoR
50	24.4	7	23.4	8.35	34.3	1.9	34.9	12.48	51.2	1.8
90	47.6	6	19.6	7.02	14.7	0.9	51.9	18.54	39	1.5
100	46.1	7	18.5	6.6	14.3	0.9	42.4	15.13	32.8	1.3
150	67.5	7	41.6	14.86	22	1.4	78.3	27.95	41.4	1.7
200	91.4	7	47.4	16.92	18.5	1.2	72	25.73	28.1	1.2

TABLE XI

Key to Tables I to X

(a)	An outlying result as determined by Cochran's Test at $P<0.01$
obs.	The observed mean, the mean obtained from the collaborative trial data.
r	Repeatability (within laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material under the **same conditions** may be expected to lie with 95% probability.
S_r	The standard deviation of the repeatability.
RSD_r	The relative standard deviation of the repeatability ($S_r \times 100$/Mean)
Ho_r	The HORRAT value for repeatability is the observed RSD_r divided by the RSD_r value estimated from the Horwitz equation using the assumption $r=0.66R$.
R	Reproducibility (between-lab variation). The value below which the absolute difference between two single test results obtained with the same method on the identical test material under **different conditions** may be expected to lie with 95% probability.
S_R	The standard deviation of the reproducibility.
RSD_R	The relative standard deviation of the reproducibility ($S_R \times 100$/MEAN).
Ho_R	The HORRAT value for reproducibility is the observed RSD_R value divided by the RSD_R value calculated from the Horwitz equation.

APPENDIX II

The Determination of Tetracyclines at Residue Levels in Animal Tissues and Milk

1 Scope

The method permits the trace residue determination of tetracycline, chlortetracycline and oxytetracycline in animal tissue and fluids, and honey.

2 Principle

Tissue is extracted into a buffer and the tetracyclines retained on a Chelating Sepharose column, loaded with copper ions. Residual copper and organic contaminants are removed by the use of a XAD-2 resin column, the tetracyclines being eluted with methanol. Quantification is by HPLC with UV detection.

3 Reagents

Chemicals and solvents are analytical grade reagents except where stated. Deionised double distilled water is used throughout.

3.1 Tetracycline free base

3.2 Chlortetracycline HCl

3.3 Oxytetracycline HCl

3.4 Succinate Buffer, *p*H 4.0: dissolve 5 g of succinic anhydride in 900 ml of water, adjusted to *p*H 4.0 with 0.1 mol/l sodium hydroxide and made up to 1 l with water.

3.4.1 Succinic anhydride

3.4.2 Sodium hydroxide solution, 0.1 mol/l

3.5 EDTA-Succinate Buffer: dissolve 5 g of succinic anhydride in 900 ml of water, adjusted to *p*H 4.0 with 0.1 mol/l sodium hydroxide. Add 37.2 g of EDTA disodium salt and make to 1 l with water.

3.5.1 Ethylene diamine tetra acetic acid, disodium salt (EDTA)

3.6 Methanol, redistilled

3.7 Ethanol

3.8 Acetonitrile

3.9 Oxalic Acid, 0.01 mol/l: weigh out 1.26 g of oxalic acid and make up to 1 l with water.

3.9.1 Oxalic acid

3.10 Chelating Sepharose Fast Flow, in 20% ethanol

3.11 Amberlite XAD-2

3.12 Copper Sulphate Solution: weight out 5.0 g of copper sulphate and make up to 1 l with distilled water.

3.12.1 Copper sulphate

3.13 β-mercaptopropionic Acid Solution: weigh out 0.5 g of β-mercaptopropionic acid and make up to 10 ml with methanol.

3.13.1 β-Mercaptopropionic acid, (Thiolactic acid)

3.14 Acetone

3.15 HPLC Mobile Phase: To 500 ml of 0.01 M oxalic acid add 500 ml of acetonitrile. Mix and pass through a 0.45 μm filter, assisted by vacuum. Further de-gas using ultrasonication in conjunction with reduced pressure.

4 Apparatus

4.1 Centrifuge tubes, 250 ml.

4.2 Centrifuge tubes, 50 ml.

4.3 Glass columns, 200 mm x 20 mm i.d., fitted with sintered glass frit and stopcock.

4.4 Glass filter funnel, 250 ml.

4.5 Conical flasks, 250 ml.

4.6 Pear shaped flasks, 50 ml.

4.7 Round bottomed flasks, 250 ml.

4.8 Bulb pipettes, 5 ml.

4.9 Bulb pipettes, 10 ml.

4.10 Vials, low volume for autosampler.

4.11 All glass filter holder, Millipore, 47 mm.

4.12 Homogeniser, Ultra Turrax or equivalent.

4.13 Vortex mixer, Whirlimixer (Fisons) or equivalent.

4.14 Centrifuge, MSE high speed 18 or equivalent.

4.15 Ultrasonic bath, L&R 140S or equivalent.

4.16 Rotary evaporator, plus water bath, at 40°C, Buchi or equivalent.

4.17 Filter paper, 15 cm, Whatman type 541.

4.18 Safety pipetters, Gilson Pipetman, 0.2 ml.

4.19 Safety pipetters, Gilson Pipetman, 1.0 ml.

4.20 Membrane filter, 0.45 μm "Durapore", for use with an all glass filter holder, Millipore, 47mm.

4.21 High Performance Liquid Chromatography

4.21.1 Pump, LKB 2150 pump or equivalent.

Mobile phase was pumped at 0.4 ml/min.

4.21.2 Column, 20 cm x 3 mm i.d. Chromsep (Chrompack) cartridge column assembly packed with Lichrosorb RP8 with integral (10 mm x

2.1 mm i.d.) guard column packed with pellicular (30-40 µm) reverse phase.

4.21.3 Detector, UV detection at 350 nm (Severn Analytical, SA6510 or equivalent).

4.21.4 Data/handling, electronic integrator (Spectra Physics 4290 or equivalent).

4.21.5 Injection, Autosampler (Waters WISP 712 or equivalent), 10 µl injection volume.

5 Column Preparation

5.1 Chelating Sepharose

Thoroughly mix the suspension of Chelating Sepharose. Take approximately 5 ml of aliquot and place in a 200 mm × 20 mm (i.d.) glass column, allow to settle (bed height required 15 mm) and remove the excess liquid. Pour 2 × 10 ml of copper sulphate solution through the column. Vortex mix the column after the first 10 ml of copper sulphate solution has been added, to ensure an even coating. Then pour 15 ml of succinate buffer through the column.

NB: after use, columns are rinsed with 15-20 ml of water. They may be stored in 20% aqueous ethanol at 4°C. Before use, the aqueous ethanol is drained from the column. The column is reloaded with copper sulphate solution and the cycle continued as before. If channels form in the Chelating Sepharose bed, the column should be vortex mixed to ensure even dispersion before loading sample.

5.2 Amberlite XAD-2

Take 100 g of Amberlite XAD-2, which is sufficient to produce six columns, and wash with about 300 ml of acetone, 300 ml of methanol and 300 ml of water.

The aqueous slurry is packed into glass columns, 200 mm × 20 mm (i.d.), to a bed height of 100 mm. Resin is prepared by washing in sequence with 100 ml acetone, 100 ml methanol and 200 ml water. After use, columns can be regenerated by the same procedure. Redisperse the column packing by inverting the column several times to ensure an even distribution. Remove the excess liquid before use.

6 Standards

6.1 Tetracycline Standards

Solutions of tetracyclines should be stored at 4°C. Stock solutions should be freshly prepared each week and working standards on each day of use.

6.2 Stock Standard (1 µg/µl)

For each stock standard, weigh out 100 mg of the appropriate tetracycline standard, make up to 100 ml with methanol, and store at 4°C. Prepare weekly.

6.3 Intermediate Standard (10 ng/µl)

Take 1 ml of each stock standard, dilute to 100 ml with water. Prepare weekly.

6.4 Working Standard (1 ng/µl)

Take 1 ml of intermediate standard (10 ng/µl), dilute to 10 ml.

Spiking solutions should be made up with water. Hplc standards should be made up with hplc mobile phase. Both solutions should be prepared daily.

7 Samples

Samples are stored at -20°C until required.

8 Procedure

Extractions should be performed on batches of up to 8 samples per day.

Weigh 10 g of finely sliced tissue, or 10 ml of milk, into a centrifuge bottle and add 40 ml of succinate buffer. Place in an ultrasonic bath (3 mins). Homogenise (2 min) and centrifuge (5 min) at 12,000 r.c.f. Filter the supernatant through Whatman 541 filter paper and load onto a prepared Chelating Sepharose column.

Re-extract the remaining residue using a further 40 ml of succinate buffer and load onto the sepharose column as before. Re-extract the tissue residue once more, using 20 ml of succinate buffer. Add the filtrate to the sepharose column.

Allow sufficient time for the bed to settle down, then wash the column sequentially, with 10 ml of water, 30 ml of methanol and 2 × 10 ml of water. The flow rate should not exceed 4 ml/min.

To elute the tetracyclines, pass 40 ml of EDTA-succinate buffer through the column followed by a further 10 ml EDTA-succinate buffer (4 ml/min). Collect and combine both fractions.

Load the EDTA-succinate fractions directly onto a prepared XAD-2 resin column. Allow the bed to settle down, then pass 2 × 100 ml of water through the column and discard eluate.

Elute by passing 100 ml of methanol (4 ml/min) through the column. Discard the first 10 ml of liquid, and collect the remainder.

Reduce the methanol to a small volume by rotary evaporation and quantitatively transfer the extract to a pear shaped flask with 3 × 2 ml of methanol. Add 0.1 ml of 5% β-mercaptopropionic acid solution to the residue and remove the methanol by rotary evaporation, azeotroping with acetonitrile if necessary. The temperature must not exceeded 40°C.

Redissolve the residue in 0.5 ml of HPLC mobile phase. Care should be taken to ensure the complete recovery of the sample residue. Both vortex mixing and ultra sonication should be used to ensure that the residue is dissolved. Transfer the extract to an autosampler vial.

HPLC is performed on 10 µl of the extract.

9 Interpretation of Chromatographic Data

9.1 Identification of Analyte

Identification of suspect peaks is made by the comparison of the retention times of the analytes in spiked samples, or in the standard solution, with those of the suspect peaks in the sample.

9.2 Calculation of Results

9.2.1 Initial screening: a minimum of 3 injections (10 µl) of standard tetracycline (1ng/1µl) are carried out to determine average peak height (10 ng injection = 50 ng/g (ppb) tissue concentration).

9.2.2 Calculation: the concentration of the sample (ng/g or ppb) is given by:

$$\text{Sample Concentration (ng/g)} = \frac{Pk(\text{Sample})}{Pk(\text{Standard})} \times 50$$

Where:

Pk (sample) is the peak height of the sample.

Pk (standard) is the peak height of the standard.

50 is the concentration of analyte, in ng per g of tissue, equivalent to a standard injection containing 10 ng of analyte.

For suspect samples above 100 ng/g the concentration of the reference standard should be adjusted to the next highest suitable concentration. The concentration of the standard injection in the calculation above will need to be adjusted by a similar factor.

Sample results are not normally corrected for recovery.

9.3 Evaluation of the method prior to use

Before adopting this method, the inter- and intra-batch precision should be evaluated. Batches of up to six samples are spiked at the 0.05 mg/kg level (500 µl of 1 ng/1µl working standard) with each analyte (batches should include one blank sample). Batches should be analysed on each of three separate days. Recovery of analyte should fall within the range 60-90%. The Relative Standard Deviation should fall within the range 5-15% for inter- and intra-batch precision.

APPENDIX III
Comments from Participants

Laboratory 1:

The standards used were: tetracycline, chlotetracycline hydrochloride and oxytetracycline dihydrate; no allowance was made for their different forms. The XAD column was regenerated with 100 mL of acetone and methanol followed by 400 mL of water, degassed solutions were used to prevent air bubbles coming out of solution.

The first 10 mL of eluate was discarded. The evaporation was performed in a single 100 mL pear shaped flask. It was necessary to discard the methanol from the rotary evaporator reservoir before the final water could be evaporated.

They did not possess a MSE high speed 18 centrifuge, centrifugation was performed at 6000 rpm.

TC and CTC peaks were preceded by peaks, believed to be breakdown products. These peaks represented 70-90 % of the main peak in the case of TC, and 70- 85 % in the case of CTC. When the results are corrected for these areas, assuming they are breakdown products, the results increase by a factor of 1.88 and 1.77 respectively.

Laboratory 2:

Identified several problems.

They did not possess a MSE high speed 18 centrifuge.

Section 2.1 it is not clear which types of standard material is to be used, free base or hydrochloride.

Sections 2.2, 3.1, 3.2 & 4 are ambiguous and need clarification.

Problems with chromatography, used modified mobile phase consisting 0.01M oxalic acid : CH_3N, 2:1.

Length of time to rotary evaporate at sections 5.9 & 5.10 is excessive, better to discard the first 10 mL of eluate.

Each sample was injected using a syringe as a Waters WISP 712 was not available.

Laboratory 5

3.2.1 Succinate buffer *p*H 4.0 had a limited shelf life, had to be prepared every three days. Suggest that *p*H is adjusted with .25M NaOH to reduce volume of alkali required.

EDTA succinate buffer exhibited limited shelf life (3-5 days).

Rotary evaporation at 40 °C to remove all water co-eluted from the XAD column with methanol results in poor recovery of tetracyclines.

Recommend **5.8 & 5.9** be redrafted to include the use of refractive index to identify relevant eluate fraction.

Observed sudden column failure similar to that of Laboratory 2.

Method does not give adequate instructions on the pre-cleaning of Sepharose and XAD columns. Impressed with the procedure once fully accustomed to the techneques involved.

Laboratory 6

Used a modified mobile phase of:

55 : 20 : 25; oxalic acid(0.01M *p*H 2.5): methanol: acetonitrile

J. Assoc. Publ. Analysts 30, 79-88

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No.V 35

Method for the Determination of Papain in Raw Meat by Immunoassay

Correspondence on this method may be sent to Roger Wood, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich, NR4 7UQ

1. Scope and Field of Application

The method allows the determinations of papain in raw meat.

2. Definition

Papain content: the content of papain as determined by the method specified.

3. Principle

Essentially, specific antibody attached to the solid phase act as a capture antibody. Standards (within the range 0 - 1 mg/kg) and test extract solutions are then incubated with this antibody-solid phase and any papain present is captured. Enzyme-antibody conjugate, (horseradish peroxidase labelled specific antibody) is then incubated with the solid phase and finally the enzyme substrate is added which produces a chromophore, the intensity of which is proportional to the amount of papain present in the test solution.

The Double Antibody Sandwich ELISA system employed is shown diagrammatically in Figure 1.

4. Reagents

4.1 Kit Components

4.1.1 Standard Extracts

Six vials containing 2 ml standards supplied for use in the assay as calibration standard meat extracts. They represent the following papain concentrations:-

0, 0.05, 0.1, 0.25, 0.5, 1.0, mg/kg papain.

4.1.2 Antibody Coated Microwells

Foil laminated bag containing six 2 × 8 stripwells (total 96 wells) each precoated with papain antibody and held in a plastic frame and a desiccant bag.

4.1.3 Wash Solution Concentrate

One bottle containing 50 ml of wash solution concentrate comprising a 20 fold concentrate of Tris buffered saline with 0.05% Tween 20 and 0.01% thiomersal.

4.1.4 Conjugate

One vial containing 25 ml anti-papain antibody-enzyme conjugate, ready for use.

4.1.5 Substrate

One vial containing ABTS substrate in citrate-phosphate buffer containing H_2O_2, ready for use.

4.1.6 Stop Solution

One vial containing 9 ml of citric acid stop solution, ready for use.

4.1.7 Calculations

Three sheets of pre-labelled graph paper/work sheet.

4.2 Sample Preparation

Phosphate buffered saline (PBS) is required for extraction of meat samples, (90 ml/sample). A suitable PBS solution can be prepared as follows:-

Sodium chloride	16.0 g
di-Sodium hydrogen orthophosphate (anhydrous (heat in a little distilled H_2O to dissolve)	2.3 g
Monobasic potassium phosphate (anhydrous KH_2PO_4)	0.4 g
Potassium chloride	0.4 g

Dissolve in 2 litres of distilled water. The pH of this solution is *p*H 7.2 - 7.4.

5. Apparatus

General laboratory glassware and:

5.1 Pipette, 50-200 μl (Gilson)

5.2 Pipette, 100-1000 μl (Gilson)

5.3 ELISA plate reader, (Dynatech)

5.4 ELISA well washer, (Dynatech) - useful but not essential

5.5 Multi-channel pipette, 50-200 μl - not essential

6. Procedure

6.1 Schematic Representation of Procedure

A schematic representation of the steps involved in the determination is given below:

Time	Procedure	Volume	Description
5 minutes	addition	200 µl	pipette standard extracts and sample extracts into appropriate wells
1 hour	incubation		incubate at room temp
5 minutes	wash		wash 6 times with working wash solution
5 minutes	addition	200 µl	pipette conjugate solution
1 hour	incubation		incubate at room temp
5 minutes	wash		wash 6 times with working wash solution
1 minute	addition	200 µl	pipette substrate solution
10 minutes	incubation		incubate for 10 - 12 mins until top standard reaches 1.2 absorbance units swirl plate every 2 mins
1 minute	addition	50 µl	pipette stop solution and swirl plate to mix
5 minutes	read plate		read absorbance on platereader at 410 - 420 nm

Total assay time is about 2.5 hours

6.2 Preparation of the Sample

6.2.1 Extraction of Meat Samples

To the homogenised meat samples (labelled A - J), add a total of 90 ml PBS from a measuring cylinder, (100 mls final volume). To achieve dispersal of the meat, add about 5 ml of this volume first and disperse the sample with a spatula. Add a further 5 ml and stir. When about 20 ml has been added in this way the remaining volume can be added intotal. Replace the caps and shake vigorously for about 10 seconds to ensure complete dispersal of the sample. Allow to stand at room temperature for 1.5 hours and agitate for a few seconds every 15 minutes (not critical). Loosen the screw caps and allow the extracts to stand undisturbed for 30 minutes, (to allow for sedimentation of the solids). Withdraw about 5 ml of the aqueous phase with disposable Pasteur pipettes and place in the stoppered sample containers supplied. Store at +4°C until required (3 days max).

6.3 Preparation of Kit Materials

6.3.1 Wash Solution Concentrate

Dilute the wash solution concentrate 1 to 20 with distilled water. The complete contents (50 ml) of the bottle can be diluted to 1 litre or smaller quantities can be diluted as required.

6.3.2 Kit Reagents

The kit reagents (and test extracts) must be at room temperature before the immunoassay is commenced. Remove the vials from the kit box and leave on the bench for about 2 hours to equilibrate. Invert each vial several times before use to mix contents; DO NOT SHAKE. When the assay is completed the vials should be returned to the kit box and stored at +4°C.

6.4 ELISA Procedure

6.4.1 Cut open one end of the foil laminated microwell bag and remove the plate. Check that the desiccant bag shows blue. Remove four 2 × 8 stripwells and replace with the desiccant in the foil bag. RE-SEAL IMMEDIATELY with a heat sealer or with the sticky tape supplied. It is important that the remaining wells are kept away from moisture.

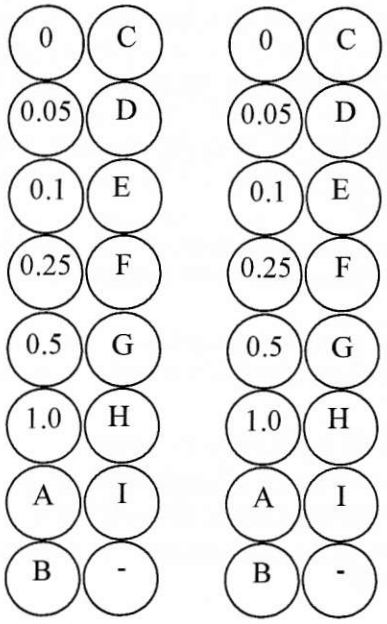
Number the two 2 × 8 stripwells remaining with a felt pen and place in the middle of the frame.

6.4.2 Samples and standards must be treated in identical ways. With a 200 µl pipette, transfer 200 µl of each standard and each sample extract (in duplicate) into the appropriate wells, as indicated in the format given in Figure 1. Pipette in an orderly sequence using a fresh tip for each sample. The zero papain standard represents the sample blank.

This stage should be completed within 5 minutes.

FIGURE 1.

Format for Standard and Sample Extract in Microwells (Section 6.4.2)



Standard Extracts:
0, 0.05, 0.1, 0.25, 0.5, 1.0

Test Extracts
A-J

Carefully cover with cling-film or a plate lid and allow to incubate at room temperature for 1 hour.

6.4.3 Wash wells with diluted wash solution

The wash procedure is performed to remove unbound reagents from the wells. This involves filling the wells 3/4 full with wash solution and then emptying. This is repeated to give 6 washes in total. Washing can be performed most simply using one of the disposable Pasteur pipettes provided; emptying the wells by inversion over a sink and apply a flicking action to effectively remove well contents. Alternatively, an 8-channel pipette can be used to fill the wells or an automatic washing system can be used, if available.

After the 6th wash the wells should be patted onto a tissue to remove excess wash solution.

6.4.4 With a 200 μl pipette, transfer 200 μl of CONJUGATE solution into all wells, (in the same order as before). Cover and allow to incubate at room temperature for 1 hour.

6.4.5 Wash wells with diluted wash solution 6 times as before.

6.4.6 With a 200 μl pipette, transfer 200 μl SUBSTRATE solution into all wells, (in the same order as before). To avoid contamination **DO NOT** pipette directly from the substrate vial. Transfer 8 ml into a clean disposable container and pipette from the latter. **DO NOT** return any unused substrate to the vial.

Allow the wells to incubate at room temperature for 10 to 12 minutes until the 1 mg/kg standard reaches 1.2 absorbance units, (relative to the zero papain standard). Chromophore development is temperature dependant; at a room temperature of 20°C this will take 12 min. **DO NOT** leave under the plate reader during the incubation stage as heat from the lamp will increase the reaction rate. Swirl the well holder fairly vigorously every 2 minutes to prevent product inhibition occurring at the surface of the solid phase. This is achieved most easily with the frame on the bench.

6.4.7 Pipette 50 μl of STOP solution into each well, (in the same order as before) and swirl the frame for 30 seconds to thoroughly mix the well contents.

NB There will be an increase of about 0.3 absorbance units for the top standard upon addition of stop solution because acidification enhances chromophore intensity.

Visually check that the zero papain wells show no colour.

6.4.8 Immediately, measure the absorbance of each well on an ELISA plate reader fitted with a filter within the range 410 - 420 nm.

i.e Zero the ELISA plate reader on air and measure the absorbance of each standard and test sample well.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

Particular care should be taken when handling the substrate solution as ABTS (2,2'-Azinobis(3-ethylbenzthiazoline sulphonic acid) is reported to be toxic.

8 Expression of Results

Subtract the average of the standard zero papain readings from each other readings. Construct a calibration curve by plotting the averaged standard values against concentration of papain (mg/kg) on the log/lin graph paper provided. Use a 'Flexi-curve' to draw the line, which

should pass through all 5 points on the graph, (see example of typical curve, Appendix 2).

As the standards and unknown meat samples have been extracted and assayed in identical ways the concentration of papain in the unknown samples is determined directly by interpolation on the standard curve and expressed as mg papain per kg meat.

9. General Notes

Kit components and test sample extracts should be kept at +4°C when not being used. However, they should be allowed to warm to room temperature before use. Allow at least 2 hours for temperature equilibration. Excess quantities of reagents are supplied with each kit. There are sufficient materials supplied to perform the pre-trial assay twice, (total of two 2 × 8 strips) and the trial assay twice, (total of four 2 × 8 strips). The immunoassay is technically simple to perform.

As with other immunoassay techniques the following general requirements apply:-

a. Take care not to cross-contaminate reagents.
b. Use a new pipette tip for each different sample, standard and reagent.
c. Do not allow pipette tips to come into contact with the surface of liquid within the wells.

10. References

10.1 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16.

10.2 JG Sargent, R Wood, Determination of Papain in Raw Meat Collaborative Trial, J.Assoc. Publ. Analysts 1992 **28(4)**, 155-170

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V0 of this series[1].

A1. Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised below (Table 1). When papain is at levels of approximately 0.08, 0.25, 0.5, 0.8; r may be taken as 0.03, 0.07, 0.08, 0.15 mg/kg respectively.

A2. Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data below (Table 1). When papain is at levels of approximately 0.08, 0.25, 0.5, 0.8; R may be taken as 0.04, 0.09, 0.24, 0.34 mg/kg respectively.

A3. Trueness (Bias)

The collaborative trial was based on the analysis of samples of homogenised beef spiked with papain. The values obtained by participants agreed well with the accepted "true" value, see Table 1.

A4. Limit of Detection

This limit has not been established.

A5. Statistical Data Derived from the Results of Interlaboratory Tests.

Participants in the collaborative trial[2] at 18 laboratories each analysed two sample of chilled homogenised beef samples as a pre-trial check. They then each analysed 10 samples once in the trial proper. These comprised 5 different samples, 4 sets of blind duplicate and 2 blank samples.

Table 1 summarises the statistical data; the papain levels were expressed in mg/kg.

TABLE 1
Statistical Analysis of Papain in Raw Beef

Sample	1	2	3	4
Number of laboratories retained after eliminating outliers	18	18	18	18
Number of laboratories	0	0	0	0
Number of accepted results	36	36	36	36
Mean observed value $\bar{x}$ (mg/kg)	0.086	0.255	0.522	0.814
Accepted "true" value	0.08	0.25	0.48	0.82
Repeatability				
Standard deviation S_r (mg/kg)	0.012	0.025	0.027	0.053
Relative standard deviation RSD_r(%)	13.44	9.99	5.26	6.49
Repeatability r [2.8× S_r] (mg/kg)	0.032	0.071	0.077	0.15
Reproducibility				
Standard deviation S_R (mg/kg)	0.014	0.033	0.085	0.12
Relative standard deviation RSD_R (%)	6.40	12.92	16.3	14.82
Reproducibility R [2.8× S_R(mg/kg)	0.039	0.092	0.24	0.34

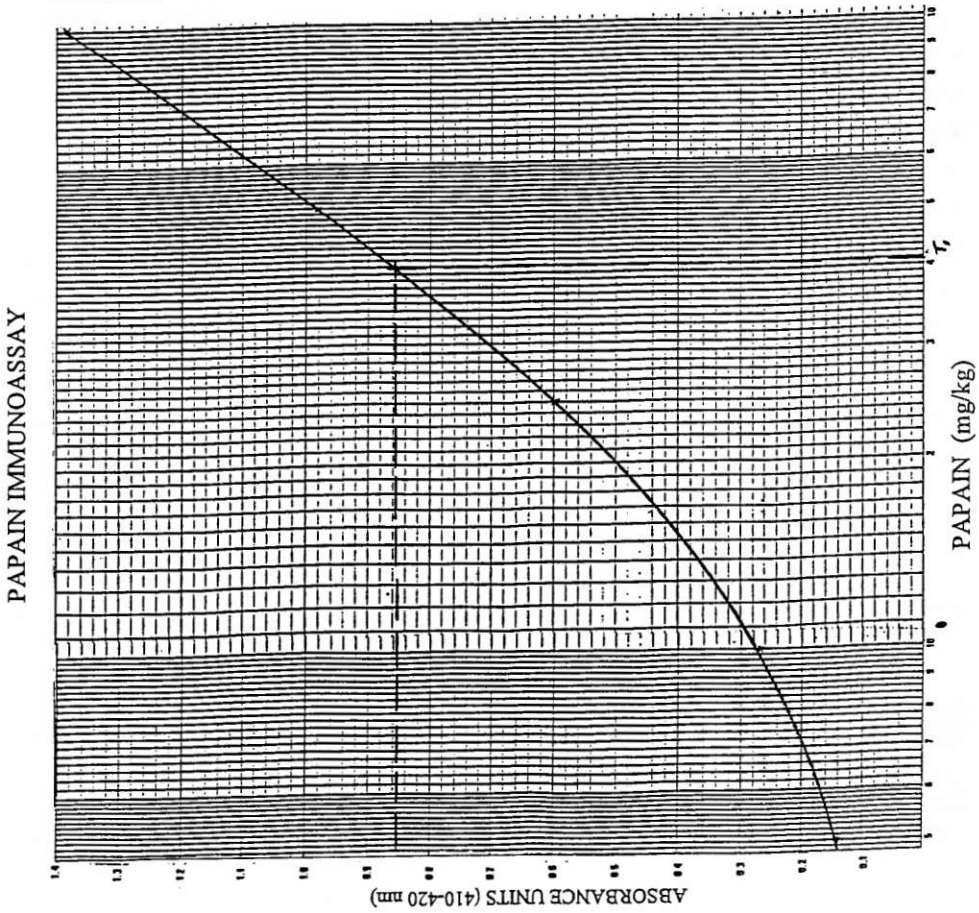
A6. KEY TO TABLE 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

APPENDIX II

Standards	Absorbance		Mean
mg/kg	1	2	
0.05	0.14	0.14	0.14
0.10	0.27	0.26	0.27
0.25	0.61	0.58	0.60
0.50	0.99	0.95	0.97
1.00	1.39	1.36	1.38

Sample	1	2	Mean	Papain mg/kg
T_1	0.86	0.84	0.85	0.41



J. Assoc. Publ. Analysts 30, 89-100

The Annual Report of the Council of the Association of Public Analysts for 1993

Presented at the Old Ship Hotel, Brighton at the Annual General Meeting of the Association on 23rd. April 1994 by the Honorary Secretary, Dr. Peter Clare

Introduction

This report records the activities of the Council of the Association of Public Analysts and its members and discusses events that influence their development for the year ending December 31st. 1993. This year has been marked by the continued development of a series of initiatives concerned with professional standards for the Association and its members. The Council of the Association meets on five occasions each year and in addition to elected members and officers of Council, the Editor of the Journal of the Association and the Public Relations Officer of the Association have regularly attended. Members of Council and of the Association continue to be members of committees of the Royal Society of Chemistry, the Ministry of Agriculture Fisheries and Food, the Local Authorities Coordinating Body on Trading Standards (LACOTS), the Food Law Enforcement Practitioners (FLEP) which is an international organisation covering the European Union and the Free Trade Area, the British Standards Institute (BSI), the European Committee for Standardization, (CEN) and the Codex Alimentarius. In addition Public Analysts serve as scientific advisors to the Association of Metropolitan Authorities and to the Association of County Councils.

Annual Conference 1993

The Annual Conference of the Association in May 1993 was held at the Swallow Hotel, South Normanton, Derbyshire . The theme was "Public Analysts Meeting the Need for Food Control ". Notable amongst the deliveries were those from Dr.S.Benn of the Royal Society of Chemistry, Mr.R.B.Radcliffe of the Institute of Food Science and Technology and Dr.H.Denner of the Ministry of Agriculture, Fisheries and Food, ('MAFF). These speakers dealt in turn with Communication for chemists, particularly via the Parliamentary process, and the role of the Royal Society of Chemistry, the food industry's view of food law enforcement and aspects of the expected competence of official laboratories.

The President of the Association, Mr.A.J.Harrison OBE reviewed the performance of laboratories in the Food Analysis Performance Ansessment Scheme of MAFF. And Mr. Cockbill of MAFF summarised the Ministry's view of the future needs for organisation of the food law

enforcement system. Both he and Mr.J.K.Humble of LACOTS, the Local Authorities Coordinating Body on Trading Standards were able to comment on the APA Policy Document which was concerned with the Future of Food Law Enforcement in the UK and which was published in April 1993.

The Coordinator of Scientific Affairs of the Association, Mr.E.B.Reynolds, summarised the involvement of Public Analysts on outside bodies, a useful and enlightening delivery, which highlighted the diverse role that scientists play in the maintenance and propagation of proper standards in all facets food and consumer goods production and trade. Mr.R.A.Stevens, Public Analyst, reviewed current developments concerning the labelling of foods.

Guest of Honour at the Annual Dinner was Professor Duncan Graham, one time Chief Executive to Humberside County Council.

Education and Training

The Training Committee of the Association, under the chairmanship of Mr.N.Harrison, has continued in its role of providing the forum for professional training of Public Analysts and their staff and the production of training guides. At a meeting on the" Role of the Expert Witness ", which was held in June at Bristol, speakers comprising a Crown Prosecutor, a Barrister, Solicitors and Public Analysts presented expert views of their respective courtroom roles and provided illustrative accounts Of their experiences. A meeting in Leicester on" Statistics for Analytical Chemists - highlighted the application of statistical techniques to analytical science and included deliveries by Central and Local Government scientists. The Annual Training School of the Association was held at the University of Reading and with the objective to provide the next generation of Public Analysts with information necessary to complement their studies in preparation for the statutory examination Mastership in Chemical Analysis, (MChemA).

The Training Committee has during recent years produced a series of training guides designed in the main to assist in the preparation of candidates for the MChemA examination. This year a considerable amount of effort has been devoted to the preparation of a training guide on the subject of certificate and report writing and it is anticipated that this will be available during 1994. In addition a guide to "Audio Visual Resources" has been published. This guide provides an account. of the teaching aids presently available which relate to the theory and practice of analytical science likely to be practised in Public Analysts' laboratories.

1993 also witnessed the introduction of the Record of Professional Experience and Training ".This document is a "log book" which has a dual purpose, namely to assist candidates who are registered with the Royal Society of Chemistry for the MChemA examination in covering all areas of the syllabus and to provide the examiners and the RSC with evidence that the candidate is thoroughly prepared for each examination module.

Professional Liaisons

During the year the Vice President, Mr.M.Barnett, has co-ordinated activities with the specific purpose of developing communications both within the membership of the Association and to outside organisations. Laboratory reporters responsible for the provision of information to the APA Bulletin have been identified. Mr. P. Lenartowicz, the Publicity Officer of the Association has planned and designed a series of information sheets which will detail the statutory role of Public Analysts, demonstrate the work carried out in their laboratories and provide an improved Public relations image for the Association and its members. The information sheets are designed to be collectable and will be widely distributed. (The first of this series has been published in March 1994.)

In April the Association produced a policy document entitled" The Future of Food Law Enforcement in the United Kingdom ". This document addressed many of the potential changes resulting from statutory proposals for community based legislation concerned with the " Official Control of Foodstuffs " and the reorganisation of Local Government. This document has been distributed widely. The recommendations of the Association, which were commended for consideration by those concerned are reproduced in the Appendix to this report, and included the introduction of the concept of the Food Control Unit, based at the level of Local Authority and staffed with scientists and field inspectors, these personnel having qualifications appropriate to anticipated demands of the European Community food control directives.

In July the President was able to address the Quality Standards panel of LACOTS on this policy document. He proposed that a Code of Practice would be a suitable mechanism for achieving the essential coherence between the professionals necessary to preserve the food control functions of the Food Safety Act 1990.

Food Quality

The Council has initiated the production of an on going exercise entitled " Guide Levels for Food Quality ". These guide levels will be produced as a consequence of pooling information concerning the composition of foods already available in the laboratories of Public Analysts and will have the benefit of presenting this data and underlining and recording trends in compositional standards that have occurred during recent years. Not only will members of the Association benefit from this exercise in that. the data will provide bench marks for food standards, but also a useful pool of information will develop from Which can be drawn information for release to outside organisations illustrating the work of Public Analysts. Dr.R.Ottie has marshalled the development of this exercise with data concerning fish fingers, other coated fish products and aspects of minced beef including lean minced beef. The data so far drawn concerning the quality fish fingers, for example, has indicated a disturbing trend. Producers of this popular family food are now manufacturing an article which contains in the main between 50% and 60% of fish. And yet it is a mere 7 years ago that the Food Advisory Committee in their report on Coated and Ice Glazed fish recommended a minimum fish content of fish fingers of 60%. In most recent years a minimum of 50% of fish in fish fingers has become the more likely norm for commercial quality.

Scientific Affairs

A Science Writer has been appointed, to complete, edit and review where necessary the analytical procedures contained in the Validated Enforcement Methods Service, VEMS. Supporting the Science Writer and the members of the working groups responsible for drafting methods, is an experienced NAMAS assessor to ensure that documented procedures are suitable for accreditation purposes. It is anticipated that this process will result in methods having a uniform style and content acceptable to all public Analysts Laboratories and their third party assessors.

MAFF has two areas of investigatory activities which are directly relevant to the statutory role of Public Analysts. These are in the fields of Food Surveillance and Food Authenticity. Public Analysts serve on a number of the committees that contribute to the progress of these MAFF based activities and recently the President has developed a mechanism to enable all PA laboratories to be more involved with the ongoing scientific development of these Food Authenticity studies.

The Coordinator of Scientific Affairs, continues to represent the Association in the standards making activities of BSI and CEN and also is involved with the Sampling and Analysis activities of the Codex Alimentarius Commission.

During the year the reins of the Analytical Quality Assurance subcommittee of the Association have been gathered by Mr. N. Michie. It has been six years since the first protocol on this subject was published and the experience gained during this interval is in need of incorporating into a revised version.

The Analytical Methods Committee of the Royal Society of Chemistry has now published the results of studies carried out in to the composition of beef. Public Analysts and their laboratories were to the fore in this exercise which provided data from authenticated beef samples which will be used for enforcement purposes where the composition of beef products is being investigated for the protection of consumer interests.

Appendix

The Recommendations of the Association of Public Analysts concerning the Future of Food Law Enforcement in the United Kingdom

1) Enforcement of food control in the UK should continue to be invested at Local Authority level and should bevested in Food Control Authorities (FCA's)

2) Food Control should be undertaken by Food Control Units (FCU's) based on the Public Analyst Service together with appropriately qualified inspectors.

3) The FCU's must be large enough to be economically viable and to include sufficient specialist staff to provide a comprehensive" one stop shop" service to the authorities served.

4) The FCU's based on laboratories would continue to serve the same number of authorities as currently, however these numbers may change following Local Government Review,

5) Whilst each Laboratory would be headed by a Public Analyst for food control purposes, Local Authorities may also wish to recognise in the title the broader spectrum of scientific services customarily provided to contributory Local Authorities.

6) A number of FCU's could be administered collectively, if required, to meet the needs of any regional council of other strategic requirements following Local Government Review.

7) Field Inspectors should either be part of, or attached to, the laboratory to ensure the closest coordination of enforcement effort essential to efficient, effective and economic operation. these personnel, having appropriate qualifications and specialist skills, may be drawn from existing local authority sources.

8) An appropriate qualification for the field inspector, incorporating aspects of Public Analyst, Trading Standards and Environmental Health Services training, should be developed within the period specified within the Additional Measures Directive

9) The training of Public Analysts, the only persons statutorily qualified for their duties under the Food Safety Act 1990 should continue to be carried out under the auspices of the Association of Public Analysts and examined by the Royal Society of Chemistry.

10) All sampling undertaken by inspection teams operating within the whole food chain (i.e. within the terms of Article 4 of the Food control Directive 89/397/EEC) would be official samples and must be submitted to the Public Analyst or by agreement to another food analyst

11) Laboratories providing specific areas of expertise will be recognised within the Public Analyst System to make the most cost effective use of the facilities and expertise of the service.

12) The services of Central Government experts with access to unique facilities and other specialists though not qualified as a Public Analyst should be used by the Public Analyst in appropriate cases. These experts would include Food Analysts approved by the Minister under the provisions of the Food Safety Act 1990 following consultation with the Association of Public Analysts.

13) Arrangements for microbiological examination for enforcement purposes should be reviewed to recognise the responsibilities of the Public Analyst and the epidemiological expertise of the Public Health Laboratory Service

14) Preliminary screening or other examinations undertaken in unofficial laboratories must be eliminated to ensure the most cost effective use of resources.

Annual Statistics for the Public Analyst Scientific Service 1993

Prepared and Presented by Mr Paul Lenartowicz

Introduction

The following report relates to the local authority work undertaken by Public Analysts' Laboratories during the calendar year 1993.

It is emphasised that the figures relate only to numbers and therefore are not a direct measure of the amount of work involved: some samples only require minimal analysis and interpretation whilst others, even apparently similar samples, can result in a virtual research project in order to certify with confidence that they are or are not satisfactory. This recognition of need and matching of analytical response and interpretation with attendant quality assurance is one of the fundamental differences between the Public Analyst service and mere test houses.

Data

Responses were received from all but two of the Public Analysts' Laboratories in the United Kingdom, corresponding to a declared population of 54.3 million against an approximate total population of 56 million. The data has been corrected to 56 million but as the Scottish figures have been shown in the past to be significantly different from the bulk, the correction for population has been made separately then the figures combined. Detailed figures appear in the Appendix.

Data received from one of the Channel Islands' laboratories appears for comparative purposes on the charts, but has not been included in calculations of United Kingdom statistics

Food Work

Food sampling has increased substantially compared with the slight fall noted in the preceding three years' annual reports, both in terms of formal (up by 44% over 1992) and informal samples (up by 42% over 1992). This may be a consequence of pressure on local authorities to achieve the World Health Organisation's recommended minimum sampling rate of 2.5 samples per 1000 population: in fact the mean total number of food samples per 1000 population was 1.81 in 1993, against 1.18 in 1992. It is notable that the Scottish authorities continue to sample consistently above the recommended minimum of 2.5, a rate achieved by only five of the English and Welsh

authorities. The area with the lowest level of sampling, whilst substantially better than last year's lowest, samples only 0.85 per 1000 population. Excluding Scotland (with a mean rate of 4.1) from the figures reduces the mean for the remainder of the UK to 1.57.

Chart I: Total Food Samples per 1000 Population

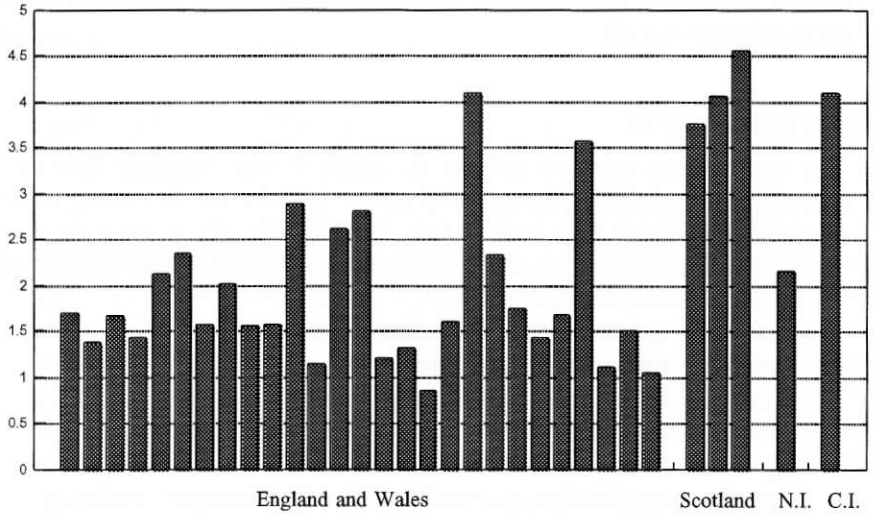
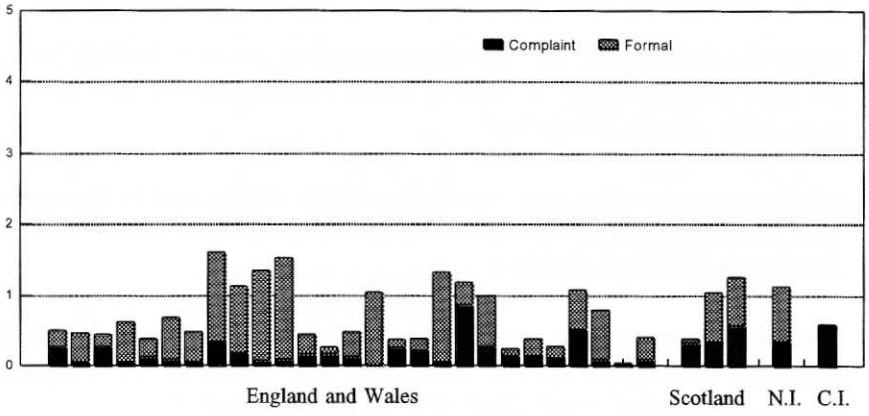


Chart I illustrates the spread of samples.

The above statistics, however, are not the whole story as they include informal samples as well as those taken in accordance with the Food Safety Act. If the informal samples are discounted the picture looks very different indeed, with a total of only 0.68 official samples per 1000 population and no areas achieving the 2.5 level.

Chart II shows the situation with respect to Official Food Act samples

Chart II: Official Food Samples per 1000 Population



Food Factory Inspection

A total of 1105 days were spent on food factory inspection during the year, slightly down on the previous years 1188. From individual laboratories' returns it is clear that the use of the Public Analyst in factory inspections has only been adopted to any significant degree in a very small number of authorities. One laboratory accounts for three quarters of the time spent on factory inspection, with over half of the Association's laboratories having had no involvement at all.

Non-food Work

The major areas other than food in which Public Analysts' laboratories served local authorities during 1993 are as follows, in decreasing order of numbers of samples:

Environmental investigations (including pollution, water, tip leachates, atmospheric samples etc.)
Water analysis (other than environmental)
Workplace monitoring and analysis
Consumer Safety and Trade Descriptions
Radiation monitoring
Agricultural samples (Fertilisers and Animal Feeds)
Miscellaneous (including coroners/toxicology samples, building materials, drugs, etc.)

In addition there were 255 call-outs to emergency incidents involving chemicals (ranging from fires to chemical spillages), utilising the 24-hour-a-day back-up service provided to the emergency services to advise and/or provide analytical facilities to help deal with any incident that may be a threat to the public or the environment. On average this equates to one call for assistance somewhere in the UK every 34 hours.

Accreditation and Proficiency testing schemes

Eighteen (55%) of the U.K. member laboratories are accredited by NAMAS for aspects of food analysis, most of which also hold accreditation for one or more other aspects of their work.

Of the remaining laboratories most are well into preparation for accreditation and it is anticipated that all will be accredited before the deadline set by the Additional Measures Directive.

Participation in various proficiency testing schemes is an increasingly important area of laboratories' work, and one which can be a significant burden of work that is not reflected in the sample statistics.

For food alone, full participation in the Ministry of Agriculture, Fisheries and Food's Food Analysis Performance Assessment Scheme (to become a mandatory requirement for Official Laboratories) has now reached thirty two circulations of samples annually, which if they do not coincide with 'routine' samples for similar analysis still require full set-up, calibration and quality assurance procedures for each sample.

Conclusion

With respect to food samples it is pleasing to note an increase in the number of samples submitted during 1993.

However, whilst the move towards greater rates of sampling is to be welcomed it must be recognized that if the number of formal samples (to the exclusion of informal samples) were to be increased to sufficient extent to achieve a sampling rate of 2.5 per 1000 population for official samples, local authorities would incur substantial increases in sampling as well as analytical costs, the formal sampling of food being both time consuming and a highly skilled operation unlike the mass purchase of informal samples. It is to be hoped that the implications of food sampling and analysis will be taken into account in the consideration of food law enforcement .provisions as local authorities are restructured, possibly into smaller food authorities, a move which also coincides with the implementation of the Additional Measures Directive.

The lack of any increase in utilisation of Public Analysts' services in food factory inspection is a matter for concern, the potential value of the Public Analyst's input having been recognised in statutory Codes of Practice under the Food Safety Act.

Given the ever increasing complexity of modem analysis, of food in particular, and the increasing burdens of accreditation and proficiency testing, it is ever more important for the maximum use to be made of the resources available to local authorities within their Public Analysts' laboratories. lt is hoped that the increased food sampling in 1993 continues apace in 1994 with a corresponding reduction in samples 'screened' out by unofficial laboratories which lack the qualifications, knowledge, experience and back-up to provide the quality of analysis and interpretation of a Public Analyst, and which remove the base of normal foods with which the Public Analyst can compare individual samples.

APPENDIX I

Data from total population of 54.3 million corrected to 56 million

Foods - all formal	29556
Foods- all informal	63245
Foods - Home Authority (if identified)	3222
Foods - Port Health	1557
Foods - Complaints	8662
Foods - Bacteriological	3587
Milks (all)	5791
Mineral and other bottled waters	478
Drinking waters	23977
Swimming Pool Waters	4690
Pollution water, effluents, tip leachates	60253
Other waters	1446
Atmospheric samples	29584
Soils	3220
Workplace monitoring (excl. asbestos.)	1951
Asbestos (bulk & airborne)	18879
Feeding Stuffs - Agriculture Act	2581
Feeding Stuffs - Medicines Act	211
Fertilisers	609
Toys (Safety) Regulations	5097
Cosmetics (Safety) Regulations	1206
Other Consumer Safety Act work	4406
Trade Descriptions samples	711
Building Materials	1734
Coroners/toxicology	2951
Radiation monitoring	9005
Other Miscellaneous	12050
Total Foods	**101463**
Total Agriculture Act + Medicines Act feeds	**3401**
Total Waters (incl. environ.)	**93144**
(Total waters excl. environ.)	**32891**
Total Consumer Safety etc.	**11421**
Total environmental sample	**102062**
Total workplace samples	**20830**
Total other samples	**16735**
TOTAL SAMPLES	**321693**
Emergency callouts	255
Food factory inspection	1105
Other factory inspection	5
Food samples per 1000 population:	
Formal (= official)	0.53
Complaint (=official)	0.15
Total Official	0.68
Informal (= unofficial)	1.13
Total foods	**1.81**