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The Determination of Acidity, Apparent Reducing Sugar and Sucrose, Hydroxymethylfurfural, Mineral, Moisture, Water-insoluble Solids Contents in Honey; Collaborative Trial

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The results of a collaborative trial, involving 18 laboratories, of the proposed EC methods of analysis for the determination of apparent reducing sugar content, apparent sucrose content, moisture, water-insoluble solids content, mineral content, acidity and hydroxymethylfurfural in honeys are reported. The results indicate that the proposed methods of analysis for the determination of mineral content, moisture, acidity, apparent reducing sugar content and water-insoluble solids content are satisfactory while those for hydroxymethylfurfural and apparent sucrose content require further investigation before they could be recommended as suitable for inclusion in any legislation on honey.

The European Community (EC) Council of Ministers has adopted a Directive on the harmonisation of the laws of the member states relating to honey[1]. This Community Directive was translated into legislation into England and Wales as "The Honey Regulations 1976"[2] and by similar legislation in Scotland[3] and Northern Ireland[4]. Amongst the compositional criteria prescribed in the Council Directive are requirements relating to the concentrations of acidity, apparent reducing sugar (calculated as invert sugar) and apparent sucrose, hydroxymethylfurfural, mineral content (ash), moisture and water-insoluble solids.

The levels prescribed are:

Acidity	not more than 40 milli-equivalents of acid per kg
Apparent reducing sugar, calculated as invert sugar:	
Blossom honey	not less than 65 per cent.
Honeydew honey and blends of honeydew honey and blossom honey	not less than 60 per cent.

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Apparent sucrose content:	
In general	not more than 5 per cent.
Honeydew honey and blends of honeydew honey and blossom honey, acacia, lavender and *Banksia menziesii* honeys	not more than 10 per cent.
Hydroxymethylfurfural content	not more than 40 mg/kg
Mineral (ash) content:	
In general	not more than 0·6 per cent.
Honeydew honey and blends of honeydew honey and blossom honey	not more than 1 per cent.
Moisture content:	
In general	not more than 21 per cent.
Heather honey and clover honey	not more than 23 per cent.
Water-insoluble solids content:	
In general	not more than 0·1 per cent.
Pressed honey	not more than 0·5 per cent.

Methods of Analysis being Collaboratively Tested

There have been discussions in the EC Working Group on Methods of Analysis of Honey on the methods to be prescribed to enforce the above provisions. Methods included in the appropriate EC Working Document were taken as the basis for the methods being tested in this trial; they are based on classical procedures. Many of them originated from discussions in the Codex Co-ordinating Committee for Europe, which has produced the Codex Standard for Honey (European Regional Standard)[5].

Participants were asked to familiarise themselves with the methods by using a practice (pre-trial) sample. In the light of their experiences, and as a result of more detailed work carried out in the co-ordinating laboratory, the methods were slightly modified; the modifications made to the original methods are given below.

1. *Determination of acidity*. The method was modified to include instructions that the alkali solution should be added in 0·05 ml portions rather than 0·1 ml portions throughout the titration. To avoid inconsistencies in graphic interpretation the calculation of results was altered from the original method with results being obtained by plotting a graph of pH change against volume of sodium hydroxide solution rather than pH against volume of sodium hydroxide solution.

2. *Determination of apparent reducing sugar and apparent sucrose contents*. An additional check on the Luff–Schoorl reagent involving standardisation with a solution of sucrose (Appendix II) (6.1.5), as described by the International Commission on Uniform Methods of Sugar Analysis[6], was included.

3. *Determination of hydroxymethylfurfural (HMF)*. The original method used the following formula:

$$\text{mg of HMF per kg of honey} = \frac{\text{absorbance}}{\text{cell path length (cm)}} \times 192$$

The method was modified to include calibration using a standard solution of hydroxymethylfurfural. This calibration is then used to calculate the results rather than the above formula.

4. *Determination of mineral content*. No amendments.

5. *Determination of moisture*. No amendments.

6. *Determination of water-insoluble solids*. Additional instructions on the washing, drying, cooling and weighing of the sintered glass crucible were included in the method.

The methods of analysis used by participants in the trial are given in Appendices I–VI. The generalised sample preparation instructions for honey as developed by the EC were also given to participants though they were not required to use them. They are reproduced as Appendix VII.

Collaborative Trial Organisation, Samples and Results

PARTICIPANTS

Nineteen laboratories, including the co-ordinating laboratory, participated in the collaborative trial (16 U.K. public analyst laboratories, the government laboratories of Jersey and the Isle of Man and the Eastern Health Board, Dublin).

SAMPLES

The samples were prepared in and the trial co-ordinated from the County Laboratory, Lancashire. Four honeys of different origin were obtained, which on analysis in the co-ordinating laboratory, were shown to be of typical composition.

Each sample was blended and subjected to repeat analysis in the co-ordinating laboratory to verify homogeneity before being packed in lots of 125 g in polystyrene containers and dispatched simultaneously so that analysis by the collaborating laboratories could be commenced at the same time.

Each sample was sent to participants in coded blind duplicate so that each participant received eight samples. The samples sent to participants were as follows:

Tasmanian leatherwood honey	coded 1 and 7
Rumanian acacia honey	coded 2 and 5
Australian light amber honey	coded 3 and 8
Spanish orange blossom honey	coded 4 and 6

RESULTS

Each participant was asked to analyse each sample once only and to report the single result as a percentage by weight on the sample as received. The results are given in Tables I–VII.

Statistical Analysis of The Results

The results were statistically analysed according to procedures outlined by the British Standards Institution[7]. Significant differences between pairs of individual results were identified using Cochran's test and the extremes of magnitude

of pairs of results were identified by Dixon's test. Outlying results are marked in the tables of results.

The values of the means, repeatabilities and reproducibilities, each as defined according to the British Standards Institution procedure[7], were calculated and these are also given in the tables.

Discussion of the Results Obtained

Very few outlying values were identified for any of the determinations except that for hydroxymethylfurfural.

DETERMINATION OF ACIDITY

The prescribed level of acidity in any sample is not more than 40 milli-equivalents of acid per kg. The mean values for the honeys used in the trial were significantly below this value; this is probably the case with most honeys available for retail sale in the U.K. The precision of the method at the lower concentration levels ($\bar{x}$ = 6·5/7·0) of the trial samples may seem at first to be unsatisfactory. The very small additions of titrant required at the end point are thought to be the main source of error at these levels. However, at the higher levels of concentration ($\bar{x}$ = 13·5) there is little change in the absolute values of r and R, thus suggesting that at a level of about 40 milli-equivalents per kg the precision of the method will be acceptable.

DETERMINATION OF APPARENT REDUCING SUGAR CONTENT

The precision of the apparent reducing sugar content procedure is of the order that would be anticipated for this determination. The procedure being tested, the Luff–Schoorl procedure, is not, however, that favoured by U.K. analysts, even though it had been improved by incorporating the standardisation of reagent instructions proposed by ICUMSA. The favoured U.K. procedure, the Lane and Eynon constant volume method, is being compared with the Luff–Schoorl method in a collaborative trial presently being organised by the U.K. Ministry of Agriculture, Fisheries and Food as part of its collaborative trial programme. Observations that were made on the possible sources of error in the use of the Luff–Schoorl reagent are reproduced in Appendix VIII.

APPARENT SUCROSE CONTENT

The precision values for the apparent sucrose levels are of the same magnitude, in absolute terms, as those for apparent reducing sugar. However, when the precision values are expressed as percentages of the mean sucrose level (i.e. as a coefficient of variation for repeatability and reproducibility) they appear to be unsatisfactory, and the method could not be recommended for inclusion in the legislation.

In honey, the sucrose level is determined by the Luff–Schoorl method using the small difference between the two much larger quantities of reducing sugar and total sugar thus giving rise to relatively large errors in the apparent sucrose content. A method which quantifies the sucrose directly would be preferred.

DETERMINATION OF HYDROXYMETHYLFURFURAL CONTENT

A number of laboratories clearly had difficulty with this determination. More outliers were identified than with any of the other determinations. There was, except at low levels of HMF, a substantial difference between the repeatability and reproducibility values observed, thus suggesting that there were variations in the calibration of the method in different laboratories. Furthermore, it is apparent that certain laboratories experienced difficulties with the analysis of all samples. These aspects of the method require further investigation and the possibility of an alternative method must be considered.

DETERMINATION OF MINERAL CONTENT

The level of ash in all the samples tested was very much lower than the prescribed limit of 0·6 g/100 g. The results obtained are satisfactory and the method can, therefore, be recommended.

DETERMINATION OF MOISTURE CONTENT

The results obtained are satisfactory and the method can, therefore, be recommended.

DETERMINATION OF WATER-INSOLUBLE SOLIDS CONTENT

The level of water-insoluble solids in all the samples tested was very much lower than the prescribed limit of 0·1 g/100 g. The results obtained are as would be expected at the concentration levels being considered and so the method can, therefore, be recommended.

Conclusions

In all, seven of the EC methods of analysis associated with the honey directive were collaboratively tested. A pre-trial involving the participating laboratories using draft methods, and additional work within the co-ordinating laboratory, proved useful in identifying modifications needed to be made to the methods.

A subsequent trial involving nineteen laboratories using the final draft (modified) methods showed that of the seven methods, five (acidity, apparent reducing sugar, moisture, mineral and water-insoluble solids content) gave satisfactory repeatability and reproducibility figures and so could be recommended for enforcement analysis. The other two methods proved to be unsatisfactory. In the case of apparent sucrose content, the repeatability and reproducibility of the method proved to be inadequate while the method for the determination of hydroxymethylfurfural gave results with large interlaboratory variations. On the basis of statistical analysis of the results and from comments received from participants with respect to weaknesses in the methodologies, these two methods could not be recommended as enforcement methods. Further investigation to improve or replace them is, therefore, necessary.

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Appendix I: Determination of Acidity (Potentiometric Titration)

1. SCOPE AND FIELD OF APPLICATION

The method determines the acidity of honey expressed in milli-equivalents of acid per kg.

2. DEFINITION

The acidity content: the acidity as determined by the method specified.

3. PRINCIPLE

A plot of the neutralisation curve of honey is obtained by titration of a sample with sodium hydroxide solution. The acidity is calculated from the total titrant used at the equivalence point.

4. REAGENTS

4.1 *Sodium hydroxide solution* 0·05 M (carbonate free).

4.2 *Water*, carbon dioxide-free prepared by boiling and cooling distilled water immediately prior to use.

5. APPARATUS

5.1 pH meter.

5.2 Magnetic stirrer.

5.3 Analytical balance.

5.4 Volumetric flask 50 ml.

5.5 Beaker, 50 ml.

5.6 Pipettes 25 ml.

5.7 Burette, capable of being read to 0·05 ml.

6. PROCEDURE

6.1 Accurately weigh approximately 5 g of honey. Dissolve in a few ml of water, pour into a 50-ml volumetric flask (5.4), and make up to volume with water. Pipette 25 ml from the flask into a beaker (5.5).

6.2 Place a magnetic stirrer (5.2) in the beaker, stir the liquid gently and titrate potentiometrically with sodium hydroxide solution (4.1).

Add the sodium hydroxide in increments of 0·05 ml only. Note the pH immediately after every addition of alkali solution.

6.3 Plot the neutralisation curve of change of pH (on the ordinate axis) against the volume of sodium hydroxide solution (on the abscissa). Determine from the graph the pH of neutralisation i.e. at the peak.

Note: the volume of alkali solution plotted on the abscissa for a given change of pH should be the mean of the two volumes over which the pH change occurs.

7. EXPRESSION OF RESULTS

7.1 *Formula and Method of Calculation*

Express the acidity as milli-equivalents of sodium hydroxide necessary to raise the pH of 1000 g of honey to the neutralisation point.

$$\text{Acidity} = \frac{1000 \times V \times M}{m} \text{ Milli-equivalents/1000 g}$$

where

m = mass in g of the test sample (= 0·5 × weight of sample taken (6.1),
M = molarity of the sodium hydroxide solution,
V = volume in ml of the sodium hydroxide added to obtain the pH at the equivalence point.

Note: This method is the same as that described in CAC/12—1969, Codex Alimentarius Commission Recommended European Regional Standard for Honey.

Appendix II: Determination of the Apparent Reducing Sugar Content, Calculated as Invert Sugar, and the Apparent Sucrose Content (Luff–Schoorl Procedure)

1. SCOPE AND FIELD OF APPLICATION

This method determines the apparent reducing sugar content, calculated as invert sugar, and the apparent sucrose content of honey.

2. DEFINITION

Apparent reducing sugar content: the content of reducing sugar as determined by the method specified and expressed as invert sugar.

Apparent sucrose content: the content of sucrose as determined by the method specified.

3. PRINCIPLE

The sample (clarified if necessary) is heated under standard conditions with a copper II solution which is partially reduced by reducing sugars. The excess copper II is subsequently determined iodometrically and the reducing sugars calculated and expressed as invert sugar. The sugar in the sample is then inverted by acid or enzymic hydrolysis and the total reducing sugars expressed as invert again determined. The difference in concentrations of invert sugar is multiplied by 0·95 to give the apparent sucrose content.

4. REAGENTS

4.1 *Carrez solution I*

Dissolve 23·75 g of zinc acetate trihydrate ($Zn(CH_3COO)_2.3H_2O$) or 21·95 g of zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) and 3 ml of glacial acetic acid in water amd make up to 100 ml with water.

4.2 *Carrez solution II*

Dissolve 10·6 g of potassium hexacyanoferrate trihydrate (II) ($K_4[Fe(CN)_6]3H_2O$) in distilled water and make up to 100 ml with water.

4.3 *Luff–Schoorl reagent*

Prepare the following solutions:

4.3.1 Copper II sulphate solution: dissolve 25 g of iron-free, copper II sulphate pentahydrate ($CuSO_4.5H_2O$) in 100 ml water.

4.3.2 Citric acid solution: dissolve 50 g of citric acid ($C_6H_8O_7.H_2O$) in 50 ml water.

4.3.3 Sodium carbonate solution: dissolve 143·8 g of sodium carbonate (Na_2CO_3) in approximately 300 ml of hot water. Allow to cool.

4.3.4 Add the citric acid solution (4.3.2) to the sodium carbonate solution (4.3.3) in a 1 litre volumetric flask with gentle swirling. Stir until effervescence ceases and then add the copper sulphate solution (4.3.1). Make up to 1 litre with water and mix well. Allow to stand overnight and then filter if necessary. Check

the molarity of the reagent thus obtained (0·1 M in Cu; 1 M in $Na_2 CO_3$) by the method described in 6.1.

4.4 *Sodium thiosulphate solution 0·1 M.*

4.5 *Starch solution:* add a slurry of 5 g of soluble starch in 30 ml of cold water to 1 litre of boiling water. Boil for 3 min, allow to cool, and if necessary add 10 mg of mercuric iodide as a preservative. Smaller volumes of the starch solution may be prepared.

4.6 *Sulphuric acid solution 3 M.*

4.7 *Potassium iodide.*

4.7.1 *Potassium iodide solution 30 per cent. (m/v).*

4.8 *Pumice chips*, boiled in dilute hydrochloric acid washed free of acid with water and then dried.

4.9 *Isoamyl alcohol.*

4.10 *Sodium hydroxide solution 0·1 M.*

4.11 *Hydrochloric acid solution 0·1 M.*

4.12 *Solution of phenolphthalein in ethanol 1 per cent. (m/v).*

4.13 *Solution of acetic acid 20 per cent. (m/v).*

4.14 *Solution of invertase (B-fructosidase):* weigh 1 g of invertase into a stoppered flask and pipette 200 ml of doubly distilled water into the flask. This solution can be kept for about a week at 4°C and is sufficient for ten analyses.

4.15 *Hydrochloric acid solution 6·34 M.*

4.16 *Sodium hydroxide solution 5 M.*

5. APPARATUS

5.1 Conical flask fitted with a reflux condenser, 300 ml capacity.

5.2 Volumetric flasks 100 ml, 200 ml and 250 ml capacity.

5.3 Drying oven, electrically heated, thermostatically controlled at a temperature of 54 ± 1°C.

5.4 Stop-watch.

5.5 Pipettes, 10 ml, 25 ml.

6. PROCEDURE

6.1 *Standardisation of the Luff–Schoorl reagent* (4.3).

6.1.1 Add 3 g of potassium iodide and 25 ml of 3 M sulphuric acid (4.6) to 25 ml of Luff–Schoorl reagent (4.3). Titrate with 0.1 M sodium thiosulphate (6.4) using starch solution (4.5) as indicator added towards the end of the titration. If the

volume of 0·1 M sodium thiosulphate used is not 25 ml the reagent must be diluted accordingly or made up afresh.

6.1.2 Pipette 10 ml of the reagent into a 100 ml volumetric flask and dilute to volume with water. Pipette 10 ml of diluted reagent into 25 ml of 0·1 M hydrochloric acid (4.11) in a conical flask and heat for one hour in the boiling water bath. Cool, make up to the initial volume with freshly boiled water and titrate with 0·1 M sodium hydroxide (4.10) in the presence of phenolphthalein (4.12) as indicator. The volume of 0·1 M sodium hydroxide (4.10) used must be between 4·5 and 5·5 ml.

6.1.3 Titrate 10 ml of the diluted reagent (6.1.2) with 0·1 M hydrochloric acid (4.11) in the presence of phenolphthalein (4.12) as indicator. The end point is characterised by the disappearance of the violet colour. The volume of 0·1 M hydrochloric acid (4.11) used must be between 9·5 and 10·5 ml.

6.1.4 The pH of the Luff–Schoorl reagent must be between 9·3 and 9·4 at 20°C.

6.1.5 Check on the Luff–Schoorl reagent.

Dissolve 9·500 g of pure sucrose (e.g. BDH "Analar" grade) in water. Mix, and make up to volume with water in a 500-ml volumetric flask. Pipette 25 ml of this solution into a 250-ml volumetric flask. Heat the solution to 65°C over a water bath. Remove the flask from the water bath and add 10 ml of 6·34 M hydrochloric acid (4.15).

Allow the solution to cool naturally for 15 min and then bring to 20°C, neutralise with 5 M sodium hydroxide solution and make up to 250 ml. Cool to ambient temperature.

Determine the reducing sugar in 25 ml of this solution as in Section 6.4.

This aliquot should require 19 ± 0·1 ml of sodium thiosulphate 0·1 M solution.

6.2 *Inversion of the Sample Solution*

6.2.1 Prepare a solution of honey in a 250 ml volumetric flask by dissolving 50 g of well-mixed honey in water and making up to volume with water.

6.2.2 Transfer 10 ml of the solution, prepared according to 6.2.1, to a 100 ml volumetric flask and make up to the mark.

6.2.3 Invert a 25 ml portion of the diluted honey solution (6.2.2) in a 100 ml volumetric flask using procedure 6.2.3.1 or 6.2.3.2.

6.2.3.1 Place 25 ml of the solution obtained in 6.2.2 and 25 ml of water in a 100 ml volumetric flask; heat the solution to 65°C over a boiling water bath. Remove the flask from the water-bath and add 10 ml of 6·34 M hydrochloric acid (4.15). Allow the solution to cool naturally for 15 min and then bring to 20°C and neutralise with 5 M sodium hydroxide solution (4.16) using litmus paper as indicator.

Cool to ambient temperature, *or*

6.2.3.2 Place 25 ml of the solution obtained in 6.2.2 and 25 ml of water in a 100 ml volumetric flask; add four or five drops of acetic acid (4.13) and 20 ml of invertase solution (4.14). Stir, close with a cotton-wool plug and place in a drying oven (5.3) at 54 ± 1°C for 2–3 h. Remove from oven and cool.

6.3 *Clarification of the Sample Solution*

6.3.1 Add 2 ml of Carrez solution I (4.1) and 2 ml of Carrez solution II (4.2) to the solution obtained according to 6.2.3. Shake vigorously after each addition. Make up to volume (100 ml) with water. Filter the liquid through a dry filter and discard the first portions of the filtrate. Use the succeeding fractions for determining invert sugar in accordance with the Luff–Schoorl method described under 6.4.

6.3.2 Repeat the steps described under 6.3.1 with 25 ml of the solution prepared in accordance with 6.2.2 with 45 ml of water added. On the last fractions of the filtrate determine the invert sugar content according to the Luff–Schoorl method described in Section 6.4.

6.3.3 Dilute solution 6.3.1 and 6.3.2 so that 25 ml of solution contains at most, 60 mg of reducing sugars expressed as invert sugar.

6.4 *Titration by the Luff–Schoorl method*

6.4.1 Pipette 25 ml of Luff–Schoorl reagent (4.3) into a 300 ml conical flask (5.1) add exactly 25 ml of the filtrate obtained as under 6.3.1. (as diluted according to 6.3.3). Add two pumice chips (4.8). Fix a reflux condenser to the conical flask (5.1) and immediately place the apparatus on a ceramic wire gauze over a bunsen flame. The gauze shall have a hole cut in the same diameter as the base of the conical flask.

Heat the liquid to boiling point over a period of approximately 2 min and simmer gently for exactly 10 min. Cool immediately in cold water and after 5 min titrate as follows: Add 10 ml of potassium iodide solution (4.7.1) and immediately add with caution (because of effervescence) 25 ml of 3 M sulphuric acid solution (4.6). Titrate with 0·1 M sodium thiosulphate solution (4.4) until the solution is almost colourless then add a few ml of starch solution (4.5) and continue the titration until the blue colour disappears.

Note: A small volume of *iso*-amyl alcohol (4.9) may be added before acidifying with sulphuric acid to reduce foaming.

6.4.2 Carry out the same titration again with the filtrate obtained in accordance with section 6.3.2 (as diluted according to 6.3.3).

6.4.3 Carry out a blank test replacing the 25 ml of the 6.3.3 solution with 25 ml of water.

7. EXPRESSION OF RESULTS

7.1 *Formulae and Method of Calculation*

The reducing sugar content, expressed as invert sugar; in the solutions obtained at 6.3.1 and 6.3.2 (as diluted according to 6.3.3) are calculated in the following way: subtract the number of ml of 0·1 M sodium thiosulphate solution used for the determination from the number of ml of sodium thiosulphate solution used for the blank test. Obtain the concentration of invert sugar corresponding to the difference in volumes from the Luff–Schoorl table of values (Table IX).

Calculate the reducing sugar contents, before and after inversion, as a percentage of the original sample. Then:

(a) *the apparent reducing sugar content*, expressed as invert sugar, is the content of reducing sugars determined before inversion of the sample solution, calculated as a percentage of the original sample;

(b) *the apparent sucrose content* is

$$(m - m_1) \times 0{\cdot}95$$

where

m is the total reducing sugar content, expressed as invert sugar, determined after inversion of the sample solution, calculated as a percentage of the original sample, and

m_1 is the total reducing sugar content, expressed as invert sugar, determined before inversion of the sample solution, calculated as a percentage of the original sample.

TABLE IX

REDUCING SUGAR EQUIVALENTS BY LUFF–SCHOORL METHOD. TABLE OF VALUES FOR 25 ML OF LUFF–SCHOORL REAGENT

Difference in volumes of 0·1 M $Na_2S_2O_3$ soln used	Glucose, fructose invert sugars $C_6H_{12}O_6$	
ml	*mg*	Difference
1	2·4	
2	4·8	2·4
3	7·2	2·4
4	9·7	2·5
5	12·2	2·5
6	14·7	2·5
7	17·2	2·5
8	19·8	2·6
9	22·4	2·6
10	25·0	2·6
11	27·6	2·6
12	30·3	2·7
13	33·0	2·7
14	35·7	2·7
15	38·5	2·8
16	41·3	2·8
17	44·2	2·9
18	47·1	2·9
19	50·0	2·9
20	53·0	3·0
21	56·0	3·0
22	59·1	3·1
23	62·2	3·1

Appendix III: Determination of Hydroxymethylfurfural (HMF)

1. SCOPE AND FIELD OF APPLICATION

The method determines the HMF content of honey.

2. DEFINITION

The hydroxymethylfurfural content: the HMF content as determined by the method specified.

3. PRINCIPLE

Spectrophotometric determination using barbituric acid and *p*-toluidine solutions.

4. REAGENTS

4.1 *Barbituric Acid Solution*

Transfer 500 mg of barbituric acid to a 100 ml volumetric flask using 70 ml water. Place the flask on a very hot water bath until the barbituric acid dissolves, allow to cool and make up to volume with water.

4.2 *Para-toluidine Solution*

Weigh 10·0 g of *p*-toluidine and dissolve in approximately 50 ml of *iso*propanol by heating gently on a water bath. Transfer the solution to a 100 ml volumetric flask with *iso*propanol and add 10 ml of glacial acetic acid. Allow to cool and make up to the calibration mark with *iso*propanol. Store the solution in the dark. Do not use for at least 24 h.

Note: Care should be taken when handling *p*-toluidine.

4.3 *Water (Oxygen-free)*

Bubble oxygen-free nitrogen gas into boiling water for several minutes. Allow the water to cool before use.

4.4 Hydroxymethyl furfural, pure, for preparation of standard solution (6.2.2).

5. APPARATUS

5.1 Spectrophotometer calibrated to read at 550 nm.

5.2 Volumetric flasks, 50 ml and 100 ml capacity.

5.3 Pipette, 2 ml.

6. PROCEDURE

6.1 *Preparation of the Test Sample*

Weigh 10 g sample of honey and dissolve it *without heating* in 20 ml of oxygen-free water (4.3). Pour the entire contents into a 50 ml volumetric flask (5.2) and make up to the volume with water (4.3) (designate: honey solution). The sample should be analysed as soon as it has been prepared.

6.2 *Photometric Determination*

6.2.1 Sample determination. Take two test tubes and pipette into each of them 2·0 ml of honey solution; (6.1); then add to each tube 5·0 ml of the *p*-toluidine solution (4.2). Pipette into one of the tubes 1 ml of water (4.3) and into the other

1 ml barbituric acid solution (4.1). Agitate both tubes. The tube containing water is the blank.

Add the reagents quickly so as to complete the operation within a minute or two. Read off the extinction of the solution in the sample tube compared with the blank tube at 550 nm using a 1 cm cell, as soon as the maximum absorbance value is reached.

6.2.2 Standard determination. The method should be calibrated using a standard solution of hydroxymethylfurfural (HMF) (4.4). Check the purity of the HMF standard by spectrophotometric assay at 284 nm, E (molar) = 16·830.

Prepare a solution of HMF in water so that a 10 μg standard of HMF in 2 ml of water can be used in place of the honey solution in 6.2.1. Carry out the colour reaction as in 6.2.1 and measure the absorbance as in 6.2.1.

7.1 *Formula and Method of Calculation*

The following formula can be used to calculate an approximate figure for HMF:

$$\text{mg of HMF per kg of honey} = \frac{\text{absorbance}}{\text{cell path length (cm)}} \times 192$$

For calculation of the mg HMF per kg honey using a standard determination, the following formula can be used:

$$\text{mg of HMF per kg of honey} = \frac{\text{absorbance of sample solution}}{\text{absorbance of standard solution}} \times 25$$

Note: This method is the same in principle as that described in CAC/12-1969 Codex Alimentarius Commission Recommended European Regional Standard for Honey and is based on a paper by J. H. Turner, P. A. Roberts, C. L. Barrich and R. H. Cotton, *Anal. Chem.*, 1954, **26**, 898.

Appendix IV: Determination of Mineral Content (Ash) (Incineration at 600°C)

1. SCOPE AND FIELD OF APPLICATION

The method determines the ash content of honey.

2. DEFINITION

Ash content: the content of ash as determined by the method specified.

3. PRINCIPLE

The residual mass of a test portion is determined after incineration in an oxidising atmosphere at 600°C and calculated as a percentage by mass of the sample.

4. REAGENTS

4.1 *Olive oil*

4.2 *Dilute hydrochloric acid, approximately 7 g HCl per 100 ml.* Carefully add, with stirring, 100 ml concentrated hydrochloric acid (HCl, density 1·64 g/ml) to 500 ml water and mix.

5. APPARATUS

5.1 Incineration dishes made of platinum or silica.

5.2 Electric muffle furnace, air-ventilated, temperature controlled by thermostat at 600°C with a differential no larger than 25°C, fitted with a pyrometer.

5.3 Infra-red ray lamp.

5.4 Desiccator, containing an efficient desiccant, e.g. dried silica gel.

6. PROCEDURE

6.1 *Preparation of the Incineration Dish*

Clean the incineration dish (5.1), whether new or not, with boiling dilute hydrochloric acid (4.2). Rinse it free from acid with a large quantity of water.

Heat for 30 min in the muffle furnace (5.2).

Remove it from the furnace, allow it to cool to ambient temperature in the desiccator (5.4) and weigh it to the nearest 0·1 mg (m_1).

6.2 *Test Portion*

6.2.1 Weigh into the prepared incineration dish (6.1), to the nearest 1 mg, about 5–10 g of honey (m_0).

6.2.2 Place the dish and contents (6.2.1) in the muffle furnace (5.2) and heat gently until the sample becomes black and dry. Care must be taken to remove risk of loss through foaming and excessive swelling of the mass. An infra-red lamp (5.3) may be used to aid carbonisation of the sample prior to putting it on the muffle furnace. The use of initial charring may be essential to prevent excessive foaming. The addition of a few drops of olive oil (4.1) may also help to prevent excessive swelling.

6.2.3 Ignite the dish at 600°C until no further apparent change in colour in the residue ash occurs.

6.2.4 Remove the dish from the furnace, place it in the desiccator (5.4) and allow it to cool to ambient temperature.

6.2.5 Weigh the dish and residue to the nearest 0·1 mg.

6.2.6 Repeat 6.2.3, 6.2.4 and 6.2.5 until the difference in two successive weighings is less than 0·1 mg. Let the final weight be m_2.

7. EXPRESSION OF RESULTS

7.1 *Formula and Method of Calculation*

The ash content, calculated as a percentage by mass of the prepared sample, is given by:

$$\frac{100 \times (m_2 - m_1)}{m_o}$$

where:

m_o is the mass of the test portion, in g (6.2.1),

m_1 is the mass of the prepared dish, in g (6.1),
m_2 is the mass of the dish and residue, in g (6.2.6).

7.2 *Repeatability*

The difference between the results of two determinations, when carried out simultaneously or in rapid succession by the same analyst on the same sample, shall not exceed 10 mg of ash when calculated on 100 g of sample.

Note: This method is the same in principle as that described in CAC/12-1969. Codex Alimentarius Commission Recommended European Regional Standard for Honey.

Appendix V: Determination of Moisture (Refractometric Procedure)

1. SCOPE AND FIELD OF APPLICATION

This method determines the moisture content of clear and normally coloured honeys.

2. DEFINITION

Moisture content: the content of moisture as determined by the method specified.

3. PRINCIPLE

The refractive index of a test portion is determined at 20°C and converted into moisture content by reference to tables showing concentration as a function of refractive index.

4. APPARATUS

4.1 Refractometer, capable of being read to unity in the fourth decimal place over the refractive index range 1·4700 to 1·5100, provided with means for the circulation of water about the prisms and a thermometer, the bulb of which is immersed in the circulating water stream. The thermometer shall have a certificate of accuracy at 20°C.

4.2 Light source for 4.1 consisting of a sodium lamp of the type recommended by, and adjusted in accordance with the instructions of, the manufacturer of the refractometer.

4.3 Water bath, controlled by a thermostat at 20°C with a differential no larger than 0·5°C, fitted with a pump for circulating water about the prisms of the refractometer (4.1).

4.4 Glass or plastic rod with an angled, flattened end, as required for applying the test portion to the prism of the refractometer (4.1).

5. PROCEDURE

Measure the refractive index of the sample at 20°C in the refractometer (4.1).

6. EXPRESSION OF RESULTS

6.1 *Method of Calculation*

Calculate the moisture content using the refractive index values shown in Table X. The moisture is expressed as percentage by mass.

Note: This method is based on Chataway's refractometric method (*Canada J. Res.*, 1932, **6**, 540) as revised by Wedmore (*Bee Wld.*, 1955, **36**, 197).

6.2 The following correction to the refractometer reading must be used if a temperature of 20°C is not employed.

6.2.1 Temperature above 20°C—add 0·00023 per °C.

6.2.2 Temperature below 20°C—subtract 0·00023 per °C.

6.3 This method is the same in principle as that described in CAC/12-1969, Codex Alimentarius Commission Recommended European Standard for Honey.

TABLE X

CONVERSION TABLE FOR ESTIMATION OF MOISTURE CONTENT

Refractive index (20°C)	Moisture content *per cent.*	Refractive index (20°C)	Moisture content *per cent.*
1·5044	13·0	1·4875	19·6
1·5038	13·2	1·4870	19·8
1·5033	13·4	1·4865	20·0
1·5028	13·6	1·4860	20·2
1·5023	13·8	1·4855	20·4
1·5018	14·0	1·4850	20·6
1·5012	14·2	1·4845	20·8
1·5007	14·4	1·4840	21·0
1·5002	14·6	1·4835	21·2
1·4997	14·8	1·4830	21·4
1·4992	15·0	1·4825	21·6
1·4987	15·2	1·4820	21·8
1·4982	15·4	1·4815	22·0
1·4976	15·6	1·4810	22·2
1·4971	15·8	1·4805	22·4
1·4969	16·0	1·4800	22·6
1·4961	16·2	1·4795	22·8
1·4956	16·4	1·4790	23·0
1·4951	16·6	1·4785	23·2
1·4946	16·8	1·4780	23·4
1·4940	17·0	1·4775	23·6
1·4935	17·2	1·4770	23·8
1·4930	17·4	1·4765	24·0
1·4925	17·6	1·4760	24·2
1·4920	17·8	1·4755	24·4
1·4915	18·0	1·4750	24·6
1·4910	18·2	1·4745	24·8
1·4905	18·4	1·4740	25·0
1·4900	18·6		
1·4895	18·8		
1·4890	19·0		
1·4885	19·2		
1·4880	19·4		

Appendix VI: Determination of Water-insoluble Solids (Gravimetry)

1. SCOPE AND FIELD OF APPLICATION

This method determines the water-insoluble solids content of honey.

2. DEFINITION

Water-insoluble solids content: the content of water-insoluble solids as determined by the method specified.

3. PRINCIPLE

The water-insoluble solids content is determined by gravimetry after drying for 1 h at 135°C.

4. APPARATUS

4.1 Analytical balance, capable of weighing to 0·1 mg.

4.2 Sintered glass crucible pore size (15–40 μm).

4.3 Drying oven electrically heated, thermostatically controlled at a temperature of 135 ± 1°C.

5. PROCEDURE

5.1 Accurately weigh 20 g of honey and dissolve it in a suitable volume (*ca* 200 ml) of water at 80°C and mix well.

5.2 Filter through a previously dried and weighed sintered glass crucible (4.2). The sintered glass crucible should be allowed to reach ambient temperature in a desiccator containing an efficient desiccant (e.g. dried silica gel) prior to weighing.

5.3 Wash carefully with warm water at 80°C until free from sugars. Thorough washing of the sintered crucible with warm water is essential (use Mohr's test to check that washing is complete, see note 2).

5.4 Dry the sintered crucible for one hour at 135°C in the oven (4.3), allow to cool in a desiccator and weigh to an accuracy of 0·1 mg.

5.5 Repeat the drying until constant weight is obtained.

6. EXPRESSION OF RESULTS

6.1 *Formula and Method of Calculation*

The water-insoluble solid content as a percentage of the sample is given by:

$$\frac{m}{m_1} \times 100$$

where

m_1 = initial mass, in g, of the test sample,

m = mass, in g, of the dried insoluble solids obtained from the test sample.

Note:

1. This method is the same in principle as that described in CAC/12-1969, Codex Alimentarius Commission Recommended European Regional Standard for honey.
2. Add phloroglucinol solution (1 per cent. in ethanol) to filtrate in test tube. Mix. Run a few drops of concentrated sulphuric acid down sides of tube. Colour is produced at interface if sugars present.

Appendix VII: Sample Preparation Instructions

1. PREPARATION OF THE SAMPLE FOR ANALYSIS

1.1 *General*

The mass of the sample presented to the laboratory for analysis shall be at least 200 g.

1.2 *Liquid Honey or Pressed Honey*

If the sample is free from granulation, mix carefully by stirring or shaking. If the honey is granular, place the closed container on a water bath, taking care not to immerse it, and heat for 30 min at 60°C; further heat, if necessary, at 65°C until complete liquefaction. Occasionally shake the container. Mix carefully and allow to cool rapidly as soon as the sample liquefies. Do not heat honey which has to be used for the determination of hydroxymethylfurfural content or diastase activity. If the honey contains foreign matter (e.g. wax, twigs, bees or particles of honeycombs) heat the sample to 40°C on a water bath and strain the honey through cheesecloth in a hot-water jacketed funnel before sample preparation.

1.3 *Comb Honey*

Remove the upper part of the combs, if they are sealed, and completely separate the honey from the combs by passing through a sieve; the mesh of the sieve is formed by wires woven to form square appertures of size 0·500 mm. If part of the wax or comb passes through the sieve, heat the sample as described under 1.2 and strain the honey through the filter. If the comb honey is granular heat it until the wax liquefies, stir, allow to cool and remove the wax.

1.4 *Containers*

The prepared sample shall always be kept in an air-tight and moisture-tight container.

Appendix VIII: Luff–Schoorl Reagent—Possible Sources of Error in Apparent Reducing Sugar and Sucrose Methods

Participants observed an inconsistency in the values for the appropriate titres in the preparation and subsequent standardisation of the Luff–Schoorl reagent in the trial; this may have lead to errors in the estimation of apparent reducing sugar content giving rise to the observed variability in trial results for the

method. It was found from calculation that the reagent stoichiometries are correct for obtaining the required titres in the standardisation (i.e. copper, carbonate and bicarbonate concentrations). However, the use of non-standardised sodium thiosulphate solution for the standardisation of the Luff–Schoorl reagent (copper content) is questionable as it is known to be unstable in solution, being succeptable to light and bacterial degradation. In addition, if freshly-boiled distilled water is not used in the preparation of the reagent, then dissolved carbon dioxide will be present thus also causing slow decomposition. It is suggested that if the thiosulphate reagent is to be kept for several days then a preservative should be added (e.g. 10 mg of mercuric iodide per litre or a few drops of chloroform). It is also recommended that the thiosulphate solution be standardised using potassium iodate (or bromate) immediately prior to use and that iodate-free iodide be specified in 4.7 (Appendix II) since a trace of iodate may liberate iodine from iodide in sufficient quantity to affect the thiosulphate titre during the Luff–Schoorl standardisation.

TABLE I

COLLABORATIVE DETERMINATION OF ACIDITY OF HONEY

	Acidity (*milli-equivalents/kg*)			
	Sample Codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	13·0, 11·0	11·0, 13·0	18·0, 20·0	16·0, 19·0
2	4·9, 5·7	5·9, 5·9	12·2, 11·9	12·1, 12·3
3	12·1, 12·6	6·7, 7·4	17·5, 17·1	15·8, 16·7
4	4·6, 5·8	6·5, 5·8	12·5, 12·0	12·2, 12·6
5	4·5, 6·3	5·4, 6·4	11·8, 11·6	11·8, 13·4
6	4·8, 6·1	4·9, 6·0	10·6, 11·0	11·7, 12·5
7	6·3, 6·4	5·4, 6·4	12·8, 13·2	12·9, 12·9
8	4·5, 8·5	5·5, 2·5	12·5, 14·0	12·2, 12·4
9	4·4, 6·4	5·6, 5·2	13·8, 13·0	12·7, 12·9
10	4·7, 6·3	6·6, 5·1	12·9, 11·9	12·0, 12·7
11	5·9, 7·9	7·0, 8·9	15·0, 14·9	12·0, 15·0
12	5·3, 1·6	3·5, 2·3	3·5†, 3·0†	2·8†, 2·9†
13	15·8*, 7·5*	10·9, 8·7	13·1*, 20·3*	24·7*, 20·1*
14	5·1, 5·1	5·3, 3·9	11·9, 11·4	11·8, 12·0
15	5·4, 5·4	5·2, 5·7	11·2, 11·1	11·3, 11·3
16	7·0, 9·0	7·0, 7·0	18·0, 16·0	15·0, 14·0
17	7·0, 13·3	7·9, 5·9	12·8, 11·8	11·8, 13·2
18	4·8, 6·3	6·0, 6·0	11·1, 11·7	11·9, 10·8
19	11·9, 12·9	8·0, 9·9	16·9, 15·6	19·6, 20·9
Mean($\bar{x}$)	7·0	6·5	13·5	13·5
Repeatability (r)	4·7	2·9	2·0	2·6
Reproducibility (R)	8·5	6·2	7·1	7·1

* Results rejected by Cochran's Test $p < 0{\cdot}05$[7]. Values not used in calculation of mean, repeatability or reproducibility.

† Results rejected by Dixon's test $p < 0{\cdot}05$[7]. Values not used in calculation of mean, repeatability or reproducibility.

TABLE II

APPARENT REDUCING SUGAR CONTENT OF HONEY (EXPRESSED AS INVERT SUGAR)

	Apparent reducing sugar (*g/100 g*)			
	Sample Codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	71·4, 71·7	71·6, 71·2	71·4, 71·4	72·8, 72·4
2	76·1, 76·5	74·8, 74·7	76·8, 75·0	76·4, 77·0
3	74·4, 74·2	74·5, 74·1	72·5, 73·5	74·8, 75·0
4	68·0*, 73·3*	68·6*, 65·9*	67·7*, 71·2*	69·7, 75·9
5	72·8, 72·6	73·4, 71·8	73·0, 73·0	74·3, 74·7
6	75·0, 74·4	73·8, 73·2	74·8, 74·8	76·6, 74·4
7	73·7, 73·8	72·5, 72·9	73·8, 73·3	74·9, 74·5
8	72·5, 75·0	70·9, 73·3	74·1, 72·5	75·8, 74·1
9	75·7, 76·0	75·4, 74·3	75·5, 75·4	76·7, 76·4
10	75·2, 75·5	75·5, 74·1	77·1, 75·8	76·6, 76·5
11	73·9, 73·4	73·2, 73·8	73·4, 73·6	75·3, 74·4
12	75·0, 73·6	73·3, 73·7	71·5, 73·7	75·2, 71·5
13	74·3, 76·6	71·1, 72·4	73·3, 73·4	73·1, 75·8
14	73·5, 72·7	72·9, 72·1	73·8, 73·3	74·9, 73·5
15	74·6, 74·1	74·6, 73·6	75·0, 74·6	76·4, 76·0
16	74·4, 74·7	73·4, 73·4	74·6, 75·2	76·0, 76·4
17	75·3, 74·1	73·9, 73·9	74·1, 74·1	73·6, 75·6
18	74·9, 74·4	73·9, 74·4	74·8, 74·5	75·6, 75·7
19	75·3, 75·4	75·8, 74·3	75·8, 77·0	76·9, 72·3
Mean ($\bar{x}$)	74·4	73·5	74·2	74·9
Repeatability (r)	2·0	2·0	1·9	4·5
Reproducibility (R)	3·6	3·4	4·1	4·7

* For key, see Table I.

TABLE III

APPARENT SUCROSE CONTENT OF HONEY

	Apparent sucrose (*g/100 g*)			
	Sample Codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	4·1, 3·9	1·8, 1·9	2·9, 3·2	2·9, 3·2
2	0·4, 0·0	0·0, 0·0	0·0, 0·0	3·5, 0·9
3	0·9, 1·3	3·1, 3·4	3·2, 2·1	3·5, 4·3
4	3·2, 2·8	5·6, 4·5	5·3, 1·8	3·4, 3·5
5	1·9, 1·3	2·6, 4·1	3·9, 1·9	2·9, 2·4
6	2·8, 1·6	2·3, 0·8	1·4, 2·8	1·7, 3·7
7	2·2, 1·8	4·0, 3·1	2·7, 2·2	4·4, 3·9
8	1·5, 0·0	0·8, 0·0	0·0, 3·2	1·6, 2·4
9	2·0, 1·5	3·1, 4·3	2·4, 2·7	3·1, 2·8
10	4·5, 2·9	5·7, 6·2	2·8, 3·0	4·4, 4·6
11	1·5, 1·5	2·9, 2·1	2·3, 2·2	2·8, 2·4
12	4·3, 2·1	1·8, 3·1	4·5, 2·5	2·4, 1·7
13	0·6, 1·0	2·9, 3·9	1·6, 3·0	4·3, 4·3
14	1·1, 1·1	2·7, 3·2	1·4, 1·1	1·9, 3·2
15	2·2, 3·1	2·2, 4·0	1·8, 2·7	2·2, 2·6
16	2·3, 2·6	3·6, 4·6	2·7, 2·2	2·9, 3·6
17	1·7, 2·1	3·8, 2·7	1·5, 2·1	3·4, 2·7
18	1·7, 3·1	4·4, 2·6	2·4, 3·3	2·9, 3·7
19	0·0, 1·4	0·9, 3·9	0·5, 1·3	1·7, 4·7
Mean ($\bar{x}$)	2·0	3·0	2·3	3·1
Repeatability (r)	1·9	2·5	2·9	2·3
Reproducibility (R)	3·3	4·4	3·2	2·7

TABLE IV

HYDROXYMETHYLFURFURAL (HMF) CONTENT OF HONEY

	Hydroxymethylfurfural (*mg/kg*)			
	Sample codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	10·0, 10·0	20·0, 19·0	27·0, 30·0	46·0, 48·0
2	12·1, 11·7	20·3, 19·6	42·7, 44·3	51·4, 51·9
3	12·9, 8·3	23·9*, 30·4*	32·7, 26·2	49·6, 48·4
4	15·8*, 43·5*	21·8*, 29·3*	60·0, 52·3	70·0, 70·0
5	1·82, 13·0	19·5, 20·6	38·6, 45·7	51·4, 52·3
6	11·8, 11·6	20·6, 19·9	37·9, 36·0	49·9, 50·8
7	11·3, 11·1	20·0, 20·3	42·0, 40·9	50·8, 50·0
8	12·4, 12·1	23·8, 23·6	49·7, 49·2	63·0, 58·6
9	5·0, 10·0	17·0, 18·0	36·0, 36·0	44·0, 44·0
10	10·1, 14·7	21·3, 22·1	44·5, 49·0	51·7, 55·0
11	11·9, 14·1	19·8, 19·4	46·0, 44·1	51·7, 57·3
12	10·4, 21·0	10·2*, 36·0*	2·3*, 49·0*	63·0, 65·0
13	8·4, 6·0	6·0†, 0·8†	3·0†, 0·8†	11·5†, 1·7†
14	17·3, 18·0	27·4, 26·4	56·4, 54·4	65·2, 64·1
15	11·8, 11·9	22·3, 20·8	42·7, 41·9	50·1, 51·6
16	11·0, 14·0	23·0, 23·0	48·0, 50·0	58·0, 59·0
17	14·2, 15·7	22·2, 25·2	47·4, 48·8	60·7, 59·5
18	12·6, 10·4	20·9, 18·8	42·0, 41·4	49·1, 52·2
19	9·1, 1·3	8·3†, 9·2†	23·5, 14·6	36·6*, 25·5*
Mean ($\bar{x}$)	11·4	21·2	41·8	54·8
Repeatability (r)	9·4	2·5	8·2	4·6
Reproducibility (R)	11·0	7·0	27·9	20·2

* For key, see Table I.

TABLE V

MINERAL CONTENT (ASH)

	Ash (*g/100 g*)			
	Sample codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	0·19, 0·20	0·04, 0·04	0·18, 0·18	0·08, 0·08
2	0·25, 0·26	0·03, 0·063	0·22, 0·24	0·08, 0·11
3	0·19, 0·20	0·02, 0·029	0·17, 0·18	0·06, 0·06
4	0·29, 0·22	0·08, 0·04	0·20, 0·20	0·08, 0·08
5	0·15, 0·20	0·05, 0·04	0·17, 0·16	0·09, 0·06
6	0·15, 0·15	0·07, 0·01	0·16, 0·14	0·04, 0·03
7	0·22, 0·23	0·04, 0·03	0·17, 0·18	0·07, 0·07
8	0·30, 0·21	0·07, 0·06	0·20, 0·20	0·08, 0·09
9	0·21, 0·18	0·02, 0·04	0·10, 0·15	0·14, 0·03
10	0·26, 0·32	0·08, 0·07	0·25, 0·27	0·11, 0·12
11	0·25, 0·24	0·06, 0·05	0·20, 0·20	0·08, 0·09
12	0·19, 0·28	0·03, 0·06	0·27, 0·27	0·12, 0·08
13	0·20, 0·29	0·18†, 0·10†	0·23, 0·21	0·16, 0·11
14	0·21, 0·23	0·04, 0·045	0·18, 0·18	0·07, 0·09
15	0·22, 0·26	0·03, 0·03	0·20, 0·19	0·08, 0·07
16	0·17, 0·18	0·04, 0·03	0·16, 0·12	0·04, 0·07
17	0·31, 0·32	0·07, 0·07	0·27, 0·27	0·10, 0·14
18	0·29, 0·31	0·02*, 0·10*	0·23, 0·23	0·10, 0·06
19	0·20, 0·17	0·04, 0·04	0·13, 0·15	0·04, 0·03
Mean ($\bar{x}$)	0·23	0·05	0·20	0·08
Repeatability (r)	0·09	0·04	0·04	0·05
Reproducibility (R)	0·14	0·05	0·13	0·09

* For key, see Table I.

TABLE VI

MOISTURE CONTENT

	Moisture content (*g/100 g*)			
	Sample codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	15·8, 15·8	17·5, 17·5	17·4, 17·3	15·9, 15·9
2	16·0, 16·0	17·9, 17·9	17·3, 17·4	16·2, 16·2
3	16·3, 16·3	17·4, 18·1	17·4, 17·7	16·2, 16·3
4	15·9, 16·0	17·9, 17·9	17·3, 17·3	15·9, 16·1
5	15·9, 16·0	18·1, 18·0	17·4, 17·5	16·2, 16·1
6	19·8*, 16·1*	17·9, 18·1	17·3, 17·4	16·3, 16·5
7	15·9, 15·9	17·8, 17·9	17·5, 17·5	15·8, 15·8
8	16·4, 16·2	18·0, 18·2	17·4, 17·4	16·2, 16·2
9	15·8, 15·6	17·5, 17·6	17·1, 17·1	15·8, 15·8
10	15·8, 15·6	17·7, 17·8	17·1, 17·2	16·2, 16·3
11	15·9, 15·9	17·9, 17·8	17·4, 17·4	16·0, 16·0
12	16·2, 15·9	18·5, 18·3	17·7†, 18·0†	16·5*, 17·0*
13	14·7, 16·4	16·7, 17·4	16·5†, 16·7†	14·9†, 15·0†
14	15·8, 15·0	17.8, 17·6	17·1, 17·1	15·8, 15·8
15	16·0, 15·8	17·8, 17·9	17·4, 17·5	16·0, 16·0
16	15·9, 16·0	17·8, 17·9	17·3, 17·4	16·0, 16·0
17	15·7, 15·8	17·8, 18·0	17·4, 17·4	15·8*, 16·2*
18	16·0, 15·8	17·8, 17·8	17·2, 17·3	15·3*, 15·9*
19	15·6, 15·8	15·6*, 17·8*	15·3*, 17·3*	15·9, 15·9
Mean ($\bar{x}$)	15·9	17·8	17·3	16·0
Repeatability (r)	0·28	0·53	0·21	0·17
Reproducibility (R)	0·92	0·87	0·40	0·55

* For key, see Table I.

TABLE VII

WATER-INSOLUBLE SOLIDS

	Insoluble solids (*g/100 g*)			
	Sample codes			
Laboratory	(1,7)	(2,5)	(3,8)	(4,6)
1	0·017, 0·018	0·003†, 0·002†	0·029, 0·031	0·004, 0·005
2	0·017, 0·009	0·005, 0·007	0·030, 0·029	0·003, 0·009
3	0·119†, 0·149†	0·012, 0·011	0·108*, 0·169*	0·028, 0·023
4	0·101, 0·020	0·000, 0·020	0·030, 0·030	0·010, 0·010
5	0·028, 0·016	0·009, 0·000	0·026, 0·032	0·008, 0·000
6	0·028, 0·022	0·005, 0·007	0·018, 0·026	0·007, 0·008
7	0·031, 0·020	0·002, 0·012	0·029, 0·034	0·006, 0·002
8	0·020, 0·030	0·010, 0·003	0·030, 0·040	0·001, 0·010
9	0·050*, 0·020*	0·010, 0·010	0·030, 0·030	0·010, 0·010
10	0·160*, 0·060*	0·007, 0·020	0·030, 0·050	0·030, 0·030
11	0·070*, 0·040*	0·030†, 0·020†	0·080†, 0·070†	0·020, 0·030
12	0·030, 0·030	0·020, 0·010	0·020, 0·030	0·010,* 0·080*
13	0·003, 0·011	0·008, 0·005	0·036, 0·009	0·009, 0·002
14	0·013, 0·014	0·000, 0·000	0·026, 0·029	0·000, 0·005
15	0·020, 0·020	0·010, 0·010	0·020, 0·030	0·010, 0·010
16	0·020, 0·030	0·010, 0·010	0·050, 0·030	0·040*, 0·020*
17	0·026, 0·024	0·008, 0·007	0·036, 0·039	0·008, 0·007
18	0·020, 0·030	0·010, 0·020	0·040, 0·030	0·070*, 0·010*
19	0·018, 0·026	0·018†, 0·036†	0·017, 0·027	0·019, 0·015
Mean($\bar{x}$)	0·021	0·009	0·031	0·011
Repeatability (r)	0·016	0·016	0·023	0·010
Reproducibility (R)	0·021	0·016	0·023	0·026

* For key, see Table I.

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Book Reviews

HPLC IN FOOD ANALYSIS. Edited by R. Macrae. Academic Press, London, 1988. 464 pp. Price £37.00. ISBN 0-12-464781-2.

The second edition of *HPLC in Food Analysis* reflects the large amount of use which the technique now enjoys, as well as reflecting the volume of research going on in the field. The volume is some 150 pages longer than the first edition which appeared in 1982. The first four chapters of the first edition have been condensed into a single chapter, thus providing a concise and clear introduction to those who have no background in HPLC.

In addition to analyses which are now well established, additional chapters on polynuclear aromatics and nitrosamines, pesticide residues, and natural pigments have been incorporated. The chapter on Natural Pigments in Foodstuffs covers an area of analysis which takes on added importance when one considers today's emphasis on labelling of foodstuffs with the word "natural". The chapter on the Determination of Synthetic Food Colours is of interest in the light of the public interest in food additives. Many people in the public analyst service have probably at some time questioned the need for the high levels of azo-dyes used by some food manufacturers.

The familiar fields of analysis for vitamins, carbohydrates, food additives and amino acids are also covered in this edition together with a chapter on the determination of mycotoxins.

In the chapter devoted to Pesticide Analysis the technique is said to be of particular advantage when dealing with determinations of pyrethroids and carbamates which are not amenable to GLC determination.

The final chapter deals with the possibility of the mass spectrometer as a detector in LC but points out the difficulties of interfacing LC to the MS and the high cost of dedicated LC/MS system at £100,000 to £120,000.

The text is clear and readable with a wide bibliography up to 1986. At £37.00, the volume is good value.

D. G. LLOYD

HUMAN RISK ASSESSMENT. The role of animal selection and extrapolation. Edited by M. V. ROLOFF. Taylor and Francis, London, 1987.

This book records the proceedings of a conference on Human (toxicological) Risk Assessment held in St Louis, Missouri in October 1985. The relevance of animal testing of drugs, chemicals and food ingredients is broadly discussed and scrutinised by internationally recognised experts in many branches of Toxicology, and by Legislators. The opening chapter of the book "Animal selection and

extrapolation—the problem defined" by one of the grand old men of toxicology, G. E. Paget, itemises the scientific problems besetting the assessors of toxicological data and the emotive and political forces which can influence decision-making processes. This well written chapter sets the tone of the book.

Part 1 deals with the approaches adopted by the American federal legislators (FDA and EPA) and the WHO with particular reference to carcinogenicity tests. The problems as seen by a consultant pathologist are amusingly but pointedly expressed in the chapter by F. Roe.

Part 2 records the major physiological and biological differences shown by the commonly used test species and man which contribute to different responses to toxic substances. Of particular interest is the role of diet and nutritional status in toxic responses.

Part 3 concerns itself with species differences in metabolic and pharmacokinetic parameters which affect toxic responses. This is the one part of the book where analytical chemists may feel some empathy, since the importance of absolute levels of comounds and their metabolites in the biological systems is paramount in this aspect of toxicological assessment.

Part 5 concerns the variability of responses shown because of genetic differences both within and across species. Pharmacogenetic considerations in man are particularly well illustrated, and the shortcomings of the present systems used by regulatory bodies to estimate risk to man are drawn to the reader's attention.

In the Summary the problems are reiterated and suggestions made as to the roads toxicology must take to reinforce public confidence in the systems used to ensure the well being of the consumer.

This text is an excellent, but specialised, reference volume, and should be on the bookshelves of all working in the scientific fields which contribute to the assessment of human toxicological risks.

D. Howes

Analyses of Hazardous Substances in Biological Materials, Vol. 2. Edited by T. Angerer and K. H. Scholler. VCH, Weinham, FDR: 250 pp. hardback.

This series of monographs was produced for the West German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Volume I was published in its English edition in 1985. This volume offers a further selection of approved analytical methods for chemicals of major importance to health.

In all, 14 methods are detailed for monitoring contaminant levels in blood, plasma or urine. These cover a range from metallic analytes to a selection of organic metabolites.

The methods chosen include Atomic absorption by Electrothermal Atomisation and hydride techniques, Atomic emission (ICP), HPLC, Gas Chromatography, Fluorimetry, Ion-selective Electrodes and Voltametry. In addition there is a valuable 30 page dissertation on digestion procedures for the determination of metals.

Each method is detailed in an unequivocal manner and includes precision data, sampling information and its own reference list (largely of German origin). Not surprisingly, however, discussions of tolerance values are largely confined to consideration of BAT and MAK concentrations.

This series will serve as a working manual of reliable methods for the operator in the field of biological monitoring, and as such will be highly valued.

T. D. HOOD

MICRO AND SEMI-MICRO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. By D. Ishii. VCH, 1988. 200 pp., 156 figures, 200 tables. Price approx. DM 120.00/U.S. $66.00. ISBN 3-527-26636-4.

This review, edited by Daido Ishii, has contributions by the members of Nagoya University and the Japanese Spectroscopic Company. The volume is stated to be intended to give introductory and comprehensive information to people interested in miniaturisation and micro HPLC. Whilst conventional HPLC apparatus is fairly common today, using 4–5 mm diameter columns, with the micro technique the columns are a tenth of this diameter. The advantages include low consumption of mobile and stationary phases, leading to economy and the possible use of more expensive phases, an increase in mass sensitivity high resolution with long columns, easy applicability of temperature programming, and other advantages due to diminution of overall size.

It is interesting to note that the micro technique was first described at least 15 years ago, and therefore this volume is to be welcomed as being long overdue to popularise the technique. The chapters cover all parts of the HPLC apparatus and technique in a very readable manner; the illustrations of the separations achieved are admirable, and the section on the application of high speed HPLC to drugs shows that the technique must be one for the future. What other technique currently available can separate four types of diazepam and their metabolites in 30 s?

Altogether the authors of this review have opened a window to another world, and this small volume, which includes appendices on the characteristics of packing, is recommended both to students and practising chromatographers in Public Analysts Laboratories, and those engaged in research and quality control, as essential reading.

S. LANDSMAN

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Obituary

G. V. James, MBE, MSc., Ph.D., MChemA, CChem, FRSC, M.Inst.Env.Sci.

Dr George Vaughton James, who died on 21 June 1988, was the former Scientific Adviser, Public Analyst and Official Agricultural Analyst for the County of Gwent, and, prior to Local Government Re-organisation in 1974, held similar appointments for the City of Bath and County Boroughs of Cheltenham and Swindon. From 1977 to 1985 he was the Honorary Editor for this Journal.

He was born, an only son, on 12 March 1910 in Newcastle upon Tyne, and having lost his father in World War I, was brought up by his mother and grandparents. He was educated at Rutherford College and from 1927 to 1935 he was employed as an Assistant in the Analytical Laboratory at the Royal Victoria Hospital, Newcastle upon Tyne, during which time he obtained the degree of Master of Science, his thesis being an investigation into the mechanism of water softening. He then went to the Liverpool Heart Hospital where he researched methods of food analysis for three years, during which time he passed the then Institute of Chemistry Branch E Examinations in Analysis including Microscopy of Food, Drugs and Water. In 1938, Dr James performed further research on the action and excretion of the newly created sulphonamide drugs at the Bernhard Baron Research Laboratories, Queen Charlotte's Hospital, Hammersmith, where he passed the B.Sc. degree examination in physiology and prepared his Ph.D. thesis on water treatment. During his academic training, Dr James was elected in 1933 as an Associate of the Institute of Chemistry (AIC) and elevated to the grade of Fellow in 1937 (FIC).

At the outbreak of the Second World War, Dr James volunteered for the Royal Army Medical Corps and was mobilised early in 1940. Within three days he was given command of a Unit in France and as he had never experienced the rigours of military training and discipline, he confessed to being "the worst trained and most unprofessional officer in the British Army". But despite these shortcomings, he proved to be a very able officer, showing consideration and devotion to the men and patients under his command. These qualities were exemplified by his actions during the evacuation of the British Expeditionary Force at Dunkirk in 1940, where, with complete disregard for his own safety and by somewhat unorthodox means, including commandeering ambulances, bicycles and even a railway train, he succeeded in helping a large number of patients and troops to return to England. He was promoted to the rank of Major, was injured in a bomb explosion in Yugoslavia and awarded at the end of the war the MBE for his gallantry. In later years he joined the British Legion and

the Dunkirk Veterans Association where as District President he held a particular interest in the Benevolent Fund to assist former comrades.

On demobilisation, Dr James spent some time working on the formulation and effectiveness of disinfectants for a London firm, but saw his future career in the West Country, and joined the Bristol firm of analytical chemists, Cook and Barke, where he practised for many years as Principal and Consultant, while taking on his official appointments as Public Analyst.

It has been said that, apart from his love of travel, Dr James had no hobbies or interests. Practising his profession was his supreme joy, which gave him tremendous satisfaction. When he was not in his laboratory or spending time with his family, he would be passing on his experiences by writing useful papers on a variety of analytical subjects, many of which were published in this Journal. In addition he published a book entitled *Water Treatment*, a subject on which he was an undoubted authority.

Above all, Dr James was a gentleman, and this quality was no more apparent than when he appeared in Court to give evidence as an expert witness. He would politely explain the results of his analysis and their significance in relation to the case, in clear, straightforward terms, which the Bench or Jury could readily understand and would retain the same courteous demeanour even under vigorous cross examination.

It might be imagined that, with this dedication to his work, Dr James was a dull and boring fellow. This was certainly not the case, for it was a delight to have him as a travelling companion, when he would draw on a wealth of stories and experiences and relate them with his wry sense of humour, which his mischievous smile often betrayed.

His colleagues in the profession of chemistry, particularly those younger members who benefited from his advice and guidance during the development of their own careers will sadly miss him, but remember him with much affection. He is survived by his wife Joan, their two sons and two daughters.

D. J. TAYLOR