

The Determination of Reducing Sugars by Classical Procedures: Collaborative Trial

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Summary

The results of a collaborative trial involving 19 participants on the determination of reducing sugars in sugar solutions (syrups) by the Lane and Eynon Constant Volume method, the Luff-Schoorl procedure and a modified Luff-Schoorl procedure using standardised reagents, are reported. Significant differences in the mean levels of reducing sugar were observed between the Lane & Eynon and the Luff-Schoorl method and between the Lane & Eynon and modified Luff-Schoorl methods. Significant differences were found in precision between the modified Luff-Schoorl method when compared with both the Lane and Eynon Constant Volume and the original Luff-Schoorl methods. The modified Luff-Schoorl procedure performed with better precision for the samples analysed.

Introduction

Methods of analysis for sugars have been agreed by the European Community and are given in the Commission Directive 79/796/EEC laying down Community methods of analysis for testing certain sugars intended for human consumption⁽¹⁾. This Directive has been incorporated into UK legislation through The Specified Sugar Products (Amendment) Regulations (1982)⁽²⁾.

The Directive specifies methods for the determination of reducing sugars expressed as 'invert sugar' using the Lane and Eynon Constant Volume procedure (method 7) or the Luff-Schoorl procedure (method 6). Member States may choose one of these methods for incorporation into their national legislation. The UK has chosen the former.

The Lane and Eynon Constant Volume method has traditionally been preferred to the Luff-Schoorl procedure by UK analysts for the determination of reducing sugars. Among the reasons given for preference of the Lane & Eynon method to the Luff-Schoorl procedure, is that errors are known to occur in the preparation and use of the Luff-Schoorl reagent. In an attempt to rectify this, the International Commission on Uniform Methods of Sugar Analysis (ICUMSA)⁽³⁾ has recommended the incorporation of a reagent standardisation step in the Luff-Schoorl procedure.

The modified Luff-Schoorl method was tested in a recent collaborative trial conducted by MAFF and the Lancashire County Analysts Laboratory⁽⁴⁾ on the analysis of honeys, which included analysis for

apparent reducing sugar content using a modified Luff-Schoorl procedure. Errors in the estimation of apparent reducing sugar content were thought to have given rise to the observed variabilities in the trial results. Several arguments were put forward as to the cause, notably that of poor attention to proper reagent preparation and storage and especially in the use of freshly boiled distilled water and accurately standardised sodium thiosulphate solution.

In the light of this it was decided that a trial to assess the relative merits of three methods of analysis for the determination of reducing sugars in sugar solutions be carried out. It was anticipated that the results would show which of the methods is the most precise.

Methods of Analysis being Collaboratively Tested

The methods tested in this trial were as follows:

- (1) Method 7 of EC Directive 79/796/EEC (Lane and Eynon constant volume modification method): 'LE'. [See Appendix I for outline of method]
- (2) Method 6 of EC Directive 79/796/EEC (Luff-Schoorl method): 'LS' [See Appendix II for outline of method]
- (3) A modified Luff-Schoorl procedure including a standardisation step, proposed by the British National Committee (BNC) of ICUMSA: 'BNC' [See Appendix III for outline of method]
- (4) Method 2 of EC Directive 79/796/EEC for dry matter (or an equivalent method if difficulties are experienced with this method, stating where differences arise). [See Appendix IV for outline of method]

Familiarisation of the methods by participants through the analysis of a pre-trial sample was not carried out as participating analysts carry out these, or similar analyses, on a routine basis.

Collaborative Trial Organisation, Samples and Results

Nineteen laboratories agreed to participate in the collaborative trial (15 UK Public Analyst Laboratories, the Government Laboratories of Jersey and the Isle of Man, The British Sugar Corporation and Tate and Lyle plc).

Samples

The samples comprised four typical commercial products prepared in bulk by Tate and Lyle plc London and consisted of the following:

- Sample A: Glucose Syrup - with an estimated dextrose equivalent value of 42
- Sample B: Glucose Syrup - with an estimated dextrose equivalent value of 63
- Sample C: Black Treacle - commercial product
- Sample D: Golden Syrup - commercial product

Sub-samples were prepared by warming each bulk sample in its container in hot water and pouring the warm solutions into 250 ml wide-mouthed pvc screw-topped bottles. Approximately 200 ml of each sample was despatched to participants with instructions to analyse each in duplicate. Differences in the visual characteristics of each sample were so great as to effectively prevent provision of the samples as blind duplicates.

Results

The results obtained in the trial are reported in Tables I- V

Statistical analysis of the results

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

Repeatability and Reproducibility

Calculations for repeatability (r) and reproducibility (R) as defined by that Protocol⁽⁵⁾ were carried out on those results remaining after removal of outliers for each method/sample calculation. These are also given in Tables I - VI and have been summarised in Table V. The results from the analysis for dry matter content have been included primarily as a check for the expression of the reducing sugar content on a dry matter basis.

The values of the within-laboratory precision parameter, the repeatability (r), calculated for the methods under examination in this trial, are of the order that would be anticipated for this type of determination (in the range 0.63 - 1.64). The values for r predicted by the Horwitz equation⁽⁶⁾ (using the approximation $r = 0.66R$) are in the range 1.5 - 2.2 .

Analysis of within-laboratory variance ratios (calculated as r_1^2 / r_2^2) using a 2-sided F-test show that only the observed difference in r between LS/BNC for sample B is significant ($P < 0.01$). There are no significant differences in the repeatability values for samples A, C, and D between any of the three methods.

The values of the between-laboratory precision parameter, the reproducibility, (R), calculated for all three methods are marginally higher than would be anticipated for this type of determination (in the range 1.88 - 4.38). The values of R derived from the Horwitz equation⁽⁶⁾ are in the range 2.3-3.3 .

Analysis of the between-laboratory variance ratios (calculated as $(2R_1^2 - r_1^2) / (2R_2^2 - r_2^2)$) using a 2-sided F-test, show that no significant differences in R exist between any of the methods for the results of the analyses of samples A and B. However, the results from the analyses of samples C and D show some significant differences in R . Significant differences were found between BNC/LE ($p < 0.05$) from the analysis of

sample C and between BNC/LS ($p < 0.05$) and LE/LS ($p < 0.01$) for the analysis of sample D.

The results of the analyses suggest that the modified Luff-Schoorl method (BNC) exhibits better precision than the other two methods for samples C and D.

Comparison of means

The mean values obtained from each method/sample set were compared by significance testing using a 2-sided Student t-test. From the analysis of the results from samples A and B significant differences in mean values obtained

were found between the LE/LS and the LE/BNC (in all cases at the $p < 0.01$ level), but not between the LS/BNC methods. Significant differences in mean values were found between the LE/LS and between LE/BNC methods from analysis of sample C ($p < 0.05$). Results of the analysis of sample D showed a significant difference ($p < 0.05$) between LE/LS methods.

The results indicate that there are significant differences in results between LE/LS and between LE/BNC methods, the LE method giving higher results, but not between LS/BNC methods.

Dry Matter Content

The respective dry matter contents were used to calculate the Dextrose Equivalents of samples A and B, and thus compare with the anticipated values. The Dextrose Equivalents of samples A and B were expected to be around 42 and 63 % respectively; these agreed with the found Dextrose Equivalents (average of values obtained by the three methods) which were 44 and 64 % respectively.

The data in tables III-VIII were converted to 100 % dry matter basis and re-analysed for statistical outliers. Analysis of the converted data did not then include the original outliers as valid data, these outliers were therefore assumed not to have been caused by variations in the samples' respective dry matter contents as received in the laboratory.

TABLE I
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC DRY MATTER

(g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	81.08(a)	77.84(a)	82.48 (a)	80.33 (a)	80.66	80.89	83.91	84.06
2	81.81	81.47	83.08	82.97	81.77	81.82	84.46	84.49
3	81.62	81.04	82.81	81.71	81.37	81.81	84.46	84.12
4	80.70	80.80	82.40	82.60	81.60	81.50	83.80	84.10
5	82.16	82.08	83.39	83.28	82.10	81.89	84.28	84.54
6	84.70	85.0 *	85.30	85.1 *	81.60	80.6 *	85.70	85.2 *
7	79.50	78.50	82.40	81.10	79.60 (b)	78.80 (b)	82.30 (b)	81.6 (b)
8	81.67	81.02	82.88	82.41	81.64	82.02	84.58	84.47
9	84.21	84.47	85.03 (b)	84.91 (b)	83.31 (b)	83.46 (b)	84.80	84.90
10	81.20	81.10	82.57	82.49	81.39	81.33	84.05	84.00
11	80.90	80.91	82.42	82.42	81.15	81.24	84.00	84.00
12	83.94	83.11	89.40 (b)	89.12 (b)	81.70 (a)	79.83 (a)	84.54	83.84
13	88.84 (b)	88.94 (b)	88.93 (b)	89.43 (b)	82.12	82.03	92.86 (b)	92.87 (b)
14	81.47	81.33	82.84	82.70	81.73	81.51	83.27	83.42
15	81.81	81.40	82.90	82.90	81.30	81.30	84.20 (a)	83.1 (a)
16	81.10	81.00	82.30	82.50	80.50	80.50	83.40	83.50
17	81.10	81.10	82.30	82.50	81.40	81.30	84.50	84.50
18	81.40	81.50	82.90	82.90	81.20	81.20	84.50	84.30
19	91.30	91.4 *	90.30	90.9 *	83.80	83.8 *	89.20	89.8 *
Mean	81.51		82.60		81.44		84.17	
r	0.87		1.00		0.38		0.50	
S_r	0.309		0.356		0.137		0.178	
RSD_r %	0.38		0.43		0.16		0.21	
R	3.43		1.32		1.27		1.22	
S_R	1.224		0.470		0.452		0.434	
RSD_R %	1.50		0.57		0.56		0.52	

For Key See Table VI Note : Sample B, 23.5% of original data removed as outliers

TABLE II
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC Lane and Eynon		Invert Sugar(g/100g)						
Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	36.47	36.61	54.06	54.06	35.98	35.98	49.84	49.65
2	36.44	36.25	53.53	53.84	35.73	36.15	49.39	49.58
3	36.61	36.16	54.62	54.90	37.06	37.03	50.41	50.44
4	36.50	36.30	55.00	54.10	37.60	37.10	50.20	49.70
5	37.29	36.94	53.97	54.41	36.90	36.92	49.44	49.69
6	36.70	35.90	54.60	54.47	36.29	36.49	49.88	49.97
7	36.70	35.90	54.30	54.60	35.50	36.20	49.1 (a)	51.50 (a)
8	36.88	36.69	54.20	55.05	38.40	38.80	50.29	50.29
9	36.96	37.35	55.03	55.06	36.22	36.44	50.27	50.12
10	35.90	36.00	52.97	52.79	36.90	37.20	48.90	48.80
11	35.50	35.40	52.40	53.20	40.40	39.70	48.70	49.40
12	34.20	35.20	52.70	51.30	34.70	34.90	51.50	52.00
13	36.86	36.13	54.94	54.54	43.91	44.79	49.39	49.76
14	36.70	37.10	55.20	55.20	36.10	36.40	50.10	50.00
15	42.0 (b)	42.8 (b)	62.4 (b)	62.80 (b)	41.10	41.50	57.80	57.60
16	36.70	36.60	54.30	54.40	37.50	37.40	49.90	50.00
17	36.10	36.20	54.10	54.10	36.90	37.00	50.10	50.20
18	37.00	37.30	54.70	54.80	38.00	37.90	50.90	50.40
19	36.60	36.10	53.60	53.30	36.90	36.40	49.90	49.90
Mean	36.42		54.12		37.16		49.97	
r	0.84		1.03		0.71		0.60	
S_r	0.300		0.369		0.254		0.216	
RSD_r %	0.82		0.68		0.68		0.43	
R	1.79		2.50		4.38		1.87	
S_R	0.64		0.893		1.563		0.667	
RSD_R %	1.76		1.65		4.21		1.335	

For Key See Table VI

TABLE III
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC Luff-Schoorl

Invert Sugar (g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	35.34	35.40	52.32	51.93	35.30 (a)	32.50 (a)	48.34	48.26
2	35.32	34.82	52.24	52.37	36.09	36.07	52.34	52.10
3	35.18	34.87	51.32	51.99	36.59	36.45	47.62	47.63
4	34.60	34.90	52.40	52.70	35.60	36.10	48.20	49.40
5	36.40	36.02	52.61	52.91	36.02	36.29	49.29	49.12
6	34.79	34.33	51.88	51.02	34.34	34.33	49.39	49.60
7	37.70	36.70	54.20	54.30	36.30	35.70	50.90	51.30
8	34.82	35.12	51.03	51.29	36.11	35.89	49.00	49.06
9	36.79	37.22	52.95	53.10	37.10	36.95	49.69	49.70
10	36.20	35.70	52.40	52.70	35.90	36.40	50.00	49.70
11	35.20	34.30	50.30	50.50	34.60	33.90	45.60	45.60
12	35.30	35.70	52.20	52.70	34.60	35.40	50.60	51.30
13	34.23	34.51	51.05	50.86	37.75	38.41	48.07	47.66
14	35.10	33.40	51.30	51.00	35.80	36.80	48.40	47.90
15	43.30 (b)	43.80 (b)	63.90 (b)	64.20 (b)	42.00 (b)	42.40 (b)	59.10 (b)	58.90 (b)
16	35.20	35.10	52.00	52.10	36.20	36.00	49.20	49.50
17	35.60	36.00	52.20	52.60	36.60	36.20	49.00	49.30
18	35.30	34.90	51.70	51.30	39.10	39.20	49.80	49.50
19	32.90	33.70	50.10	50.20	35.10	34.50	47.10	47.30
Mean	35.24		51.94		36.13		49.07	
r	1.25		0.74		0.98		0.82	
S_r	0.448		0.263		0.350		0.29	
RSD_r %	1.27		0.51		0.97		0.59	
R	2.81		2.84		3.49		4.31	
S_R	1.004		1.013		1.247		1.54	
RSD_R %	2.85		1.95		3.45		3.14	

For Key See Table VI

TABLE IV
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method	Modified Luff-Schoorl (BNC)						Invert Sugar (g/100g)	
Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	34.94	35.88	53.50	52.58	36.32	36.17	50.54	50.18
2	35.27	35.50	52.46	52.48	35.89	36.27	50.30	50.97
3	34.83	35.83	52.37	53.08	36.11	36.67	50.04	50.45
4	34.90	34.30	52.50	53.00	37.30	36.40	50.20	50.60
5	34.49	34.05	52.12	51.95	35.23	35.37	49.53	49.13
6	34.64	34.22	52.12	50.98	35.22	35.22	56.77 (a)	50.77 (a)
7	36.10	34.30	53.80	51.70	36.30	36.40	50.90	51.10
8	35.01	35.40	51.27	51.27	36.27	35.68	48.86	49.08
9	37.09	37.50	53.42	53.37	37.40	37.26	50.10	49.96
10	35.90	36.30	52.40	52.90	35.80	35.80	48.80	48.60
11	34.60	34.30	51.70	52.90	35.20	36.10	47.30 (a)	49.60 (a)
12	35.10	34.20	52.90	51.70	35.20	36.30	49.50	49.90
13	34.86	34.65	50.67	50.41	36.65	36.14	50.45	51.04
14	35.60	34.70	52.70	53.80	35.50	34.90	49.00	48.50
15	44.90 (b)	44.80 (b)	64.80 (b)	64.40 (b)	42.70 (b)	42.20 (b)	58.00 (b)	57.70 (b)
16	35.80	35.70	52.80	53.00	36.50	36.80	50.20	50.40
17	34.50	34.60	52.80	52.80	35.40	34.80	50.20	49.90
18	34.00	34.40	53.60	53.00	35.70	36.50	48.50	47.30
19	34.80	34.20	51.60	52.00	39.00	38.50	50.20	50.40
Mean	35.07		52.43		36.17		49.84	
r	1.37		1.64		1.11		0.94	
S_r	0.491		0.585		0.397		0.335	
RSD_r %	1.40		1.12		1.10		0.67	
R	2.37		2.41		2.59		2.52	
S_R	0.845		0.862		0.925		0.901	
RSD_R %	2.41		1.64		2.56		2.52	

For Key See Table VI

TABLE V

Method of	Sample	Mean	r	S _r	RSD _r	R	S _R	RSD _R	n
Lane & Eynon	A	36.43	0.84	0.30	0.82	1.79	0.64	1.76	18
	B	54.12	1.03	0.37	0.68	2.50	0.89	1.65	18
	C	37.15	0.71	0.25	0.68	4.38	1.56	4.21	18
	D	49.97	0.60	0.22	0.43	1.87	0.67	1.33	17
Luff-Schoorl (LS)	A	35.24	1.25	0.45	1.27	2.81	1.00	2.85	18
	B	51.98	0.74	0.26	0.51	2.84	1.01	1.95	18
	C	36.13	0.98	0.35	0.97	3.49	1.25	3.45	17
	D	49.07	0.82	0.29	0.59	4.32	1.54	3.14	18
Luff-Schoorl	A	35.07	1.37	0.49	1.40	2.37	0.85	2.41	18
	B	52.43	1.64	0.59	1.12	2.41	0.86	1.64	18
	C	36.17	1.11	0.40	1.10	2.59	0.93	2.56	18
	D	49.84	0.94	0.34	0.67	2.52	0.90	1.81	16

For key, see Table VI

TABLE VI

Key to results tables I-V

n.a.	Not analysed
r	Repeatability (within -lab 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.
R	Repeatability (between-lab variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material under different conditions may be expected to lie with 95 % probability.
S _r	The relative standard deviation of the repeatability.
S _R	The standard deviation of the reproducibility
RSD _r	The relative standard deviation of the repeatability $S_r \times 100/x$
RSD _R	The relative standard deviation of the reproducibility $S_R \times 100/x$
(a)	Cochrans outlier ($p < 0.01$) not used in calculation of statistical parameters.
(b)	Grubbs outlier ($p < 0.01$) not used in calculation of statistical parameters.
*	Different dry matter method used, results not used in calculation of statistical parameters.

Conclusions

From statistical analyses of the results from this trial, it has been shown that modification of the Luff-Schoorl procedure to incorporate reagent standardisation steps has improved the precision of the method so that it exhibits better precision than both the original Luff-Schoorl procedure and the Lane and Eynon Constant Volume method. Both the Luff-Schoorl and modified Luff-Schoorl methods gave similar values which are lower than the value obtained with the Lane & Eynon method when applied to the same sample. As these determinations are all empirical in nature, it is important that the appropriate method is selected for the estimation of the standard, notwithstanding its inherent precision. Nevertheless, in view of the increasing international recognition of the Luff-Schoorl method it is important that only the Luff-Schoorl method with the incorporated reagent standardisation step is prescribed.

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APPENDIX I

Measurement of Reducing Sugars Expressed as Invert Sugar (Lane and Eynon Constant Volume modification)

As described in method 7 of EC Directive 79/796/EEC, in which the reducing sugars content of the sample is determined by titrating a sample solution, at its boiling point, against a specified volume of Fehlings solution, using methylene blue as indicator.

APPENDIX II

Determination of Reducing Sugars Expressed as Invert Sugar (Luff-Schoorl)

As described in method 6 of EC Directive 79/796/EEC, in which the reducing sugars in the sample are heated to boiling point under standardised conditions with a copper II solution, which is partially reduced to copper I. The excess copper II is subsequently determined iodometrically.

APPENDIX III

Determination of Reducing Sugars Expressed as Invert Sugar (modified Luff-Schoorl)

As described in Appendix II above except that the Luff-Schoorl reagent is standardised as follows:

Take 25.0 ml of Luff-Schoorl reagent, transfer to a conical flask and add 25.0 ml of the standard reducing sugar solution (1.25 mg/ml), transfer to a conical flask and add 25.0 ml of the standard sugar solution. Add anti-bumping granules and 1 ml of 3 methylbutan-1-ol. Fit the flask to the reflux condenser and place on to a wire gauze under which a flame has been lit. Adjust the flame so as to bring the contents of the flask to boil in about 2 min., reduce the flame so that the solution boils gently and reflux for exactly 10 min. Cool immediately in cold water and after 5 min. titrate as follows:

Add 10 ml of potassium iodide solution and, with constant swirling of the flask, immediately add 25 ml of sulphuric acid solution carefully in small increments to prevent excessive foaming. Titrate with 0.1 N sodium thiosulphate solution until a dull yellow colour appears; add starch indicator and complete the titration.

Carry out a blank titration (without boiling) on a mixture of 25 ml of Luff-Schoorl reagent and 25 ml of water after adding 10 ml of potassium iodide and 25 ml of sulphuric acid solution.

APPENDIX IV

Determination of Dry Matter

As determined by Method 2 of EC Directive 79/796/EEC in which the dry matter is determined at a temperature of $70 \pm 1^\circ\text{C}$ using a vacuum oven at a pressure not exceeding 3.3 KPa (34 mbar). The test portions are prepared by mixing with water and Kieselguhr before drying.

References:

- 1 Council Directive 79/796/EEC, O J No L239, **22**, 22 September 1979.
- 2 The Specified Sugar Products (Amendment) Regulations (1982 SI : No 255, 1982, HMSO, London)
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- 6 Horwitz W., Anal.Chem. 1982, **54**, 67A-76A