

# A COMPARISON OF THREE METHODS FOR THE DETERMINATION OF WATER IN FINAL MOLASSES

By A. W. MACGILLIVRAY\* and D. NUROK

*Sugar Milling Research Institute*

## Abstract

The Karl Fischer and a gas chromatographic method of water determinations are described. These two methods are compared with the vacuum oven method for the measurement of water in final molasses. Karl Fischer and the vacuum oven method are in good agreement, while the G.C. method gives slightly higher results.

## Introduction

Measurement of purity is of fundamental importance in cane sugar factory control and for this reason the analytical procedures involved merit close attention. The "true purity", the ratio of sucrose to dry solids, is accepted as the best measure of exhaustion of final molasses, but in spite of this it is seldom determined industrially because of the difficulties involved in determining the dry solids accurately. The most widely used method involves extending the molasses on sand or paper and drying under vacuum for a prolonged period. This is a time-consuming procedure prone to quite large errors, and as such is not well suited to routine use. This paper describes two alternative methods for the determination of dry solids in final molasses; the Karl Fischer method, and a gas chromatographic method. The precision of these two methods is discussed, and comparisons are made between them and the vacuum oven method.

## The Karl Fischer determination of water in molasses

The use of the Karl Fischer (K.F.) method of water determination has been comprehensively described,<sup>15</sup> and there have been numerous applications in the sugar industry<sup>13,16,19</sup>. Fischer's reagent is highly specific for water, and although parasitic side reactions may diminish the reagent's affinity for water,<sup>15</sup> regular standardization with known weights of water is sufficient to ensure highly reproducible results. The end-point is readily detected conductimetrically, the "dead-stop" titration method being most commonly used.

Errors which can arise with the K.F. method are the reaction with substances other than water, the reaction with water which has entered the titration vessel from the atmosphere, and the deterioration of excess K.F. reagent at the end-point, causing a rapidly fading colour or current. Douwes-Dekker<sup>6</sup> found an average difference of 0.5% between results obtained by K.F. and by vacuum drying on final molasses, while Schiweck<sup>18</sup> found significant differences of up to 1.2% between the two methods. Both

these authors considered K.F. to be an acceptable method for final molasses. Errors due to atmospheric moisture and fading end-points can be overcome by instrumental methods, and Kviestis<sup>13</sup> has shown that reasonably stable end-points can be maintained for as long as two minutes.

## Gas chromatographic determination of water in molasses

The use of gas chromatography (G.C.) for water determination has been described for numerous industrial and academic applications. These include the determination of moisture in cheese,<sup>20</sup> in chlorophyll extracts,<sup>3</sup> in air<sup>2</sup> and in mixtures of various organic compounds<sup>4,5,12</sup>. However, no reference to the determination of water in molasses could be found.

Water may be determined indirectly by allowing it to react with either calcium carbide<sup>10</sup> or 2,2-dimethoxypropane<sup>14</sup> in which case either acetylene or acetone is analysed. Alternatively the water may be analysed directly on a polyethylene glycol<sup>2,5</sup> on teflon column or on a porous polymer bead column<sup>20,3,12</sup>.

The latter column was used for the work described in this paper.

## Experimental

### (a) The K.F. Method

The equipment used for the K.F. experimental work was a Beckman K.F. 4 Aquameter. The design of this instrument is discussed by Frediani<sup>9</sup> and by Haagen-Smit *et al.*<sup>11</sup>

Approximately 200 mg, accurately weighed, of final molasses were introduced to the titration vessel by means of a 10 ml disposable syringe, with the needle removed. While this was being done a stream of dry air was blown into the vessel, to prevent the entry of any moisture from the atmosphere. Prior to introducing the molasses about 35 ml of AR methanol were run into the vessel and titrated to dryness with K.F. reagent. The stabilized K.F. reagent<sup>17</sup> had a water equivalent of ca 4.5 mg water/ml K.F. After allowing a 10 minute period for dispersing the molasses in the methanol, the titration was initiated. The instrument was set to declare an end-point when a current of 40  $\mu$ A flowed for a period of 30 seconds. At this point the instrument would automatically cut out, and the volume of K.F. reagent titrated could be read off a digital display. The actual titration took approximately five minutes to complete, depending on the weight of molasses introduced.

The water equivalent of the K.F. reagent was determined every day, using sodium tartrate dihydrate as the primary standard.

\*Present address: Bookers Agricultural and Technical Services, Bucklersbury House, 83 Cannon Street, London EC4.

**(b) Vacuum oven method**

Molasses samples were diluted 1:4 with water. Approximately 5 g of the diluted molasses, weighed to 0,1 mg were extended on 40 g of pre-dried, acid-washed sand, and dried at 68°C and 133 mbar vacuum for 24 hours. After cooling in a desiccator the samples were reweighed, and the solids determined by difference.

**(c) Gas chromatographic method**

The method for the gas chromatographic determination of water in molasses is given in detail as it has not been described before.

**(i) Sample preparation**

The sample was prepared by weighing 1 g of final molasses into a 7 ml vaccine bottle and then pipetting in 4,5 ml of dry analar dimethylformamide. Four 4 mm ball bearings and six 3 mm square pieces of stainless steel were added and the bottle was then sealed with a rubber septum. 1 ml of analar dry n-butanol was added by syringe, the amount added being determined by weighing. The bottles were then vigorously shaken to disperse the molasses in the solvent. This took between thirty minutes and four hours depending on the viscosity of the molasses. Standards were prepared in the same way using 0,2 g water instead of molasses.

It was found that there is a rapid uptake of atmospheric moisture if the components are mixed by stirring an open bottle.

**(ii) Gas chromatography**

A Perkin Elmer F11 gas chromatograph, fitted with a 2 m × 3 mm o.d. stainless steel column packed with Chromosorb 102 was used. Porapak QS was initially tried as packing material but was found to yield irreproducible results. The oven was set at 200°C and the injection block at 210°C. Hydrogen was used as carrier gas at a flow rate of 20 ml/minute. 1,5 µl of sample were injected using a Hamilton 10 µl syringe. The time for an analysis was seven minutes.

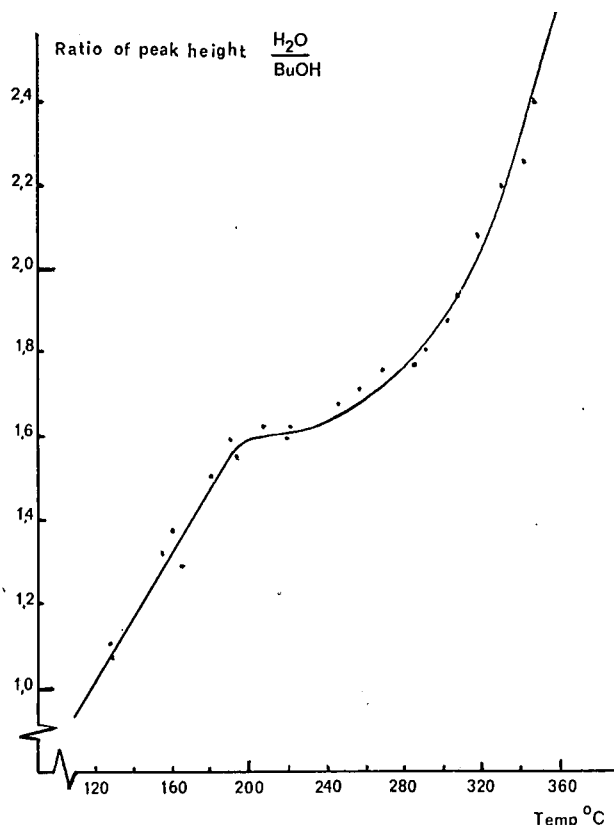
Peak heights were measured instead of peak areas as the water peak was extremely sharp. n-Butanol was used as internal standard. The constant of proportionality K,

$$K = \frac{\text{Weight BuOH}}{\text{Weight H}_2\text{O}} \times \frac{\text{Peak Height H}_2\text{O}}{\text{Peak Height BuOH}} \text{ used}$$

for calculating the amount of water present, was determined each day using two freshly prepared samples. This was to allow for any day to day variation in flow rate setting.

The setting of injection block temperature, carrier flow rate and sample size were chosen after the following short investigation to check how these operating parameters affected the results obtained. These experiments are summarised in Figures 1, 2 and 3.

Figure 1 is a plot of the ratio of peak heights (water:butanol) of a sample of molasses against injection block temperature. This ratio is directly proportional to the amount of water present. The



**FIGURE 1:** Influence of injection port temperature on peak height ratio.

increasing ratios below 200° and above 220° are respectively due to too low an injection block temperature leading to slow volatilization of components and too high an injection block setting leading to probable dehydration of sugars. Only the plateau between 200° and 220° is suitable for quantitative work. It should be noted that the upper temperature limit of the plateau is due to a chemical effect whereas the lower limit is a design effect and could be lowered, e.g. by using on-column injection.

Figure 2 is a plot of K versus carrier gas flow rate. It is seen that the value of K obtained from a standard sample varies linearly with flow rate. Thus it is essential that the determination of water be done at the same flow rate as that used to determine K. This precaution was taken during the subsequent analyses.

Figure 3 is a plot of the ratio of peak heights against sample size. This shows that the value of the ratio is constant for a sample size between 1,5 µl and 3 µl but is very sensitive to sample size for values below 1 µl.

**Results****(a) Reproducibility of the methods**

In order to test the reproducibility of the methods four samples of molasses were analysed by K.F., and one by G.C. and the fiducial limits for 5% probability were calculated. The results are given in Table I.

In order to test the interlaboratory reliability of the method, the four samples analysed by K.F. were sent to the Sugar Industry Research Institute, Mauritius, for analysis. The instrument used in their laboratory was a Metrohm K.F. Titrator E452, with an

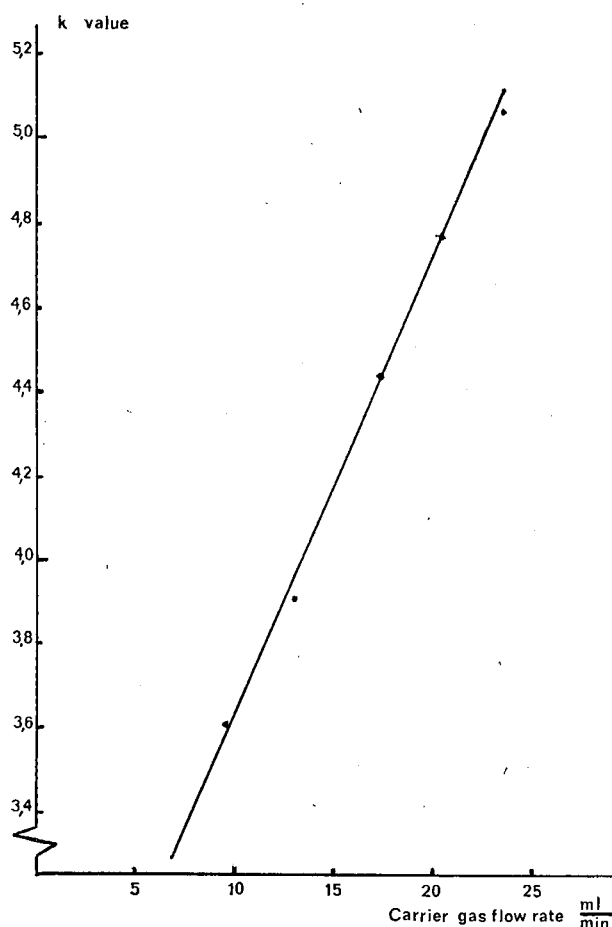


FIGURE 2: Influence of carrier gas flow rate on k-value.

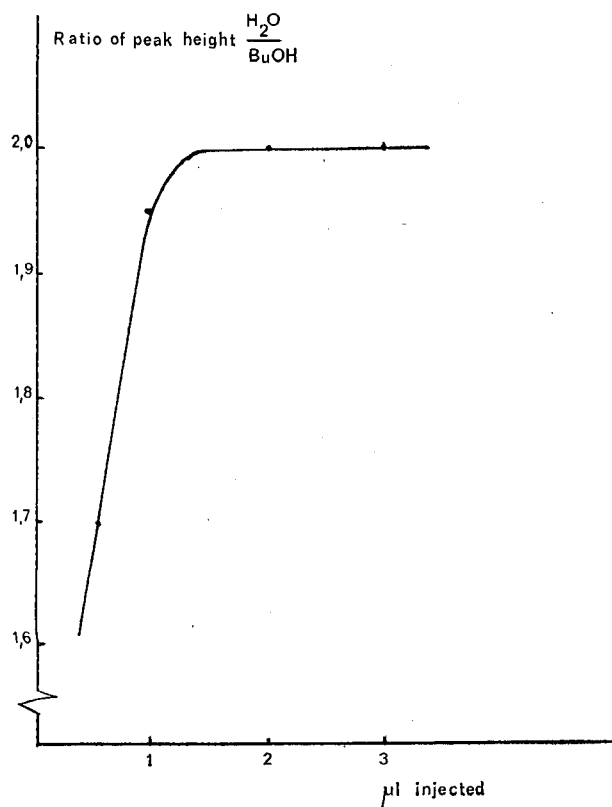


FIGURE 3: Influence of sample size on peak height ratio.

TABLE I

Sample	Karl Fischer method				G.C. Method
	A	B	C	D	E
1.	20,39	18,77	19,59	20,79	18,64
2.	20,35	19,26	19,42	20,89	18,77
3.	20,23	18,92	19,38	20,72	18,82
4.	20,37	19,14	19,28	20,84	18,65
5.	20,29	19,02	19,42	20,42	18,51
6.	20,34	19,03	—	20,71	18,75
7.					19,18
8.					18,73
9.					19,00
10.					18,92
Mean	20,345	19,02	19,42	20,73	18,80
Variance ( $\sigma^2$ )	0,0150	0,0289	0,0161	0,0288	0,0377
Std. Deviation ( $\sigma$ )	0,1225	0,1700	0,1270	0,1699	0,1942
Std. error of mean ( $\frac{\sigma}{\sqrt{N}}$ )	0,0548	0,0760	0,0635	0,0759	0,0614
t for 0,05 p Confidence	2,571	2,571	2,776	2,571	2,262
limits ( $t \times \frac{\sigma}{\sqrt{N}}$ )	$\pm 0,1409$	$\pm 0,1954$	$\pm 0,1763$	$\pm 0,1950$	$\pm 0,1389$
% moisture	20,34 $\pm 0,14$	19,02 $\pm 0,19$	19,42 $\pm 0,18$	20,73 $\pm 0,19$	18,80 $\pm 0,14$

TABLE II

Sample	A	B	C	D
SIRI	20,71	19,53	19,49	20,86
SMRI	20,34	19,02	19,42	20,73

end point of 23  $\mu$ A for 20 seconds. The results under these conditions are recorded in Table II.

Although two of the results were outside the calculated confidence limits from our internal tests, the agreement is quite good as an inter-laboratory comparison. A further interlaboratory comparison has been made, using purely routine results obtained during the past season in our laboratory and in the Huletts' Central Laboratory. Weekly composite samples of final molasses which were routinely analysed at the SMRI once a month were also analysed by Huletts on 67 occasions during the season. It must be emphasised that the two laboratories were analysing samples which had been composited completely independently at the factories concerned, and it is likely that a considerable part of the difference between results was due to sampling errors. The Huletts' Laboratory used a Metrohm E452 K.F. Titrator, with the same end-point as that used at the Sugar Industry Research Institute, Mauritius.

Analysis of variance shows that the interlaboratory error is not significant. This is a definite improvement compared to results using the dry solids by vacuum oven, and in fact of all the analyses performed in common by the two laboratories on final

TABLE III  
Comparison of dry solids between Hulett's R & D (H) and SMRI (S)

Mill Laboratory	EM		FX		AK		DL		ME	
	H	S	H	S	H	S	H	S	H	S
May	83,12	82,12	82,89	81,81	82,75	82,80	84,58	84,96	83,59	82,85
June	83,47	82,29	83,30	82,75	82,63	82,56	83,56	83,34	82,95	82,32
July	81,38	80,48	83,02	82,95	83,13	81,70	83,99	83,01	83,65	83,59
August	81,35	80,81	82,36	81,69	82,50	81,23	83,66	83,44	82,85	81,59
September	80,13	80,10	81,18	81,45	83,51	82,29	85,31	83,35	82,36	82,41
October	80,45	78,62	82,44	82,40	83,46	82,75	83,75	83,14		
November	79,74	79,15			82,39	80,96	82,92	83,76		
December	79,75	80,04					82,84	82,85		

TABLE III (continued)  
Comparison of dry solids between Hulett's R & D (H) and SMRI (S)

Mill Laboratory	PG		GH		RN		SZ		UK	
	H	S	H	S	H	S	H	S	H	S
May	81,18	80,37	82,72	81,15	82,50	80,63			77,55	76,54
June	80,08	78,63	81,20	80,79	83,18	82,28	79,54	79,20	78,39	76,60
July	79,80	79,53	81,14	80,30	83,05	82,83	79,78	78,55	76,21	77,17
August	80,67	80,15	77,58	76,24	82,72	83,20			78,09	77,27
September			79,70	79,38	81,90	82,00			77,74	76,39
October	80,76	80,72	78,46	79,81			75,67	75,35		
November	80,14	80,19	79,48	79,10						
December	80,29	80,58								

TABLE IV

Sample	Moisture % Molasses			Differences		
	KF	GC	Vacuum	a-b	a-c	c-b
	(a)	(b)	(c)			
1	19,01	19,34	18,45	-0,33	+0,56	-0,89
2	17,25	18,59	17,35	-1,34	-0,10	-1,24
3	11,99	12,66	12,25	-0,67	-0,26	-0,41
4	18,05	18,61	18,00	-0,56	-0,05	-0,61
5	19,95	20,25	20,17	-0,30	-0,22	-0,08
6	18,03	18,44	18,05	-0,41	-0,02	-0,39
7	17,18	17,60	17,37	-0,42	-0,19	-0,23
8	21,39	21,28	20,70	+0,11	+0,69	-0,58
9	16,40	16,80	17,27	-0,40	-0,87	+0,47
10	18,15	18,54	18,35	-0,39	-0,20	-0,19
Average	17,74	18,21	17,80	-0,47	-0,06	-0,36

molasses, the Karl Fischer moisture shows the best agreement.

(b) Comparison between G.C., K.F. and vacuum oven methods

Ten samples of final molasses were analysed by each method. For the K.F. method each sample was analysed three times independently; for G.C. two independent sub-samples of the molasses were taken, and each sub-sample was analysed in duplicate. For the oven method a single dilution of each molasses was made, and three replicate analyses made on each. The results are shown in Table IV.

Applying Student's t test it is found that the difference between the G.C. method and the other two methods is significant at the 5% level. There is no significant difference between the K.F. and the vacuum oven method.

Discussion

Comparing the three methods analytically, both K.F. and G.C. have the advantage of being fairly rapid determinations in relation to the vacuum oven method. The G.C. method involves a sample preparation stage which may be quite lengthy depending upon the viscosity of the sample to be analysed, but this

could be overcome by dissolving a number of samples simultaneously in a shaker. A similar technique has been described for K.F. work<sup>1</sup> but although there may have been a saving in time when this was tried, a slight deterioration in precision was found. Both methods are objective in that the results are arrived at automatically, but a certain amount of operator skill is necessary to achieve reproducible results.

Table I gives an indication of the precision to be expected from the two methods. The results for the K.F. method are similar to those quoted by Stachenko<sup>19</sup> and by Vignes,<sup>21</sup> and represent an improvement over the precision of the vacuum oven method.<sup>7</sup> The G.C. Method showed an equally good degree of precision, but as the results can be influenced by a number of operational parameters, great care has to be exercised in order to achieve this level.

The gas chromatographic value for water was found to be consistently higher than the Karl Fischer value. The temperature of the injection block may have been slightly too high, resulting in additional water due to sucrose decomposition.

Alternatively the increment may have been due to water of crystallisation in a compound such as calcium magnesium aconitate hexahydrate that was liberated at 210° in the injection block but which was not available under Karl Fischer conditions due to the insolubility of the hydrate in methanol.

No absolute method for the determination of water in molasses exists, and it is therefore impossible to say which of the two methods gives the true result. However, in a purely practical sense the Karl Fischer method would be easier and cheaper to initiate in a laboratory than the G.C. method. The good agreement between the K.F. and the vacuum oven does not confirm the work of Douwes-Dekker or Schiweck, and cannot be used as an argument in favour of K.F. However, the method has the advantage of being more

convenient while giving essentially the same results, hence there would be no problem in introducing it as a standard method.

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