

HYDROCHLORIC ACID/RESORCINOL VERSUS PHENOL/SULPHURIC ACID FOR MONITORING TRACE SUGARS

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Abstract

The two most commonly used quantitative trace sugar tests in the South African sugar industry are based on the colour formed when sucrose is reacted with hydrochloric acid/resorcinol, or with phenol/sulphuric acid. Wide differences in the results when the two methods were used on some condensate samples were shown to be due to volatile compounds which interfered with the hydrochloric acid/resorcinol test. Following modifications to this test procedure, the results were found to be comparable with the phenol/sulphuric acid test data.

Introduction

The analysis of condensates in sugar factories for relatively small amounts of sugars is important where they are used for boiler feed water because the sugars decompose in the boilers giving rise to foaming, severe drop in pH and in extreme cases can lead to serious damage of the boiler.

Continuous monitoring of the condensates used for boiler feed water is effected in many factories by measuring the conductivity of the water. This indirect method relies on the fact that, associated with any sugar from entrainment or leaking tubes, will be inorganic salts which will increase the conductivity of the condensates. In some instances, condensates may be contaminated with impurities such as ammonia which cause an appreciable increase in conductivity although no sugars are present. To make allowance for this when setting up the conductivity monitor, it is necessary to have a reliable measure of the sugars content which is not affected by interference by impurities which can be tolerated in the boiler feed water.

Three methods of analysing water for traces of sugars have been evaluated to establish which is the most suitable for use in factory laboratories. These were:

- the HCl/resorcinol method,
- the phenol/sulphuric method and
- the cysteine hydrochloride/carbazole method.

Evaluation of various trace sugar tests

Requirements of an ideal trace sugar test are that:

- other substances do not interfere,
- it should have a large linear range,
- it should be simple and quick to perform and
- the reagents should be stable.

Hydrochloric acid/resorcinol trace sugar test¹

This test has a large linear range and an excellent linear relationship between sucrose concentration and absorbance measured at 520 nm. Its response to fructose is much greater than glucose and this should be borne in mind when analysing samples such as effluent, where significant quantities of glucose polymers may be present and which would otherwise record as "sucrose". A disadvantage of this test is that the samples require heating in a boiling water bath and this adds to the complexity of the test. Further, the colour development is sensitive to changes in temperature so special care needs to be exercised. A further disadvantage is that the reagents change slowly with time and thus it is essential for a calibration curve to be drawn for every analysis.

Phenol/sulphuric acid trace sugar test²

This test gives an excellent linear relationship between sucrose concentration and absorbance at 490 nm. The test is simple to perform but has a lower linear range (0-60 ppm sucrose) than the hydrochloric acid/resorcinol test (0-200 ppm). The reagents are stable for long periods. All carbohydrates will form colours in this test, so care must be taken to ensure that this method is applicable to the sample to be analysed.

Cysteine hydrochloride/carbazole trace sugar test³

This test produces an intense colour at low sucrose concentrations but it has two major disadvantages:

- the correlation between sucrose concentration and absorbance at 558 nm shows more scatter than the above two tests, and
- the reagents have to be prepared daily.

Comparison of these trace sugar tests

The above three methods were performed on fresh or frozen condensate samples from various mills. The results are listed in Table 1.

TABLE 1
Comparison of trace sugar tests

Sample	ppm Sugars by		
	HCl/resorcinol at 520 nm	Phenol/sulphuric acid	Cysteine HCl/carbazole
Vapour 2 (MS)	13	8	7
Vapour 1 (MS)	13	3	2
Boiler feed (MS)	0	2	3
Exhaust (DL)	3	1	1
Juice heater (AK)	21	7	8
Boiler feed (DL)	8	2	3

The results of all three methods are sometimes comparable but for some samples, the difference between the hydrochloric acid/resorcinol test and the other two tests was excessive. As was noted earlier, the cysteine hydrochloride/carbazole test has two major disadvantages so it was not evaluated any further. Further comparisons of condensate samples obtained from Amatikulu are shown in Table 2.

TABLE 2
Comparison of hydrochloric acid/resorcinol and phenol/sulphuric acid trace sugar tests

Sample	ppm Sugars by	
	Hydrochloric acid/resorcinol at 520 nm	Phenol/sulphuric acid
Vapour 1 evaporator A	38	9
Vapour 1 evaporator B	46	12
Vapour 1 pan A	38	7
Vapour 1 pan B	8	5
Exhaust, new Kestner	5	6
Exhaust, old Kestner	41	10
Combination	33	7
Boiler feed	10	3

These results showed a very poor correlation between the two methods and this indicates that further investigative work is necessary.

The trace sugar tests which have been discussed were not entirely specific to sucrose and could be influenced by other components present in the condensate samples. To further validate these trace sugar tests, a reference method that would determine sucrose specifically, was needed. High pressure liquid chromatography (HPLC) was selected as the reference method, being considered sufficiently accurate and reproducible to be used for this purpose.⁴ An added advantage was that sample preparation was simple. The results from condensate samples which were analysed by the two trace sugar tests and by HPLC are listed in Table 3.

TABLE 3

Comparison of trace sugar tests to HPLC

Sample	ppm Sugars by		
	HCl/resorcinol at 520 nm	Phenol/sulphuric	HPLC
Vapour 1 (DL)	19	4	2
Vapour 2 (AK)	23	7	4
Pan A(1) (ME)	19	4	0
Pan A(2) (ME)	16	4	0
Pan B (ME)	25	11	8

The results showed that in the hydrochloric acid/resorcinol test, the sucrose concentration was over estimated to a much greater degree than in the phenol/sulphuric acid test. More comparative data which show the same trends are listed in the Appendix in Table 7 (a), (b) and (c).

Obviously there were interfering substances in the condensate samples that affected the hydrochloric acid/resorcinol test much more than the phenol/sulphuric acid test. Since the larger differences were found on vapour condensate, it seems reasonable to assume that the interference may be due to volatile compounds. To test this supposition, two condensate samples were freeze-dried (pan A (1) and pan B (ME)), reconstituted with distilled water to their original volume and analysed again using the three methods. The results obtained are listed in Table 4.

TABLE 4

Analysis of freeze-dried samples

Sample	ppm Sugars by		
	HCl/resorcinol at 520 nm	Phenol/sulphuric	HPLC
Pan A(1), (original)	19	4	0
Pan A(1), (freeze-dried)	4	4	0
Pan B, (original)	25	11	8
Pan B, (freeze-dried)	12	8	8

The results indicate that the difference between the two trace sugar tests can be explained by the influence of volatile compounds on the hydrochloric/acid/resorcinol test. The figures for the freeze-dried samples showed good agreement with the HPLC figures.

To further substantiate these findings, six samples of condensate from Mount Edgecombe were analysed and the results are shown in Table 5.

The satisfactory agreement of the three methods after freeze-drying confirms that the reason for the over estimation in the hydrochloric acid/resorcinol test was due to volatile compounds present in the condensate.

TABLE 5

Comparison of original and freeze-dried samples

Sample	ppm Sugars by				HPLC
	HCl/resorcinol at 520 nm		Phenol/sulphuric		
	Original	Freeze-dried	Original	Freeze-dried	
Evaporator 4	61	35	37	33	33
Mirrlees Quad Return condensate	26	3	4	1	0
Pan A	19	0	5	2	0
Boiler feed	17	2	5	2	0
Vapour condensate	12	2	3	1	0
	4	1	2	2	0

All the data presented so far showed that the trace sugar concentration obtained by the hydrochloric acid/resorcinol test at 520 nm must be viewed with caution and that the phenol/sulphuric acid test provides data which is more reliable although it was also affected by some interference.⁵

Establishing the cause for the different results

It was shown above that the difference between the two trace sugar tests can be explained by the presence of volatile compounds. It was also noted that when the hydrochloric acid/resorcinol test was performed on samples showing large differences between the two tests, a distinct pink, colour, instead of the normal reddish-brown colour developed. To gain more insight into this interference, visible spectra of the interfering pink colour (Figure 1) and the reddish-brown colour (Figure 2) obtained from a standard sucrose solution, were plotted.

In Figure 1, the absorption maximum of the interfering pink colour occurred at 555 nm. In Figure 2, the absorption maximum for a standard sucrose solution occurred at 480 nm (reddish-brown colour). All the absorbance readings obtained above for the hydrochloric acid/resorcinol test were taken at 520 nm which is the wavelength recommended for South African sugar factories.¹ Obviously these two spectra show that this is wrong, because the closer the wavelength is to 555 nm to determine sucrose, the greater will be the degree of over estimation, because of the influence of the 555 nm peak. To avoid this, the 480 nm wavelength should be used for the hydrochloric acid/resorcinol test. This wavelength has been recommended previously.⁶

The results obtained from Mount Edgecombe condensate samples are listed in Table 6. They were measured at 480 nm as well as 520 nm for the hydrochloric acid/resorcinol test, the phenol/sulphuric acid test and HPLC data.

TABLE 6

Influence of the wavelength on the hydrochloric acid/resorcinol test

Sample	ppm Sugars by			
	HCl/resorcinol at 520 nm	HCl/resorcinol at 480 nm	Phenol/sulphuric	HPLC
Evaporator 4	54	30	32	29
Boiler feed	10	3	3	0
Mirrlees quad	17	4	5	0
Vapour condensate	20	5	2	0
Pan A	21	5	5	0
Return condensate	12	3	5	0

These results showed a dramatic improvement in the correlation between the three methods when 480 nm was chosen as the wavelength for the hydrochloric acid/resorcinol test. More comparative data are listed in the Appendix in Table 8.

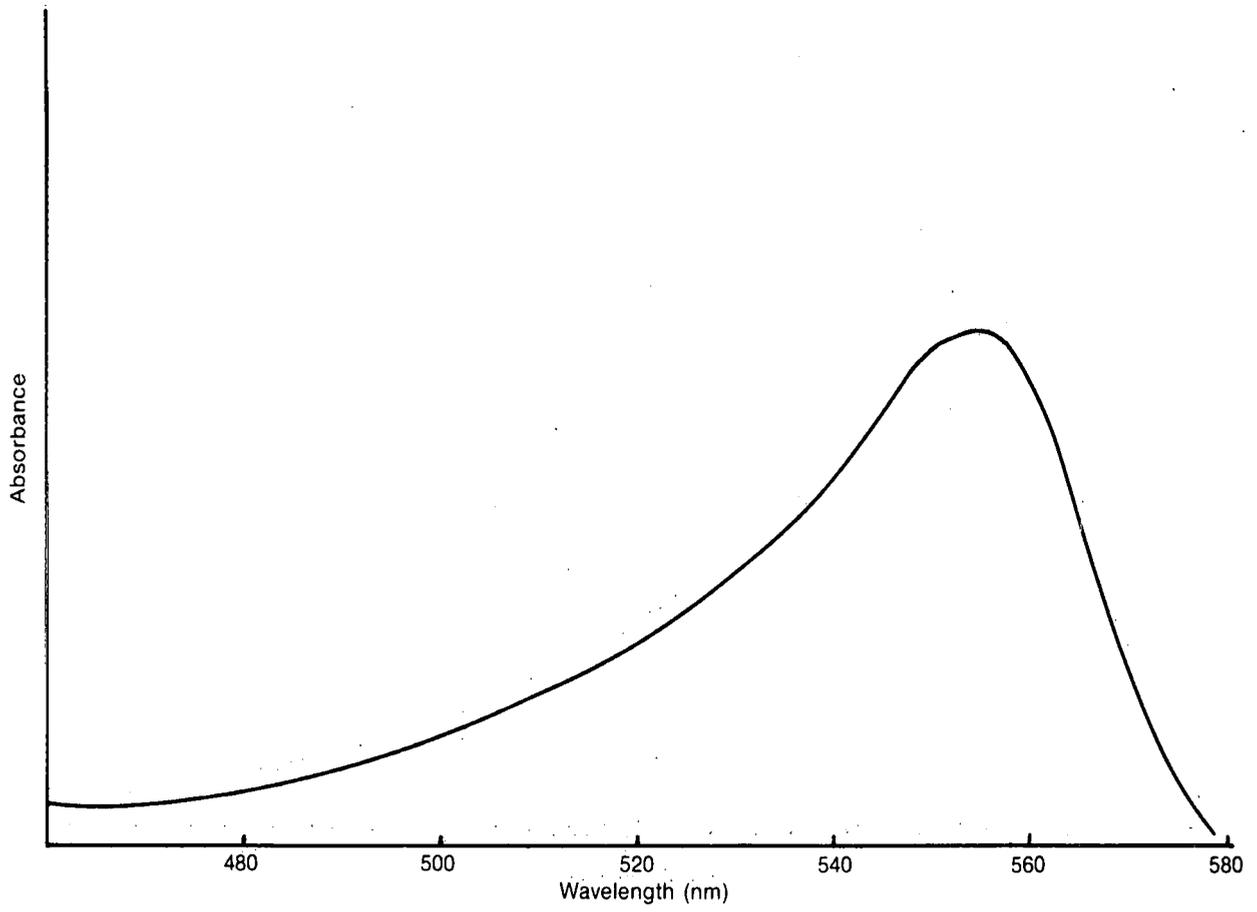


FIGURE 1 Visible spectrum showing typical interference (pink colour)

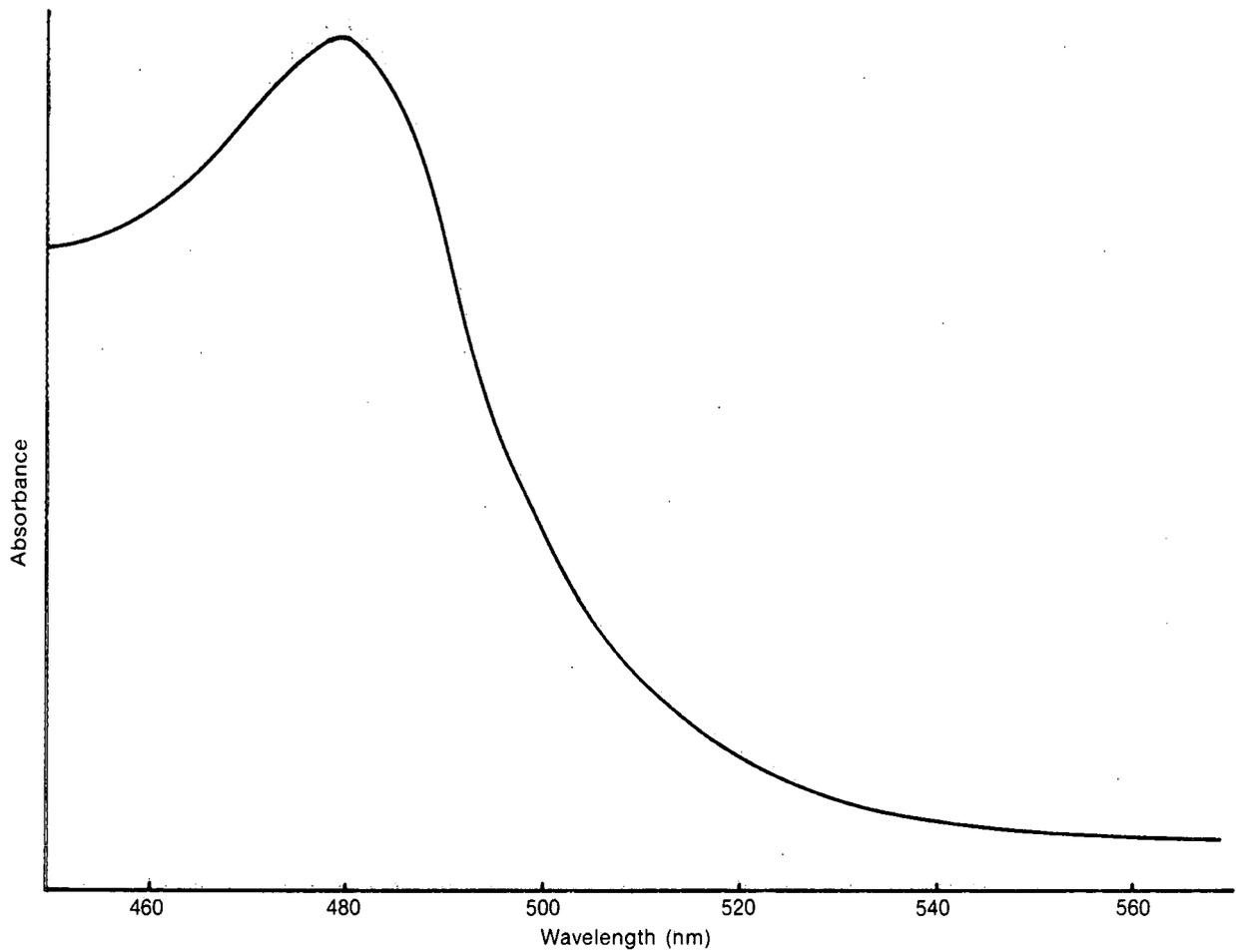


FIGURE 2 Visible spectrum showing typical sucrose presence (reddish brown colour)

As yet, the identity of the large peak observed at 555 nm has not been established but research is progressing and will be subject of another paper.

Conclusions

Certain volatile compounds in condensates can affect the hydrochloric acid/resorcinol test much more than the phenol/sulphuric acid test. By selecting a wavelength of 480 nm for the hydrochloric acid/resorcinol test, the correlation between the two trace sugar tests can be dramatically improved. Further, these two tests show good agreement with a more specific method (HPLC) to determine sucrose. The phenol/sulphuric acid test has been recommended as the quantitative test to be used in all Tongaat-Hulett factory laboratories since its suitability for this purpose has been demonstrated in this paper.

REFERENCES

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6. Gray, D. J. S. (1950). Critical factors in the resorcinol reaction for the determination of fructose. *The Analyst* 75 (891): 314-317.

APPENDIX

TABLE 7
Additional comparative data

Sample	ppm Sugars by		
	HCl/resorcinol at 520 nm	Phenol/sulphuric	HPLC
(a)			
Evaporator 4	61	37	33
Mirrlees Quad Return condensate	26	4	0
Pan A	19	5	0
Boiler feed	17	5	0
Vapour condensate	12	3	0
	4	2	0
(b)			
Evaporator 4	62	32	29
Mirrlees Quad Return condensate	23	5	0
Pan A	20	5	0
Boiler feed	27	5	0
Vapour condensate	10	3	0
	3	2	0
(c)			
Evaporator 4	68	56	46
Mirrlees Quad Return condensate	17	6	0
Pan A	19	3	0
Boiler feed	22	9	0
Vapour condensate	11	6	0
Evaporator 4 (2)	4	0	0
Evaporator 4 (3)	50	13	11
Evaporator 4 (4)	66	30	24
	81	42	35

TABLE 8
Influence of wavelength chosen

Sample	ppm Sugars by			
	HCl/resorcinol at 520 nm	HCl/resorcinol at 480 nm	Phenol/sulphuric	HPLC
Evaporator 4	61	33	37	33
Return condensate	23	2	5	0
Vapour condensate	8	0	2	0
Evaporator 4 (2)	50	10	13	11
Evaporator 4 (3)	66	22	30	24
Evaporator 4 (4)	81	34	42	35