Rapid Automatic Analysis of Acids in Juice Using a Discrete Analyzer

Mari Klemm, Leena Kaski, Sari Hartikainen, Annu Suoniemi-Kähärä
Thermo Fisher Scientific, Oy, Vantaa, Finland

Overview

An automated, discrete photometric system was used to simultaneously measure a multiple acid test panel for acetic acid, L-ascorbic acid, citric acid, D-isocitric acid, D-lactic acid, L-lactic acid, and total acids from each sample. The analytical performance of the system was evaluated and the precision studies demonstrate the repeatability and reproducibility of the methods used. A within run CV% as low as 0.5% was achieved for citric acid analysis. The analyzer was capable of performing an acid test panel for 100 samples in 4 hours and 15 min.

Introduction

Acids play a significant role in the taste, color, and microbial stability of juice. Analysis is essential to ensure that it fulfills the required quality and authenticity characteristics. In various phases of the production process, from raw materials to finished products, the health standards and uniform quality are constantly confirmed. For example, the ratio of citric acid to D-isocitric acid is commonly used as a marker to detect the quality of citrus juices. In addition, ascorbic acid is widely used as an additive to prevent the oxidation of food.[1-4]

Thermo Scientific™ Gallery™ and Gallery™ Plus automated discrete analyzers measure several acids from a single sample without the need for extra method changeover time. Most homogenous samples can be measured without pretreatment. Since the necessary analysis steps are automated true walk-away time is provided for the operator. The methods used are either colorimetric or enzymatic and some analysis is done by automating traditional titration methods.

Laboratories choose automated methods for ease-of-use and optimized application resulting in smaller volumes of reagents required and enabling low costs per test. Discrete cell technology offers faster, reproducible results for ascorbic acid, citric acid, D-isocitric acid, and total acid testing. Routine analysis of acetic and lactic acid can also be performed with these analyzers.

In this study, measuring ranges and performance data for the system reagents used with Gallery analyzers are described. From performance data, the correlation graphs illustrate the quality control sample concentration plotted against a theoretical calculated concentration.

Precision studies demonstrate the repeatability and reproducibility of the methods used. In less than ten minutes after insertion of test samples into the analyzers, results are obtained.

Materials and Methods

Thermo Scientific system reagents are ready-to use. Some reagents are available in lyophilized or powdered format which requires reconstitution and ensures a longer shelf life. Reagent volumes are optimized for the system application; typically, an average of 300 test can be reported from each kit.

Reagent vials are bar-coded. Embedded in the barcode is the material lot number and expiration date and as part of its programming, the instrument sounds an alarm when the reagent is almost finished and will automatically calibrate after the insertion of a new reagent vial. In addition to the reagent's on-board stability, the instrument has the capability to fully trace reagents and store results, including associated calibrations and reagent lot data.

Instruments and Applications

Thermo Scientific Gallery and **Gallery Plus** analyzers are pre-programmed with the methods to test various acids in their application menus. Application parameters are adapted to test less than 300 µL of total volume where the sample and first reagent are usually blanked to eliminate any color interference.

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries.
This information is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others.

Measuring ranges

Measuring ranges for each acid kit are shown in Table 1. Applications are designed to incorporate automated dilutions to achieve these ranges.

TABLE 1. Measurement ranges of acid kits.

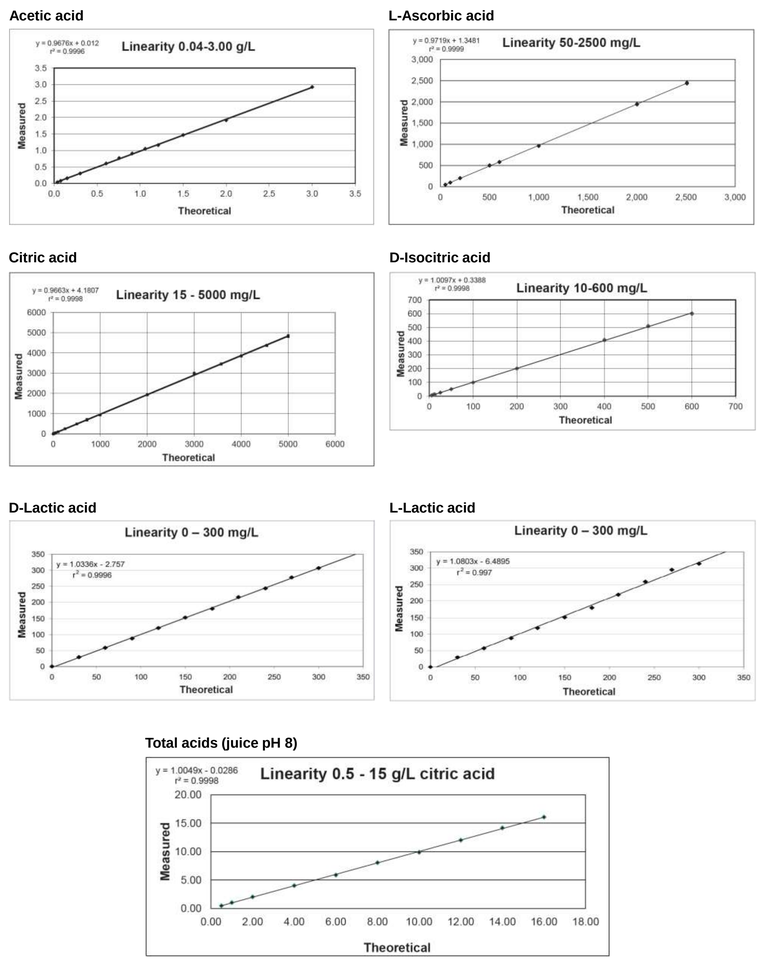
Kits	Test limit low	Test limit high
Acetic acid	0.04 g/L	3.0 g/L
L-Ascorbic acid	50 mg/L	2500 mg/L
Citric acid	15 mg/L	5000 mg/L
D-Isocitric acid	10 mg/L	600 mg/L
D-Lactic acid	25 mg/L	1600 mg/L
L-Lactic acid	20 mg/L	1600 mg/L
Total acids (Juice pH 8)	0.5 g/L as citric acid	15 g/L as citric acid

Results and Discussions

Examples of Calibration Curves

Linearity performance curves for each method using water based solutions are presented in Figure 1. The anlayzers are designed with an automated dilution function, allowing for easy insertion of additional data points if required. The image plots linearity comparing both measured and theoretical values.

FIGURE 1. Linearity performance curves for each method .



Precision Studies

Results of the precision studies performed with the Gallery analyzer are shown in Table 2. Usually, three sample levels with at least 10 replicates were tested every day for 5 days. The assay was calibrated daily between batches.

Analysis Speed

An automated operating system allow laboratories to simultaneously measure multiple analytes while reducing total analysis time and increasing efficiency. The Gallery Plus analyzer is capable of performing an acid test panel for 100 samples in 4 hours and 15 minutes. The panel included tests for acetic acid, L-ascorbic acid, citric acid, D-isocitric acid, D-lactic acid, L-lactic acid, and total acids from each sample. First results are available in less than 17 minutes. In comparison, if only total acids are tested, the Gallery Plus instrument completes 100 tests in 35 minutes with the first results available in less than 6 minutes.

TABLE 2. Method precision data for Gallery analyzers

Acetic acid

	Mean 0.25 mg/L		Mean 0.42 mg/L		Mean 0.94 mg/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.003	1.3	0.005	1.1	0.018	2.0
Between run	0.002	0.9	0.003	0.8	0.013	1.4
Total	0.004	1.6	0.006	1.4	0.023	2.4

L-Ascorbic acid

	Mean 118 mg/L		Mean 496 mg/L	
	SD	CV %	SD	CV %
Within run	1.014	0.9	4.080	0.8
Between run	0.523	0.4	0.836	0.2
Total	1.141	1.0	4.164	0.8

Citric acid

	Mean 61 mg/L		Mean 126 mg/L		Mean 3779 mg/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	1.4	2.2	1.2	1.0	20.8	0.5
Between run	0.4	0.6	1.0	0.8	60.8	1.6
Total	1.4	2.3	1.6	1.3	64.3	1.7

D-Isocitric acid

	Mean 39 mg/L		Mean 168 mg/L		Mean 554 mg/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.222	0.6	0.816	0.5	4.918	0.9
Between run	0.712	1.8	0.625	0.4	2.286	0.4
Total	0.745	1.9	1.028	0.6	5.424	1.0

D-Lactic acid

	Mean 31 mg/L		Mean 77 mg/L		Mean 172 mg/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.791	2.6	0.884	1.1	5.049	2.9
Between run	0.872	2.8	1.143	1.5	0.426	0.2
Total	1.177	3.8	1.445	1.9	5.067	2.9

L-Lactic acid

	Mean 26 mg/L		Mean 125 mg/L		Mean 182 mg/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.662	2.0	1.605	1.3	2.681	1.5
Between run	0.789	3.0	1.570	1.3	1.631	0.9
Total	1.029	4.0	2.246	1.8	3.138	1.7

Total acids (Juice pH 8)

	Mean 1.70 g/L		Mean 2.51 g/L		Mean 4.28 g/L	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.017	1.0 %	0.015	0.6 %	0.027	0.6 %
Between run	0.036	2.1 %	0.048	1.9 %	0.026	0.6 %
Total	0.040	2.3 %	0.050	2.0 %	0.037	0.9 %

Conclusion

Thermo Scientific Gallery and Gallery Plus automated discrete analyzers have the capability to measure several acids from a single sample without the need for extra method changeover time. These methods cover exact enzymatic measurements but, titration methods are also designed for discrete analysis use. Since the analyzer can automate sample dilutions, methods are designed to accurately measure large concentration ranges. Methods were repeatable; a within run CV% as low as 0.5% can be achieved for citric acid analysis. When comparing the calculated concentration to measured concentrations, an r^2 = 0.997 or better was obtained.

All necessary analysis steps are automated, providing true walk-away time for the operator. Results are fully traceable and designed for ease of use. Optimized applications result in the use of very low volumes of reagents which enable low costs per test. Discrete cell technology offers rapid quality and authenticity testing for juice samples. The analysis of 100 samples using the total acids test requires only 35 minutes.

References

1. Beutler, H. O. Methods of Enzymatic Analysis. L-Ascorbate and L-Dehydroascorbate. 3rd ed; Bergmeyer, H. U., Ed.; VCH Publishers (UK) Ltd., Cambridge, UK, 1988: Vol. VI, pp 376-385.
2. International Federation of Fruit Juice Producers (A.I.J.N), IFUMA22, Determination of Citric Acid (enzymatic).
3. Compendium of International Methods of Analysis, Citric acid, Method OIV-MA-AS313-09 : R2009 1.
4. IHS Engineering 360, Standard: DS - DS/EN 1137, Fruit and Vegetable Juices – Enzymatic determination of citric acid (citrate) content-NADH spectrometric method.

analitica

Thermo SCIENTIFIC