DRAFT TANZANIA STANDARD

Determination of organic acids in fruit juices

TANZANIA BUREAU OF STANDARDS

0. Foreword

Organic acid analysis is one of most basic analyses in food and beverage industry. Organic acids impact the taste, colour and microbiological stability and shelf-life extension of fruit juices. Juices often contain more than one acid and each contribute to total acidity of the juice.

Routine analytical methods express the acidity in terms of the predominant acid instead of total acidity in a particular juice. Organic acids content in juices can be more accurately determined by using High Performance Liquid Chromatography (HPLC).

In the preparation of this standard assistance was drawn from TZS 570:2015 Determination of organic acids in fruit juices published by Tanzania Bureau of Standards

In reporting the result of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off, it shall be done in accordance with TZS 4, *Rounding off numerical values.*

1.0 Scope

This Tanzania standard prescribes method for the determination of organic acids in fruit juices

2.0 Normative References

For the purpose of this standard the following references shall apply:

- TZS 4, *Rounding off numerical values.*
- TZS 59, *Water - Distilled quality - Specification*

3.0 Principle of the method

Juice is eluted through disposable cartridge to remove interference, filtered and injected into liquid chromatographic system; quinic, malic and citric acid are separated by using reversed-phase LC columns in HPLC with UV or PDA detection.

4.0 EQUIPMENT, APPARATUS AND REAGENTS

4.1 Equipment and apparatus

i. HPLC
ii. Column: C18, or equivalent, 250mm x 4.6mm, 5µm particle size;
iii. SPE cartilage 5µm particle size, 10 cm long
iv. Filter membrane 0.45 µm
v. Analytical balance (50g Capacity)
vi. Volumetric flasks 100mL, 250mL, 1000mL
vii. Beakers 100mL, 250mL and 1000mL
viii. Pipettes
ix. pH Meter

x. Stirrer
xi. Measuring cylinders

4.2 Reagents

i. Potassium phosphate monobasic (KH_2PO_4)
ii. Acetonitrile (CAN)– HPLC grade
iii. Methanol- HPLC grade
iv. 85% Phosphoric acid

5.0 PROCEDURES

5.1 Stock preparation (2 mg/ml)

Accurately weigh about 0.200 g analytical chemical standard grade of quinic, malic and citric acids. Dilute combined acids to 100 ml volumetric flask with distilled water, sonicate for 15 minutes to dissolve and then filter.

5.2 Working Standard preparation

Prepare 50mg/L, 100mg/L, 150mg/L, 200mg/L and 250mg/L from stock solution standards (1000mg/l) to the respective concentration above in 100mL volumetric flasks. Calibration curves with good linearities can be obtained for the concentration ranges between 5 - 1000 mg/L for malic quinic and citric acids.

5.3 Sample preparation

Filter 10 ml of sample through filter membrane of 0.45 µm, when the sample is clear and not turbid then inject 5-20 µl. When the sample is turbid and not clear it has to pass through SPE as follows:

- Condition SPE by eluting 10 ml Acetonitrile/Water (50:50) through 10 ml Leur- Lock syringe.
- Remove syringe and pass 10 ml air through cartridge.
- Elute 10 ml of turbid sample through conditioned cartridge, discard first 4-5 ml and collect next 4-5 ml for injection in HPLC
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5.4 Preparation of buffer solution 0.2M (pH 2.4)

Weigh Accurately 27.2 gram of Potassium hydrogen phosphate add about 800mls of distilled water in 1000mls volumetric flask, and adjust to pH 2.4 by using phosphoric acid and then fill to the mark.

5.5 Sample injection in HPLC

Inject working standard solutions and sample into HPLC

6.0 HPLC Conditioning

i. Mobile phase: 100 % Methanol or Methanol: Water (70:30) then followed by water and then phosphate buffer. Reverse order at end of working day, never let methanol come into contact with phosphate buffer.

ii. Elution: Low pressure gradient/ Isocratic

iii. Column: C18; 250mm x 4.6mm, 5µm;

iv. Detection: 214 nm

v. Flow rate: 0.8 ml/min

vi. Injection volume: 5- 20µL

7.0 Calculation with calibration curve

7.1 If a dilution is made for the sample, the final result shall be:

Result (mg/L) = HPLC concentration reading X Dilution factor

If the analyzed sample is solid, the final result shall be calculated as follows:

$$\text{Results (mg/Kg)} = \frac{\text{HPLC concentration reading X Dilution factor X Total volume (or in mg/L)}}{\text{Weight taken (in g) or Volume taken (in ml)}}$$

8.0 Calculation without calibration curve

$$= \frac{PA_{SAMPLE}}{PA_{STANDARD}} \times \frac{\text{Injection Volume}_{STANDARD}}{\text{Injection Volume}_{SAMPLE}} \times \text{Concentration of standard}$$

WHERE:

PA_{sample}= Peak Area or Height of sample

$PA_{STANDARD}$ =Peak Area or Height of standard