

HIRANUMA APPLICATION DATA	Automatic Titrator	Data No.	A6	Jun 6, 2017
FOOD	Continuous measurement of acid (citric acid) and amino nitrogen in grapefruit juice			

1. Abstract

Successive measurement of acid (citric acid) and amino nitrogen in grapefruit juice and its titration process will be introduced.

The amino nitrogen is determined by Formol method which is simplified method of Van Slyke method. Firstly, measure the acid (citric acid) in sample solution by the titration with sodium hydroxide standard solution until the pH reaches 8.1 as described in the formula (1). After that, add sodium hydroxide standard solution to adjust the pH to 8.4. Add neutral buffered formalin solution to react amino nitrogen with formalin for generating carboxylic acid (formula (2)). Titrate the carboxylic acid with sodium hydroxide until the pH reaches 8.4, and measure amino nitrogen based on formula (3). Figure 1 shows measurement flowchart on this procedure.

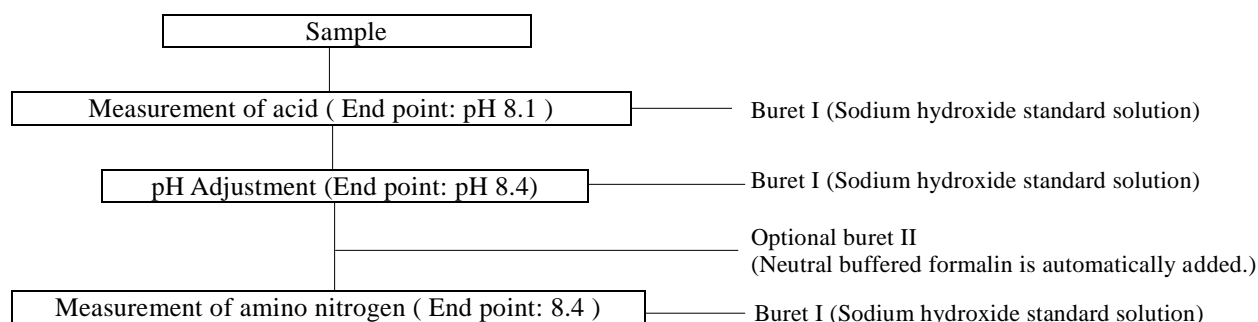
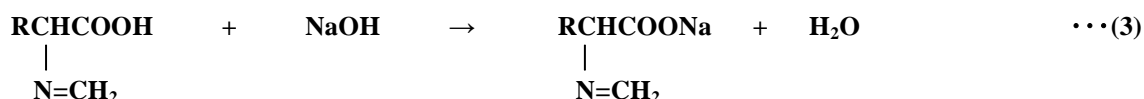
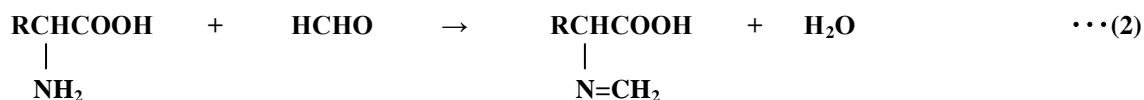
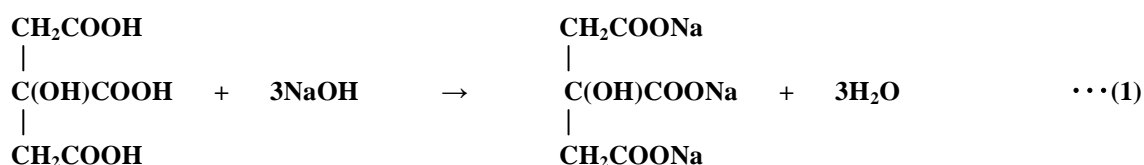


Figure 1 Flowchart of measurement

2. Configuration of instruments and reagents

(1) Configuration

Main unit	:	Hiranuma Automatic Titrator COM series
Option	:	One buret
Electrodes	:	Glass electrode GE-101B Reference electrode RE-201Z Thermistor electrode TE-403

※Glass reference combination electrode can be used instead of glass electrode and reference electrode. The glass reference combination electrodes are listed below.

- GR-501BZ (Fixed sleeve type)
- GR-511BZ (Movable sleeve type)

(2) Reagents

Titrant : 0.1 mol/L sodium hydroxide standard solution
 Additive solution : Neutral buffered formalin solution 15mL
 (Formalin adjusted to pH 8.4 by adding 1 mol/L or 0.1 mol/L sodium hydroxide solution)

3. Measurement procedure

(1) pH calibration

The pH calibration should be performed with neutral phosphate (pH 6.86) and borate (pH 9.18) pH standard solution.

(2) Measurement of acid

- i) Dispense 5 g of sample into a 200 mL beaker and accurately weigh it.
- ii) Add pure water to make about 100 mL of solution.
- iii) Immerse the electrodes and start to titrate with sodium hydroxide standard solution. Perform it until the pH reaches 8.1.
- iv) Add 0.1 mol/L sodium hydroxide standard solution to adjust the pH to 8.4.
- v) After the above process, 15 mL of neutral buffered formalin solution is automatically dispensed.
(Option: Buret)
- vi) Titrate with 0.1 mol/L sodium hydroxide standard solution until the pH reaches 8.4.
- vii) Measure the blank with the procedure ① ~ ③ without sample.

4. Measurement conditions and results

Examples of titration conditions

Measurement of blank

Cndt No.	1	ConstantNo.	1	Mode No.	14
Method	Set	Size	0.0 g	Pre Int	0 sec
Buret No.	1	Blank	0.0 mL	Del K	0
Amp No.	1	Molarity	0.1 mol/L	Del Sens	0 mV
D. Unit	pH	Factor	1.005	Int Time	3 sec
S-Timer	5 sec	K	0.0	Int Sens	3 mV
C.P. mL	0.0 mL	L	0.0	Brt Speed	2
Direction	↑	Unit	mL	Pulse	8
D.P. mL	0.0 mL	Formula	D		
End Point pH	8.10 pH	Digits	3		
Over mL	0.0 mL	Auto In Pram.	Non		
Max.Vol.	1 mL				

Measurement of sample

(1) Titration of acid (citric acid) with sodium hydroxide standard solution

Cndt No.	2	ConstantNo.	2	Mode No.	20
Method	Set	Size	4.6569 g	Pre Int	0 sec
Buret No.	1	Blank	0.016 mL	Del K	5
Amp No.	1	Molarity	0.1 mol/L	Del Sens	0 mV
D. Unit	pH	Factor	1.005	Int Time	3 sec
S-Timer	10 sec	K	64	Int Sens	3 mV
C.P. pH	6.50 pH	L	0	Brst Speed	4
Direction	↑	Unit	%	Pulse	40
T Timer	15 sec	Formula	(D-B)*K*F*M/(S*10)		
D.P. pH	0 pH	Digits	3		
End Point pH	8.10 pH	Auto In Pram.	Non		
Over mL	0.0 mL				
Max.Vol.	20 mL				

(2) Addition of sodium hydroxide standard solution to adjust the pH to 8.4

Cndt No.	3	ConstantNo.	3	Mode No.	21
Method	Set	Size	4.6569 g	Pre Int	0 sec
Buret No.	1	Blank	0.0 mL	Del K	0
Amp No.	1	Molarity	0.0 mol/L	Del Sens	0 mV
D. Unit	pH	Factor	0	Int Time	3 sec
S-Timer	0 sec	K	0	Int Sens	3 mV
C.P. mL	0.00 mL	L	0	Brst Speed	2
Direction	↑	Unit	mL	Pulse	20
D.P. mL	0.0 mL	Formula	D		
End Point pH	8.40 pH	Digits	3		
Over mL	0.0 mL	Auto In Pram.	Non		
Max.Vol.	20 mL				

(3) Dispense neutral buffered formalin solution.

Cndt No.	4
Method	Disp
Buret No.	2
S-Timer	0 sec
Disp Vol.	15.00 mL

(4) Titration of amino nitrogen with sodium hydroxide standard solution

Cndt No.	5	ConstantNo.	5	Mode No.	5
Method	Set	Size	4.6569 g	Pre Int	0 sec
Buret No.	1	Blank	0.0 mL	Del K	5
Amp No.	1	Molarity	0.1 mol/L	Del Sens	0 mV
D. Unit	pH	Factor	1.005	Int Time	3 sec
S-Timer	30 sec	K	14	Int Sens	3 mV
C.P. pH	6.00 pH	L	0	Brst Speed	2
Direction	↑	Unit	mg/100	Pulse	40
T Timer	15 sec	Formula	(D-B)*K*M*F/(S*10)*1000		
D.P. pH	6.5 pH	Digits	3		
End Point pH	8.40 pH	Auto In Pram.	Non		
Over mL	0.1 mL				
Max.Vol.	20 mL				

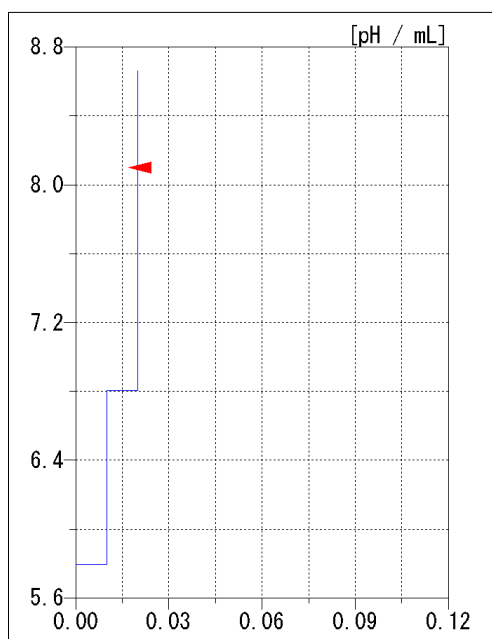
Measurement results

Measurement of blank

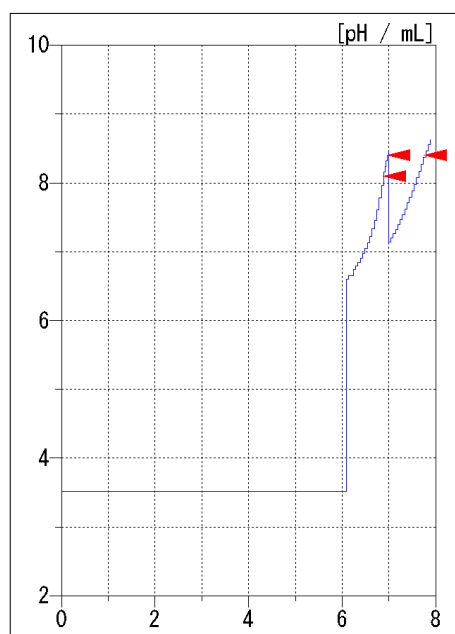
Number of measurement	Size(g)	Titer(mL)
1	—	0.015
2	—	0.017
3	—	0.017
Average (Blank)		0.016 mL

Measurement of sample

Number of measurement	Size(g)	Acid (Citric acid)		Amino nitrogen	
		Titer (mL)	Concentration (%)	Titer (mL)	Concentration (mg/100g)
1	4.6181	6.799	0.945	0.785	23.917
2	4.7046	6.922	0.944	0.796	23.806
3	4.6569	6.874	0.947	0.791	23.899
Average		0.945 %		23.874 mg/100g	
Standard deviation		0.002 %		0.060 mg/100g	
Coefficient of variation		0.162 %		0.250 %	



Measurement of blank



Measurement of sample

Examples of titration curves

5. Note

Simplified method of Van Slyke method is utilized in this article. However, please make sure whether it is applicable for your standard-compliant when using this method.

Procedure for the determination of taurine described in *Japanese Pharmacopoeia (rev. 17)* is similar to this method.

Keyword : Grapefruit juice, Amino nitrogen, Citric acid, Van Slyke method, Formol method, Formaldehyde

*Some measurement would not be possible depending on optional configuration of system.