COD 11003 20 tests
STORE AT 15-30ºC
Reagents for measurement of vanilmandelic acid concentration Only for *in vitro* use in the clinical laboratory

VANILMANDELIC ACID

BioSystems REAGENTS & INSTRUMENTS

CE

VANILMANDELIC ACID

PRINCIPLE OF THE METHOD

Vanilmandelic acid (VMA) is retained by an anionic exchange resin, being eluted thereafter once the interfering substances are washed away. The VMA is quantified spectrophotometrically as vanillin after periodate oxidation under alkaline conditions[1,2,3].

CONTENTS AND COMPOSITION

1. Reagent. 1x25 mL. Sodium phosphate buffer 0.6 mol/L pH 7.0, sodium azide 15 mmol/L.
2. Reagent. 1x120 mL. Sodium acetate buffer 0.2 mol/L pH 6.1, sodium azide 15 mmol/L.
3. Reagent. 1x170 mL. Sodium chloride 2 mol/L, sodium azide 15 mmol/L.
4. Microcolumns. 1x20. Contain a pre-weighted amount of a buffered anionic exchange resin and sodium azide 1.5 mmol/L.

A. Reagent. 1x30 mL. Potassium carbonate 3 mol/L.
WARNING: H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H335: May cause respiratory irritation. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

B. Reagent. 1 for 10 mL. Sodium *m*-periodate powder 0.12 mol/L, after reconstitution.
DANGER: H272: May intensify fire; oxidiser. H301: Toxic if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H335: May cause respiratory irritation. P301+P310: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

C. Reagent. 1 for 10 mL. Sodium metabisulfite powder 0.67 mol/L, after reconstitution.
DANGER: H302: Harmful if swallowed. H318: Causes serious eye damage. EUH031: Contact with acids liberates toxic gas.

S. Standard. 1 for 5 mL. Concentration of reconstituted standard is given on the label. The concentration value is traceable to the Standard Reference Material 925 (National Institute of Standards and Technology, USA).

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 15-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the reagent blank over 0.050 at 360 nm.
- Microcolumns: Absence of buffer over the resin bed.

AUXILIARY REAGENTS

- Concentrated hydrochloric acid (analytical grade).

REAGENT PREPARATION

Reagents (B) and (C): Dissolve the dry powder in 10 mL of distilled water. Stable for 5 months at 2-8ºC.

Standard (S): Dissolve the dry powder in 5 mL of Reagent (3) and add one drop of concentrated hydrochloric acid. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

- Spectrophotometer or photometer with a 360 nm filter (358-362).
- Thermostatic water bath.

SAMPLES

Urine. 24 hours specimens collected by standard procedures.

Keep at 2-8ºC and use within 24 hours. Samples can be stored at 2-8ºC for a maximum of 5 days or 1 month at –20ºC if adjusted to pH 1-2 with concentrated hydrochloric acid (HCl). Centrifuge or filter before testing.

PROCEDURE

Sample Preparation

1. Pipette into a test tube (Note 1):

Sample	1.0 mL
Reagent (1)	1.0 mL

Chromatographic Separation

2. Remove the upper cap of the Microcolumn (4) and then snap the tip off the bottom. Push the upper disc down to the resin surface taking care not to compress it. Let the microcolumn drain completely to waste.
3. Pour the contents of the tube (treated sample) into the microcolumn and let it drain to waste.
4. Wash the tube with 2-3 mL of distilled water and pour it into the microcolumn. Let it drain to waste.
5. Add to the microcolumn:

Reagent (2)	5.0 mL	Let the microcolumn drain to waste

6. Place the microcolumn over a test tube and pipette:

Reagent (3)	6.0 mL	Collect the eluate

7. Mix the eluate thoroughly (Note 2).

Colorimetry

8. Pipette into labelled test tubes:

	Reagent Blank	Standard	Sample Blank	Sample
Eluate	—	—	1.0 mL	1.0 mL
Reagent (3)	1.0 mL	0.9 mL	—	—
Standard (S)	—	0.1 mL	—	—
Reagent (A)	0.4 mL	0.4 mL	0.4 mL	0.4 mL
Reagent (B)	0.1 mL	0.1 mL	—	0.1 mL

9. Shake thoroughly and incubate the tubes for 30 minutes at 37ºC. Then add:

Reagent (C)	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Reagent (B)	—	—	0.1 mL	—

10. Read the absorbance (A) of the Sample Blank, Sample and Standard against the Reagent Blank at 360 nm. The absorbance is stable for at least 2 hours.

CALCULATIONS

The VMA concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample} - A_{Sample\ Blank}}{A_{Standard}} \times \frac{V_E}{V_S} \times \frac{V_{STC}}{V_{EC}} \times C_{ST} \times \frac{1}{Rec} = C_{Sample}$$

The volume of sample (V_S) is 1 mL, the volume of eluate (V_E) is 6 mL, the volume of eluate in the colorimetry (V_{EC}) is 1 mL, the volume of Standard in the colorimetry (V_{STC}) is 0.1 mL, the concentration of the Standard (Cst) is given on the label and the mean recovery (Rec) is 0.888. The following formulas are deduced for the calculation of the concentration:

$\frac{A_{Sample} - A_{Sample\ Blank}}{A_{Standard}} \times Cst$	$\times 0.676 = C_{Sample}$

The amount of VMA per 24 hours urine is calculated using the following general formulas:

mg/L VMA µmol/L VMA	$\times V_{Urine/24\ hours}$ (L) =	mg VMA/24 hours µmol VMA/24 hours

REFERENCE VALUES

Urine[6]: < 13.6 mg/24-h = < 68.6 µmol/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Control Urine (cod. 18036 and 18037) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.9 mg/L = 9.7 µmol/L.
- Linearity limit: 300 mg/L = 1500 µmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
11.2 mg/L = 56 µmol/L	5.0 %	25
43.2 mg/L = 216 µmol/L	2.5 %	25

- Reproducibility (run to run):

Mean Concentration	CV	n
11.2 mg/L = 56 µmol/L	8.6 %	25
43.2 mg/L = 216 µmol/L	8.2 %	25

- Sensitivity: 7.50 mA·L/mg = 1.50 mA·L/µmol.
- Trueness: Results obtained with samples with added VMA did not show systematic differences when compared with the theoretical concentrations. Details of the comparison experiments are available on request.
- Interferences: Gentisic (1000 mg/L), Homogentisic (2000 mg/L) and 5-Hydroxyindolacetic (50 mg/L) acids do not interfere. p-Hydroxymandelic acid (>12,5 mg/L) interferes. Some food components, drugs and substances may interfere[5].

DIAGNOSTIC CHARACTERISTICS

Vanilmandelic acid is the main end product of the catecholamines metabolism and is excreted in urine. Its measurement reflects the total production of adrenaline and noradrenaline in the body.

Increased daily excretion values of vanilmandelic acid are associated with catecholamine-secreting neurochromaffin tumors like pheochromocytomas, neuroblastomas or paragangliomas[4,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. If the sample has been stored acidified with an excess of hydrochloric acid, check and adjust the pH at 6.5 - 7.5 with diluted sodium hydroxide
2. The test can be interrupted at this point. Store the eluate in the tube sealed at 2-8ºC for a maximum of 24 hours.

BIBLIOGRAPHY

1. Wybenga D, Pileggi VJ. Quantitative determination of 3-methoxy-4-hydroxy-mandelic acid (VMA) in urine. *Clin Chim Acta* 1966; 16: 147-154.
2. Farndon JR, Davidson HA, Johnston IDA, Wells SA. VMA excretion in patients with Pheochromocytoma. Ann Surg 1980; 191: 259-263.
3. John J. Pisano , J. Richard Crout, David Abraham. Determination of 3-methoxy-4-hydroxymandelic acid in urine. Clin Chim Acta 1962; 7: 277-284.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)

Quality System certified according to EN ISO 13485 and EN ISO 9001 standards