VANILLYLMANDELIC ACID

Chromatographic – Spectrophotometric Determination
of 3-Methoxy-4-Hydroxymandelic Acid
(Vanillylmandelic Acid VMA)
in Urine

40 tests **REF 3626**

INTENDED USE

Kit for quantitative *in vitro* determination of Vanillylmandelic Acid in urine.

PRINCIPLE

VMA is adsorbed on an anionic buffer balanced resin. After interfering substances washing, VMA is eluted and spectrophotometrical defined by oxidation to vanillin, obtained by methaperiodate in alkaline medium.

REAGENTS AND COLUMNS

Kit components:	REF 3626
REAGENT 1 Phosphate buffer	**1 x 45 ml**
REAGENT 2 Sodium chloride	**2 x 220 ml**
***REAGENT 3** Carbonate potassium	**1 x 50 ml**
***REAGENT 4** Methaperiodate sodium (pre dosed)	**1 vial**
***REAGENT 5** Methasulphite sodium (pre dosed)	**1 vial**
STANDARD Vanillylmandelic acid 100 mg/L	**1 x 2 ml**

NOTICE: store it sealed; close the vial immediately after use and tighten the screw cap. The solution is ethyl alcoholic based, which increases the vanillylmandelic acid concentration during evaporation, with consequent falsely low values.

COLUMNS Chromatographic columns **40**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and columns are stable up to the expiration date on the label.

PREPARATION OF THE WORKING REAGENTS

REAGENT 4

Dissolve the contents of a vial of Reagent 4 with 25 ml of distilled water. Shake gently until complete dissolution.
STABILITY: at least 6 months at 2-8°C. Store it sealed.

REAGENT 5

Dissolve the contents of a vial of Reagent 5 with 25 ml of distilled water. Shake until complete dissolution.
STABILITY: at least 6 months at 2-8°C. Store it sealed.

SAMPLE

24 hour urine.
Collect the 24 hour urine, mix it and measure the volume.
Store at 2-8°C. If VMA is not determined within the following day, pour 10 ml of urine in a vial and, mixing well, bring the pH between 4 and 5, adding 1-2 drops of glacial acetic acid. Centrifuge or filter the urine before use.
STABILITY: one week at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	360 nm
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37°C
Method:	spectrophotometric
Linearity:	125 mg/L
Sensitivity:	1.5 mg/L
Recovery:	98 ± 2 %
C.V.:	3%

PREPARATION OF THE SAMPLE

Let the reagents reach room temperature. Reagents 1 and 3 may precipitate: shake reagents well before use.
Pipette into a test-tube:

Urine	1.0 ml
Reagent 1	1.0 ml

Mix well and make sure the pH is ranged between 6.5 and 7.5, otherwise adjust it with some drops of diluted sodium hydroxide.
The tube contents will be used in the test.

PREPARATION OF THE COLUMN

Remove the cap and put it back. This operation will create a pressure inside the column, to help the liquid leak.

Wear gloves and snap the bottom tip off. Let the liquid completely drain.

CHROMATOGRAPHIC SEPARATION

Pour the contents of the sample test-tube (2 ml) into a column and let the liquid completely flow. Discard the eluate. Pipette 3 ml of distilled water in the same test-tube and pour into the column. Let the liquid completely drain. Discard the eluate.
Pipette into the column:

Distilled water	5.0 ml	discard the eluate

Put the column on a 15-20 ml test-tube and pipette:

Reagent 2	2.0 ml	collect the eluate

Wait until the liquid is completely drained. Repeat the addition of Reagent 2 for 4 times, letting the liquid completely flow. At the end, 10 ml of Reagent 2 eluate will be obtained.

COLORIMETRIC REACTION

Mix the collected eluate and pipette into labeled test-tubes:

	Blank sample	Sample	Blank reagent	Standard
Eluate	2.0 ml	2.0 ml	---	---
Reagent 2	---	---	2.0 ml	2.0 ml
Standard	---	---	---	20 µl
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 4	---	0.25 ml	0.25 ml	0.25 ml

Mix thoroughly and incubate in a thermostatic bath at 37°C for 30 minutes. Pipette:

Reagent 5	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Reagent 4	0.25 ml	---	---	---

Mix thoroughly and read the absorbencies of the sample (As), the blank sample (Asb) and the standard (Astd) at 360 nm against the blank reagent.

CALCULATION

VMA (mg/L) = (As - Asb) / Astd x 10
VMA (mg/24h)= mg vanillylmandelic acid / L x L 24h urine

REFERENCE VALUES

24 hour urine:
adults 1 - 11 mg/24 hours
children 0.10 - 0.18 mg/Kg body weight/24 hours
VMA/creatinine ratio:
adults 1-8 mg VMA/g creatinine in urine
children 2-12 mg VMA/g creatinine in urine

NOTES

1. No particular restriction in the diet preceding the urine collection is required, as the blank sample subtracts dietary vanillin, which may give falsely high values.
2. Some drugs influence the VMA urinary excretion:
   - increased values result from administration of insulin, reserpine, epinephrine, norepinephrine
   - decreased values result from administration of morphine, pentobarbital, chloropromazine, iproniazid.
   In both cases, adjust the results as it follows:
   VMA (mg/L)=[(A360 S - A360 SB) - (A380 S - A380 SB)] / (A360 ST - A380 ST) x 10
   Perform the readings within 60 minutes from the end of the reaction.
3. The VMA/creatinine ratio in urine allows to perform the test on a single micturition and may give quite precise results, within certain limits, for screening purposes.
   Nevertheless, the 24 hour urine is preferred and it is required when the VMA/creatinine ratio is close or lightly beyond normal values limit.

REFERENCE

1. D. Wybenga et V.J. Pileggi, “Clin. Chim. Acta”, 16 (1), 147-154 (1966)

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