

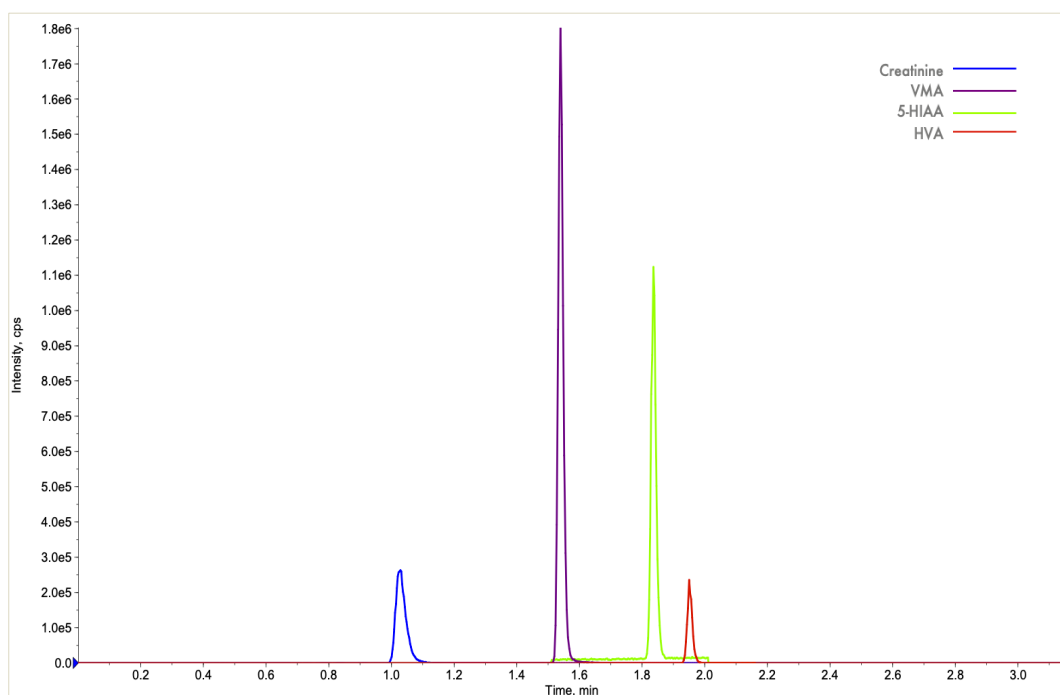
## FLOMASS<sup>®</sup> VMA, HVA AND 5-HIAA IN URINE

Catecholamines (Epinephrine, Norepinephrine and Dopamine) are neurotransmitters produced by adrenal gland. Their release into the body causes physiological changes, including increased heart rate and arterial pressure.

Catecholamines metabolism involves conversion to Metanephrines (Metanephrine, Normetanephrine and 3-Methoxytyramine), molecules linked to pheochromocytoma and others neuroendocrine tumors. Further metabolism of Metanephrines lead to formation of: VMA (Vanillylmandelic Acid), that at high levels could be a marker of pheochromocytoma, 5-HIAA (5-Hydroxyindoleacetic Acid), possible tumoral marker of carcinoid syndrome, HVA (Homovanillic Acid), useful marker in diagnosis of neuroblastoma and neuroendocrine pathologies.

Creatinine in a molecule arising from Creatine degradation reaction in muscles, involved in energy-yielding metabolism. It is released into blood flow and then undergoes to glomerular filtration, then it is secreted via urine.

24 h-Creatinine blood/plasma/urine concentration is commonly used as marker of renal function. High Creatinine levels indicate nephrons damage and renal impairment, whereas low levels can occur in case of anemia, muscular atrophy, or debilitating conditions of the organism. Creatinine levels are higher in men because of higher muscular mass.



## HPLC-MS/MS system conditions

**Ionization:** ESI negative mode

**MS/MS:** specific MRM

**Injection volume:** 1  $\mu$ l (variable according to instrumental sensitivity)

**Running time:** 3.2 min

**Column heater:** 45°C

**Column conditioning:** column should be conditioned for 5 min at chromatographic gradient initial condition. Then, run 3 blank injections (MPA only) using the gradient as above.

## Sample preparation

- Prepare a mix with 20  $\mu$ l of Internal Standard Mix + 960  $\mu$ l of Diluting Solution sufficient for the number of samples to be analyzed
- Pipette 20  $\mu$ l of urine in a vial (if human, preserved with 0.1% HCl)
- Add 980  $\mu$ l of the Mix solution obtained in previous step of the procedure
- Vortex 30 sec
- Centrifuge for 5 min at 12000 rpm
- Pipette 200  $\mu$ l of supernatant in an autosampler vial with low volume insert
- Inject 1-10  $\mu$ l according to instrumental sensitivity and analyze with HPLC-MS/MS technique

## Performance

ANALYTE	LINEARITY (ng/mL)	LLOD (ng/mL)	LLOQ (ng/mL)	CV% INTRA	CV% INTER
VMA	0.33 – 750	0.10	0.33	2.6 – 5.2	2.7 – 5.0
5-HIAA	0.46 – 750	0.14	0.46	2.8 – 6.1	4.5 – 5.7
HVA	0.49 – 750	0.15	0.49	2.8 – 5.9	4.0 – 6.0
Creatinine	11.70 – 7500	3.50	11.70	3.2 – 4.7	2.8 – 5.9

## Ordering guide

EUM19100	FloMass® VMA, HVA and 5-HIAA in Urine	100 assays
EUM19041	7-Levels Calibrators, lyophil.	2 x 7 x 0.5 mL
EUM19051	2-Levels Controls, lyophil.	2 x 2 x 0.5 mL
EUM00C17	Chromatographic Column	1 pc
EUM00A14	Precolumn	4 pcs