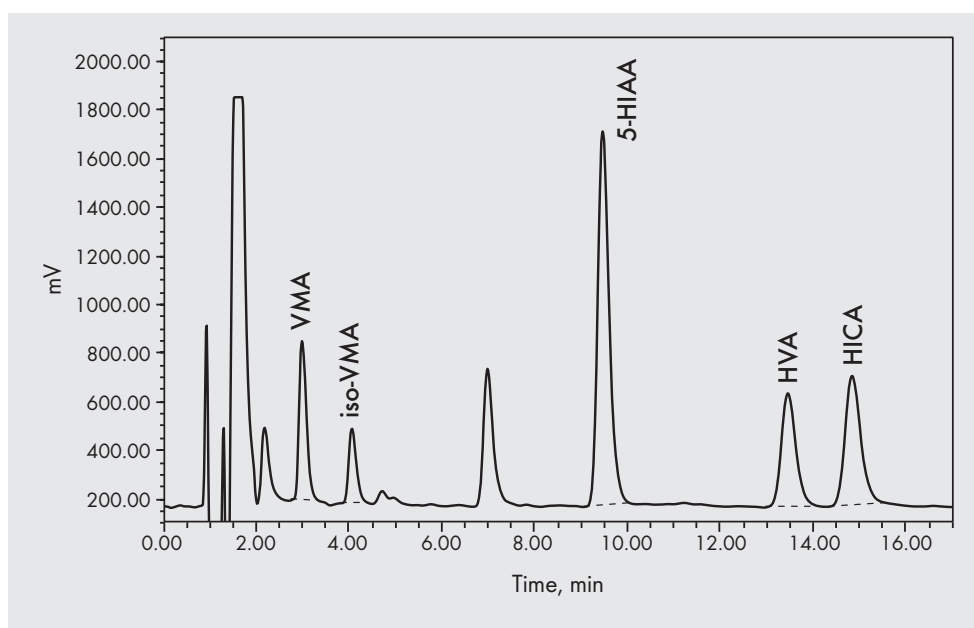


VMA, HVA and 5-HIAA in Urine

Kit for HPLC Analysis

- > No pH-adjustment necessary
- > Simplified sample preparation
- > 2 internal standards available



Neuroblastoma is a neoplastic disease of infants and early childhood and is the third most common cancer disease in children. Because of the close histological relationship of this tumor to the autonomic sympathetic nervous system, these patients excrete increased amounts of the catecholamine metabolites vanillylmandelic acid (VMA) and homovanilic acid (HVA) into urine. The measurement of VMA and HVA serves as a screening test for neuroblastoma. The concentration of 5-hydroxyindoleacetic acid (5-HIAA) in urine is a diagnostic marker for the carcinoid syndrome. This malignant proliferation of the enterochromaffin cells of the gastrointestinal tract leads to an excessive production of the tissue hormone serotonin, the major metabolite of which is 5-HIAA.

This kit allows the easy and safe routine analysis of VMA, HVA and 5-HIAA in urine with an isocratic HPLC system and an electrochemical detector.

Specifications

Linearity:	up to 78 mg/l (VMA, 5-HIAA) up to 51 mg/l (HVA)
Limit of quantification:	0.5 mg/l
Intraassay:	CV = 3.8 %
Interassay:	CV = 4.5 %
Recovery:	70 % (VMA), 82 % (HVA), 66 % (5-HIAA)
Analysis time:	< 18 min

Pre-Analytic Treatment

Specimens: urine

Determination of VMA/HVA: collect urine in 10 ml HCl 10–25 %. Stability at room temperature 7 days, at +2 to +8 °C 14 days, below -18 °C at least 3 months.

Determination of 5-HIAA: collect urine in 10 ml glacial acetic acid. Stability at +2 to +8 °C light protected up to 2 weeks.

Sample Preparation

- Add 1 ml Internal Standard (buffered solution) to 50 µl urine and mix.
- Apply sample to the Sample Clean Up Column. Draw through by vacuum or centrifugation; discard the effluent.
- Apply 3 ml Wash Buffer 1 to the Sample Clean Up Column. Draw through by vacuum or centrifugation, discard the effluent.
- Apply 3 ml Wash Buffer 2 to the Sample Clean Up Column. Draw through by vacuum or centrifugation, discard the effluent. Repeat this step once.
- Apply 2 ml Elution Buffer. Draw through by vacuum or centrifugation. Collect the eluate, add 100 µl Finisher and mix well.
- Inject 10–20 µl eluate into the HPLC system.

HPLC Parameters

For the analysis of VMA, HVA and 5-HIAA in urine any isocratic HPLC system with electrochemical detector is suitable.

Injection volume:	10–20 µl
Flow rate:	1.0 ml/min
Potential:	~ 760 mV
Column temperature:	ambient (~ 25 °C)

Ordering Information

Order no.	Product
1000/B	VMA, HVA, 5-HIAA in Urine by HPLC Kit for 100 determinations
Components available separately:	
1011	Mobile Phase, 1000 ml
1012	Mobile Phase, 10 x 1000 ml
1003/B	VMA, HVA, 5-HIAA Calibration Standard (lyoph.), 5 x 1 ml
1009	VMA, HVA, 5-HIAA Urine Calibration Standard (lyoph.), 5 x 5 ml
1004/B	Internal Standard (iso-VMA), 100 ml
1005	Wash Buffer I, 300 ml
1006	Wash Buffer II, 300 ml
1077	Elution Buffer, 200 ml
1013	Finisher, 10 ml
1008	Sample Clean Up Columns, 50 pcs.

Automated sample preparation on
the GILSON® Aspec™ available

Startup Accessories:	
1100/B	HPLC Column, equilibrated, with test chromatogram, 1 pc.
1099	Potential Optimization Mix (VMA, iso-VMA) (lyoph.), 5 x 0.5 ml
51303/B	Internal Standard (HICA), for further improvement of 5-HIAA analysis, 5 ml
15009	PEEK-encased prefilter, 5 µm, 5 pcs.
15010	PEEK prefilter housing, 1 pc.
17001	Precolumn cartridge holder 4/10, 1 pc.
17002	Precolumn cartridge 4/10, 1 pc.

Chromsystems Controls (lyoph.):	
0040	Endocrine Urine Control, Normal Range, 10 x 8 ml
0050	Endocrine Urine Control, Pathological Range, 10 x 8 ml

Accessories for electrochemical detectors:	
41203	Glassy carbon working electrode, activated and tested, 1 pc.
41211	Reference electrode Ag/AgCl, 1 pc.
41239	KCl solution, 3 mol/l, 50 ml

