Determination of urinary vanillylmandelic acid, homovanillic acid and 5-hydroxyindoleacetic acid by LC-MS/MS for clinical research

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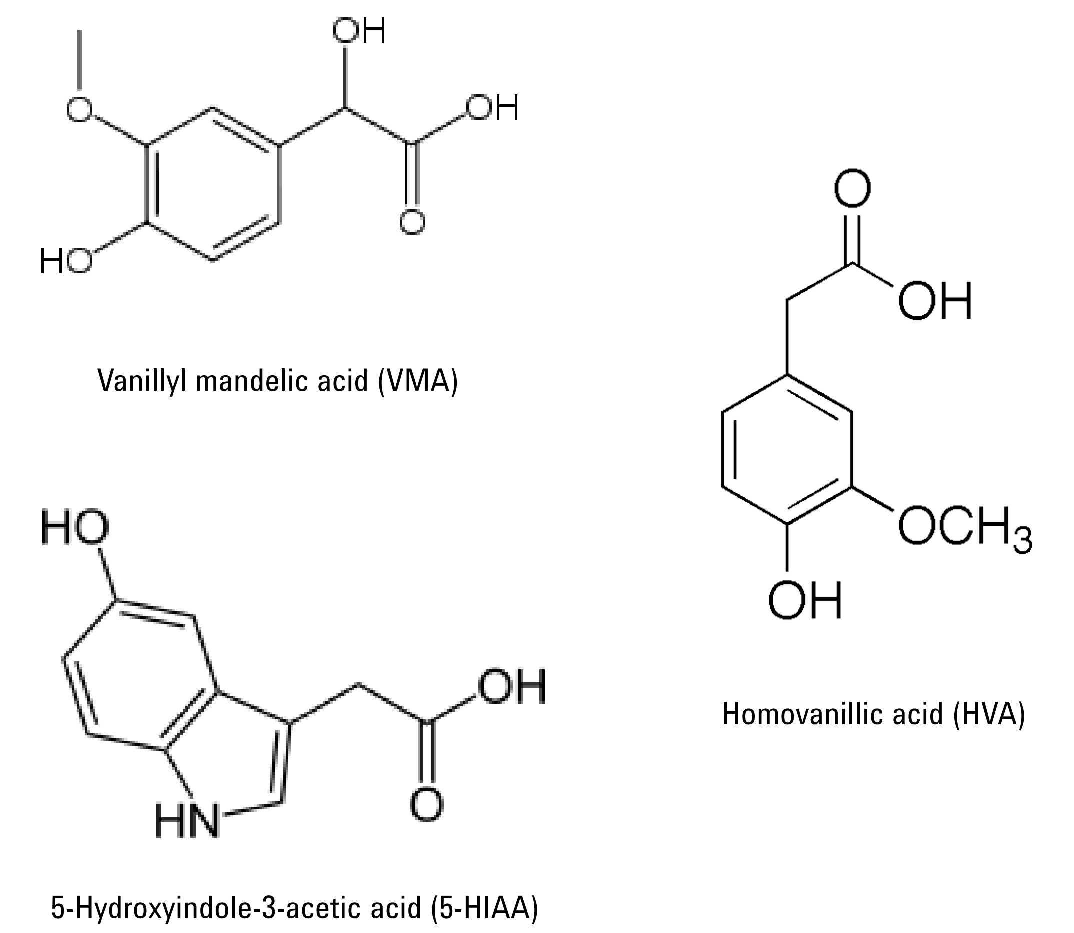
Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS method has been developed for the quantitation of vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in urine. The level of creatinine in urine can also be quantified at the same time.



Vanillyl mandelic acid (VMA)

Homovanillic acid (HVA)

5-Hydroxyindole-3-acetic acid (5-HIAA)

Figure 1. Structures of studied compounds.

A simple sample preparation involving only a dilution is used for the simultaneous determination of VMA, HVA and 5-HIAA in urine. Calibrators were created by spiking clean urine with various concentrations of each analyte. The chromatographic system consists of a pentafluorophenyl (PFP) column and a mobile phase comprised of methanol and water containing 0.2% formic acid. Quantifier and qualifier MRM transitions were monitored and deuterated internal standards were included for each analyte to ensure accurate and reproducible quantitation.

Experimental

Sample Preparation

Calibrators (Cerilliant) are prepared with clean urine matrix (Golden West Biologicals). Isotopically labelled Internal standards (Cerilliant) and BioRad Lyphocheck human urine controls were used. Calibrators, controls and urine samples are diluted 1 in 10 with 0.2% formic acid in water containing internal standards.

LC Method

Agilent 1290 HPLC binary pump, well plate sampler with thermostat, temperature-controlled column compartment

Parameter	Value
Analytical Column	Agilent Pursuit 3 PFP, 2x150mm, 3µm PN: A3051150X020
Guard Column	Agilent Meta Guard column Pursuit 3 PFP, 2 mm PN: A3051MG2
Column Temp	40°C
Injection Volume	20 µl
Autosampler Temp	4°C
Needle Wash	Flush port for 10 seconds
Mobile Phase A	0.2% Formic Acid in Water
Mobile Phase B	Methanol
Flow Rate	0.3 ml/min
1290 Pump Gradient	Time (min.) %B 0.0 15 0.5 15 2.5 60 3.0 95 6.0 95
Stop Time	6 min.
Post Time	3 min.

Table 1. LC Parameters

Experimental

MS Method

Agilent 6460 QQQ with JetStream technology

Ion mode:	AJS ESI(-)
Gas temperature:	325 °C
Drying gas (nitrogen):	10 L/min
Nebulizer gas (nitrogen):	50 psi
Sheath gas (nitrogen):	300 °C
Sheath flow:	11 L/min
Capillary voltage:	3500V
Nozzle voltage:	1500V
Q1/Q3 Resolution:	0.7 unit
Dwell time:	20 msec
Delta EMV:	200V

Source conditions and MRM transitions (table 2) were determined and optimized automatically using Agilent Optimizer software.

Compound	Prec Ion	Prod Ion	Frag (V)	CE (V)	CAV (V)
HVA*	181.1	137	70	4	2
HVA	181.1	122	70	16	2
HVA-D5	186.1	142.1	70	4	2
VMA*	197	137	100	24	2
VMA	197	138	100	10	2
VMA-D3	200.1	140	100	24	2
5-HIAA*	190.1	146.1	70	6	2
5-HIAA	190.1	144	70	24	2
5-HIAA-D5	195.1	151.1	70	8	2

Table 2: MRM Transitions table (*Quantifier)

Results and Discussion

Chromatographic separation of all analytes (figure 2) is achieved in less than four minutes through the use of a pentafluorophenyl (PFP) column. The separation of interferences present in urine from HVA and 5-HIAA are especially critical. Without proper separation by retention time, these compounds can cause interferences leading to inaccurate quantitation.

Absolute ion suppression and matrix effect were observed but were compensated for by the internal standards (table 3).

Commercially available quality controls (QC) material (BioRad) were used to measure the accuracy and precision of this method. Results (table 4) show agreement with expected values and excellent precision at both levels.

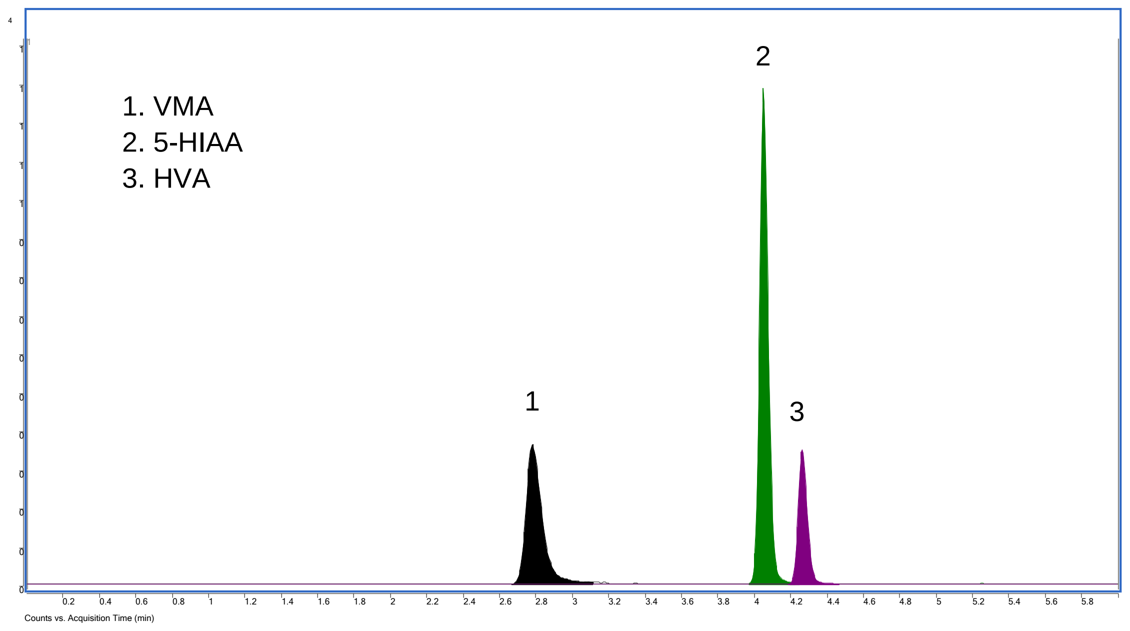


Figure 2. Chromatography for VMA. 5-HIAA and HVA

Compound	Matrix effects % (n = 10)		Accuracies % With ISTDs corrections (n = 10)		
	Average	SD	Range	Average	SD
VMA	91.5	6.4	95.6-108.3	100.0	3.9
5-HIAA	93.2	3.3	94.6-115.2	100.0	5.8
HVA	91.3	1.5	92.9-103.9	100.0	3.1

Table 3. Ion suppression and matrix effects

Results and Discussion

Compound	Level 1			Level 2		
	Range (HPLC)	Measured	CV (%)	Range (HPLC)	Measured	CV (%)
VMA	2.1-3.1	2.5	2.3	11.2-16.8	14.6	2.4
5-HIAA	2.2-3.4	2.8	2.0	20.8-31.2	27.6	2.8
HVA	1.0-1.4	1.3	5.8	13.0-19.6	15.8	3.9

Table 4. Results in mg/L (n=5) of BioRad QC run by LC/MS/MS (range determined by BioRad using HPLC)

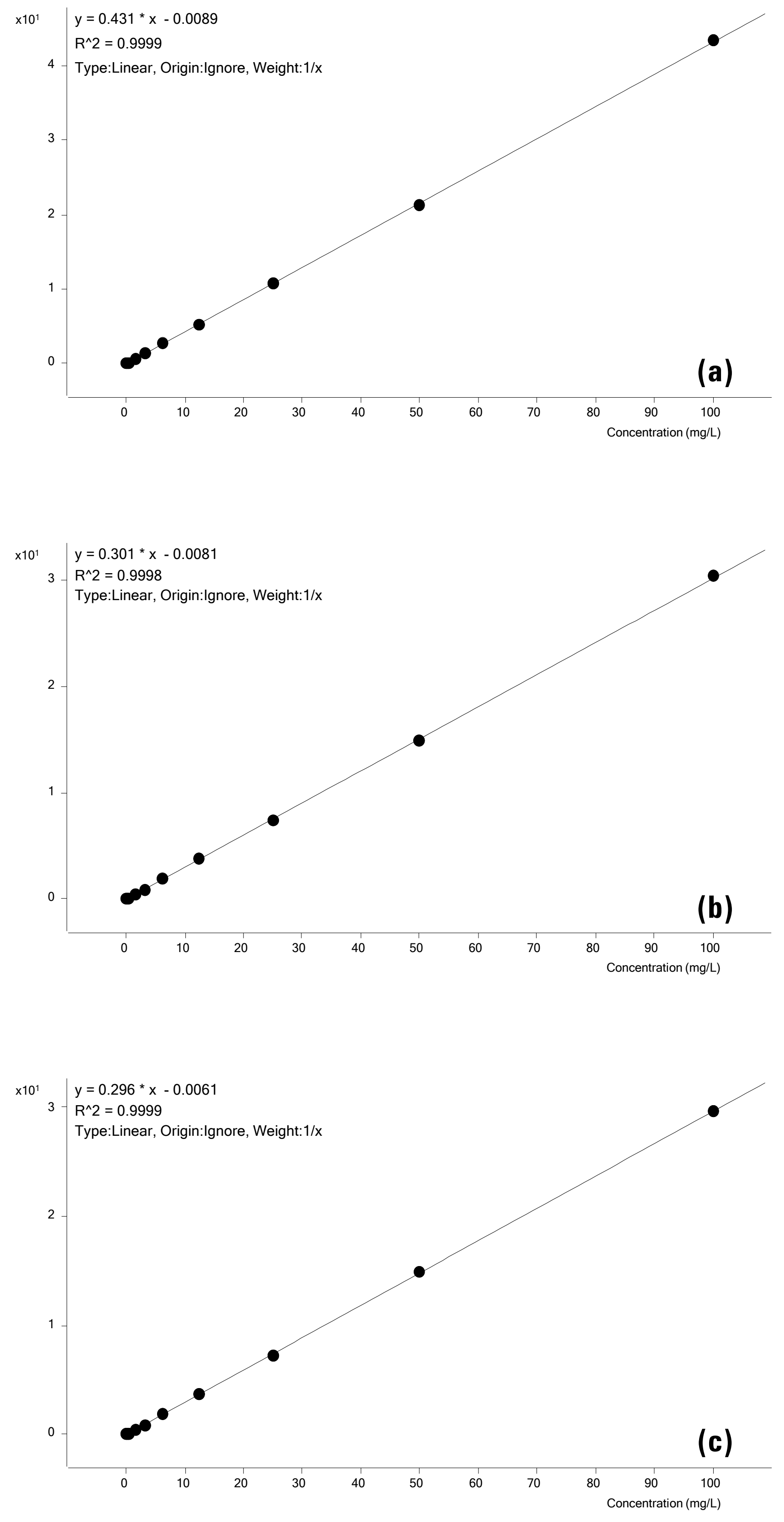


Figure 3. Calibration curves for (a) VMA, (b) 5-HIAA and (c) HVA

Compound	R^2	Concentration (mg/L)	Concentration (µmol/L)	Accuracy (%) n = 3	Intraday CV (%) n = 3	Interday CV (%) n = 5
VMA	0.9999	0.078	0.39	106.8	3.9	3.9
		12.5	63.1	100.1	1.3	1.7
		100	504.6	100.5	0.6	0.5
5-HIAA	0.9998	0.078	0.41	109.7	3.4	3.4
		12.5	65.4	99.1	2.2	1.7
		100	523.1	100.8	0.1	0.1
HVA	0.9999	0.078	0.43	102.2	4.2	3.2
		12.5	68.6	99.9	1.7	1.3
		100	548.9	100.5	0.4	0.3

Table 5: Summary of analyte performance for VMA, 5-HIAA and HVA.

Conclusions

A robust method for quantifying vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in urine with excellent reproducibility and accuracy has been developed.