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

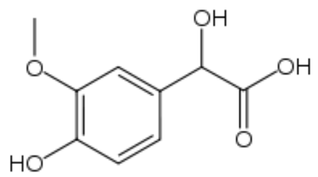
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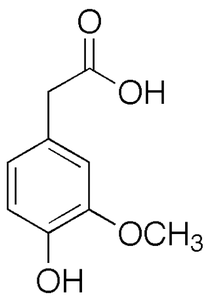
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## Introduction

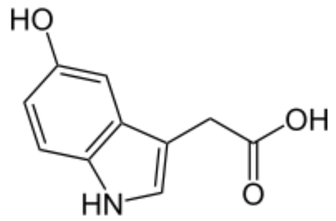
Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS method has been developed for the quantitation of vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic 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<br>PN: A3051150X020 |
| Guard Column | Agilent Meta Guard column Pursuit 3 PFP, 2 mm<br>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<br>0.0 15<br>0.5 15<br>2.5 60<br>3.0 95<br>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.

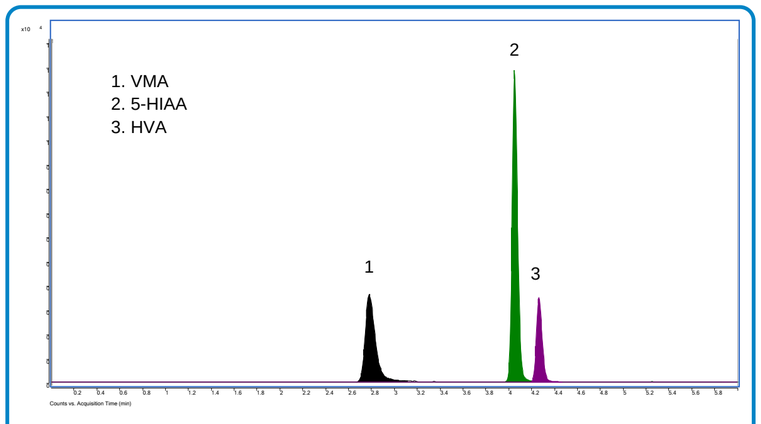



**Figure 2.** Chromatography for VMA. 5-HIAA and HVA

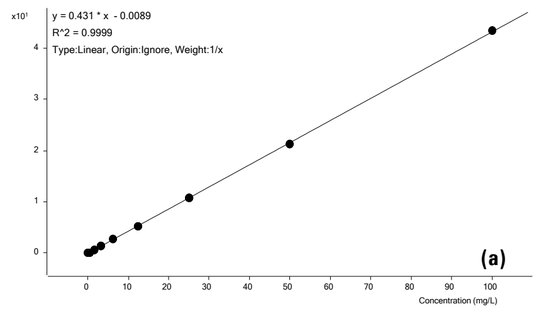
| Compound | Matrix effects % (n = 10) | | Accuracies % With ISTDs corrections (n = 10) | | |
|---|---|---|---|---|---|
| | Average | SD | Range | Average | SD |
| VMA | 91.5 | 6.4 | 95.6-108.3 | 100.0 | 3.9 |
| 5-HIAA | 93.2 | 3.3 | 94.6-115.2 | 100.0 | 5.8 |
| HVA | 91.3 | 1.5 | 92.9-103.9 | 100.0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

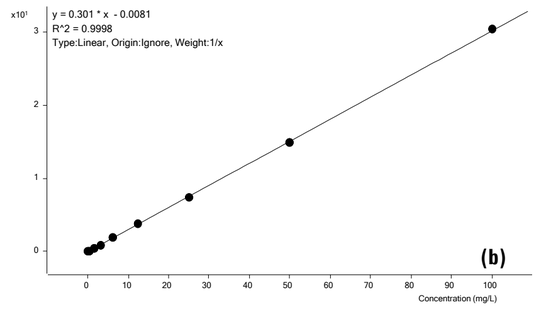
**Table 3.** Ion suppression and matrix effects

## Results and Discussion

| Compound | Level 1 Range (HPLC) | Level 1 Measured | Level 1 CV (%) | Level 2 Range (HPLC) | Level 2 Measured | Level 2 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)



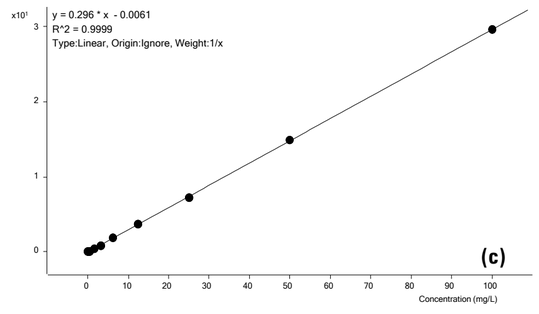




**Figure 3.** Calibration curves for (a) VMA, (b) 5-HIAA and (c) HVA

| Compound | R² | 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.

Agilent LC/MS products are for research use only and not to be used for diagnostic procedures