HPLC application note

Diagnosis of Neuroblastoma, a Childhood Cancer, by LCEC

Abstract

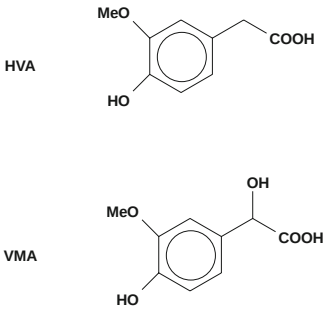
A rapid, sensitive and automated method for the diagnosis of Neuroblastoma is described. In the assay, Homovanillic acid (HMA) and Vanillylmandelic acid (VMA) in infant urine are determined by LCEC. The method is highly selective and sensitivity is in the ppm range. Each analysis is completed within 10 minutes. Results can be individually and/or group validated to assure the precision and accuracy of assays. Sample preparation is minimal, and up to 160 samples can be batched for unattended operation. The system also offers intelligent diagnostics to ensure optimal chromatographic performance and protection of precious samples.

'...the electrochemical reaction involves the selective oxidation of the vanillyl nucleus to the corresponding benzoquinone...'

Neuroblastoma (NB) is the most common solid tumour in young children and the second most common cause of infant mortality under the age of five. NB is a tumour of the sympathetic nerves or nerve ganglia. It usually occurs in the thoracic or abdominal regions close to the spine and can spread rapidly into other areas including adrenal, kidney, liver and bone.1 NB is an embryonic neoplasm estimated to be present in 0.4% of new borns. The tumour remains dormant initially but will eventually develop in approximately one in 7000 children before the age of five2. However, NB, is curable when detected early in babies less than 12 months old. In contrast, the chances of recovery dimish markedly in children over the one despite costly therapy. As NB is easily curable when detected early, mass screening programs have been initiated in Japan, UK, Canada, France and USA.

Neuroblastomas are unique tumours biochemically because they possess metabolic pathways for catecholamine synthesis and catabolism. Homovanillic acid (HVA) and vanillylmandelic acid (VMA), metabolites of dopamine, are re-excreted in excessive amounts in patients' urine and present as the most useful markers for the tumour.

Keywords:
Neuroblastoma, Homovanillic Acid (HMA), Vanillylmandelic Acid (VMA), Electrochemical Detection, Reversed Phase HPLC



Both HVA and VMA contain the electroactive vanillylmoiety, and Liquid Chromatography with Electrochemical Detection (LCEC) is the method of choice for their determination. The electrochemical reaction involves the selective oxidation of the vanillyl nucleus to the corresponding benzoquinone. The anodic current generated can then be used for accurate quantitation of the analytes. This method of LC detection is highly selective with little interference from other metabolites. The GBC LC1260 Electrochemical Detector, with its unique 'Wall Jet' design, allows shorter equilibration time and increased reliability. The use of a MicroComposite Glassy Carbon Electrode results in increased sensitivity when compared with conventional 3 mm glassy carbon electrodes. Sensitivity has also been enhanced through the use of low noise electronic circuitry with active and digital filtering. In addition, the automatic self-cleaning mode of the detector extends the electrode's operating life by avoiding contamination of the electrode surface.

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Automation of the HPLC system is accomplished by control of the LC1120 Advanced Spindle Driven HPLC Pump, the LC1260 Electrochemical Detector and robotic functions of the LC1650 Advanced Autosampler via the WinChrom Chromatography Data Management System. The system utilises a comprehensive set of validation parameters, enabling single and group validation during analyses. Command sequence can be easily pre-programmed to direct the execution of different analytical routines depending on the validation results. This ensures optimal performance of the analyser and avoids wastage of analysis time and precious samples during unattended operations. Multi-tasking of the management system also permits the running of other software programs, e.g., for report preparation, while analyses are being processed.

The LC1650 Advanced Autosampler has a maximum sample capacity of 160. The low cost LC1120/LC1150 HPLC Column Oven Option Kit delivers excellent flow rate accuracy and precision. In addition, the configuration of the system offers maximum flexibility, offering easy modification of the instrumentation for other HPLC applications.

'...the use of a MicroComposite Glassy Carbon Electrode results in increased sensitivity...'

GBC HPLC Instrumentation

LC1260 Electrochemical Detector.
LC1440 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option
WinChrom Chromatography Data Management System

Sample Preparation

Infant urine is collected on a filter paper strip. It is diluted and filtered (2 µm) before injection.

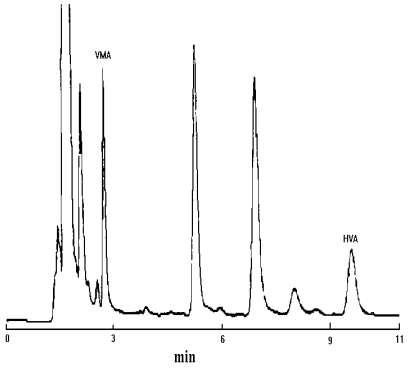


Figure 1 *Infant Urine Sample*

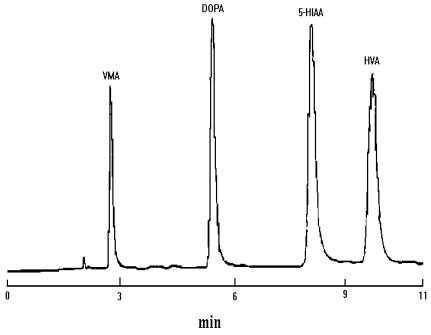
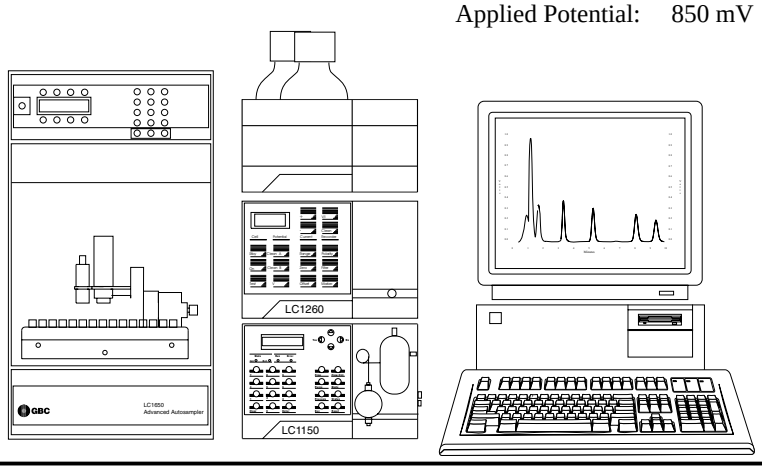


Figure 2 *15 µM Standard (5-HIAA = 5-OH-indoleacetic acid)*

Conditions

Column:	Spherisorb S5 OD S2, 150 x 4.6 mm ID
Mobile Phase:	0.05 mM Sodium Phosphate Buffer, pH 2.5, with 0.1 mM EDTA Disodium Salt/Acetone (90:1) (Helium sparging)
Flow Rate:	1.0 ml/min
Temperature:	35°C
Detection:	
Working Electrode:	3 mm Micro Composite Glassy Carbon.
Reference Electrode:	Ag/AgCl (3M KC1)
Auxiliary Electrode:	Cell Body
Applied Potential:	850 mV



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