HPLC application note

Catecholamine Analysis for the Diagnosis of Neural Crest Tumours

Abstract

A method is described for the analysis of catecholamines—dopa, dopamine, nor-adrenaline and adrenaline, by LCEC. Assay of these biogenic amines in biological fluids has been utilised for the diagnosis of neural crest tumours and other human metabolic disorders. The method is highly selective and sensitivity is at the ppm range. Each assay is completed within 6 minutes.

'...biogenic amines are secreted only by tumours occurring in embryological neural crest derived tissues...'

The analysis of catecholamines, an important class of neurotransmitters, has been utilised in clinical laboratories for the diagnosis of tumours of the neural crest and for the investigation of neurological and neuropsychiatric disorders. The specific and successful diagnosis of these tumours has been possible as these biogenic amines are secreted only by tumours occurring in embryological neural crest derived tissues. Plasma and urine samples from patients are analysed for catecholamines and their metabolites.

Three catecholamines are important for the biochemical diagnosis: Dopamine, Noradrenaline (Norepinephrine) and Adrenaline (Epinephrine). Noradrenaline is derived from dopamine by hydroxylation of its side chain, while N-methylation of noradrenaline gives adrenaline (Figure 1). Dopamine is synthesized in abnormal quantities by neuroblastomas and ganglioneuromas, resulting in elevated levels of the amines and its metabolites in the blood and urinary excretion. On the other hand, phaechromocytomas are characterised by the secretion of an excess amount of noradrenaline and adrenaline. Since none of these tumours produce any significant clinical symptoms until they reach advanced stages and intrude upon other surrounding tissues, an accurate measurement of these amines and their metabolites offers a rapid, reliable and non-invasive diagnosis.

Keywords:
Catecholamines, Dopa, Dopamine, Noradrenaline, Adrenaline, Norepinephrine, Epinephrine, Electrochemical Detection

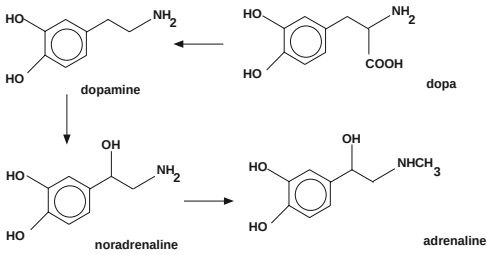

Figure 1 *Biotransformation of Catecholamines*

Traditionally, the detection of catecholamines in biological fluids has been difficult due to their low concentrations in complex matrices. The high capital and running costs for dedicated systems, such as GCMS, precludes their use in all but a few laboratories. In recent years, the advent of LCEC (Liquid Chromatography with Electrochemical Detection) has delivered the specificity and sensitivity required for the HPLC analysis of catecholamines and their 3-methylated metabolites.1 Derivatisation is not required and instrumentation is relatively inexpensive. Common to all of these biogenic amines is a 3,4-dihydroxyphenyl moiety (hence the name 'catechol') which can be selectively oxidised to the corresponding benzoquinone on the surface of the electrode (Figure 2). The anodic current generated, which is directly proportional to the number of analyte molecules in contact with the electrode surface, can be used for the accurate quantitation of the analytes.

Figure 2 *Oxidation of Catecholamines*

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'...the exceptionally low background noise level of the detector permits on-column detection of catecholamines down to low picogram levels...'

As the sensitivity and the reliability of the detector employed for the analysis are of great importance, the GBC LC1260 Electrochemical Detector has been designed with these criteria in mind. The LC1260 detector, with its unique 'Wall Jet' design, allows shorter equilibration time and increased reliability. Unlike traditional flowcells, this design also reduces the requirement for the detector flowcell to be dismantled for cleaning. Sensitivity has been enhanced through the application of low noise electronic circuitry featuring active and digital filtering. The exceptionally low background noise level of the detector permits on-column detection of catecholamines down to low picogram levels. In addition, the detector's self-cleaning mode extends the electrode operating life by avoiding contamination of the electrode surface.

Sample Preparation

Various extraction procedures are available in the literature based on cation-exchange followed by clean-up on alumina.2 Solid phase extraction procedures are also available from major SPE column manufacturers.

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
LC1440 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven
WinChrom Chromatography Data Management System

Conditions

Column: Spherisorb S5 ODS2, 150 x 4.6 mm ID

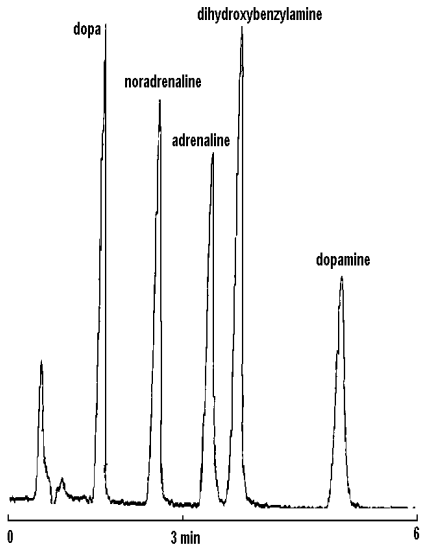


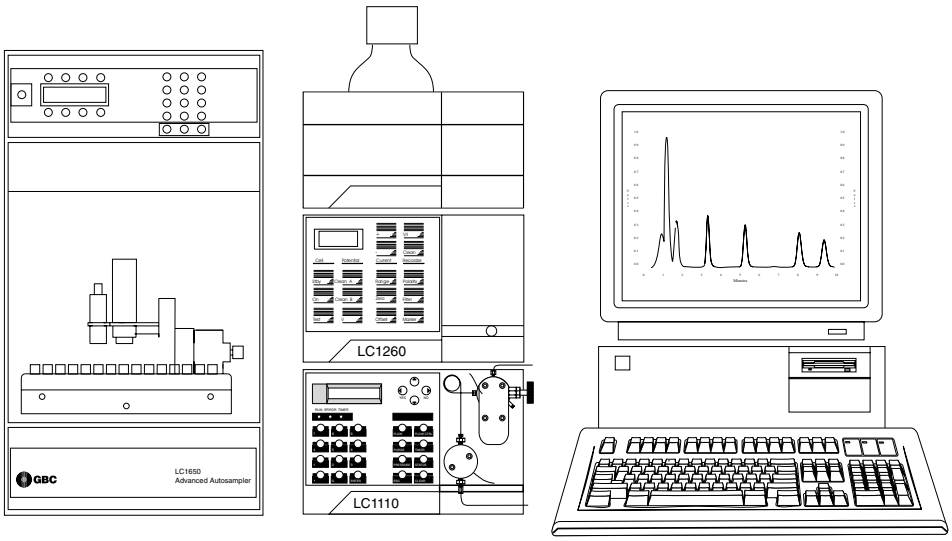
Figure 3 *Catecholamine Standards (5 picomoles on column)*

Mobile Phase: 75 mM Phosphate buffer with 1 mM sodium octyl sulfate and 0.05 mM EDTA, pH 3/ Acetonitrile (90:10) (Helium sparging)
Flow Rate: 1.0 ml/min
Temperature: 35°C
Detection:
Working Electrode: 3 mm Glassy Carbon.
Reference Electrode: Ag/AgCl (3 M KC1)
Auxiliary Electrode: Cell Body
Applied Potential: 650 mV

References

1. 'Diagnosis of Neuroblastoma, a Childhood Cancer, by LCEC', GBC Application Note B9.

2. E. Gerlo and R. Malfait, J. Chromatogr., 343, (1985), 9; G.C. Davis, P.T. Kissinger and R.E. Shoup, Anal. Chem., 53 (1981), 156; R.M. Riggin and P.T. Kissinger, Anal. Chem., 49, (1977), 2109.



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