Application Note

Catecholamine Metabolites

Introduction

Phaeochromocytoma (PCC) is a benign neuroendocrine tumour of the sympathetic nervous system that secretes catecholamines such as dopamine (fig 1), epinephrine (fig 2) also known as adrenaline, and norepinephrine (fig 3.) also known as noradrenaline causing hypertension (high blood pressure). It is easily treated by surgery to remove the tumour, if left untreated it can be fatal as the high blood pressure can lead to a stroke.

> "HILIC provides adequate retention of the polar metabolites to allow selectivity and the required senstivity to be achieved"

The University Hospital of South Manchester NHS Foundation Trust screen for this tumour by measuring plasma metanephrines using on-line solid phase extraction with a Spark Holland Symbiosis™ Autosampler interfaced to a Shimadzu VP HPLC and a Waters Xevo™ TQ triple quadrupole mass spectrometer.

Metanephrines are metabolites of catecholamines which are more stable and less prone to dietary interferences than catecholamines. The metanephrines are 3-methoxytyramine (3MT) (fig 4), metanephrine, (ME) metadrenaline (fig 5) and normetanephrine (NME) normetadrenaline (fig 6).

Fig 1: Dopamine

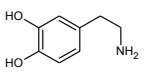


Fig 2: Epinephrine (Adrenaline)

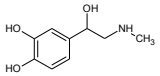


Fig 3: Norpinephrine (Noradrenaline)

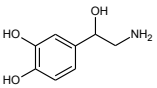


Fig 4: 3-Methoxytyramine

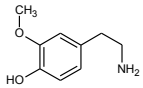


Fig 5: Metanephrine (ME) (Metadrenaline)

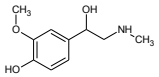
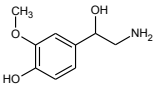


Fig 6: Normetanephrine (NME) (Normetadrenaline)



	Parent	Daughter
3MT	151.2	119.1
D4,3MT	155.3	123.1
ME	180.3	148.2
D3ME	183.3	151.1
NME	166.3	134.1
D3NME	169.3	137.1

Experimental

Column :
Fortis HILIC 50 x 2.1mm, 3µm
Competitor X HILIC 50 x 2.1mm, 3µm

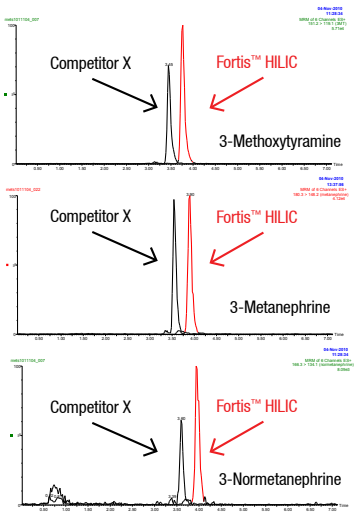
Injection Volume 100ul

Time (min)	Flow (ml/min)	% A	%B
0	0.4	5	95
0.05	0.4	5	95
4.10	0.4	20	80
4.40	0.4	20	80
4.41	0.4	5	95
7.15	0.4	5	95

A = Ammonium Formate 100mM adjusted to pH3.2 with Formic Acid
B = Acetonitrile

Result

Separation of Catecholamines is difficult due to their polar nature, the need therefore for a highly retentive polarity column is paramount. Hydrophilic Interaction Liquid Chromatography (HILIC) provides suitable retention of the metabolites in question. Allowing the selectivity and required sensitivity of the metabolites from the urine samples to be achieved. Comparison of HILIC stationary phases showed differing retention and sensitivity . HILIC's high concentration of organic modifier and simple modifiers allows the full advantages of MS as a detection technology.



Conclusion

Excellent sensitivity and peak shape were obtained for this method utilising HILIC mode. The analytes were all well retained with the greater retention on the Fortis™ HILIC column due to the higher surface area of this stationary phase. The sensitivity and peak shape was also consistently improved for each analyte on the Fortis™ HILIC column.