Application Note
Date: No. 450003H-E

Simultaneous determination of VMA, HVA, and creatinine

Neuroblastomas (NB) are catecholamine producing tumors and diagnosis is possible by analyzing for metabolic by-products of catecholamine in urine.
In this report vanillylmandelic acid (VMA), homovanillic acid (HVA) and creatinine were measured simultaneously by reversed phase HPLC and pre-processing was remarkably improved by HPLC autosampling with a micro-plate (Fig. 1).
Fig. 2 shows chromatograms of standard samples. A UV detector was used for creatinine and a coulometric detector (ECD) for VMA and HVA.
Fig. 3 shows the chromatogram from a urine test paper sample.
Specialized data processing software is being prepared.

Conditions:

Column:	Catecholpak II
Eluent:	50mMKH2PO4/ CH3CN = 2745 / 255 EDTA 10mg/L 1-Octanesulfonic acid Na 3mM pH 2.0(with H3PO4)
Wave length(UV):	235nm
Electrode potentiaL:	D1 0.2V, D2 0.25V, G 0.30V
Flow rate:	1.2ml/min
Column temperature:	45 degree celsius
Injection volume:	10ul
Sample:	STD mixture VMA,VLA,HVA:250ppb Creatinine 25ppm

4.2mm x 2 disks
↓ Eluate 200ul (1)
Shake or blend for 30min
↓
HPLC (10ul) (2)

(1) Eluent has 0.001% hibitane solution
(2) Disk shaken into micro-plate well, finally the micro-plate is placed in the auto-sampler.(with the disk still in place)

Fig. 1 Pre-preparation procedure

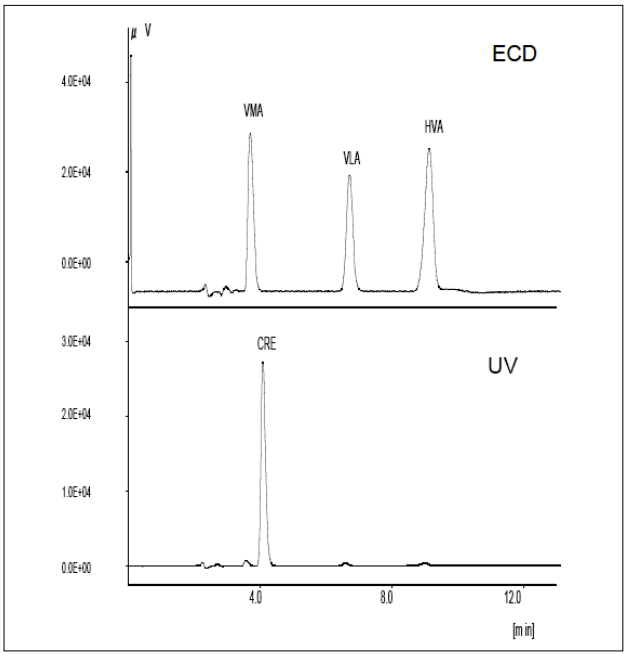

Fig. 2 Standard chromatogram

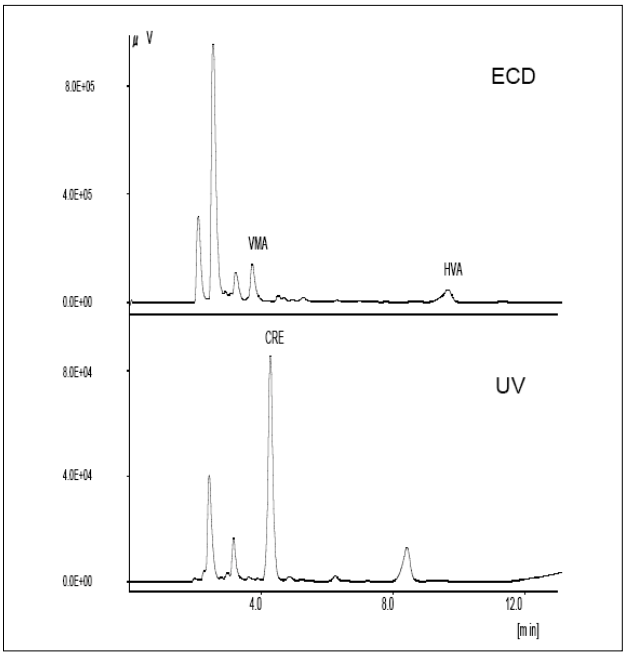

Fig. 3 Sample (6 months old urine test paper) chromatogram

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