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1. Introduction

- Determination of urinary catecholamines and their O-methylated metabolites (metanephrines) as biomarkers for various disorders and disease states is important in the clinical setting.
- The polar nature of these compounds makes them challenging to retain by reversed phase chromatography.
- In this work, analysis was successfully achieved using a novel stationary phase: ACE Excel 2 C18-PFP.
- The ACE C18-PFP has been designed to maximise selectivity and separation with multiple interaction modes including hydrophobic and π - π mechanisms.
- The ACE C18-PFP can provide enhanced retention for electron-rich aromatic analyte moieties, such as those found in catecholamines and metanephrines.
- This poster demonstrates a UHPLC-MS/MS method for the extraction, separation and quantification of both compound classes from urine.

4. UHPLC-MS/MS Results

- The separation was developed to maximise retention of norepinephrine and epinephrine. This compromised the peak shape of 3-methoxytyramine.
- Direct injection of SPE eluate no evaporation step.
- Analysis of all six analytes was achieved in <3.5 minutes.

| Norepi (Standard) 152.1 / 107.1 - Area: 1.885e5, Height: 6.047e4, RT: 0.76 min c= 4 | | ٨ | | Norepine | phrine |
|---|-----|----------|--------------------------|--------------|--------|
| | | 0.76 | | | |
| 0 | 0.5 | 1. | 0 | 1.5 | 2.0 |
| Epr(Standard) 166.1 / 10/.1 - Area: 4.764e4, Height: 1.178e4, RT: 1.03 min 1 | | ٨ | ٨ | Epine | phrine |
| 10000 - 22 5000 - 22 | | 1.03 | | | |
| 0 0 Normet (Standard) 166.1 / 134.0- | 0.5 | 1.0 | 1. | 5 2.0 | 2. |
| Area: 5.547e5, Height: 2.659e5, RT: 1.28 min | | | ٨ | Normelane | prime |
| 265 - 22 165 - | | | 1.28 | | |
| 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| Dopa (Standard) 154.1 / 91.1 - Area: 2.160e6, Height: 1.035e6, RT: 1.30 min 1.0e6 J | | | • | Dop | amine |
| 25 50e5 - 25 0000 | | | 1.30 | | |
| 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| Met (Standard) 180.1 / 148.0 - Area: 6.833e5, Height: 2.367e5, RT: 1.32 min | | | | Metane | phrine |
| 2e5 - 20 1e5 - 1e5 - | | | 1.32 | | |
| 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| 3 Methoxy A (Standard) 168.1 / 151.0 - Area: 1.236e6, Height: 1.488e5, RT: 2.92 min | | | 3 | 3-Methoxytyr | amine |
| 20 1e5 - 1e5 - 1e5 - | | | 2.92 | | |
| 1.5 | 2.0 | 2.5 T | 3.0 ime <i>,</i> min. |) 3.5 | 4.0 |

MRM Parameters:

| Analyte | | | | | | |
|---|--|--|--|--|--|--|
| Norepinephrine | | | | | | |
| D ₆ -Norepinephrine | | | | | | |
| Epinephrine | | | | | | |
| D ₆ -Epinephrine | | | | | | |
| Normetanephrine | | | | | | |
| D ₃ -Normetanephrine | | | | | | |
| Dopamine | | | | | | |
| | | | | | | |
| D ₄ -Dopamine | | | | | | |
| D ₄ -Dopamine Metanephrine | | | | | | |
| D ₄ -Dopamine Metanephrine D ₃ -Metanephrine | | | | | | |
| D ₄ -Dopamine Metanephrine D ₃ -Metanephrine 3-Methoxytyramine | | | | | | |

A UHPLC-MS/MS Method for the Separation and Low-Level **Determination of Catecholamines and Metanephrines in Urine** using a Novel C18-Based Column

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2. UHPLC-MS/MS Conditions

ACE Excel 2 C18-PFP, 100 x 2.1 mm (p/n EXL-1010-1002U) Column Shimadzu Nexera UHPLC with AB Sciex 5500 MS Instrument: Mobile phase: A: 0.25 mM ammonium formate + formic acid B: 0.25 mM ammonium formate + formic acid in Methanol Flow rate: 0.4 mL/min 40 °C **Temperature:** 7.5 µL Injection volume Gradient: MS parameters (positive mode) Curtain gas: 35 psi 50 psi Ion source gas 1 5500 V 50 psi lon source gas 2: Ion spray voltage: 700 °C lemperature: Analyte standards and deuterated internal standards spiked Sample into urine at 2-20 ng/mL, (3-methoxytyramine 50 ng/mL). SPE: EVOLUTE® EXPRESS WCX 10 mg fixed well plate, p/n 602-0010-PX01

Optimised SPE protocol:

| Step | Volume | Standard SPE | Load-Wash-Elute SPE | |
|---------------|--------|---|---------------------|--|
| Condition | 500 µL | MeOH | - | |
| Equilibration | 500 μL | 10 mM ammonium acetate | - | |
| Sample Load | 150 μL | Urine:250 mM ammonium acetate (75 μL:150 μL) | | |
| Wash 1 | 500 µL | 10 mM ammonium acetate | | |
| Wash 2 | 500 µL | IPA | | |
| Elution | 125 µL | IPA:H ₂ O (85:15 v/v) + 0.1% formic acid | | |

5. Recovery and Linearity

- Excellent recovery and linearity determined across varying clinical ranges in spiked urine:
 - 0.1 to 25 ng/mL for epinephrine
 - 0.5 to 125 ng/mL for metanephrine and normetanephrine
 - 1 to 250 ng/mL for norepinephrine and 3-methoxytyramine
 - 2.5 to 625 ng/mL for dopamine

Full SPE procedure compared to Load-Wash-Elute approach Similar recovery and reproducibility for both approaches



Transition 152.1 > 107.1 158.1 > 111.1 166.1 > 107.1 172.1 > 112.1 166.1 > 134.0 169.1 > 137.0 154.1 > 91.1 158.1 > 95.1 180.1 > 148.0

183.1 > 151.0 151.2 > 90.9

Catecholamines

Epinephrin LogP: -0.43 LogD_{pH3}: -2.95

HO

Dopamine LogP: 0.03 LogD_{pH3}: -2.25

HO

Norepinephrine LogP: -0.68

LogD_{DH3}: -3.17 LogP and LogD data obtained from www.chemicalize.com

Metanephrines Metanephrine LogP: 0.00 LogD_{pH3}: -2.80

-O NH2 но 3-Methoxytyramine LogP: 0.53 LogD_{pH3}: -2.11

Normetanephrine

LogP: -0.39 LogD_{pH3}: -3.03



84.2

Results for n=8 extracted samples

Dopamine R² = 0.991 300 350 400 450 500 550 600 Load-Wash-Elute SPE Method **Full SPE Method** % RSD Extraction % RSD Extraction Recovery Recovery 76.1 7.0 88.8 3.5 8.5 108.3 109.3 7.0 88.2 3.3 91.8 3.1 8.6 83.6 87.9 6.6 83.3 88.4 6.8 5.0

4.2

2.1

83.6

6. Conclusions

- C18-PFP.
- and retention.
- phase due to catecholamine and metanephrine polarity.
- Chromatographic separation achieved in <3.5 minutes.
- ranges.

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Retention and separation of analytes (full separation of isobaric species) was achieved by UHPLC using the novel ACE Excel 2

The use of novel LC stationary phases can help optimise selectivity

Enhanced aromatic π - π interactions between the electron-deficient PFP ring and electron-rich analyte rings, means the ACE Excel C18-PFP phase overcomes the challenge of poor retention in reversed

Simple optimised SPE protocol with direct injection of SPE eluate.

Excellent linearity and recoveries demonstrated across varying clinical