Separation and Low-Level Determination of Catecholamines and Metanephrines from Urine by UHPLC-MS/MS using a Novel C18-Based Stationary Phase

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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.

2. UHPLC-MS/MS Conditions

Column:	**ACE Excel 2 C18-PFP, 100 x 2.1 mm (EXL-1010-1002U)**	
Instrument:	Shimadzu Nexera UHPLC with AB Sciex 5500 MS	
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	
Temperature:	40 °C	
Injection volume:	7.5 µL	
Gradient:	t	%B
	0	5
	1.3	5
	1.4	95
	4.4	95

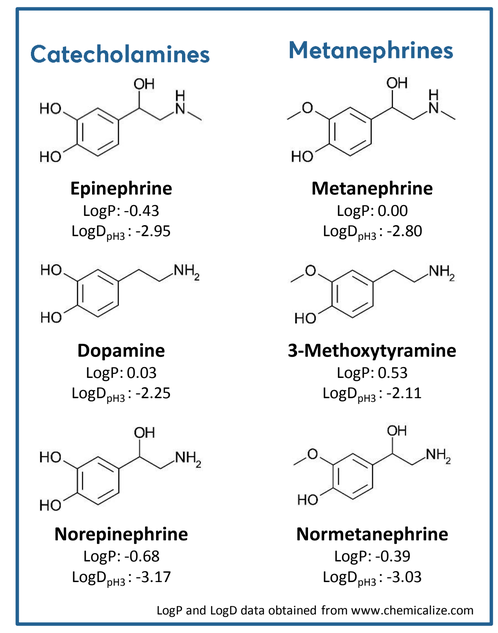
MS parameters (positive mode):

Curtain gas:	35 psi	Ion source gas 1:	50 psi
Ion spray voltage:	5500 V	Ion source gas 2:	50 psi
Temperature:	700 °C		

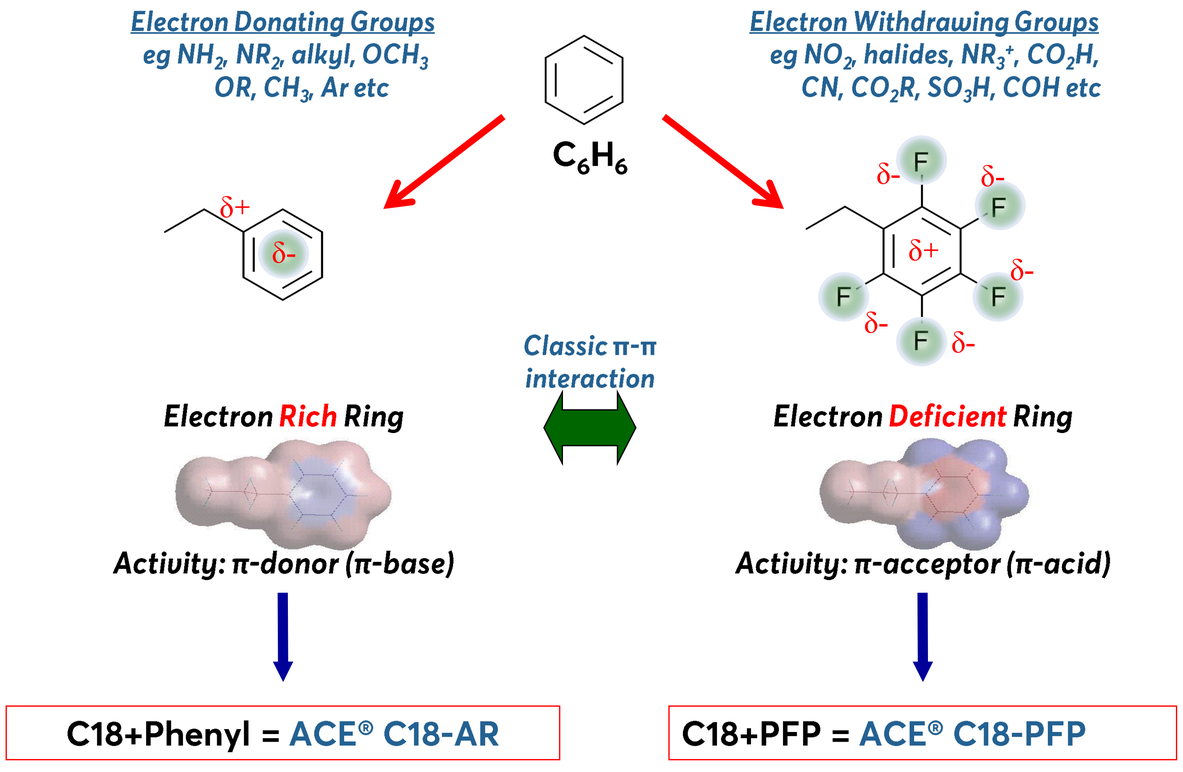
Sample: Analyte standards and deuterated internal standards spiked into urine at 2-20 ng/mL, (3-methoxytyramine 50 ng/mL).

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:150µL)	
Wash 1	500 µL	10 mM ammonium acetate	
Wash 2	500 µL	IPA	
Elution	125 µL	IPA:H_2O (85:15 v/v) + 0.1% formic acid	

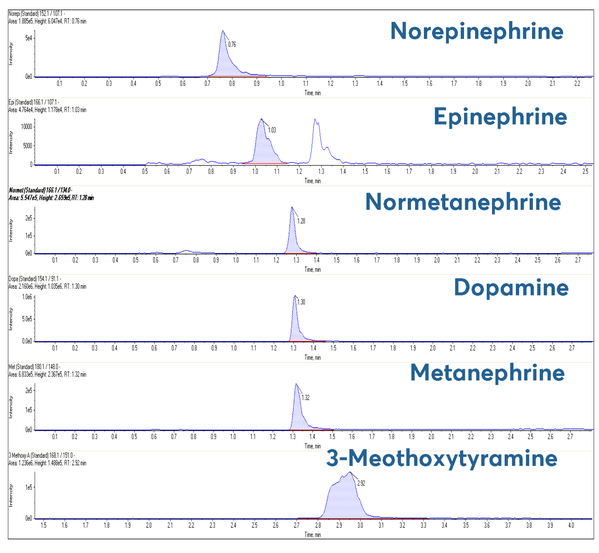


3. Rational Phase Design to Maximise Selectivity



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.

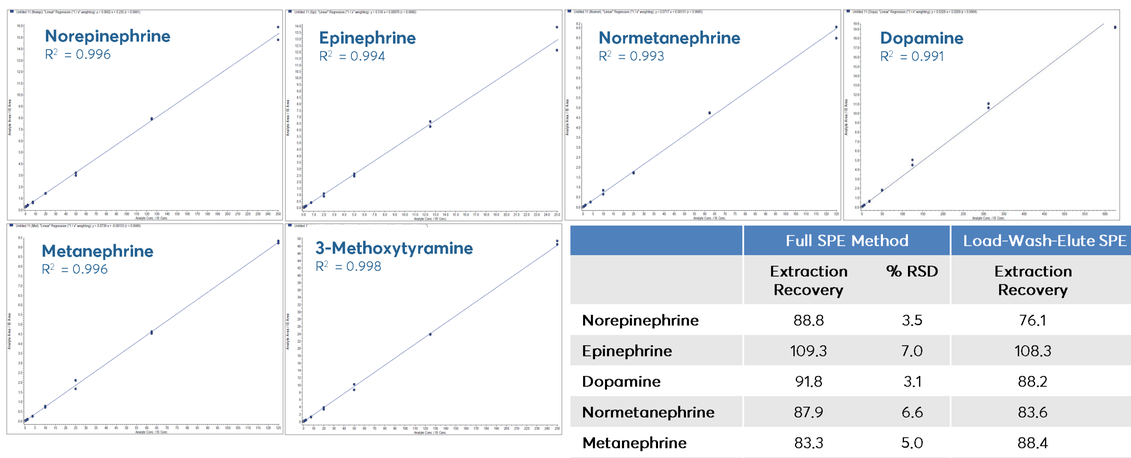


MRM Parameters:

Analyte	Transition
Norepinephrine	152.1 > 107.1
D_6-Norepinephrine	158.1 > 111.1
Epinephrine	166.1 > 107.1
D_6-Epinephrine	172.1 > 112.1
Normetanephrine	166.1 > 134.0
D_3-Normetanephrine	169.1 > 137.0
Dopamine	154.1 > 91.1
D_4-Dopamine	158.1 > 95.1
Metanephrine	180.1 > 148.0
D_3-Metanephrine	183.1 > 151.0
3-Methoxytyramine	151.2 > 90.9

5. Recovery and Linearity

- Excellent linearity determined across varying clinical ranges:
  - 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



	Full SPE Method		Load-Wash-Elute SPE Method	
	Extraction Recovery	% RSD	Extraction Recovery	% RSD
Norepinephrine	88.8	3.5	76.1	7.0
Epinephrine	109.3	7.0	108.3	8.5
Dopamine	91.8	3.1	88.2	3.3
Normetanephrine	87.9	6.6	83.6	8.6
Metanephrine	83.3	5.0	88.4	6.8
3-Methoxytyramine	84.2	4.2	83.6	2.1

Results for n=8 extracted samples

6. Conclusions

- Retention and separation of analytes (full separation of isobaric species) was achieved by UHPLC using the novel **ACE Excel 2 C18-PFP**.
- The use of novel LC stationary phases can help optimise selectivity and retention.
- 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 phase due to catecholamine and metanephrine polarity.
- Chromatographic separation achieved in <3.5 minutes.
- Simple optimised SPE protocol with direct injection of SPE eluate.
- Excellent linearity and recoveries demonstrated across varied clinical ranges.