# Low Level Determination of Catecholamines: Epinephrine, Norepinephrine and Dopamine from Plasma By UHPLC-MS/MS Using a Novel C18-PFP Column


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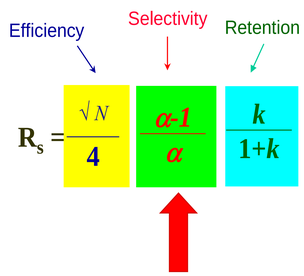
## 1. BACKGROUND

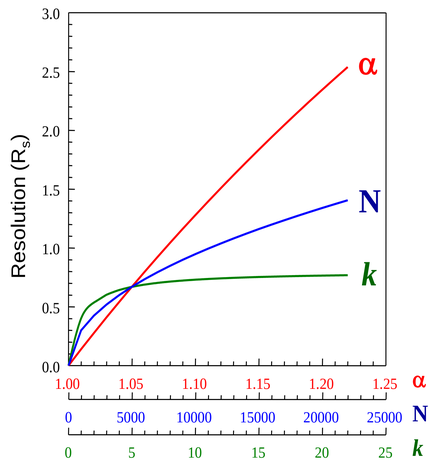
- Catecholamines are small polar molecules which can provide helpful information for monitoring various human disease states and endogenous processes. Commonly determined catecholamines within the clinical setting include dopamine, epinephrine and norepinephrine.
- Traditional analytical methods routinely include the use of electrochemical detection which can bring its own challenges. This work describes the extraction from plasma of 3 key catecholamines with subsequent quantification using UHPLC-MS/MS armed with the novel ACE Excel 1.7µm C18-PFP.
- This phase is designed to maximise selectivity and separation of closely related polar species by mechanisms that include hydrophobicity, and pi-pi interactions.

## 2. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION



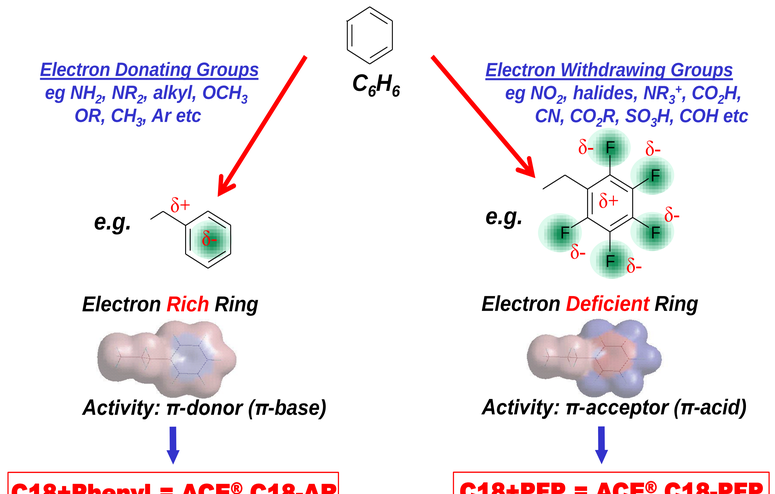

Selectivity has the greatest impact on peak resolution

**Designing Phases To Maximise Selectivity Is Therefore Powerful**




Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

## 3. RATIONAL PHASE DESIGN TO MAXIMISE SELECTIVITY




**Robust C18-Based Phases Designed With Ultra-Low Bleed For MS**

## 4. PLASMA SAMPLE EXTRACTION & INSTRUMENT CONDITIONS

**Format:** EVOLUTE EXPRESS® WCX 10mg fixed well plate, part number 602-0010-PX01.

**MS Conditions:** AB SCIEX 5500 triple quadrupole MS. Curtain gas = 35; collision gas = 7; ionspray voltage = 5500; temperature = 700C; ion source gas 1 = 50; ion source gas 2 = 50; setting time = 40 ms. Positive ions acquired in MRM mode.

| Compound | MRM Transition | DP (V) | EP (V) | CE (V) | CE (V) |
|---|---|---|---|---|---|
| Epinephrine | 166.1 > 107.1 | 148 | 8 | 24 | 16 |
| Norepinephrine | 152.1 > 107.1 | 25 | 2 | 22 | 25 |
| Dopamine | 154.1 > 91.1 | 50 | 9 | 29 | 13 |
| d6-Epinephrine | 172.1 > 112.1 | 148 | 8 | 24 | 16 |
| d6-Norepinephrine | 158.1 > 111.1 | 25 | 2 | 22 | 25 |
| d4-Dopamine | 158.1 > 95.1 | 50 | 9 | 29 | 13 |

**Column:** ACE Excel 1.7µm C18-PFP, 2.1 x 100 mm, part number EXL-1710-1002U

**Mobile Phase:** 0.25mM ammonium acetate / 0.1% HCOOH (aq)
**Mobile Phase B:** 0.25mM ammonium acetate / 0.1% HCOOH in MeOH

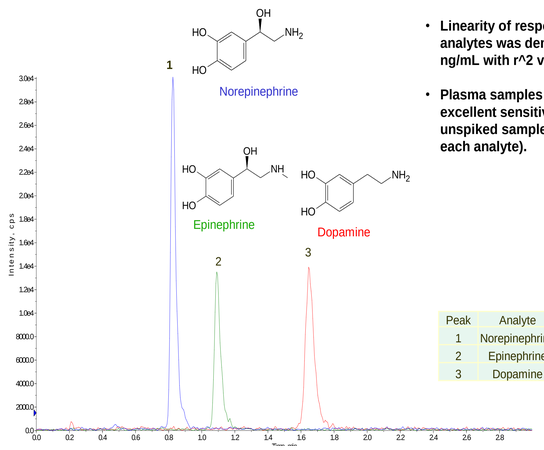
**Flow Rate:** 0.4 mL/min
**Gradient:** Start at 5 %B and hold until 1.2mins then step gradient to 95 %B and hold to 3.2mins. Switch back to 5 %B and hold for 4 mins equilibration before next injection.

**Internal Standards:** d6-Epinephrine, d6-Norepinephrine, d4-Dopamine purchased from LGC (Teddington, UK)
**Sample:** Mix plasma sample (250µL) containing internal standards with 250µL 0.05% w/w HCOOH

**Injection Volume:** 20 µL
**Temperature:** 40C

## 5. RESULTS: CATECHOLAMINES IN PLASMA BY UHPLC-MS/MS

**Note**
Dehydrated precursor ions were selected for epinephrine and norepinephrine to improve sensitivity




| Peak | Analyte | Q1 (Da) | Q3 (Da) |
|---|---|---|---|
| 1 | Norepinephrine | 152.1 | 107.1 |
| 2 | Epinephrine | 166.1 | 107.1 |
| 3 | Dopamine | 154.1 | 91.1 |

- Using the streamlined SPE process, recoveries of 81.1%, 70.8% & 77.4% with RSDs of 2.6%, 4.2% & 0.8% were achieved for epinephrine, norepinephrine and dopamine respectively.
- Linearity of response for extracted samples for all 3 analytes was demonstrated from 20 pg/mL to 1.28 ng/mL with r^2 values ranging from 0.997 to 0.999.
- Plasma samples spiked at 20 pg/mL demonstrated excellent sensitivity for all 3 analytes compared to unspiked samples (containing endogenous levels of each analyte).

## 6. SUMMARY AND CONCLUSIONS

- A rapid UHPLC-MS method for low level determination of 3 key catecholamines using the novel ACE Excel 1.7µm C18-PFP has been established.
- An extraction protocol using ISOLUTE® PLD+ Protein and Phospholipid Removal plates was developed to enable satisfactory recoveries and low level detection of the analytes from the serum matrix.
- Recoveries were found to be >70% with RSDs <10% for both analytes when spiked into serum samples across a wide range of 2 – 100 ng/mL. The method was also linear for both from 1 – 100 ng/mL in PBS/BSA with r^2 >0.99.