Low Level Determination Of Synthetic Cannabinoids (SPICE) And Metabolites From Oral Fluid Using A Novel C18-Based Stationary Phase By UHPLC-MS/MS

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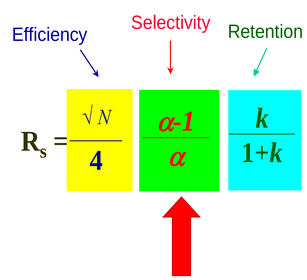
1. BACKGROUND

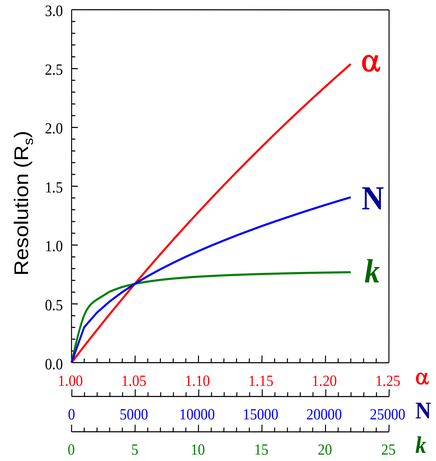
- The use of synthetic cannabinoids is becoming more wide spread and so are an important drug screening target.
- This work describes extraction and low level LC-MS/MS quantification of a range of synthetic cannabinoids and metabolites from oral fluid.
- Analysis of the extracted and enriched analytes was performed using a novel stationary phase: ACE C18-AR.
- The novel phase has been designed to maximise selectivity and separation with multiple interaction modes that include hydrophobicity and pi-pi mechanisms.

2. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION



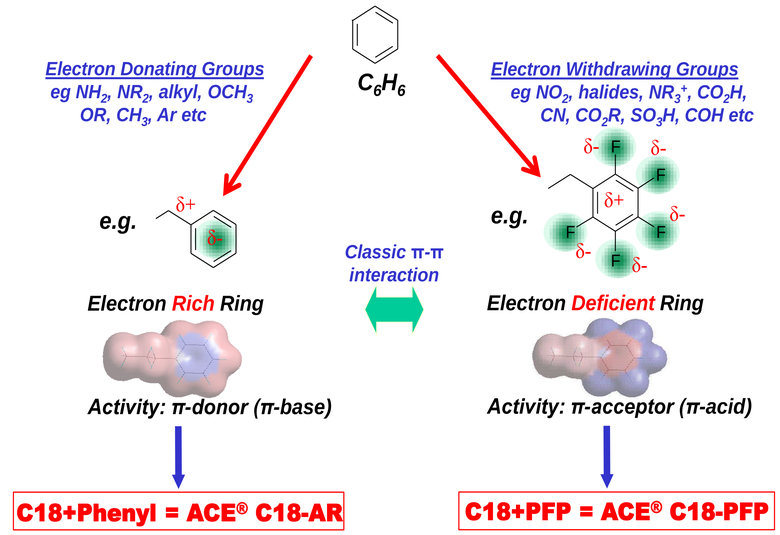
Selectivity has the greatest impact on peak resolution

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

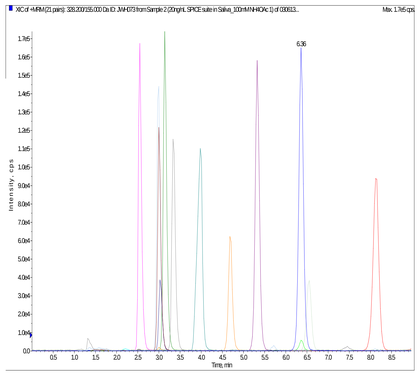


4. ORAL FLUID EXTRACTION & INSTRUMENT CONDITIONS

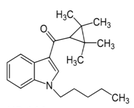
Format:	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate, part number 820-0400-PO1 ISOLUTE® SLE+ 400 µL Sample Volume Columns, part number 820-0055-BG
Oral Fluid Hydrolysis: (optional)	Add β-glucuronidase (5000 units/mL) to patient oral fluid, fortified calibration standards and/or QC standards (1 mL), in an appropriate container. Add ammonium acetate (100 mM, pH 5, 1 mL). Spike the solution with internal standard. Incubate sample as per enzyme instructions.
Sample Pre-treatment:	Mix oral fluid sample (neat or buffered, 200 µL) with ammonium acetate (100 mM, pH 5, 200 µL).
Sample Processing:	Load pre-treated oral fluid sample (400 µL) onto the ISOLUTE SLE+ 96-well plate or column. Apply a short pulse of positive pressure and allow samples to sit for 5 minutes.
Analyte Elution:	Apply ethyl acetate (2 x 700 µL). Apply short pulses of pressure and collect eluent.
Post Extraction:	Evaporate to dryness and reconstitute sample in mobile phase (500 µL).
MS Conditions:	Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface (in positive mode) for mass analysis. Ion source temperature = 500C.
HPLC Instrument:	Agilent 1200 Series HPLC / UHPLC.
Column:	ACE Excel 2 C18-AR, 2.1 x 100 mm i.d.
Mobile Phase A:	0.1% Formic Acid in Water.
Mobile Phase B:	0.1% Formic Acid in Methanol.
Isocratic Flow:	15% A: 85% B at 300 µL/min; 9 minute run time.
Injection Volume:	10 µL.
Temperature:	Ambient.

5. SYNTHETIC CANNABINOIDS: LOW LEVEL LC-MS/MS ANALYSIS

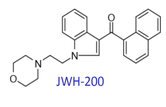
Extracted ion chromatogram for SPICE analytes fortified in neat oral fluid at 20ng/mL



Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	30	35	16
3.14	d5-JWH-018 N-(4-hydroxypentyl	363.5> 155	40	35	16
3.14	JWH-018 N- (4-hydroxypentyl	358> 155	40	30	16
3.34	JWH-018 5-pentanoic acid	372>155	40	30	16
3.98	JWH-200	385>155	40	30	16
4.69	XLR-11	330>125	30	35	16
5.32	JWH-250	336>121	40	30	16
6.36	JWH-073	328>155	40	30	16
6.37	UR-144 5-Chloro-pentyl	346.9>125	30	35	16
6.55	UR-144	312.5>125	30	35	16
8.14	JWH-018	342>155	40	30	16



UR-144



JWH-200

6. SUMMARY AND CONCLUSIONS

- Synthetic cannabinoid / SPICE drug screening protocols are becoming increasingly important in law enforcement.
- Low level quantification by UHPLC-MS/MS is achievable and an important tool for rapid screening.
- An extraction protocol using ISOLUTE® SLE+ and a separation method using the novel ACE® C18-AR were developed to enable the low level detection of a range of synthetic cannabinoids and their metabolites.
- Typical recoveries for the analytes and their metabolites at 1 ng/mL extracted from oral fluid ranged from 65-110%, %RSDs <10.