

# 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.

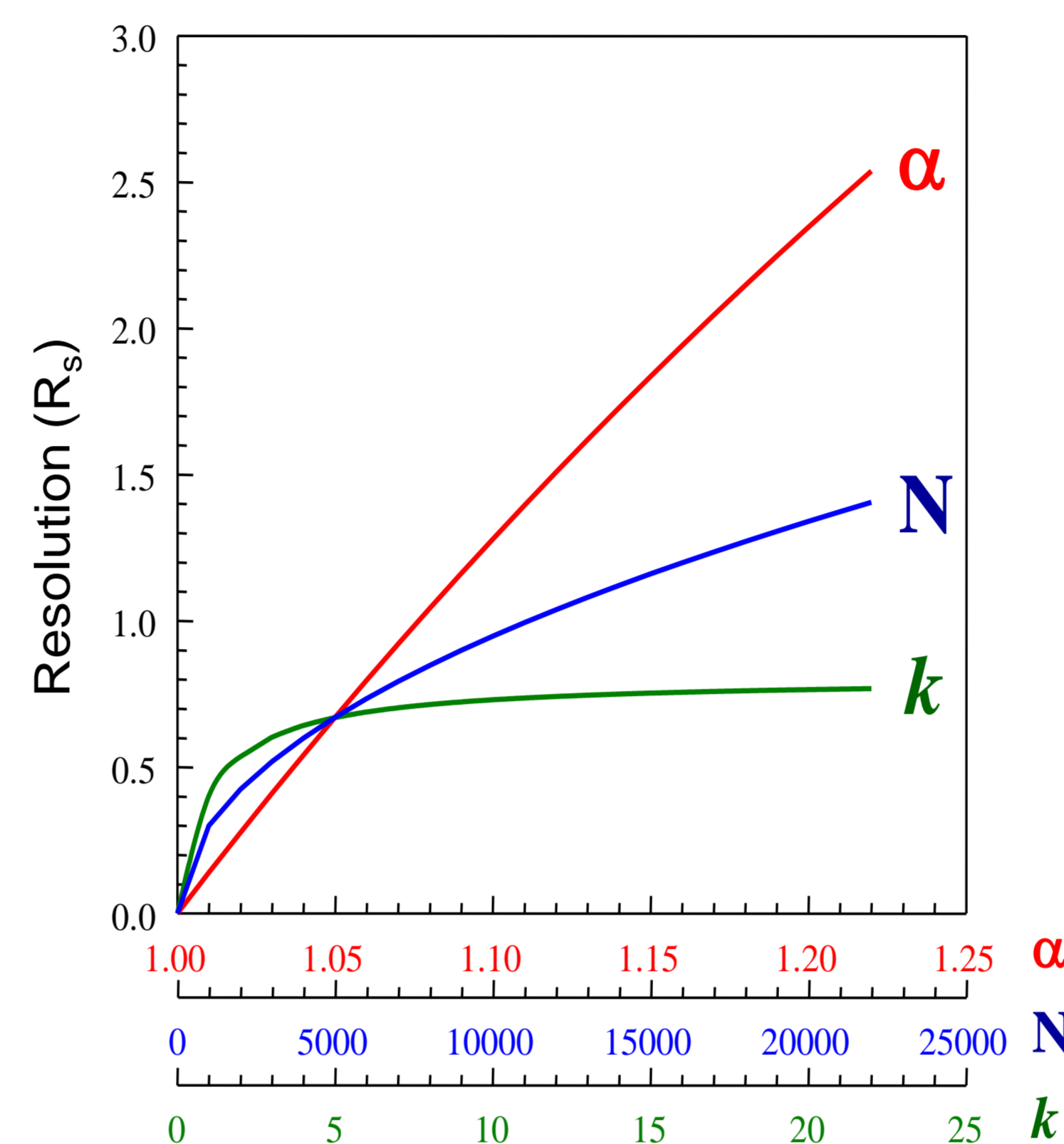
## 2. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha-1}{\alpha} \cdot \frac{k}{1+k}$$

Efficiency (N), Selectivity (α), Retention (k)

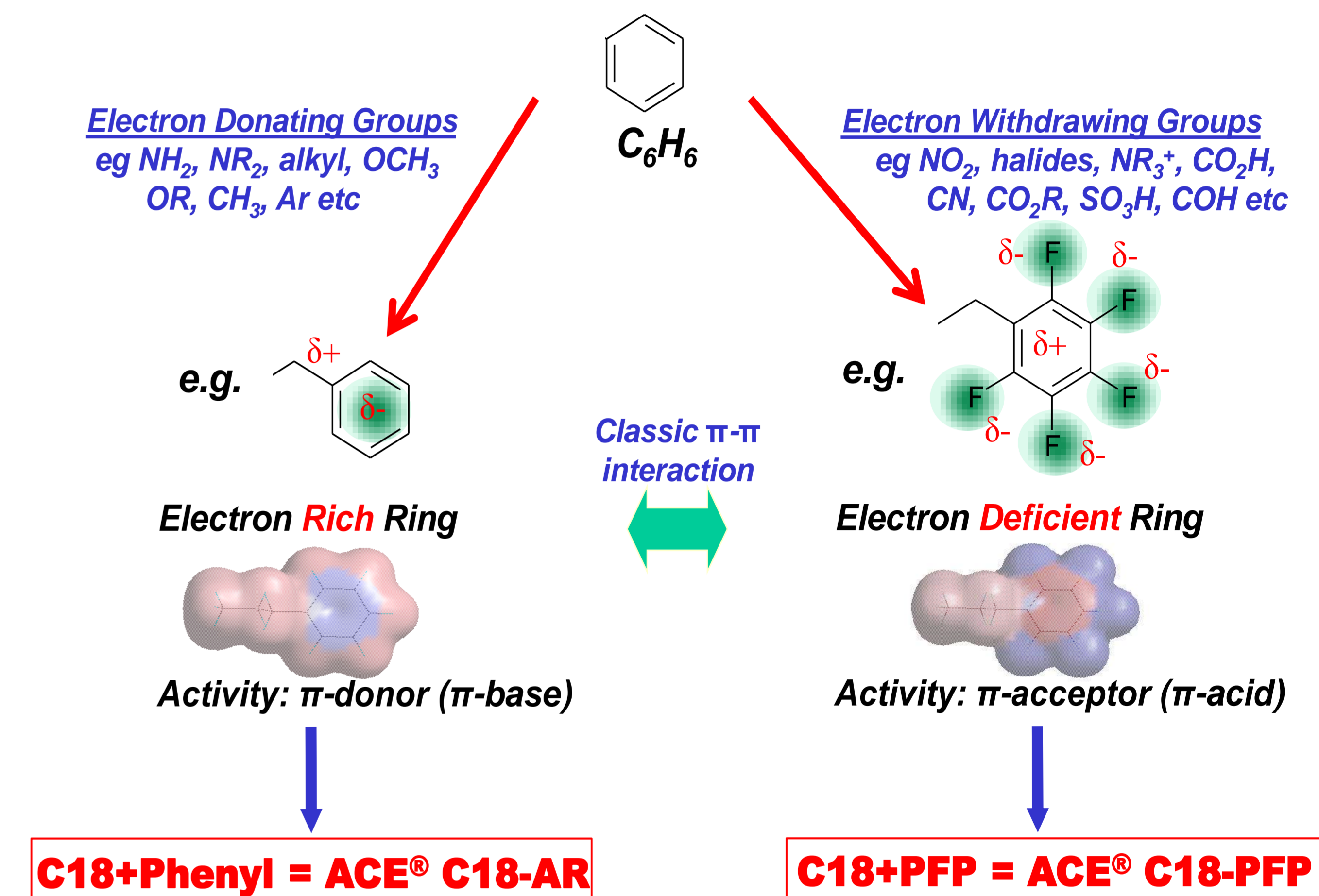
Selectivity has the greatest impact on peak resolution

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

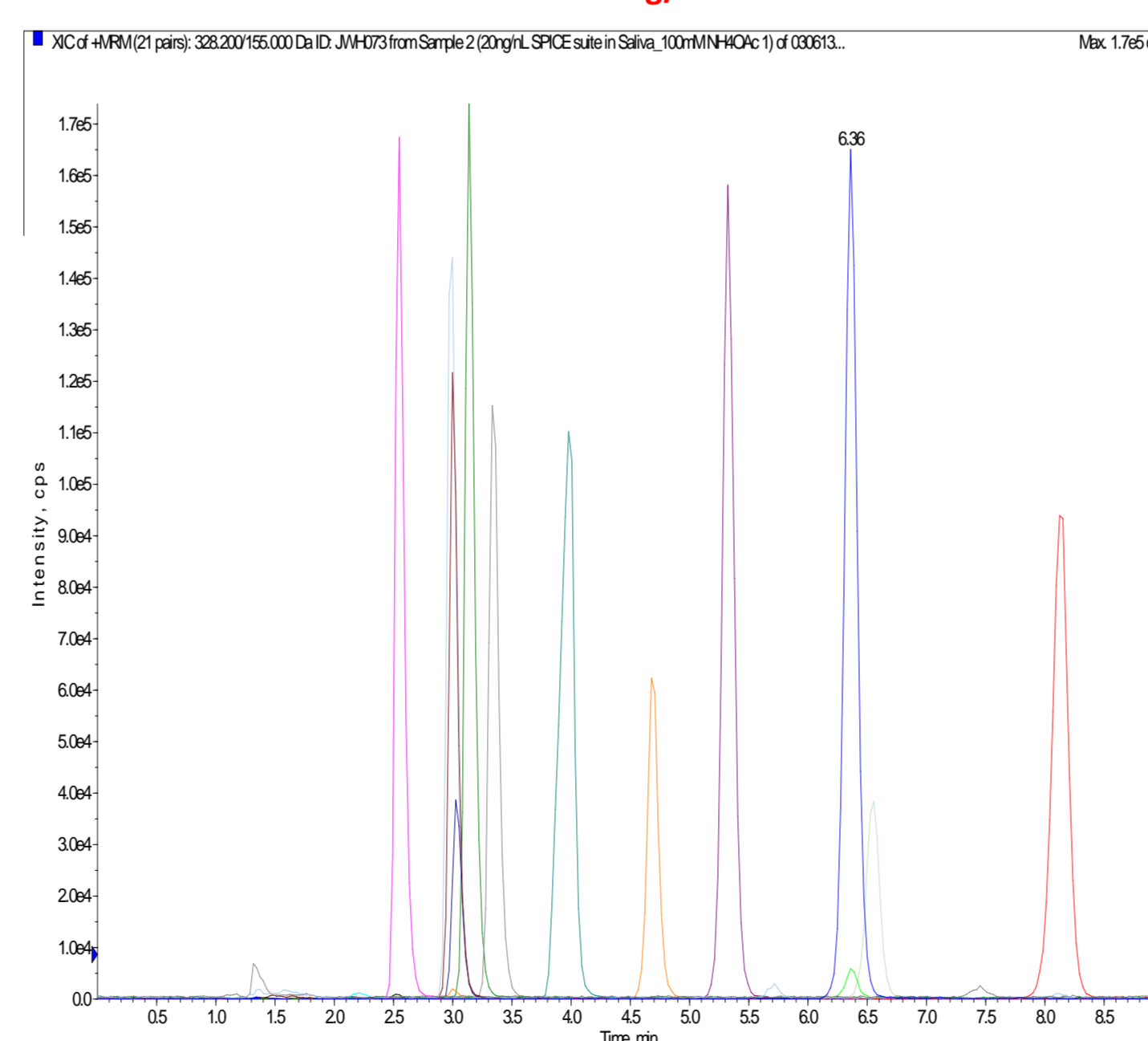


## 4. ORAL FLUID EXTRACTION & INSTRUMENT CONDITIONS

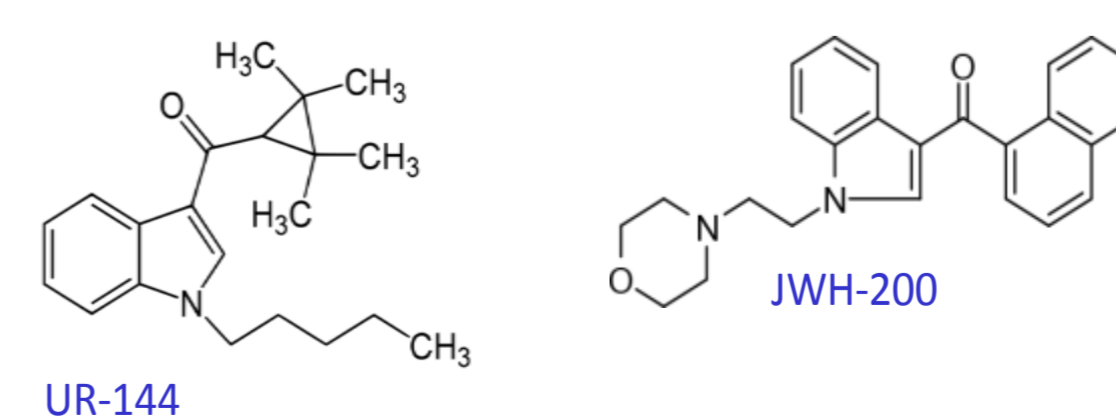
- Format:** ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate, part number 820-0400-PO1  
ISOLUTE® SLE+ 400 µL Sample Volume Columns, part number 820-0055-BG
- Oral Fluid Hydrolysis: (optional)** Add β-glucuronidase (5000 units/mL) to patient oral fluid, fortified calibration standards and/or QC 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 (CEXP)
2.55	JWH-250 N-(5-hydroxyphenyl)	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-hydroxyphenyl)	363.5>155	40	35	16
3.14	JWH-018 N-(4-hydroxyphenyl)	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



## 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**.

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