

# Use of a Novel C18-Based Stationary Phase for Human Urine Metabolite Profiling by UHPLC-High Resolution Accurate Mass Spectrometry (HRAM)

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## 1. BACKGROUND

- Urine profiling can provide **clinically useful** information.
- The **power of UHPLC** coupled to detectors such as **High Resolution Accurate Mass Spectrometry (HRAM)** is moving **clinical labs** into a new era of **sample information**.
- Human urine** is complex with a range of **polar to mid-polar** analytes present within a **highly polar matrix**. Profiling urine can help with **monitoring** or **diagnosing** various **disease states** / **endogenous metabolic** processes.
- This poster explores the use of a **1.7µm novel C18-based polar embedded** stationary phase suitable for the **retention and separation** of a wide range of **polar to mid-polar metabolites** in human urine.

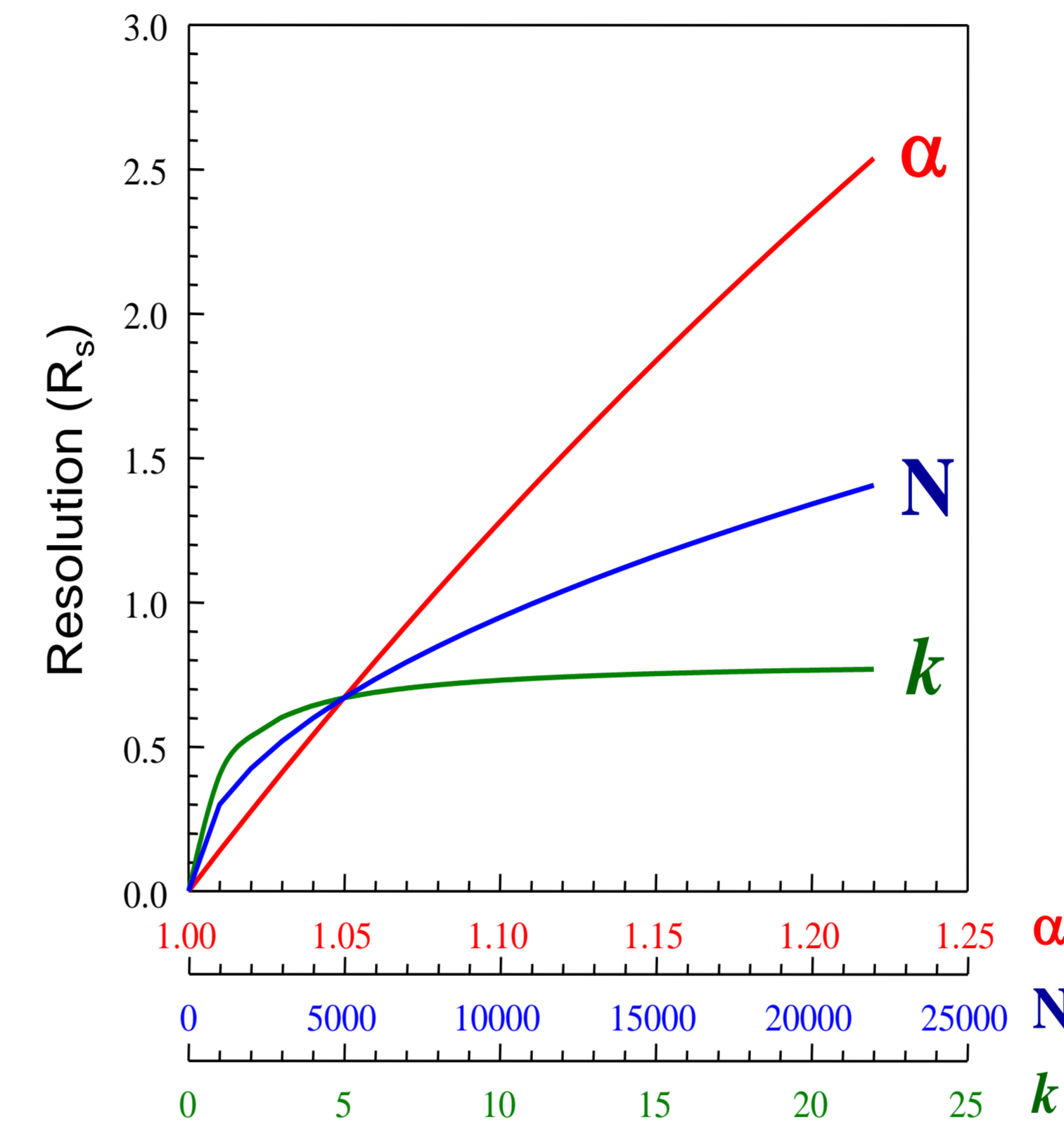
## 2. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION

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

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

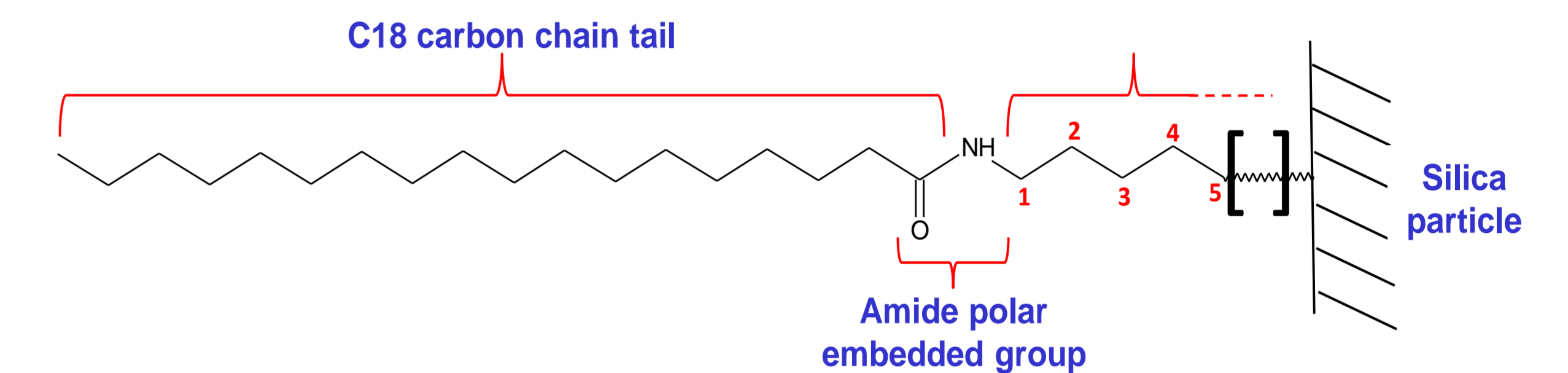
Selectivity has the greatest impact on peak resolution

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



- Ligand contains an **embedded amide moiety**:
  - Designed to **retain polar and non-polar analytes** within a single run.
  - Ideal for **H-bond donor analytes**: **acids, phenolics, amines, amides** etc.
  - Suitable for **analysis of teas, beverages** etc.
- Improved stability at low and mid pH** results from **extended carbon chain spacer**.
- C18 terminology** comes from 18 carbon 'tail'.

## 4. MATERIALS AND METHODS

Column: ACE Excel C18-Amide  
100 x 2.1 mm, 1.7µm

Part Number: EXL-1712-1002U

Mobile Phase: A: 0.01% formic acid in H<sub>2</sub>O  
B: 0.01% formic acid in MeCN

Gradient:	Time (mins)	%B
	0.0	3
	2.5	10
	8.0	100
	8.5	3
	10.0	3

Flow Rate: 0.5 mL/min

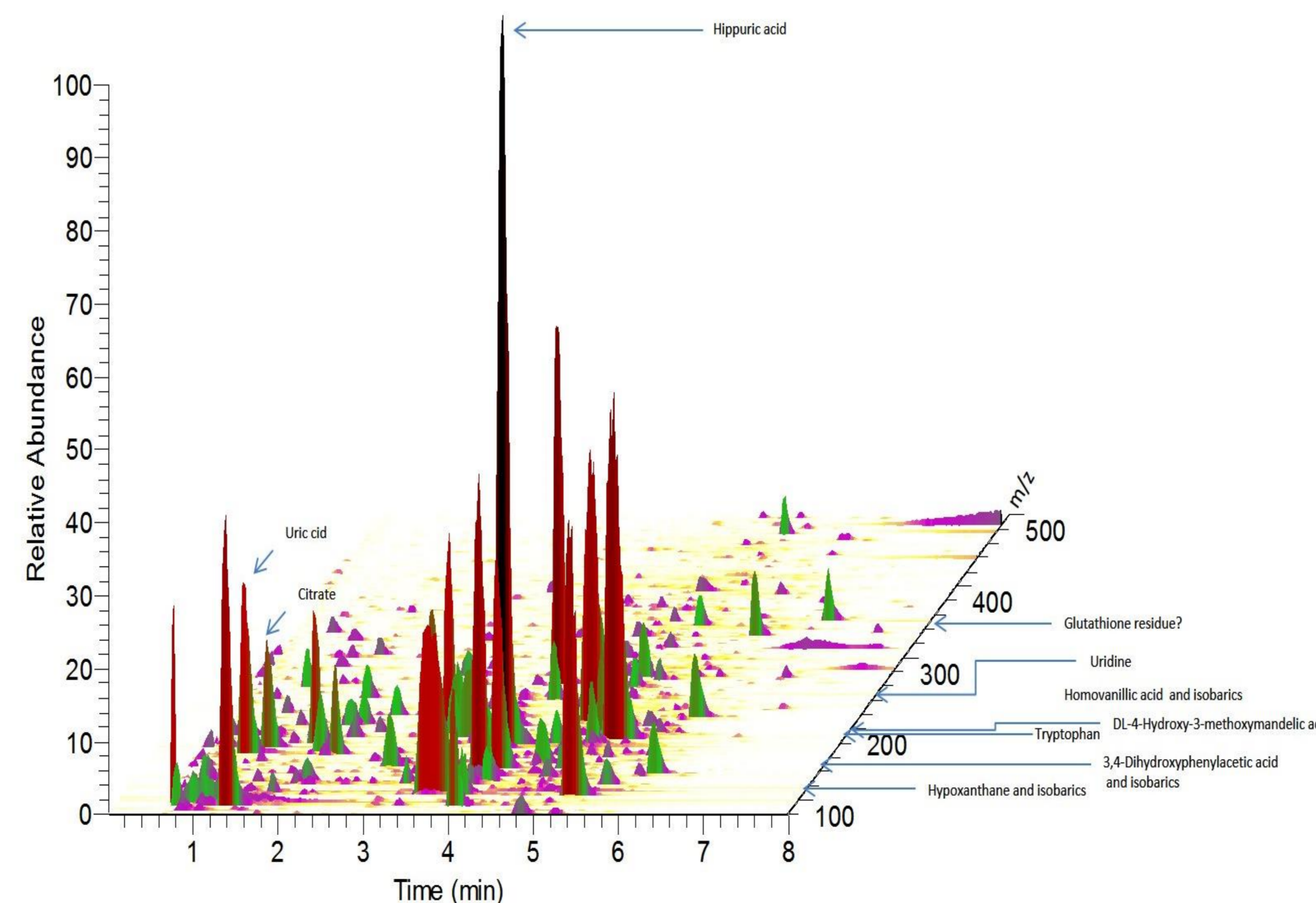
Detection: Thermo Scientific Exactive accurate mass MS.  
ESI in negative ion mode.  
Analytes between *m/z* 70-800 monitored.

Sample: Healthy adult volunteer urine. 1ml sample centrifuged at 4C at 13000rpm in microfuge vials and decanted into LC vials. Stored at -80C until analysis.



LC and MS images courtesy of ThermoScientific

## 5. HUMAN URINE METABOLITE PROFILING



## 6. SUMMARY AND CONCLUSIONS

- A **rapid UHPLC** method using a novel **ACE Excel C18-Amide, 1.7µm** column with **HRAM** detection for **profiling human urine samples** has been established.
- The new method was used to **qualitatively explore** the **metabolite profile** of human urine covering a range of **polar** analytes including **isobaric** species.
- The **novel ACE Excel C18-Amide** stationary phase demonstrated **excellent separation performance** for a range of analytes allowing **identification** of a variety of **small to medium sized** metabolites.

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