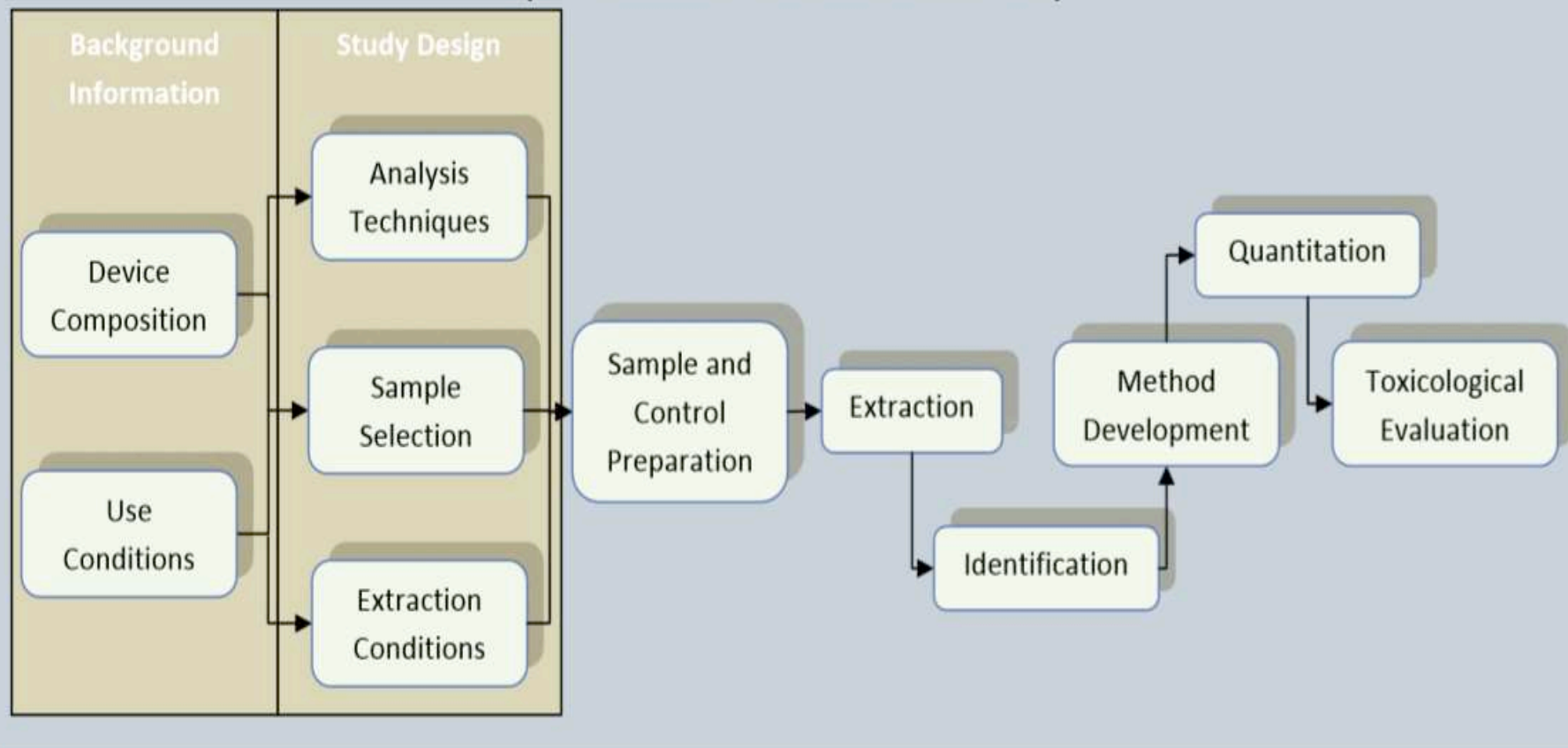


Designing an Extractables and Leachables Study

Overview

Steps in an Extractables and Leachables Study



Background Information

Materials of Construction

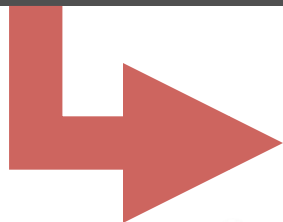
What additives are used?
Multilayer films?
Printing Inks?

Finished Packaging

Which surfaces does the product contact?
Are there interactions between components?
Is there opportunity for non-product contact components to migrate?
Are there any processing aids used?

Use Conditions

How will it be used?



How should it be extracted?

Extractables

challenge the use condition

- Temperature
- Solvent Strength
- Time

Leachables

mimic the most stringent use condition

Extraction Strategies

Cut and Cover Extraction

Simple systems

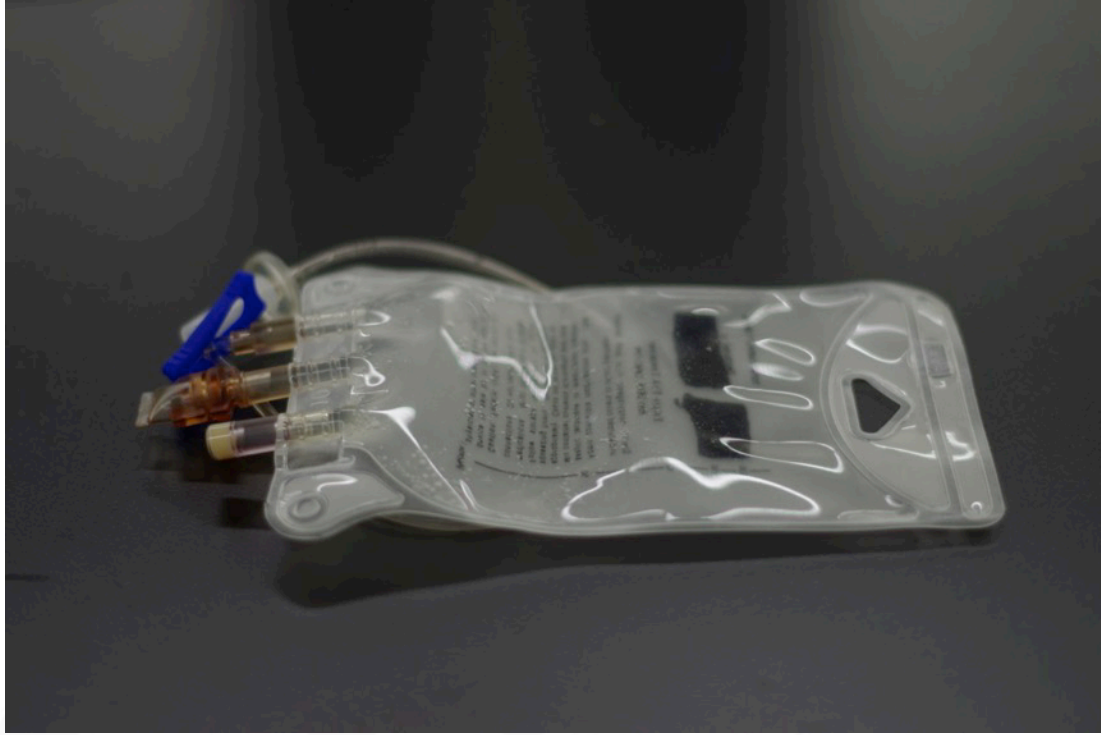
Irregularly shaped

Articles requiring two-sided
extraction

Very large containers



Extraction Strategies



Bags

Small containers

Tubing

Non-

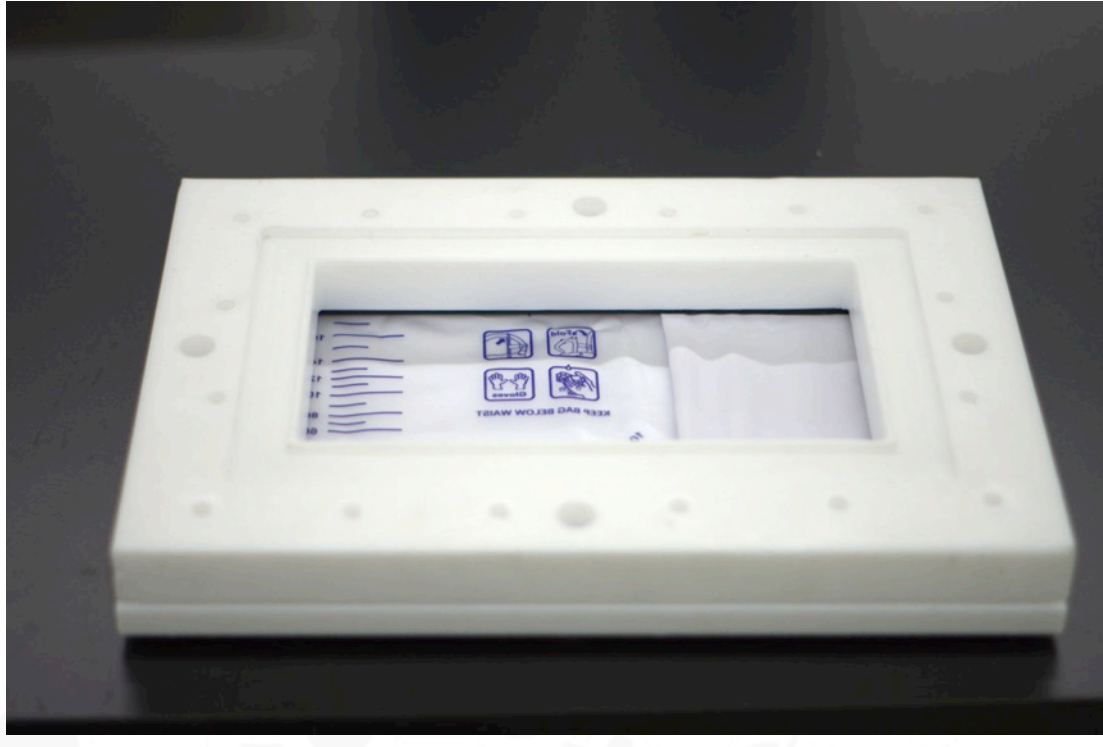
permeable

Printed

containers

Full Fill Extraction

Extraction Strategies



Multi-layer
Printed
materials
Coated
materials
Product contacts
one side

One-Sided Extraction

Extraction Strategies

Tubing
Complex
systems
In-Line filters
Connectors



Flow Through Extraction

Extraction Strategies



Direct analysis
of the entire
article

Very high
sensitivity

No risk of
volatiles loss

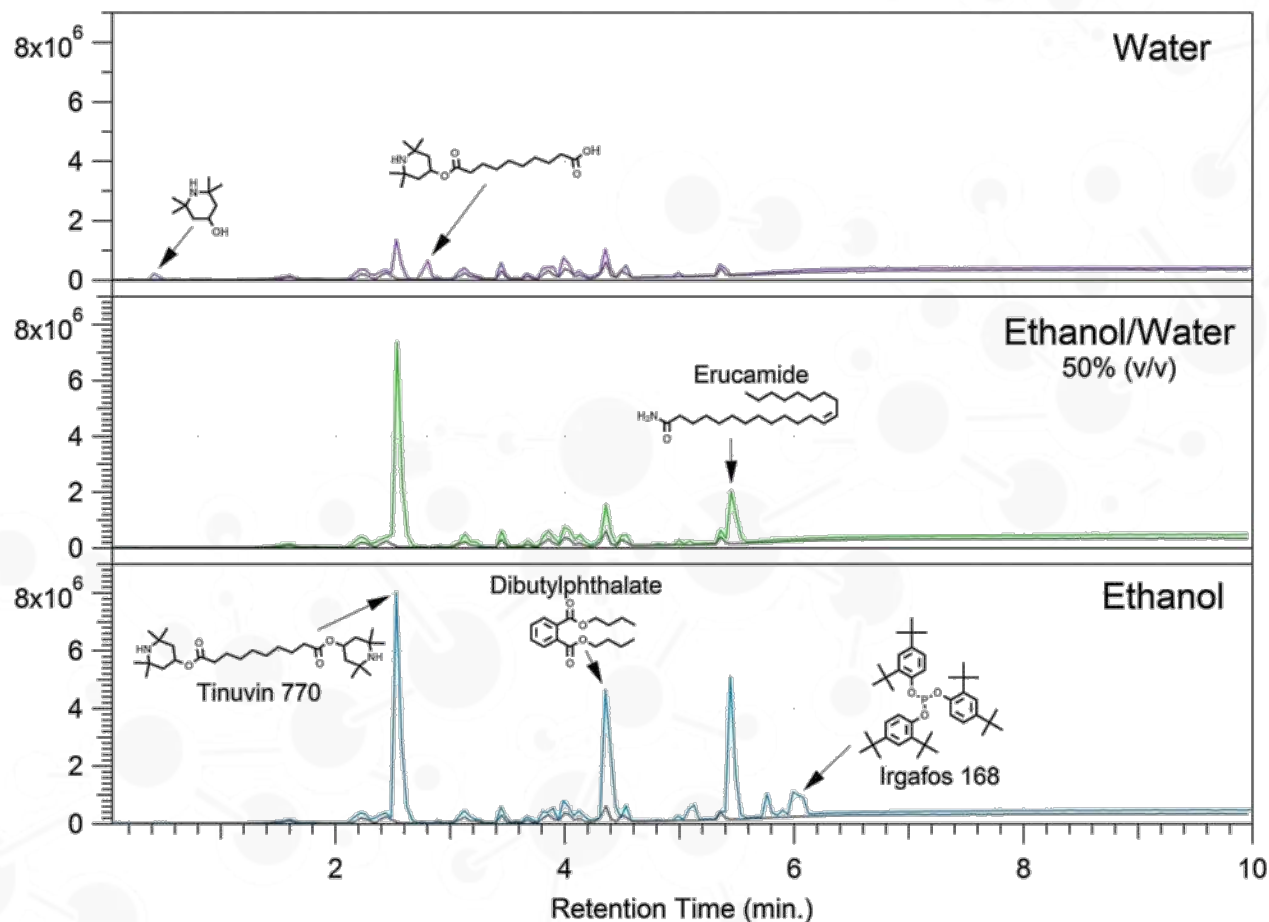
Large Volume Dynamic Headspace

Extraction Solvent

From the background information provided for a container closure system the probable extractables were:

Expected Extractable	Type	Log P	Boiling point
Erucamide	Hydrophobic	8.8	474 °C
Linear Alkanes	Hydrophobic	8.859	250-400 °C
Dibutylphthalate	Hydrophobic	4.72	340 °C
Dimethoxyethane	Volatile	-0.2	85 °C
Irganox 1010	Non-volatile	23	N/A
Irgafos 168	Non-volatile	15.5	N/A
Tinuvin 770	Basic	6.3	N/A
Stearic acid	Acidic	8.23	361 °C
Sodium benzenesulfonate	Anionic	N/A	N/A

Effect of Solvent Polarity



Agilent 1290 Infinity UHPLC; Agilent 6520 QTOF-MS
Column: Agilent Zorbax Eclipse Plus C8; 1.8 μm , 2.1 $\times$ 50 mm
Electrospray Ionization (ESI); Polarity: positive

Extract Preparation

Example:

- 250 mL per bag
- Worst case 3 bags per day
- Product contact surface area 310 cm^2
- Safety concern threshold (SCT) = $0.15 \mu\text{g}/\text{day}$

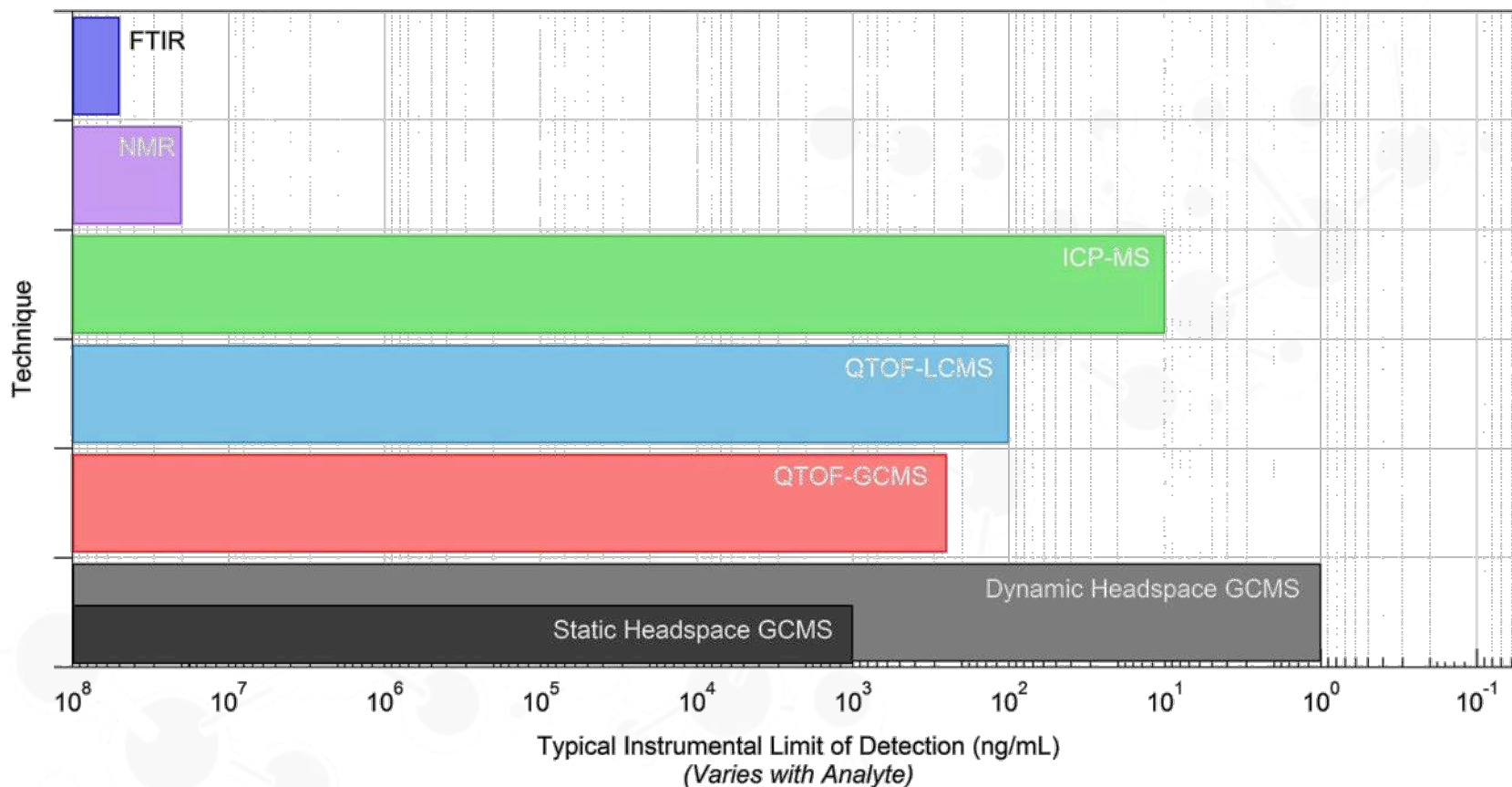
$$AET(\mu\text{g}/\text{bag}) = 0.15 \mu\text{g}/\text{day} / 3 \text{ bags}/\text{day} = 0.05 \mu\text{g}/\text{bag}$$

$$\text{Extract Vol. (mL)} = 310 \text{ cm}^2 / 6 \text{ cm}^2/\text{mL} = 52 \text{ mL}$$

$$LOD = 0.05 \mu\text{g}/\text{bag} / 52 \text{ mL}/\text{bag} \approx 1 \text{ ng/mL}$$



Qualitative Analysis



Concentration of Extracts

Extractables & Leachables can be lost during concentration



Loss depends on

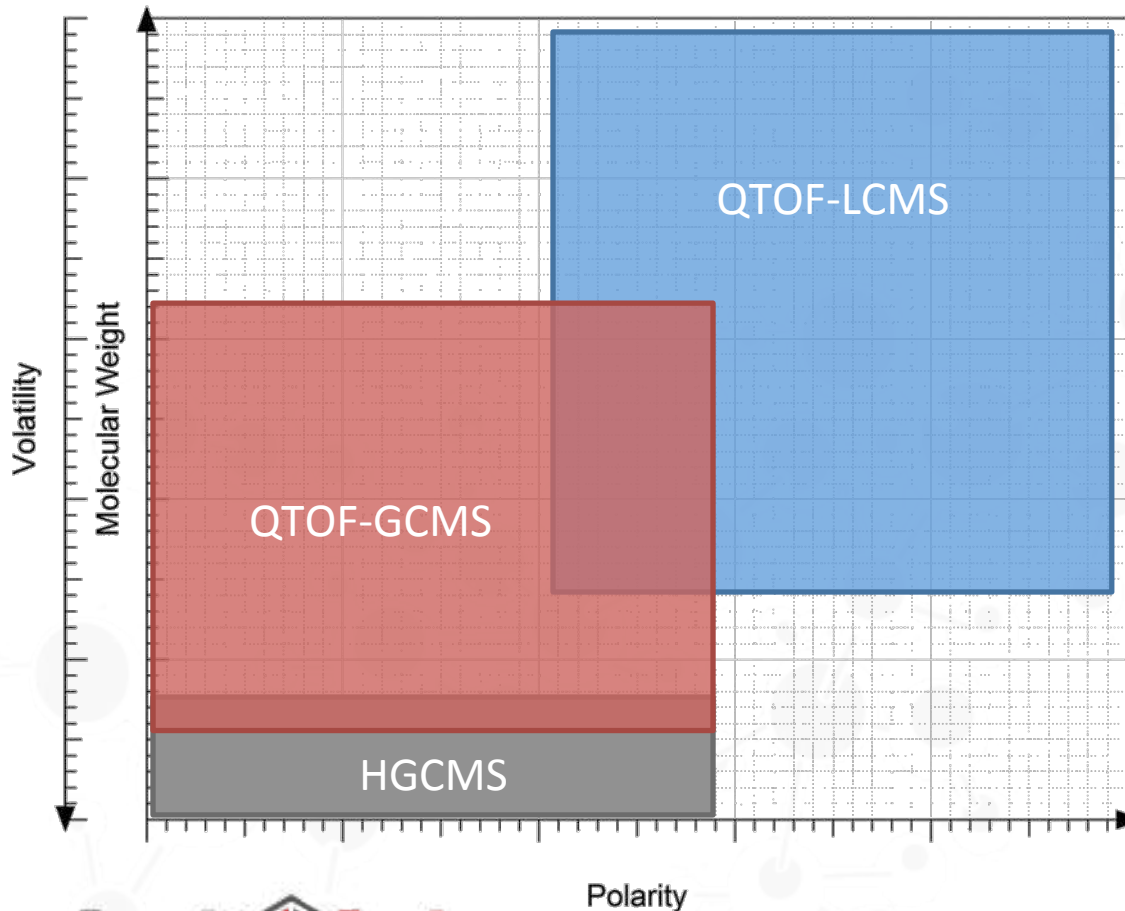
- Volatility of the analyte(s)
- Extract handling

Best Practices

- Mild conditions
- Method validation
- Headspace analysis

Qualitative Analysis

No universal analytical technique exists for E&L analysis



Headspace GCMS

- Volatiles

QTOF-GCMS

- Volatiles
- Semi-volatiles
- Non-polar analytes

QTOF-LCMS

- Polar/ionizable

ICP-MS

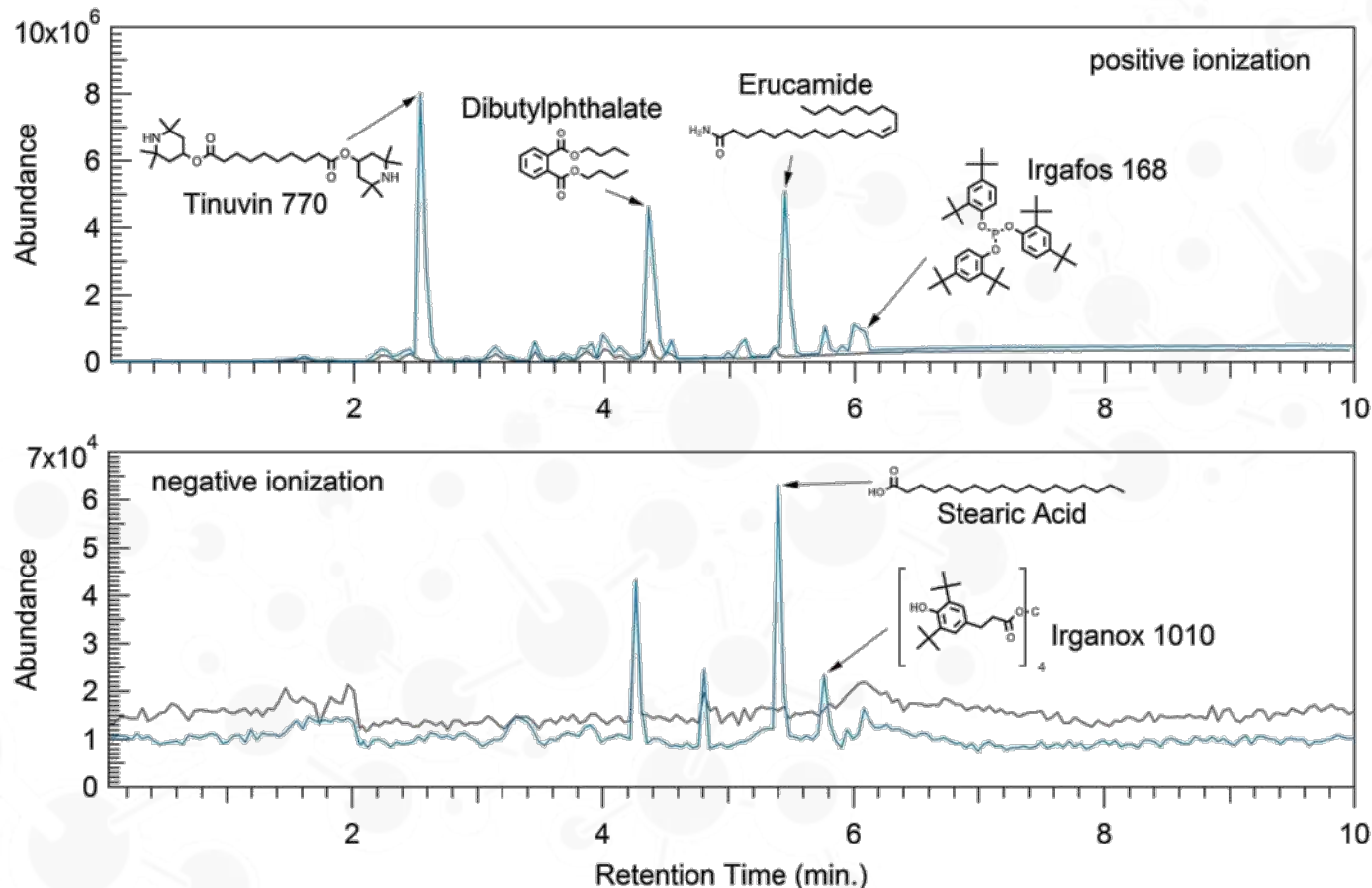
- Metals

Extraction Conditions

--Ethanol Extract--

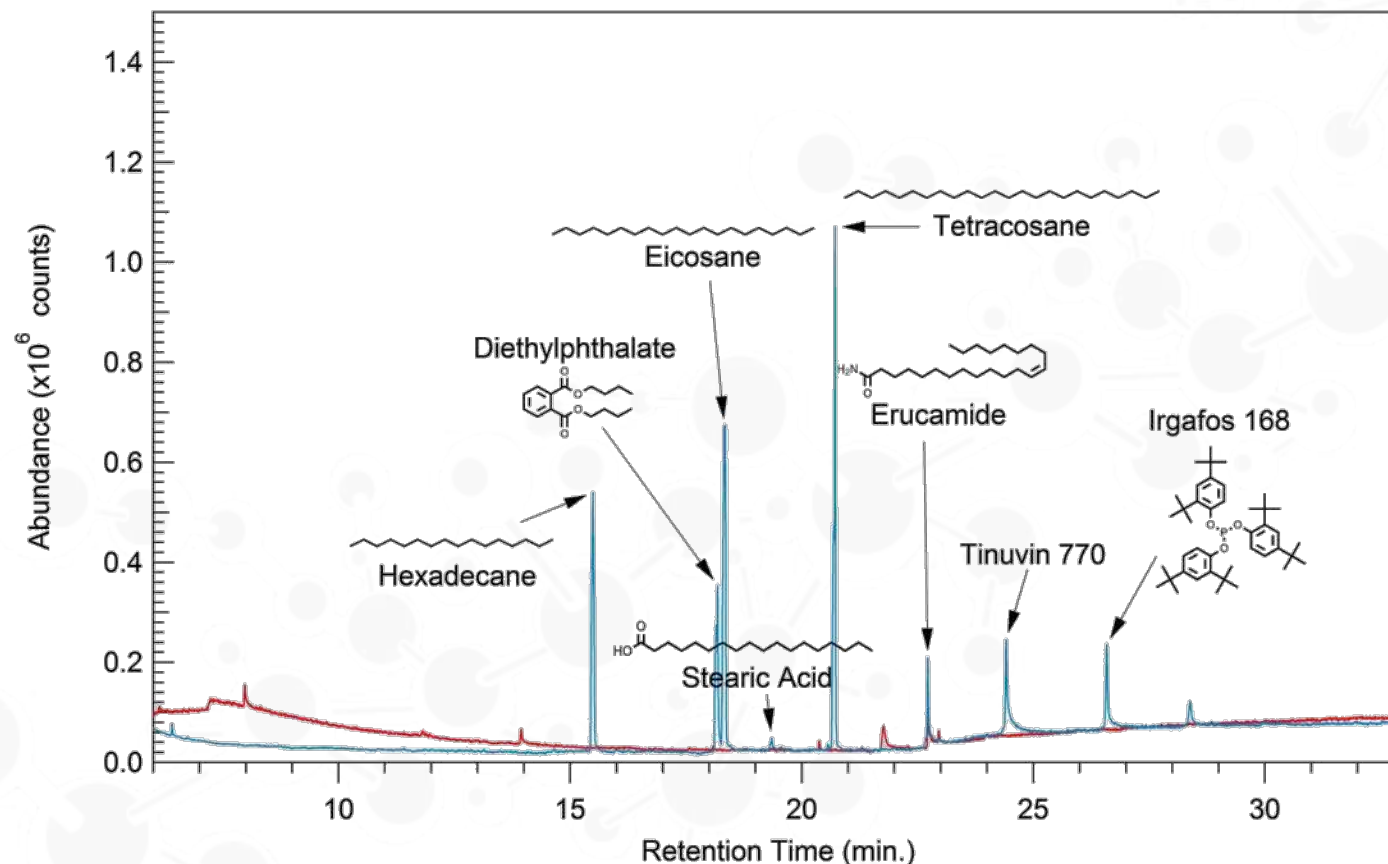
Expected Extractables	Type	Log P	Boiling point
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Tinuvin 770	Basic	6.3	N/A
Stearic acid	Acidic	8.23	361 °C
Sodium benzenesulfonate	Anionic	N/A	N/A

Qualitative Analysis



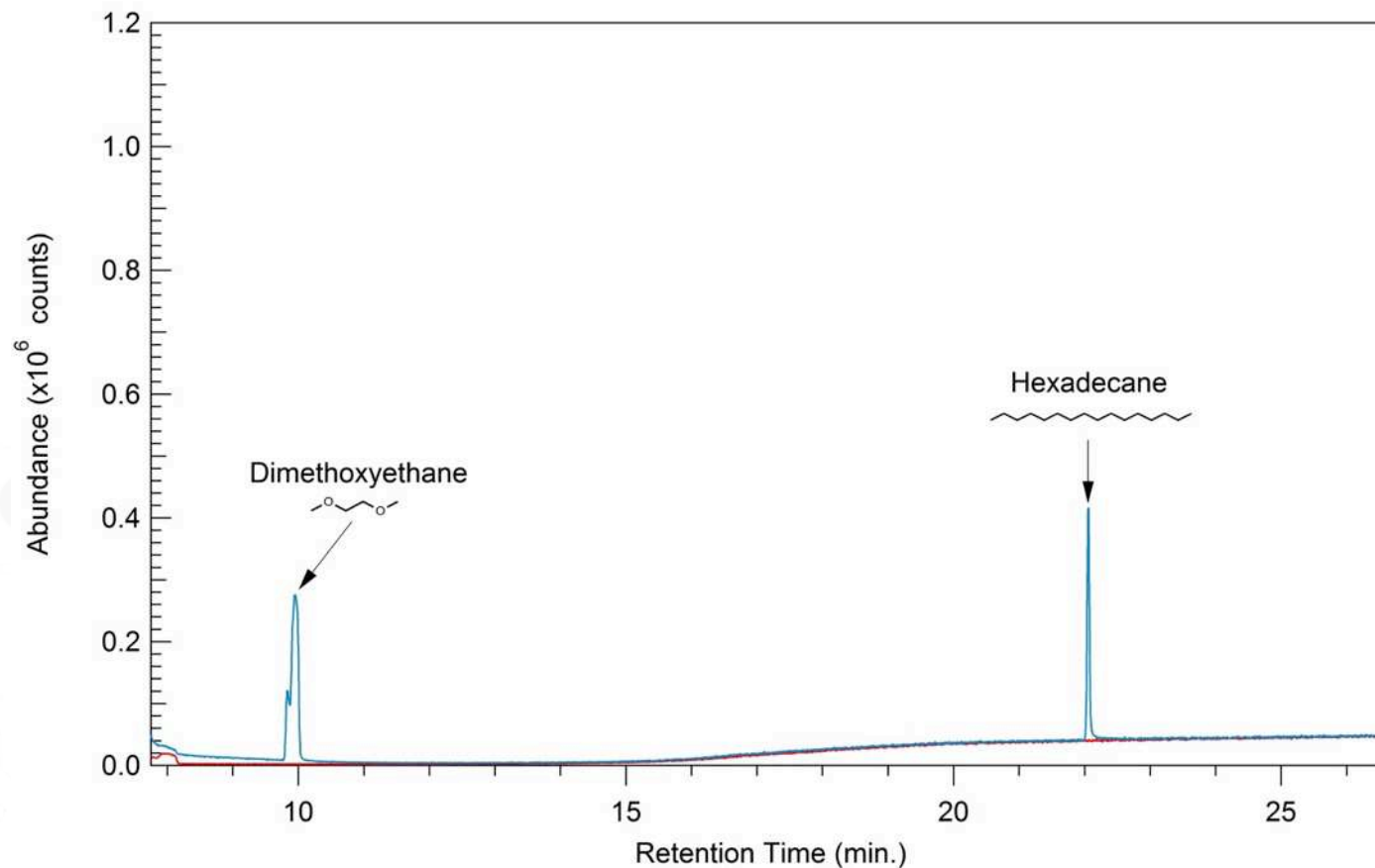
Agilent 1290 Infinity UHPLC; Agilent 6520 QTOF-MS
Column: Agilent Zorbax Eclipse Plus C8; 1.8 μm , 2.1 $\times$ 50 mm
Electrospray Ionization (ESI)

Qualitative Analysis



Agilent 7890B GC; Agilent 7200 QTOF-MS
Column: DB-5MS UI; 0.25 mm × 30 m, 0.25 µm
Electron Impact Ionization

Qualitative Analysis



Agilent 6890 GC; Agilent 5973 inert MSD
Electron Impact Ionization

Qualitative Analysis

Technique	Analyte	Boiling Point (°C)
HGCMS QTOF-GCMS QTOF-LCMS	Dimethoxyethane	85°C
	Hexadecane	286°C
	Linear Alkanes	286-400°C
	Dibutylphthalate	340°C
	Tinuvin 770	>350°C
	Irgafos 168	N.A.
	Stearic Acid	361°C
	Erucylamide	474°C
	Irganox 1010	Non-Volatile

Identification of Unknowns



Database Searches

Commercial Databases
(NIST, Wiley)

Jordi Proprietary Additive
and Oligomer Databases

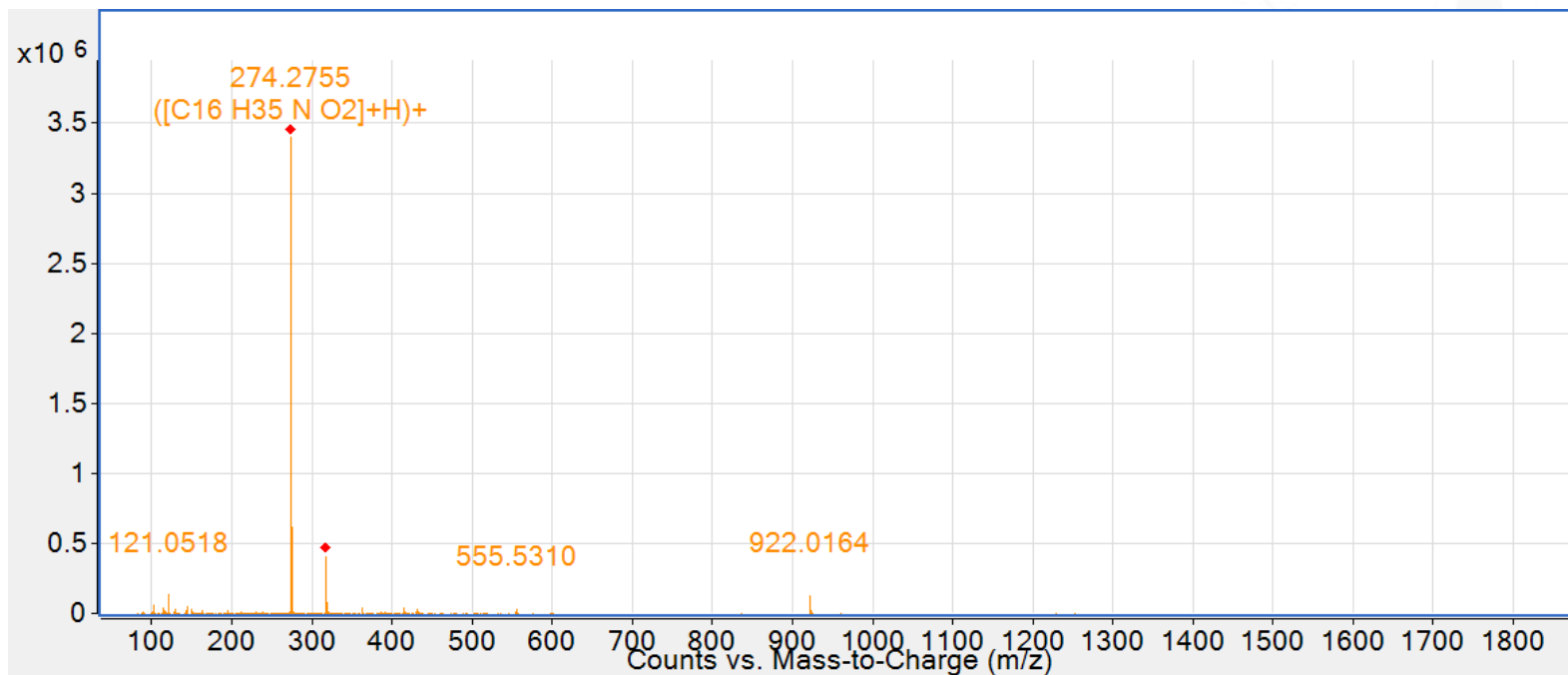
QTOF-GC / QTOF-LC

Molecular Formula
Generation (MFG)

MS/MS for QTOF -LCMS

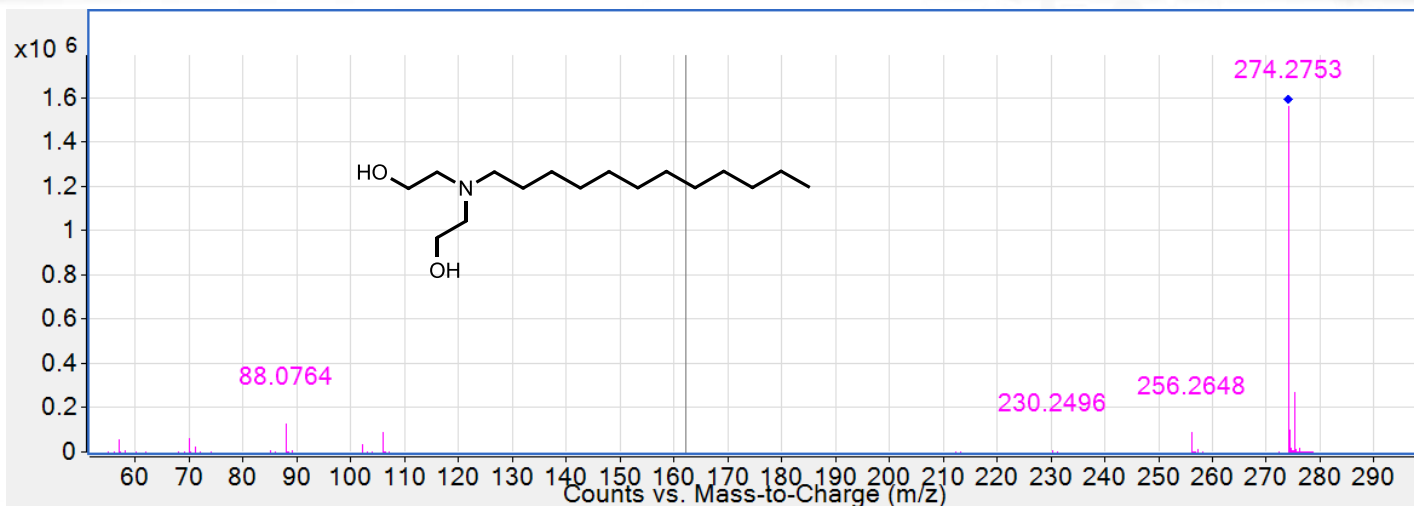
CI for QTOF-GCMS

Identification of Unknowns



m/z	Best Match	Species	Mass	Score (MFG)	Diff. (ppm)	DBE
274.2731	C ₁₆ H ₃₅ N O ₂	[M+H] ⁺	273.2660	94.66	2.73	0

Identification of Unknowns



Fragment Ion	Best Match	Score	Possible Structure
256.2648	[C ₁₆ H ₃₄ N O] ⁺	97.5	
230.2496	[C ₁₄ H ₃₂ N O] ⁺	84.16	
106.0869	[C ₄ H ₁₂ N O ₂] ⁺	99.07	
88.0764	[C ₄ H ₁₀ N O] ⁺	99.09	

Quantitative Strategies

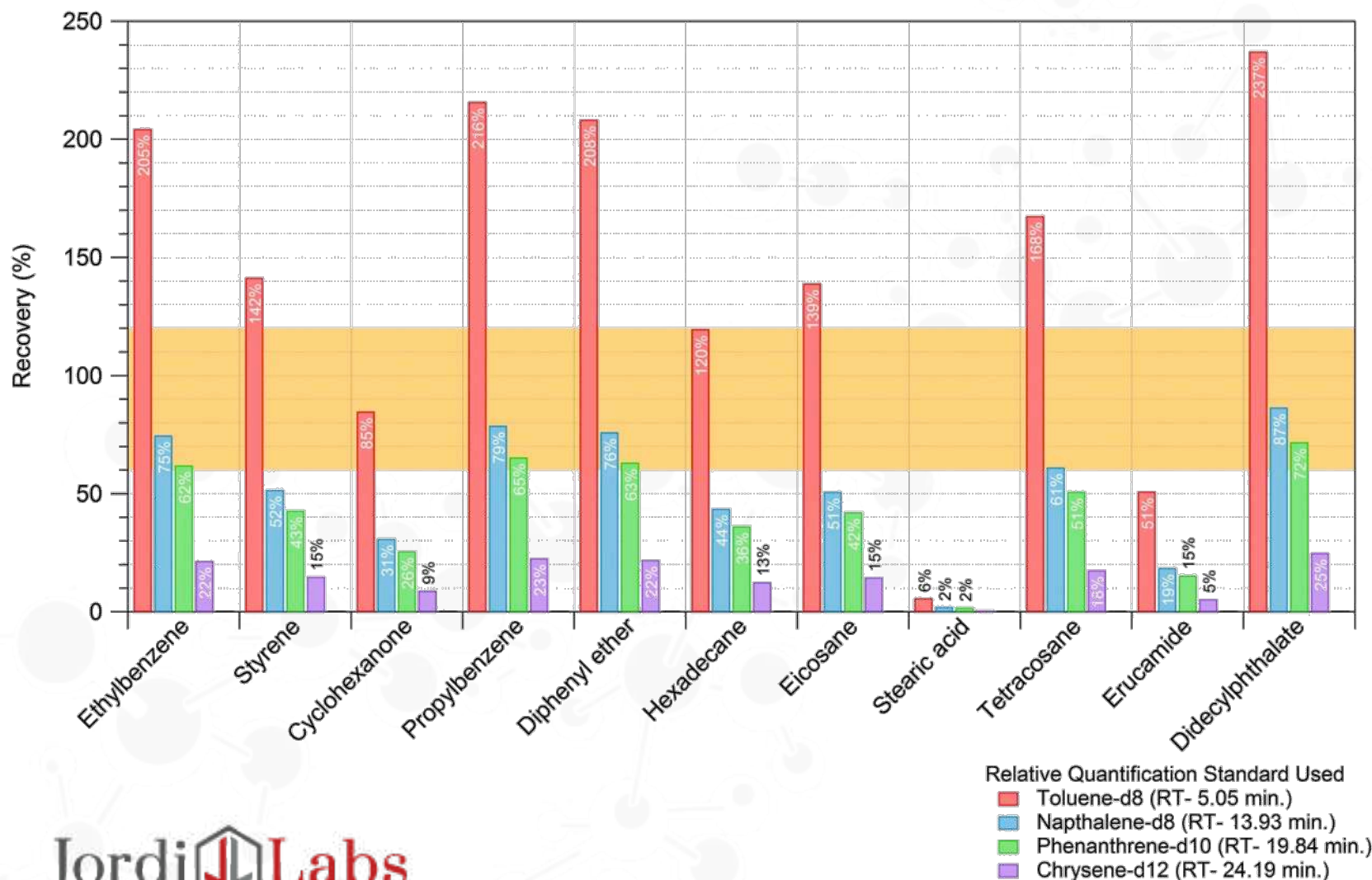


Relative Quantitation

- Quantification of compounds observed is performed against surrogate standards
- Accuracy depends on the surrogate standard used

Estimated Analyte Conc. = Observed Analyte Peak Area / Surrogate Peak Area × Surrogate Conc.

Quantitative Strategies



Quantitative Strategies

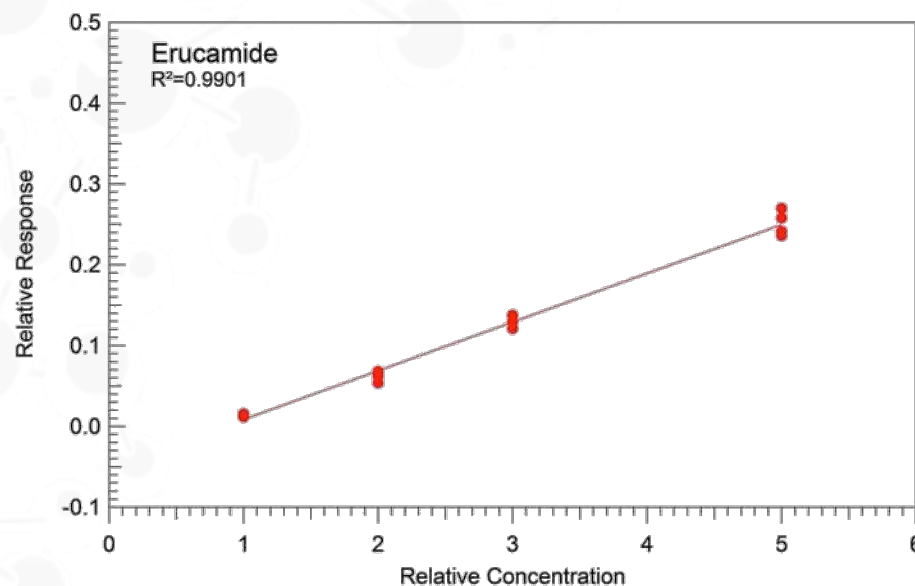
Formal Quantitation

Confirmed compounds are quantified against an analytical standard at a series of concentrations

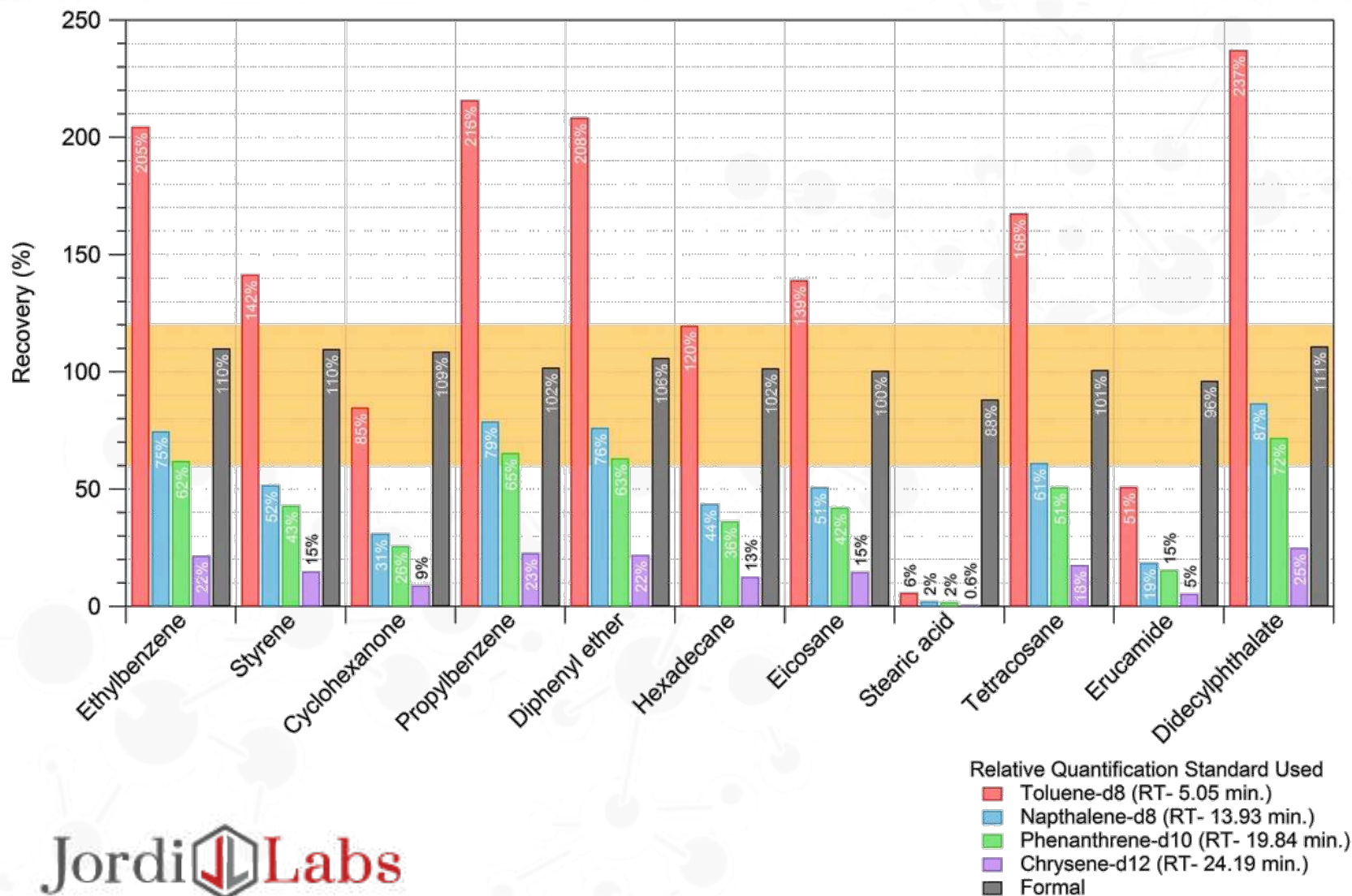
Methodologies:

- External Standardization
- Standard Addition

Requires high purity analytical standards



Quantitative Strategies



Quantitative Method Development

Quantitative Methods

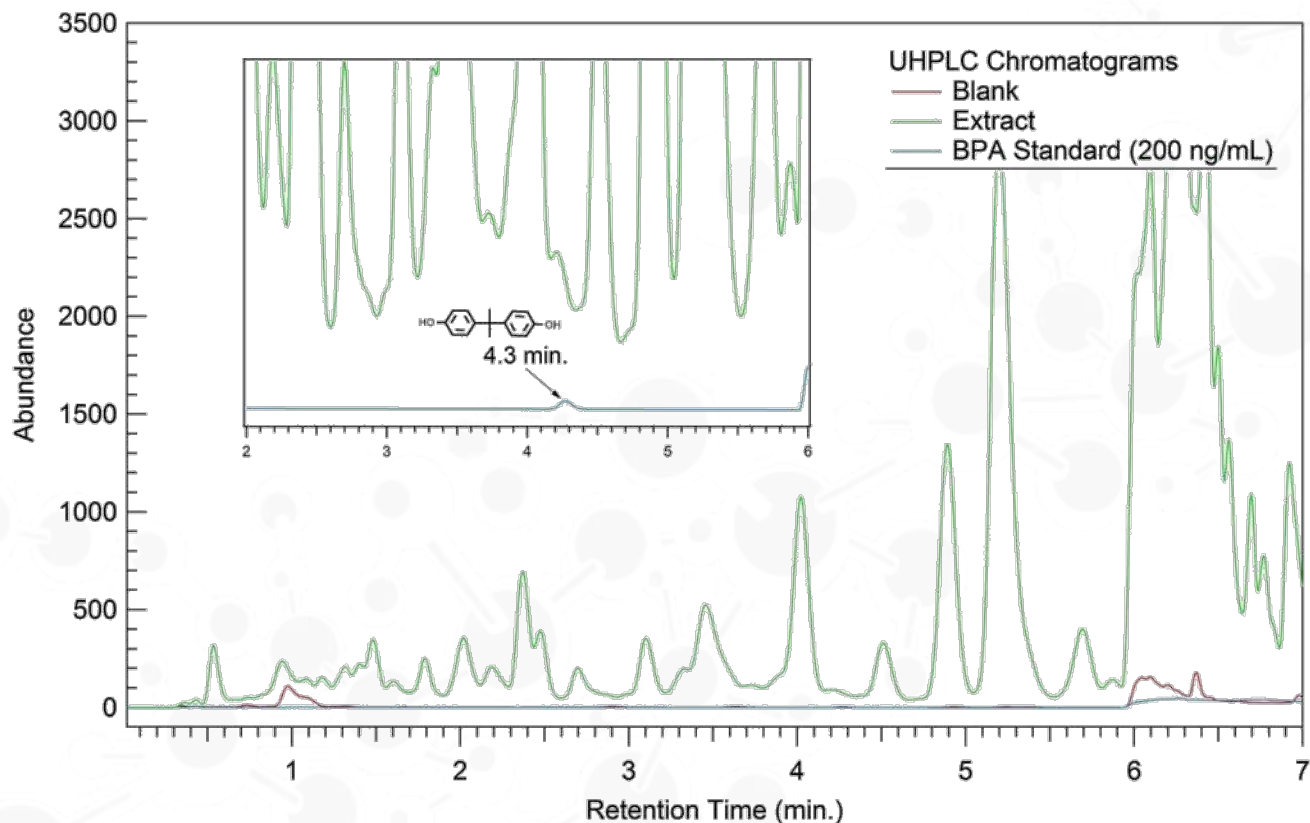
- Dynamic Headspace GCMS
- UHPLC-UV
- UHPLC-CAD
- QTOF-GCMS

In formal quantitation limit of quantitation (LOQ) can be improved using:

- Targeted MS/MS
- Large Volume Injection

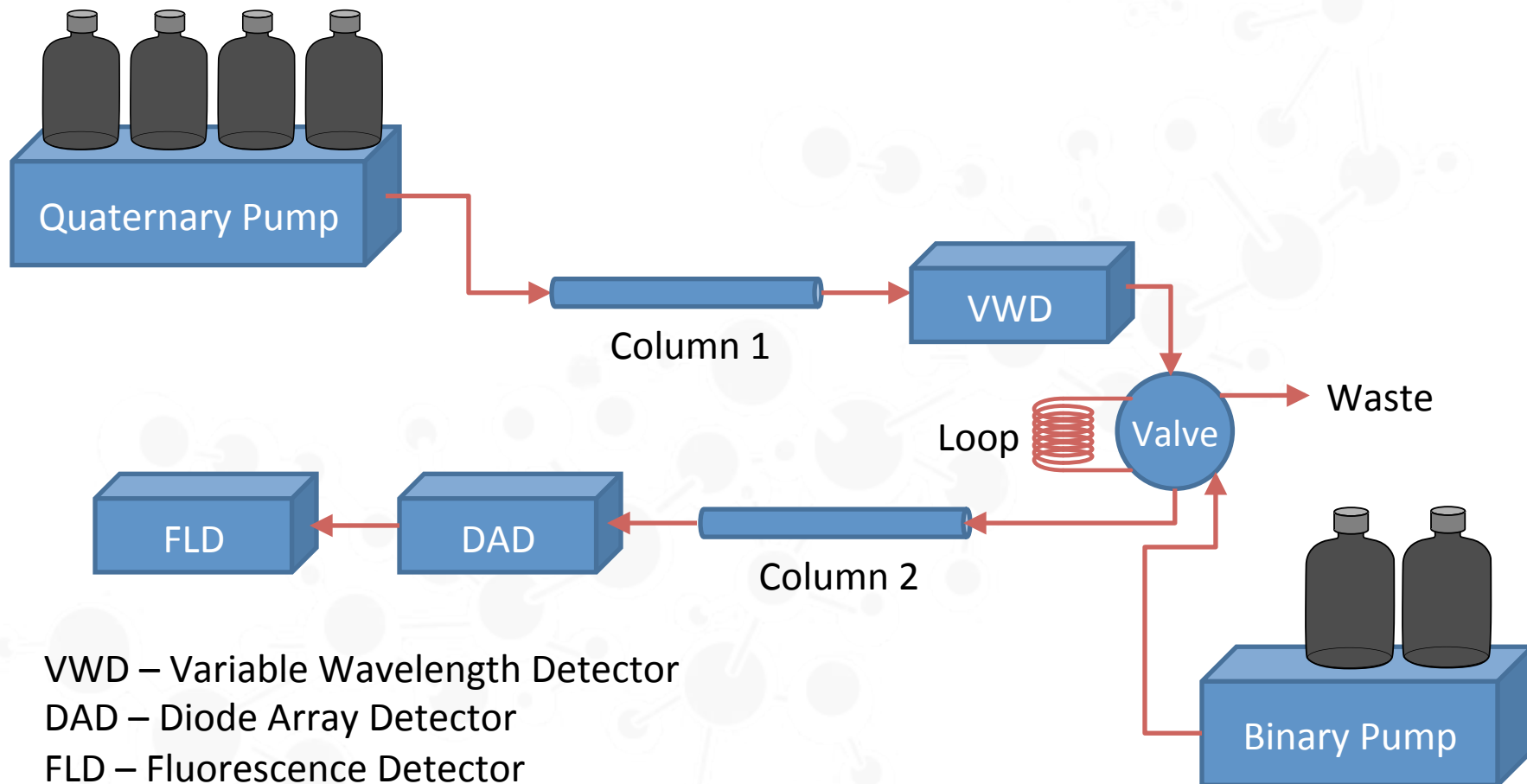


Quantitative Method Development



Agilent 1290 Infinity UHPLC
Column: Zorbax SB-C18, 1.8 μ m, 100 $\times$ 2.1 mm
Mobile Phase: H₂O – ACN Grad.
Detection: 230 nm (DAD)

2D UHPLC

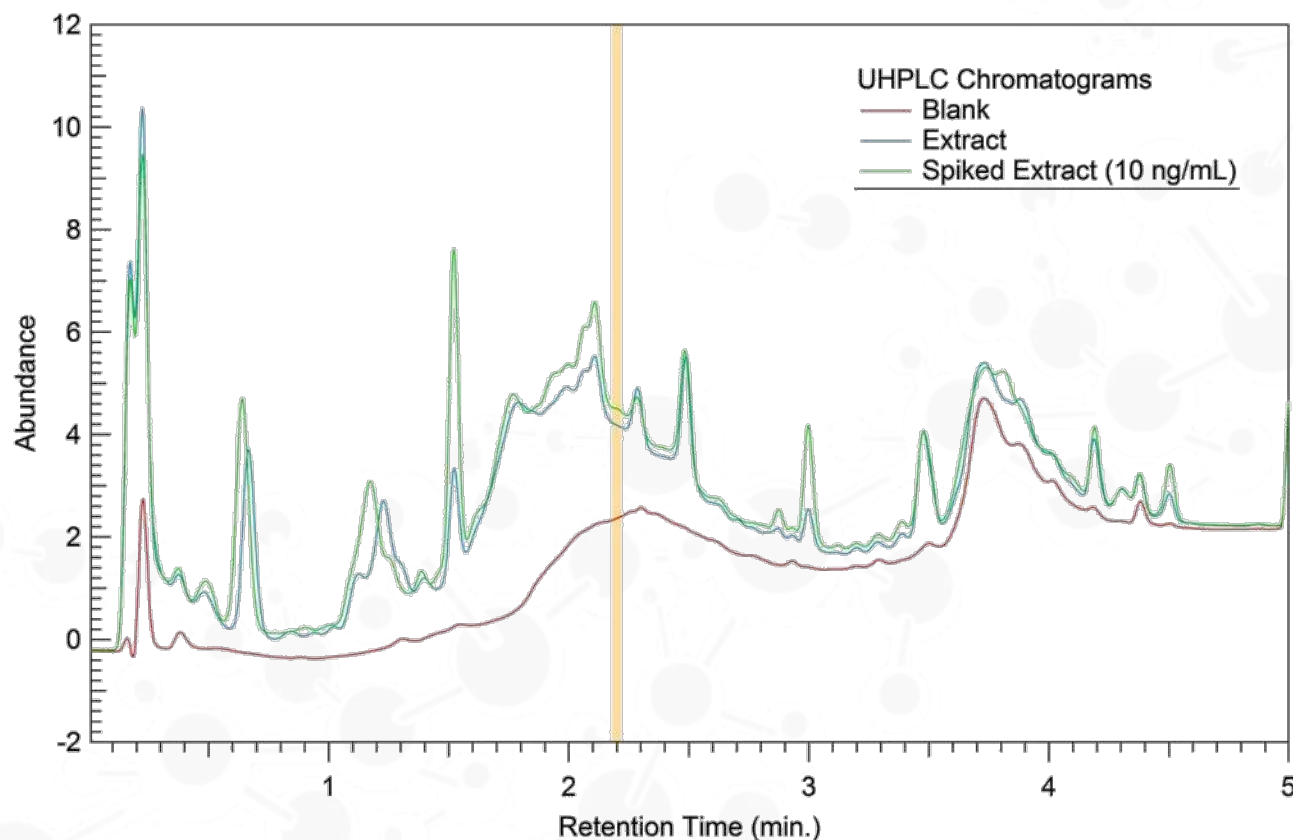


VWD – Variable Wavelength Detector

DAD – Diode Array Detector

FLD – Fluorescence Detector

Quantitative Method Development



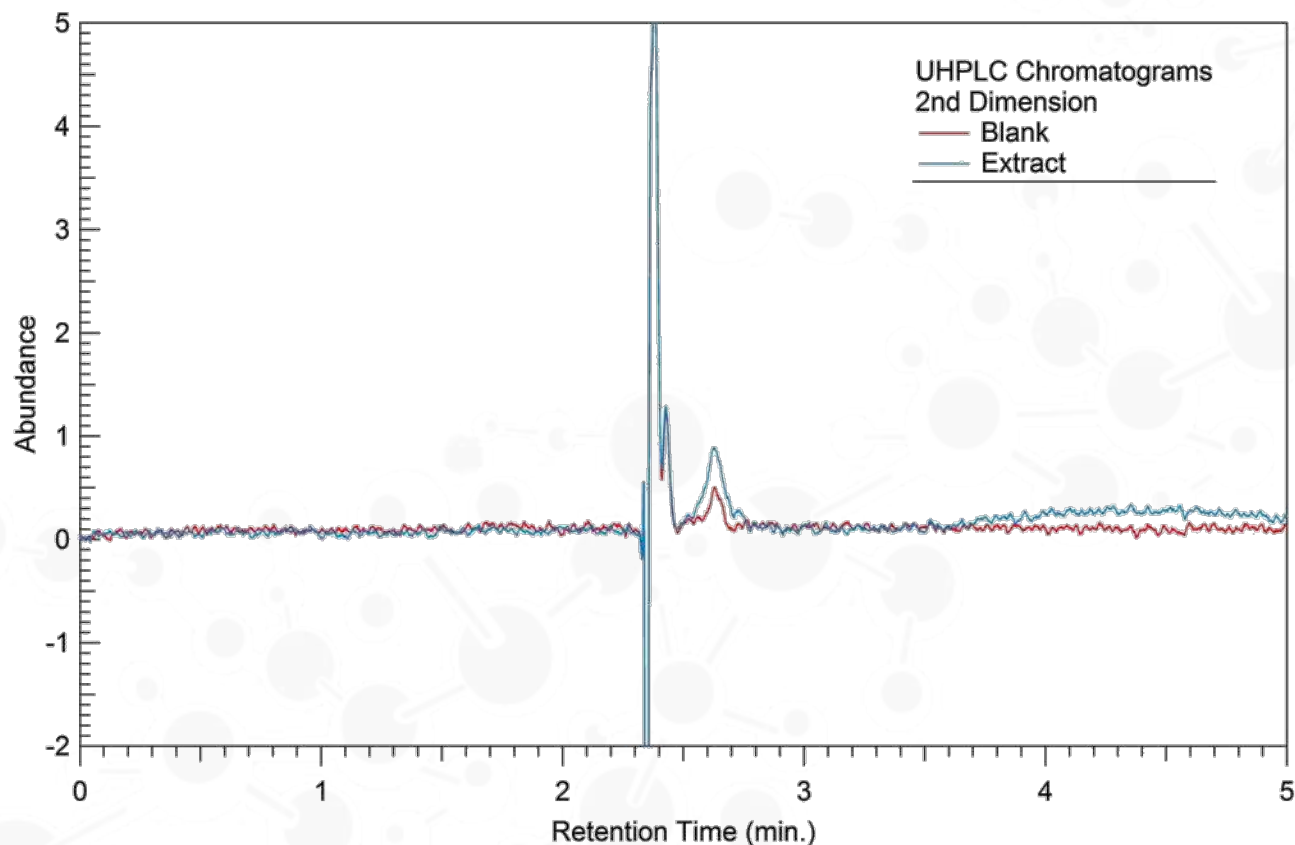
Column: Zorbax SB-C18; 1.8 μm , 2.1 $\times$ 50 mm

Mobile Phase: H_2O – ACN Gradient

Detection: 230 nm (VWD)

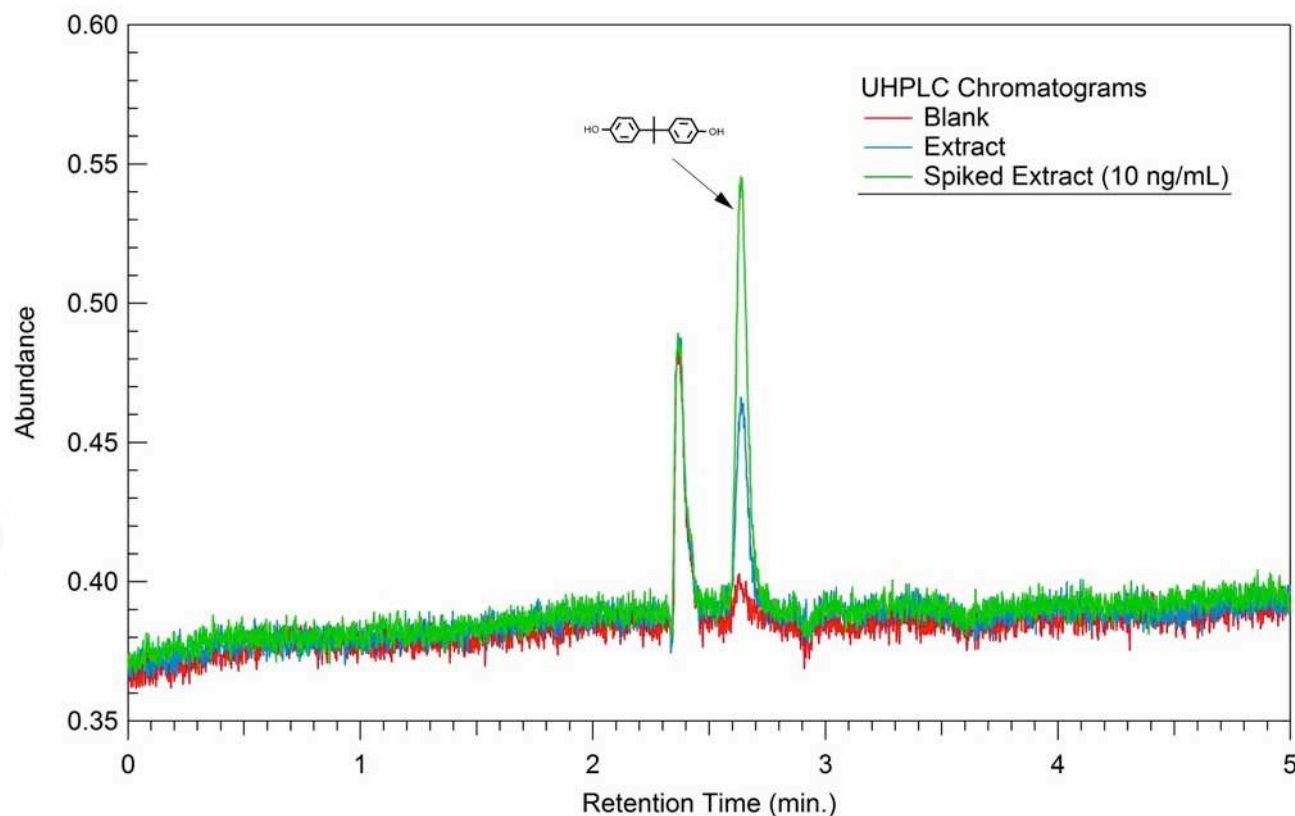
Collection Mode: Heart-cutting; 2.18-2.22 minutes (40 μL)

Quantitative Method Development



Column: Eclipse Plus Phenyl-Hexyl; 1.8 μm , 2.1 $\times$ 50 mm
Mobile Phase: H₂O – ACN Isocratic
Detection: 230 nm (DAD)

Quantitative Method Development



Column: Eclipse Plus Phenyl-Hexyl; 1.8 μm , 2.1 $\times$ 50 mm

Mobile Phase: H_2O – ACN Isocratic

Detection: Fluorescence; 225 nm excitation, 310 nm emission



A SUCCESSFUL STUDY HAS:

1. Appropriate Extraction Conditions
2. Careful Concentration of Extracts
3. Definitive Identifications
4. Accurate Quantification