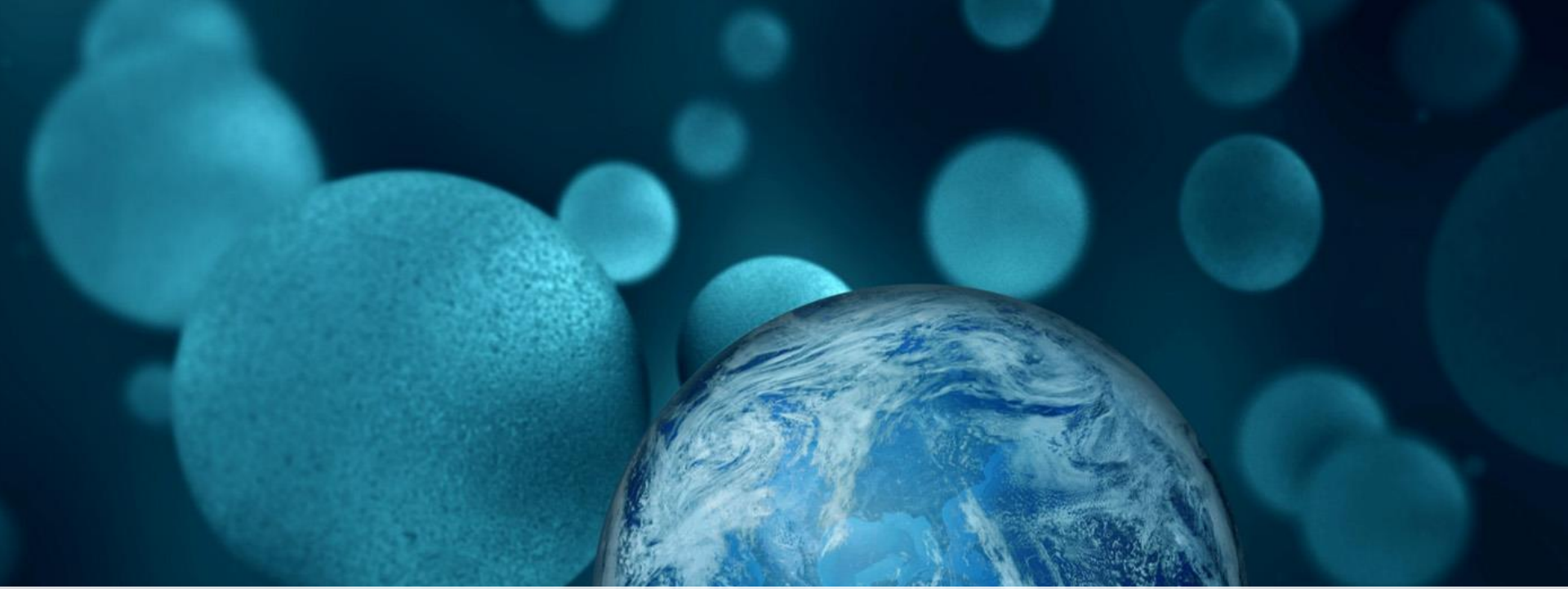

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Improvements in Sample preparation and chromatography separation products

Michal Godula, Ph.D.
Special Solutions Center EMEA

Two case studies

1. Development and optimization of method workflows for the analysis of pesticide residues in food
2. Use of UHPLC to facilitate better separations, more results, better sensitivity, easier interactions

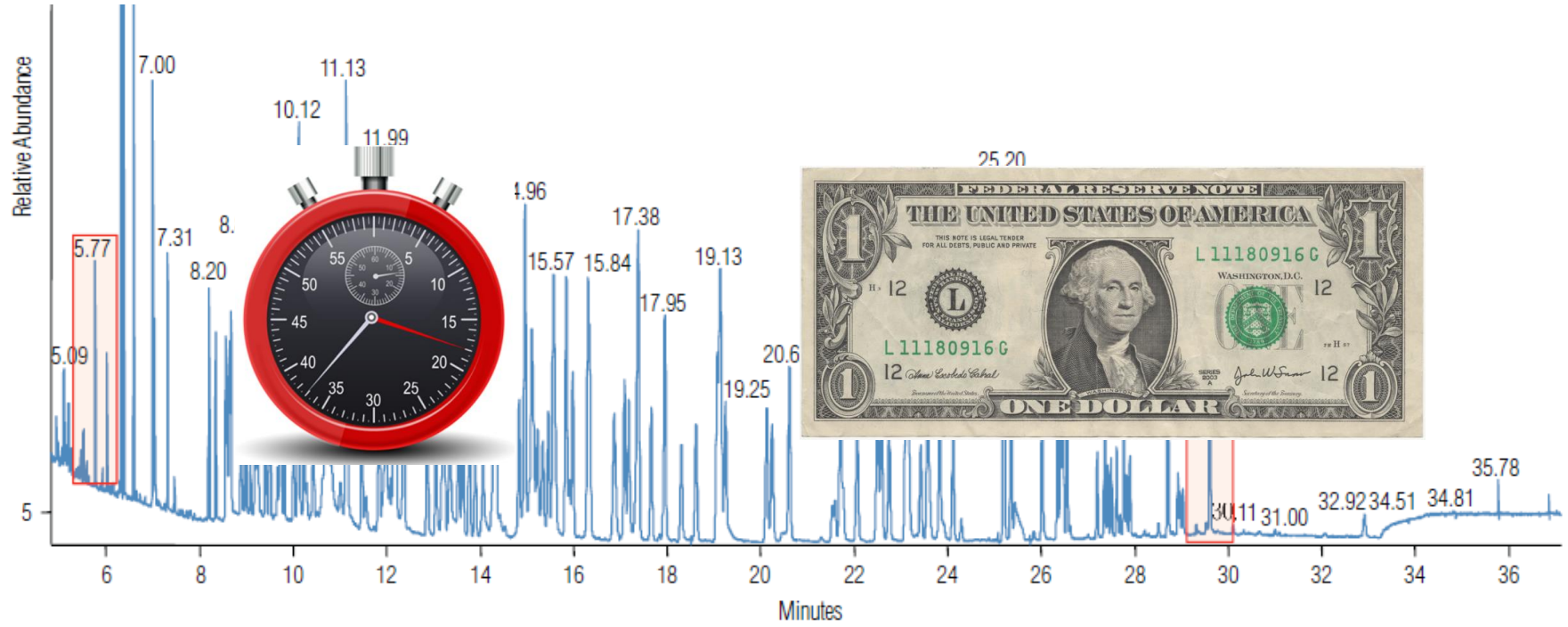
Aims

• To better understand the challenges, limitations and advantages of multi-residue workflows for pesticides residues in food and review the latest developments in consumables

• To explore how UHPLC improves existing workflows

Analytical Challenges

- Diverse and complex food matrices
- Increasingly large number of target compounds
- Low limits of detection
- High throughput (Efficient)
- Cost effective



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How Do We Overcome the Challenges?

Reproducible accurate quantification and identification results which we achieved by:

Appropriate sample preparation
Removal of matrix co-extractives to minimise matrix effects
Adjustment/buffering of pH
Solubility and stability of analytes

Chromatographic performance
Separation and shape of peak
Speed in analysis

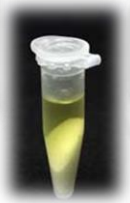
Use of high sensitivity instruments
Dilution (reduced matrix concentration)
Reduced maintenance, decreased instrument down-time

Good detector selectivity

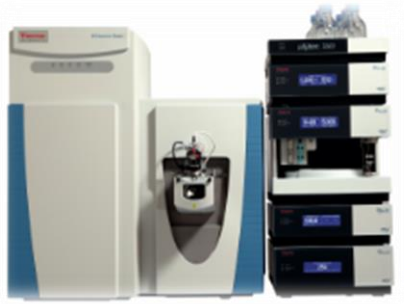
Reproducible products

Typical Workflow

1. **Sample Prep**



2. **LC-MS Analysis**



3. **GC-MS Analysis**

4. **Data Processing/Analysis**

Sample Homogenisation Before Extraction

- Unavoidable steps in the analysis
- Prerequisite to obtain representative sub-samples

Laboratory Sample

Analytical Sample

Test portion

- Pesticide residues can be lost during processing
- Critically important but receive relatively little attention
- Not usually included in validation or estimates of measurement uncertainty

Even Before Extraction…..

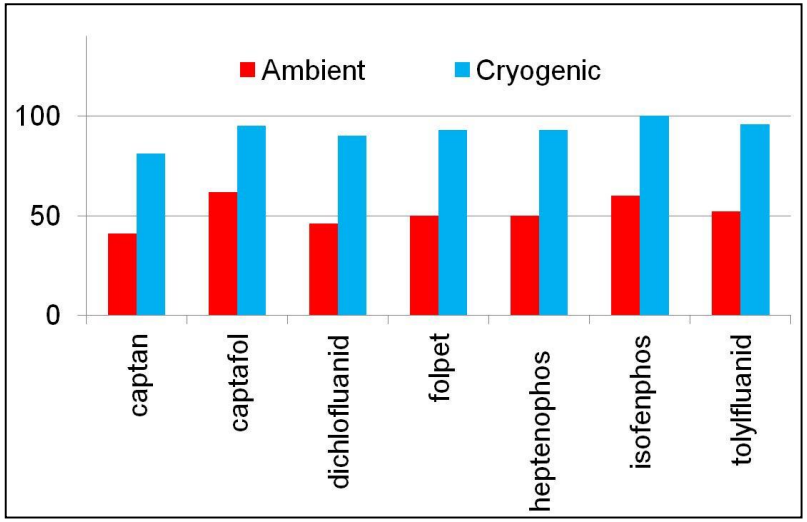
- Losses of a number of pesticides during sample processing at ambient temperatures may occur: bitertanol, dichlofluanid, captan, chlorothalonil

- Add CO_2
- Homogenise
- Frozen sample

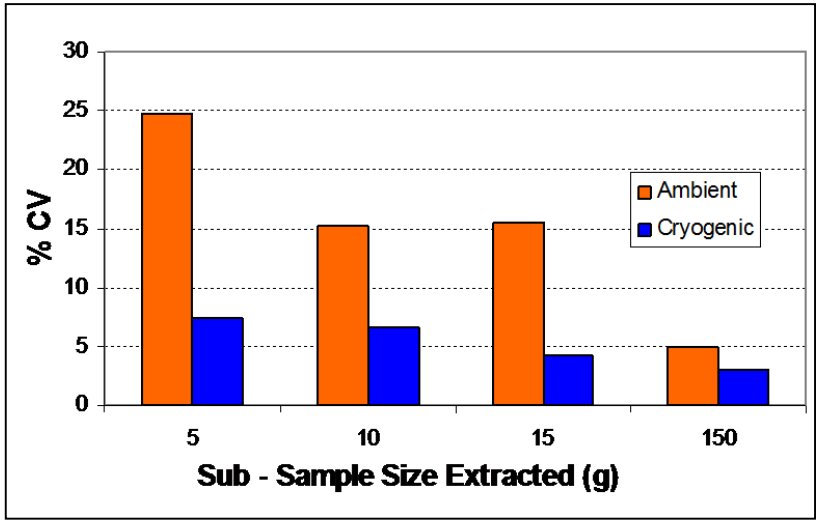
Fussell, et al. Food Addit. Contam. 2007, 24:1247-1256

Cryogenic Milling Effect on data quality

Effect on the Stability of Pesticides



Effect on the Stability of Pesticides



- Cryogenic milling improves analyte stability and homogeneity esp. improved precision for small-scale methods
- If processing at ambient tempeartures work as quickly as possible and mix continuously during withdrawing of test portions. Recognise the potential losses and report results accordingly
- Do not forget potential losses of pesticides during storage of sample and avoid freeze/thaw cycles

Fussell et al. J. Agric. Food Chem. 2007, 55, 1062-1070

Sample Extraction

Aim: high recovery of analyte and low recovery of matrix components

- Analyte compatibility
- *Solubility (like dissolves like)*
- Analyte selectivity (*minimise interfering matrix components)*
- Extraction efficiency (*incurred residues more realistic than laboratory spiked samples)*

Method	Sample size (g)	~solvent volume (mL)	Extraction solvent	Clean-up
DFG S19	25-100	>300	Acetone	L/L into DCM or ethyl acetate: cyclohexane GPC + Silica-gel (if required)
Mini Luke (Dutch Method)	15	60	Acetone/DCM/ petroleum ether mixture	Liquid/Liquid
Klein & Alder	10	20	MeOH	Chem-Elut
'SweEt' method (Swedish NFA)	1- 10	10	Acidified EtOAc	dSPE (carbon) or GPC if required
QuEChERS	1-15	10-15	Acetonitrile (other solvents also used in the same approach)	Phase separation Freezing dSPE (PSA/C18/carbon/ Zirconium oxide)
EURL QuPPe	1-15	10-15	Acidified MeOH	None

Pesticide Analysis

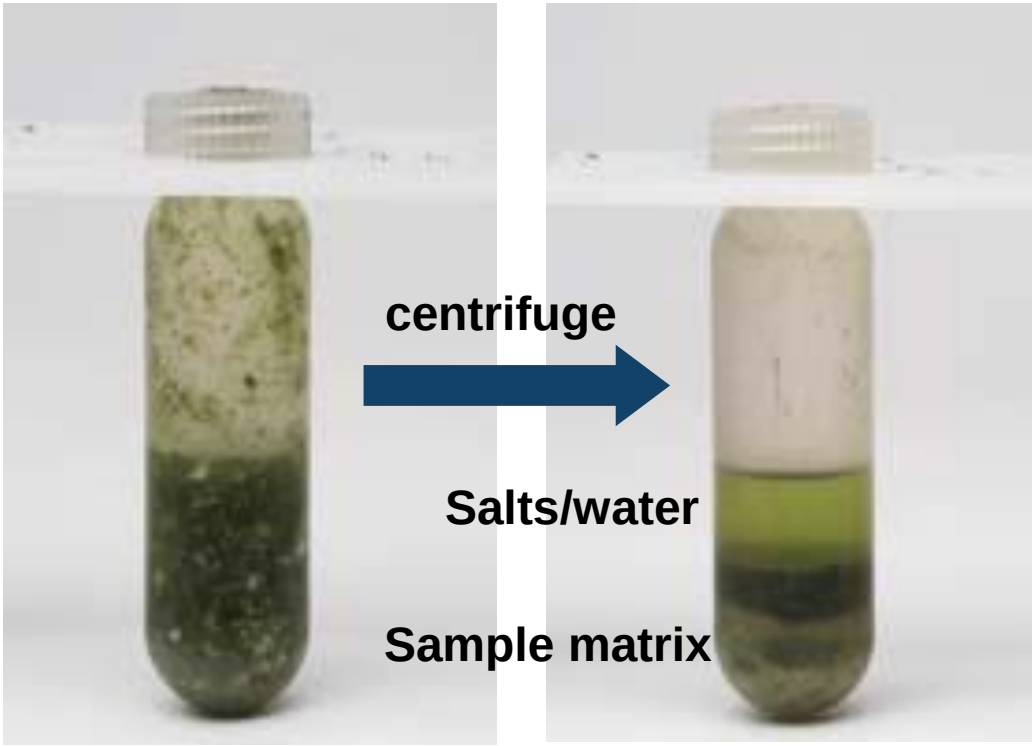
Many pesticide multiresidue methods (MRMs) are multi-facetted, QuEChERS *meets* the demands of these

Traditional MRM	QuEChERS
Blending	Shaking
Filtration	Centrifugation
Large solvent volumes	Small solvent volumes
Multiple partitioning steps	Single partitioning
Transfers of entire extract	Take aliquots (use I.S.)
Lot of glassware	Single vessel
Evaporation/Concentration	Large volume injection
Classical SPE	Dispersive SPE

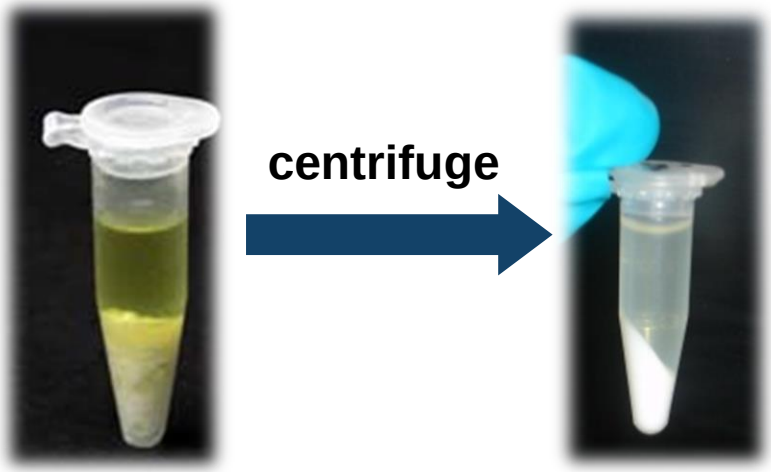
What is QuEChERS?

Extraction & Clean-up in a Tube!

1. Extraction



2. Dispersive SPE



acetonitrile supernatant containing extracted residues

QuEChERS Method Variations

Method	Description
Original QuEChERS Method – introduced in 2003	Uses Sodium Chloride to enhance extraction
Dispersive AOAC 2007.01 Method	Uses Sodium Acetate as a buffer replacing Sodium Chloride
Dual Phase Variation	Uses PSA & GCB to remove high levels of chlorophyll and plant sterols
European Version	Similar to AOAC method – uses sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate

AOAC advantages over original method

- Using sodium acetate as a buffer protects base sensitive compounds (eg. Dicofol, Captan,Tolyfluanid, Folpet, Dichlofluanid, Chlorthanonil)

AOAC disadvantages

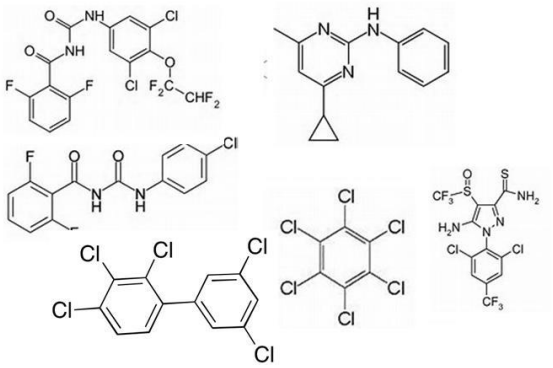
- The presence of acetic acid (PSA will absorb acetic acid, less sample clean-up, higher baseline)
- Only use this method if looking at specific compounds

Stage 2: Select the Right Product

Matrix Type	Examples	Sorbent Requirements for Clean-Up
General Matrices	• Apples • Cucumber • Melon 	$MgSO_4$, PSA Removal of excess water organic acids, fatty acids, sugars
Fatty Matrices	• Milk • Cereals • Fish 	$MgSO_4$, PSA, C18 Additional removal of lipids & sterols
Pigmented Matrices	• Lettuce • Carrot • Wine 	$MgSO_4$, PSA, C18, GCB Additional removal of pigments & sterols
High Pigmented Matrices	• Spinach • Red Peppers 	$MgSO_4$, PSA, C18, GCB, Chlorofiltr™ Additional removal of chlorophyll

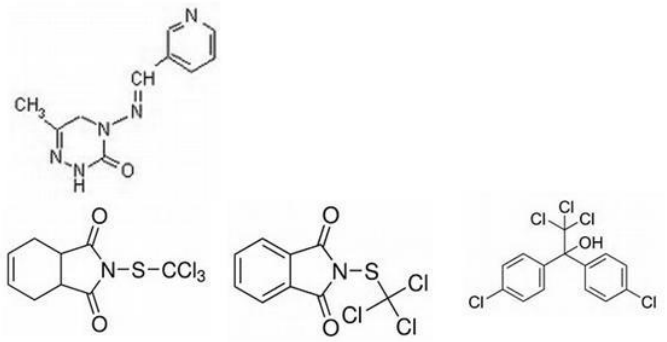
Troubleshooting QuEChERS

- Planar Pesticides loss with GCB
  - Use a product with less GCB
  - Use the Schenk method



Carbendazim	Mepanipyrm
Chlorothalonil	Pentachloroaniline
Cyprodinil	Phenmedipham
Demedipham	Phosalone
Diflubenzuron	Pymetozine
Flucycloxuron	Pyrimethanil
Hexachlrobenzen	Quinoxyfen
Hexaflumuron	Thibendazole

- Acidic compound loss with PSA
  - Decreased recovery of acephate, acrinathrin, carbaryl, chlorothalonil, diclorvos, dimethoate, mevinphos, phosmet plus others e.g pymetrozine
  - Use a product without PSA e.g. $MgSO_4$ & C18
- Loss of base sensitive pesticides
  - Addition of 0.1% acetic acid can improve stability



Captan	Dichlofluanid
Folpet	Tolylfluanid
Dicofol	Chlorothalonil

Troubleshooting QuEChERS

- **Poor extraction efficiency/recovery**
  - **Temperature of extraction** - *frozen samples take longer thaw in solvent)*
  - **Shaking time** - *matrix dependant (5-15 minutes, use mechanical shaker)*
  - **Product choice** - *verify pesticide type and that correct products are being used*
  - **Effect of pH**
  - **loss of lipid soluble pesticides** - *due removal of lipids by C18 sorbent*

- **Issues with low moisture content commodities** (e.g. Cereals, tea, etc)
  - **Rehydrate with water -** *10-20 min prior to addition of solvent*
  - **Do not exceed 20 min for cereals** - *activation of enzymes can degrade OP pesticides*
  - **Freeze milled sample overnight** - *reduce chemical interferences*
  - **Freeze supernatant** - *removes lipid/increases ease of filtering (if required)*

- **Decreased recovery of lipid soluble pesticides** (*hexachlorobenzene)*
  - **Use of C18 removes lipids**
  - **Freeze out fat**

Application examples

Application Note 20549

QuEChERS Dispersive Solid Phase Extraction for the GC-MS Analysis of Pesticides in Cucumber

Anila I Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
QuEChERS, pesticide residue analysis, cucumber, food safety

Abstract
QuEChERS dispersive SPE is a simple, fast and quantitative sample preparation method. This application demonstrates the effectiveness of this technique in the GC/MS analysis of pesticides in cucumber, using a Thermo Scientific TraceGOLD TG-5MS GC column for analysis.

The recoveries for the spiked pesticides in cucumber matrix at 50 ng/g were between 75.2 to 119.6% with relative standard deviations ranging from 2.1 to 8.9% using the QuEChERS method described in EN15662.

Introduction

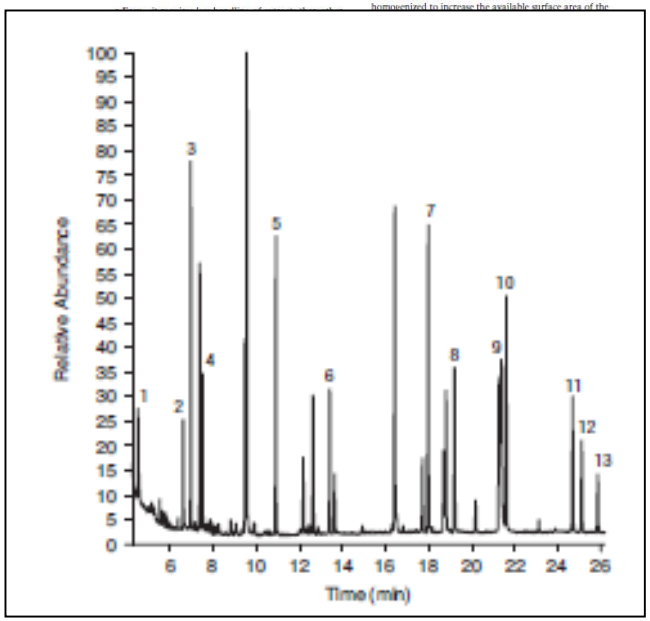
QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residue pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost. The QuEChERS methodology was developed by Anastassiades et al[1] and has become widely used in food safety analyses.

The method is:

- Quick – high sample throughput, typically 8 samples can be prepared in under 30 min

The sample preparation approach described in the European EN15662 QuEChERS procedure[2] was used for extracting pesticides from cucumber. This is a two stage process: sample extraction, followed by dispersive SPE.

In the sample extraction stage, the food sample is homogenized to increase the available surface area of the



Application Note: ANGSC PESTGRAPES 0709

QuEChERS Dispersive Solid Phase Extraction for the GC/MS Analysis of Pesticides in Grapes

Anila Khan, Luisa Pereira, Stephen Aspey, Rob Bunn, Ruth Lewis, Thermo Fisher Scientific, Runcorn, UK

Key Words
- QuEChERS
- Food Safety
- Pesticides Residue Analysis
- TRACE TR-SMS
- TRACEGuard

Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a dispersive Solid Phase Extraction (SPE) technique for extracting multi-residues of pesticides from fruits and vegetables. The advantages of this methodology are speed, ease of execution, minimal solvent requirement and cost to perform when compared with conventional solid phase extraction techniques.

The QuEChERS methodology was developed by Anastassiades *et al* and has become widely used in food safety analyses.[1] The method is:

- Quick – high sample throughput, typically 8 samples can be prepared in just under 30 min
- Easy – it requires less handling of extracts than other techniques and no laborious steps are involved
- Cheap – less sorbent material is needed and less time is need to process samples compared to other techniques
- Effective – the simple technique gives high and accurate recovery levels for a range of different compound types e.g polar pesticides, pH dependent compounds
- Rugged – the method can detect a large number of pesticides including pH dependent and polar pesticides
- Safe – unlike other techniques, it does not require any chlorinated solvents. Extraction is typically carried out using acetonitrile, which is both GC and LC amenable

The QuEChERS procedure is usually a two stage process: sample extraction, followed by dispersive SPE. In the sample extraction stage, the food sample is homogenized to maximize the available surface area of the sample for better extraction efficiencies. The homogenized sample is placed in the extraction tube containing magnesium sulphate and sodium acetate. Magnesium Sulfate ensures that upon addition of acetonitrile, a phase separation is induced between water and organic solvent with the pesticides of interest being extracted into the organic phase. When acetonitrile is poured into the extraction tube containing the homogenized sample, an exothermic reaction occurs between the magnesium sulphate and water, which can lead to low recoveries of the pesticides. This effect can be

GC analysis, and additional sample clean-up to reduce matrix effects and therefore improve method robustness.

Internal standards are used to minimize errors that might be introduced in the different steps of the QuEChERS method, as well as compensate for GC injection variability. Furthermore, adding analyte protectants such as sorbitol can be useful for labile pesticides at intermediate pH, which can be prone to decomposition in the GC injector port.

The pesticides analyzed are a mixture of organophosphate, organochlorine, pyretheroid, benzenoid, triazole and dicarboximide compounds. Lehotay reviewed the LC and GC analyses of pesticides in produce and the type of pesticide that is likely to be found in each matrix.[2] The requirements for pesticide residue analysis in fruit and vegetables are established by organizations such as World Health Organization, Japanese Food Chemical Research Foundation, EEC Directives, and the US-EPA.[3,4,5,6] These organizations establish which pesticides need to be determined in different produce and the Method Regulatory Limits (MRLs). The pesticides determined in this study are all listed by the four regulatory organizations and all have minimum MRLs of 50 ng/g (ppb). The recoveries of the pesticides in grapes are based on this value.

Goal

To demonstrate QuEChERS dispersive SPE as a simple, fast and quantitative sample preparation method for the GC/MS analysis of pesticides in grapes.

Additionally, demonstrate the suitability of the Thermo Scientific TRACE TR5-MS analytical column combined with the Thermo Scientific TRACEGuard guard column for pesticide analysis.

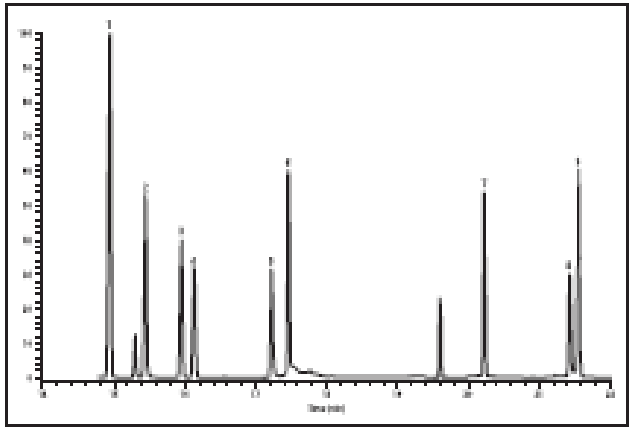


Figure 2: TIC for the GC/MS analysis of grapes spiked with 1 ng/µL of each pesticide

Technical Note: 10222

Analysis of Pesticide Residues in Lettuce Using a Modified QuEChERS Extraction Technique and Single Quadrupole GC/MS

Jessie Butler, David Steininger, Eric Phillips, Thermo Fisher Scientific, Austin, TX, USA

Key Words
- DSQ II GC/MS
- QuanLab Forms
- Food Safety
- QuEChERS
- Pesticide Residue Analysis

Introduction

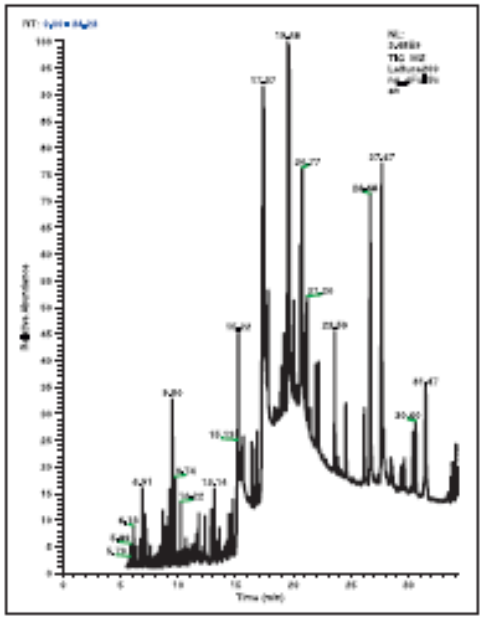
The determination of pesticides in fruits and vegetables has been simplified by a new sample preparation method, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), and published recently as AOAC Method 2007.01.[1] The sample preparation is shortened by using a single step buffered acetonitrile (MeCN) extraction and liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate ($MgSO_4$).[1] This technical note describes the application of the QuEChERS sample preparation procedure to analysis of pesticide residues in a lettuce matrix using gas chromatography/mass spectrometry (GC/MS) on the Thermo Scientific TRACE GC Ultra™ and Thermo Scientific DSQ™ II single quadrupole mass spectrometer. Thermo Scientific QuanLab Forms 2.5 software was used for data review and reporting. The MeCN extract is solvent exchanged to hexane/acetone for splitless injection with detection by electron ionization and selected ion monitoring (SIM).[2] A calibration curve was constructed in iceberg lettuce and then the precision and accuracy of the analytical method were tested by preparing matrix spikes at .5 ng/g and 50 ng/g.

Item Descriptions

TRACE TR-Pesticide (0.25 mm x 30 m, 0.25 µm with 5 m guard column)
5 mm ID liner, 105 mm long (pk of 5)
10 µL syringe
Septa (pk of 50)
Liner graphite seal (pk of 10)
Closed Exit ion Volume and ion volume holder for DSQ II
Graphite ferrule 0.1-0.25 (pk of 10)
Ferrule, 0.4 mm ID 1/16 O/V
Blank vespel ferrule for MS interface
2 mL amber glass vial, standard glass, with write-on patch (pk of 100)
Blue cap with ivory PTFE/red rubber seal (pk of 100)

Experimental Conditions

During the method validation, several experiments were



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Validated methods

10 g of sample is weighed into Quechers extraction tube

+ 20 mL of water

+ 10 mL of ACN

shaking 10 min

Centrifugation 5 min @ 5000 rpm

Injection to GC-MS (after clean-up)
or LC-MS (clean-up optional)

Further details

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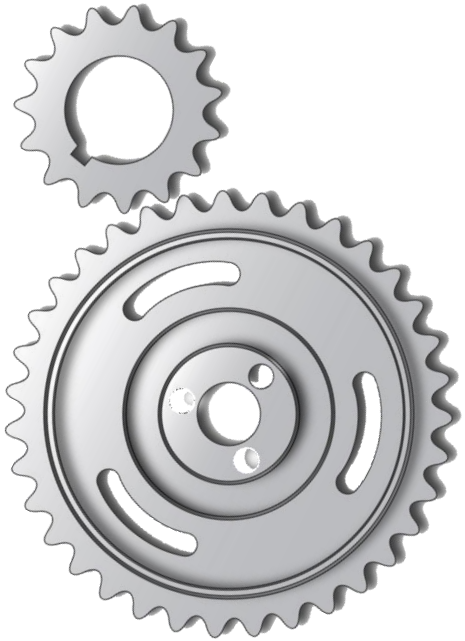
TSQ™ 8000 Evo GC-MS/MS Pesticide Analyzer

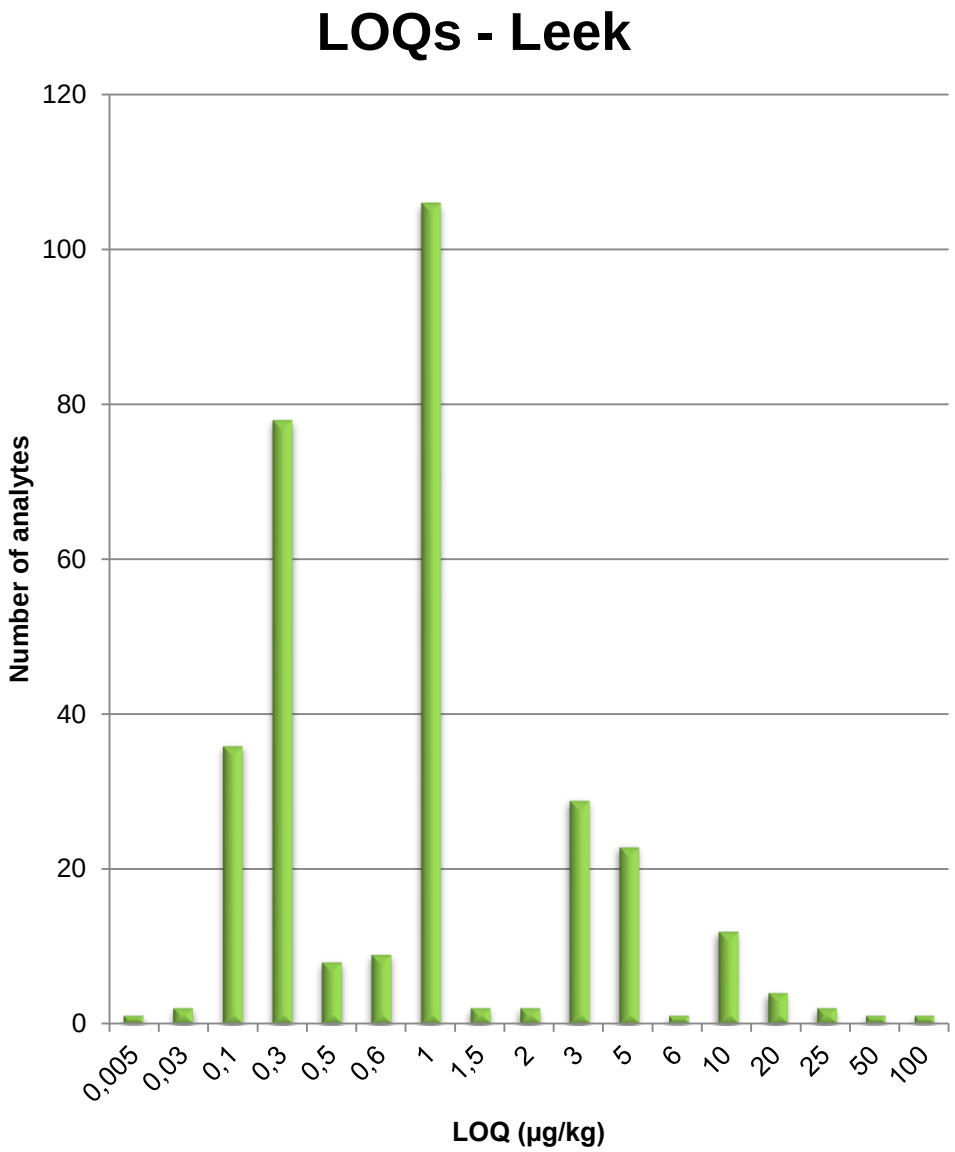
- Preconfigured performance leading TSQ 8000 GC-MS/MS system featuring the award winning TRACE 1310 GC
- QuEChERS kit for sample preparation
- Pre-loaded acquisition methods
- 1000+ Pesticide compound database
- Thermo Scientific™ TraceGOLD GC Column and consumable technology
- TraceFinder EFS Data Processing Software
- AutoSRM & timed SRM (t-SRM)
- Pesticide Analyzer Installation Guide

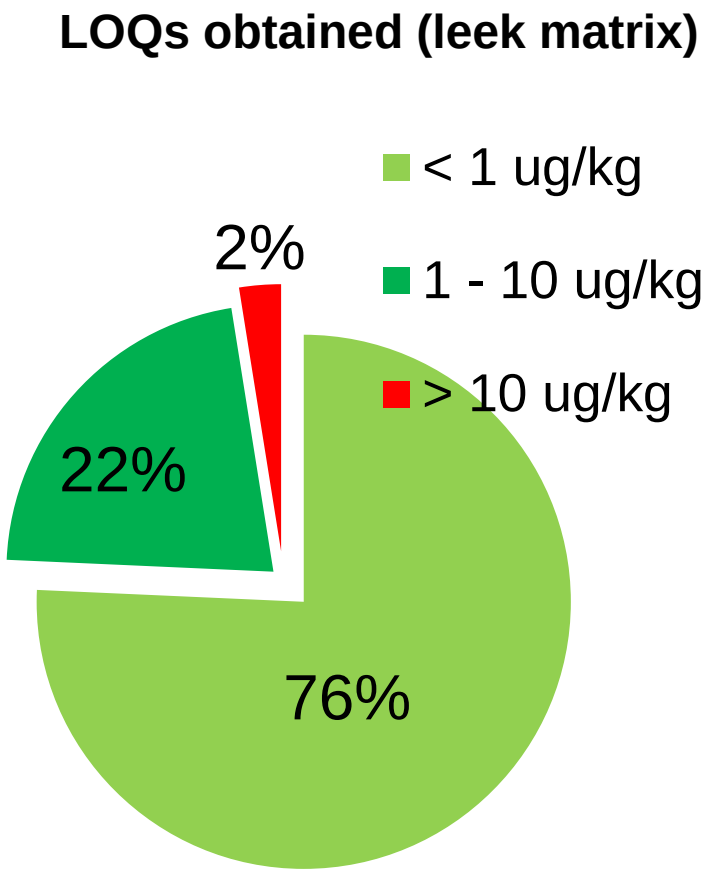
TSQ Endura™ LC-MS/MS Pesticide Explorer

- Pesticide Explorer™ package
- Package includes methods and SRM library
- 276 compounds completely validated, possible extension with SRMs available
- TraceFinder EFS Data Processing Software
- Installation Guide: get up and running fast
- Complete method support



LC-MS/MS method LOQs- Leek





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GC Method LOQ (acceptance <10 ug/kg)

Out of target range:

Amitraz (WF), Fenpropathrin (WF, LK), Tebufenocid (all), Carbofuran* (SW), Methiocarb* (WF, LK), Oxadyxil* (WF), Propargite* (WF, LK)

Application Notes and Resources

Validation of the Method for Determination of Pesticide Residues by Gas Chromatography – Triple-Stage Quadrupole Mass Spectrometry

Laszlo Hollosi, Katerina Bousova, Michal Godula
Thermo Fisher Scientific, Food Safety Response Center, Dreieich, Germany

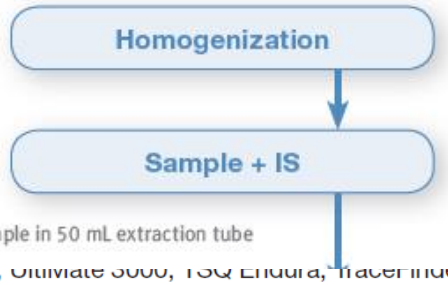
Method 63899

sive

Application Note 643

Key Words
TraceFinder, TSQ, Chromatography, GC, GC-MS, Pesticide Residues, QuEChERS, Triple Quadrupole

1. Schematic of Method



1. Weigh 10 g sample in 50 mL extraction tube

[redacted]

mple preparation through Figure 1. Samples were homogenized and extracted according to the European EN 15662 QuEChERS protocol prior to injection into the LC-MS/MS system.[1,2] The ready-to-use QuEChERS

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Using UHPLC to improve total analysis workflows

- **Better separations** 24% average increase in resolution
- **More results** 40% time saving compared to existing methodology
- **Better sensitivity** 17% average increase in peak height
- **Easier interactions** Seamless workflow with Chromeleon data processing

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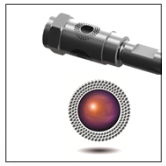
Vanquish System…Provides More Solutions!

Better integration with the world's best mass spectrometers



Maximum speed and resolution with new Thermo Scientific™ Accucore™ Vanquish™ LC columns

More throughput capacity by new rack loader

Revolutionary ease-of-use experience by new Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) features and workflows

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Accucore Vanquish Columns – New Column Technology

Accucore Columns Range Summary

17 phases / 3 particle sizes / 2 pore sizes

New Accucore Vanquish Columns

C18, 1.5 µm, 80 Å

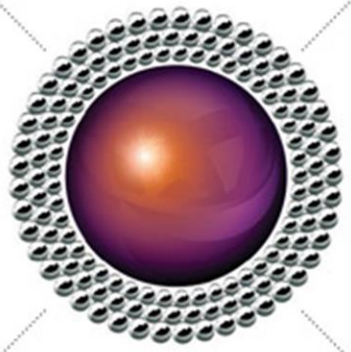
June 2011	April 2012	June 2012	September 2012	January 2013	July 2014
2.6 µm, 80 Å 6-phases	2.6 µm, 80 Å 1-phase	2.6 µm, 150 Å 2-phases	4 µm, 80 Å 2-phases	2.6 µm, 80/150 Å 5-phases	

Solid Core Particles

With a solid central core and porous outer layer, these particles generate high speed, high resolution separations

Tight Control of Particle Diameter

Enhanced selection process keeps particle size distribution to a minimum and produces high efficiency columns



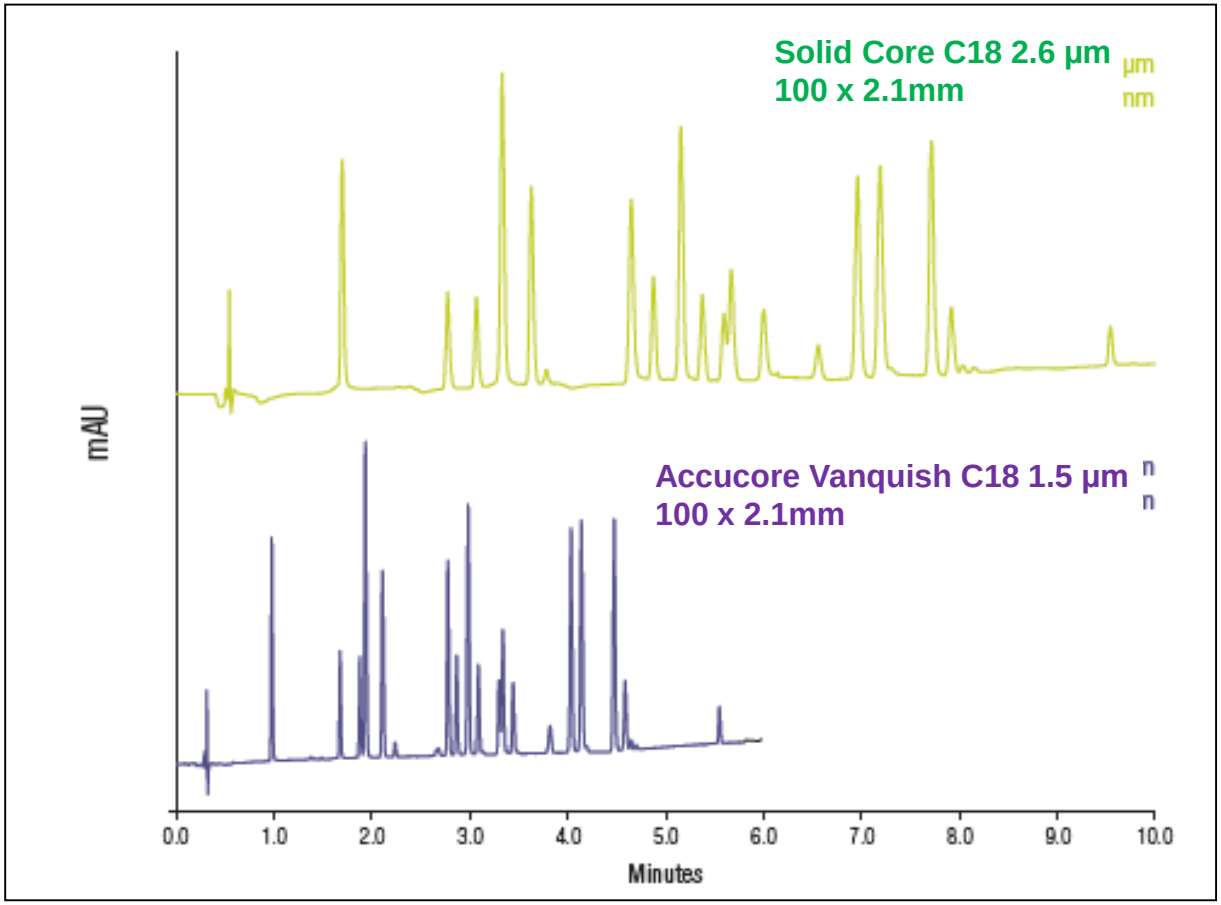
Automated Packing Process

Enhanced automated procedures ensure that all columns are packed with the highest quality

Advanced Bonding Technology

Optimized phase bonding creates a high coverage, robust phase

Why Core Enhanced technology Delivers: *More Results*



Mobile phase A:	water	
Mobile phase B:	acetonitrile	
Gradient:	Accucore Vanquish C18 1.5 µm 100 × 2.1 mm	
	Time (min)	%B
	0	20
	4	40
	7	80
	Solid core C18 2.6 µm 100 × 2.1 mm	
	Time (min)	%B
	0	20
	6.9	40
	12.1	80
Flow Rate:	Solid Core C18 2.6 µm 100 × 2.1 mm = 380 µL/min Accucore Vanquish C18 1.5 µm 100 × 2.1 mm = 650 µL/min	
Inj. Volume:	0.5 µL	
Temp.:	43 °C	
Detection:	UV at 230 nm (0.1s rise time, 50 Hz)	
Analytes:	1. Desethylatrazine	10. Diuron
	2. Metoxuron	11. Isoproturon
	3. Hexazinone	12. Metobromuron
	4. Simazine	13. Metazachlor
	5. Cyanazine	14. Sebuthylazin
	6. Methabenzthiazuron	15. Propazine
	7. Chlorotoluron	16. Terbuthylazine
	8. Atrazine	17. Linuron
	9. Monolinuron	18. Metolachlor

40% time saving and *Better Separations*

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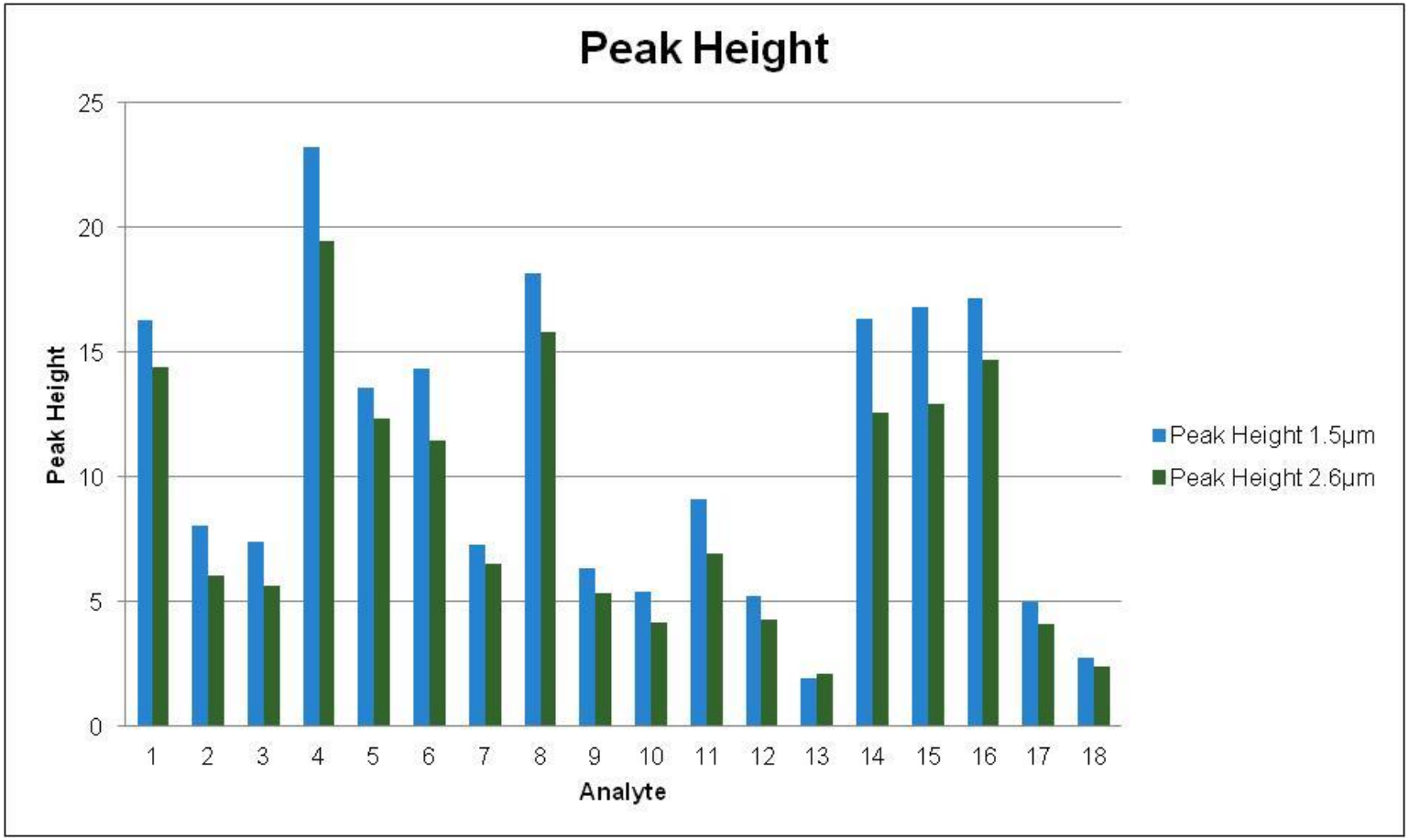
Why Core Enhanced technology Delivers: *Sensitivity*

- **Higher flow rates while maintaining better peak's and resolution**



Average increase in peak height of 17%

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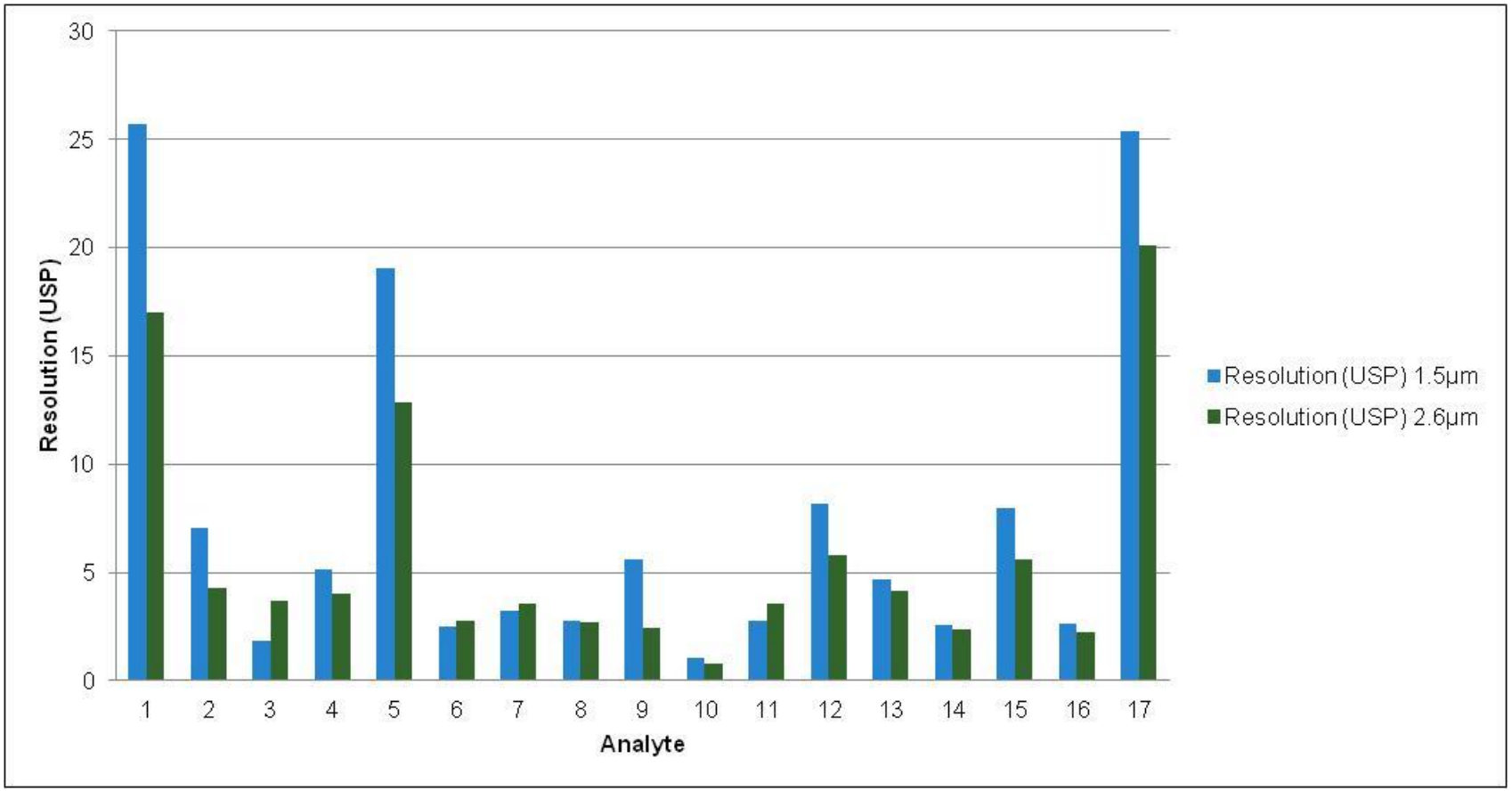
Why Core Enhanced technology Delivers: *Resolution*

- **Higher Resolution (USP) 1.5µm Vs 2.6µm**



Average increase in resolution of 24%

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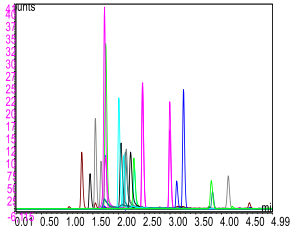
LCMS Assay – 36 Antibiotics Targeted Screening: Speed

Accucore Vanquish C18 Column, 1.5 µm, 2.1 x 100 mm

Detection of **36 antibiotics within a 5 minute** detection window, using a binary Thermo Scientific™ Vanquish™ System™ and Thermo Scientific TSQ Vantage Mass Spectrometer



Mobile phase A:	0.1% formic acid / Water
Mobile phase B:	0.1% formic acid / MeOH
Flow rate:	400 µL/min
Column Temperature:	40 °C, active pre-Heating
Injection Volume:	2 µL

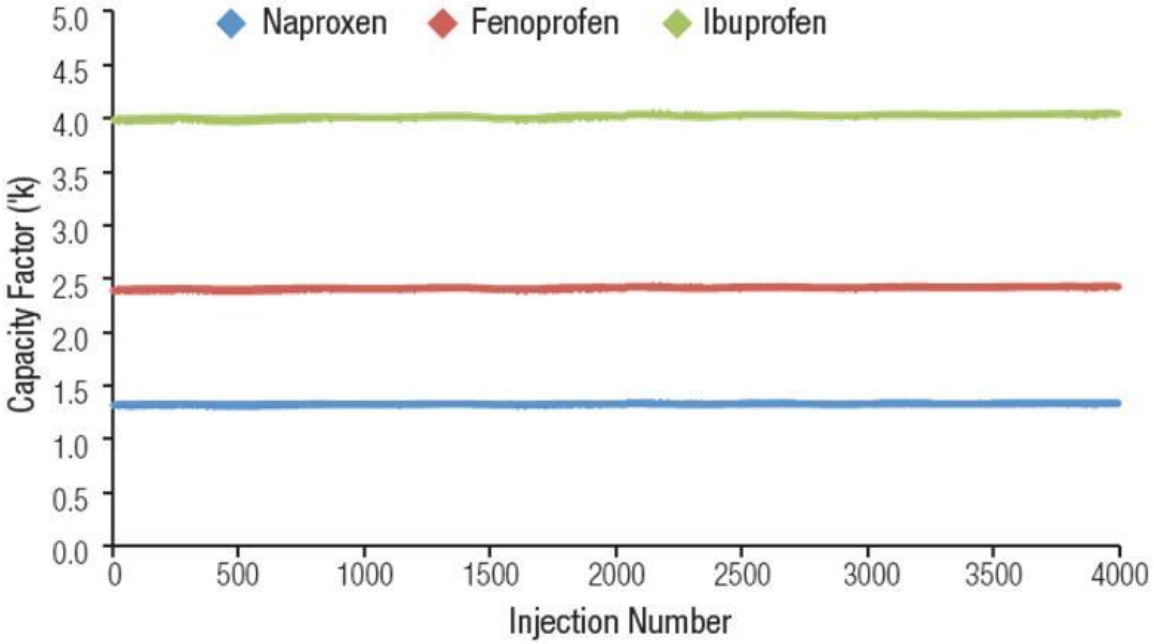
Table 1. LC Gradient Conditions

Time (min)	%B
0	10
4.375	90
5.000	90
5.125	10
8.750	10

Why Core Enhanced technology Delivers: *Stability*

- The advanced bonding and automated packing technology used in the manufacture of Accucore Vanquish UHPLC columns results in exceptionally reproducible chromatography

4000 injections @ 500 bar



%RSD for 4000 injections	Naproxen	Fenoprofen	Ibuprofen
Retention time	0.19	0.25	0.29
Capacity factor	0.41	0.42	0.41
Efficiency	2.94	2.74	2.80
Asymmetry	0.92	0.87	1.09
Peak area	0.53	0.50	0.55
Peak height	1.13	1.02	0.91
Pressure	0.53		

- RSD for retention time less than 0.3%
- RSD for peak area less than 0.6%
- RSD for peak height less than 1.2%
- Column pressure stable over 4000 injections (RSD 0.53%)

Excellent reproducibility

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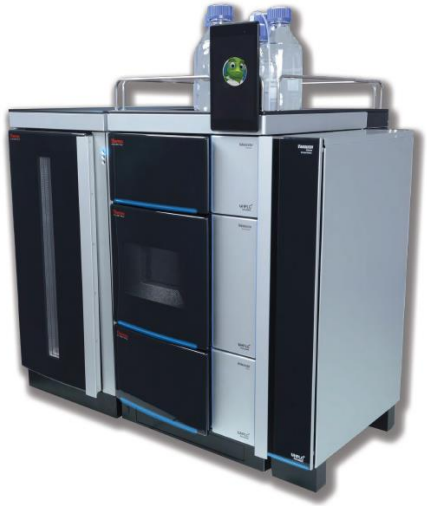
Summary

- **Accucore Vanquish and Vanquish UHPLC systems provide:**
  - **Better separations**
  - **More Results**
  - **Easier Interactions**
- **Enable our customer to achieve what was not possible before**



Accucore Vanquish UHPLC Columns Delivering Powerful Separations

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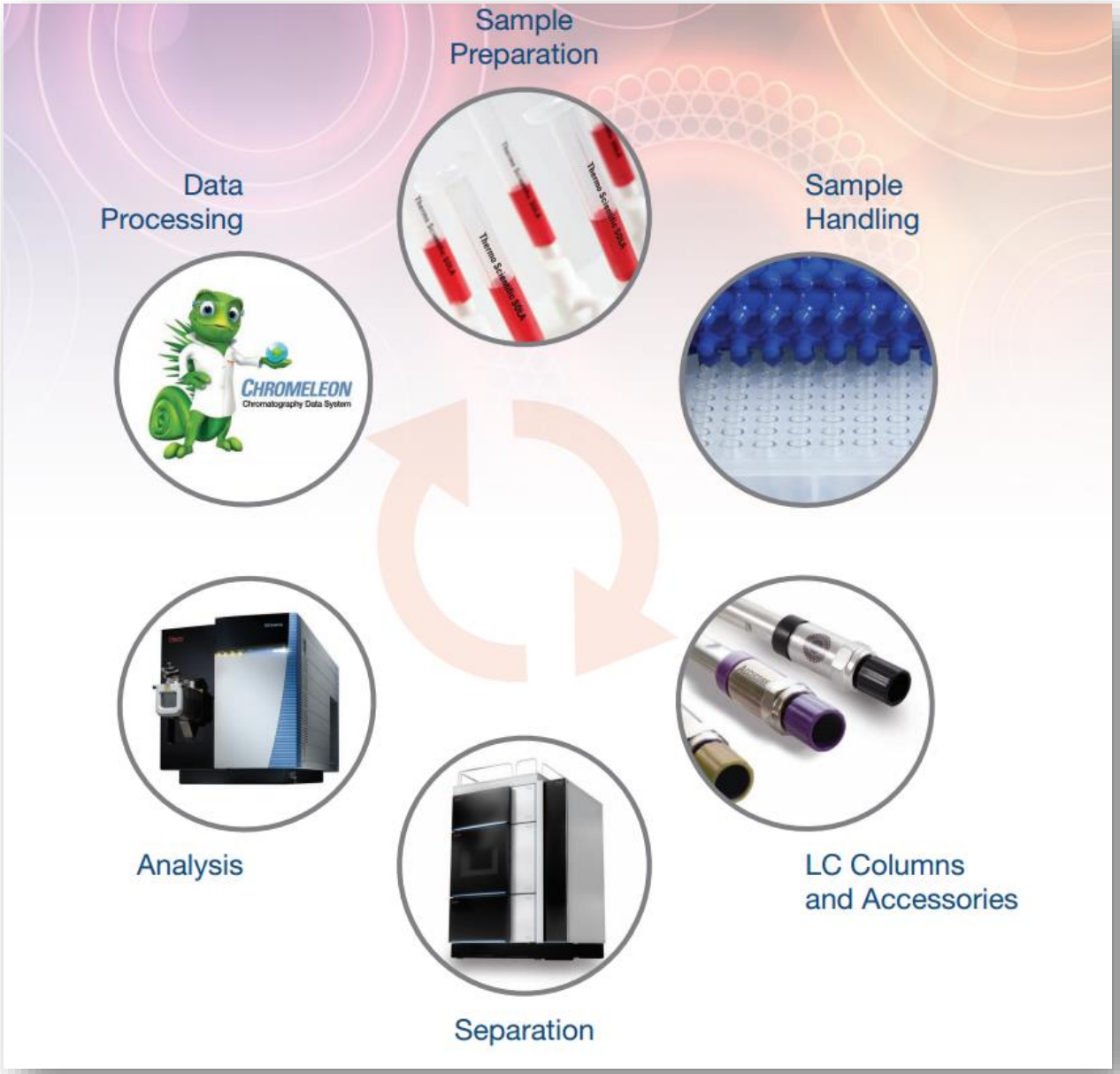

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