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GC/MS and LC/MS Solutions for Food Safety Analysis

Screening Potential Threats

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Food Safety Issues

- Caused by food contamination, or fraud
  - Related to serious health concerns
  - Have received growing public attention
  - Consumers became increasingly sensitive
  - Almost daily reports about the safety of food
  - Cause massive economical damages
- Demand for more control and certification
  - Global trade, global economy
  - Screening for more contaminants
  - Industrial quality control
  - Governmental control
  - For a steadily increasing number of samples

How can GC/MS and LC/MS analysis support the efficient control?

Workflow Solutions for Routine Food Safety Labs

- **GC-MS and LC-MS Methods**
  - Very high specificity
  - Very high sensitivity
  - Efficient multi-methods

Benefit from recent developments:

Triple Quads, Orbitrap accurate mass

Only short or no sample prep

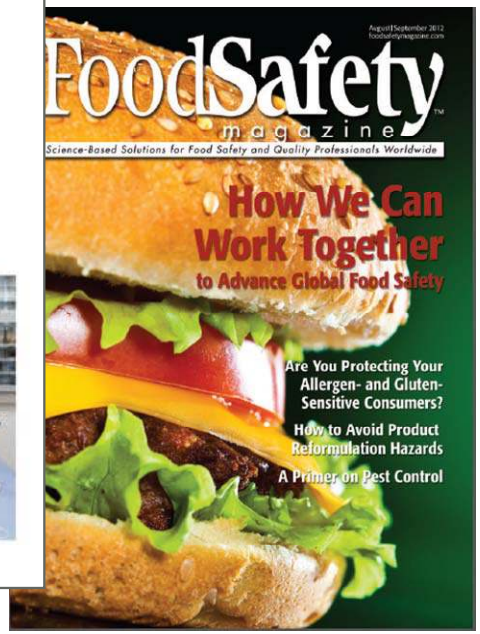
High productivity automated methods

- All methods compliant with the international regulations

- **Selected Workflow Examples**
  - Marine algae toxins
  - Pesticides
  - Dioxins
  - Processing contaminants like MCPD
  - Veterinary drugs
  - PAH in seafood


The Food Safety Response Center

Responding to the needs of the food safety community



- Data processing and reporting with the results transfer to LIMS systems

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Efficient Workflows using GC/MS and LC/MS

- **Sample preparation/extraction**
  - Should be short, automated (ASE, QuEChERS, Headspace, online-SPE)
- **Clean-up**
  - Not too much for multi methods, check recoveries (dispersive SPE, GPC, Purge&Trap)
- **Separation** (GC or LC Chromatography)
  - Recommended with short columns for short cycle times, use matrix backflush
- **Detection** (Mass Spectrometry)
  - Very high selectivity in matrix required (triple quad MRM, Orbitrap accurate mass)
- **Data Processing**
  - Control lab workflow, collect data, confirming information, QA/QC, final reporting

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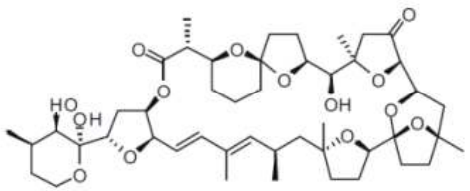
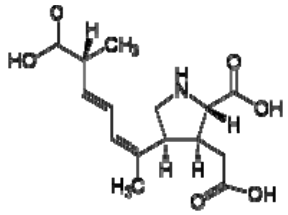
*LabOpinion Global MS Survey 2012, > 800 respondants

Marine Algae Toxins

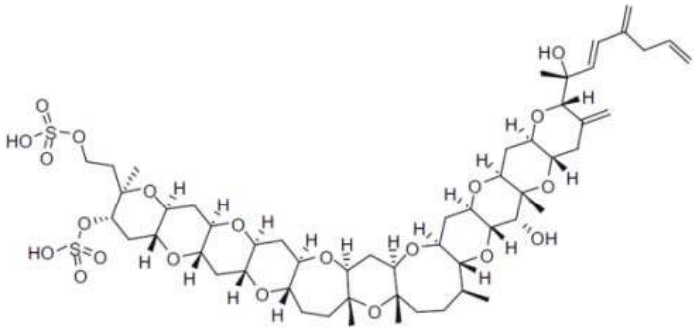
- Classification Based on Chemical Classes (> 50 structures !)
  - **PSP toxins** – saxitoxin + 20 other related toxins
  - **DSP toxins** – okadaic acid & dinophysis + pectenotoxins + yessotoxins
  - **ASP toxins** – domoic acid + 10 or more isomers
  - **AZP toxins** – azaspiracids = at least 8-hydroxy analogues
  - **NSP toxins** – brevetoxin type A & B (9 toxins)
  - **CTX toxins** – ciguatoxins
  - **Cyanobacteria** – microcystins



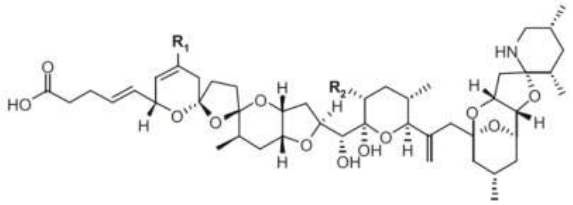
Pectenotoxin



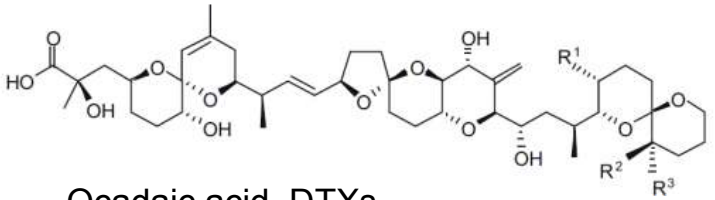
Domoic acid, ASPs



Yessotoxin



Azaspiracids



Ocadaic acid, DTXs

Marine Algae Toxins

- Shellfish
- Homogenization
- 4 g Sample
- Mixing with diatomaceous earth
- ASE Extraction, 12 min
- Centrifugation, 4000 rpm, 10 min
- Dilution and filtration
- Centrifugation, 10000 rpm, 10 min
- LC-MS/MS analysis

Acc. Solvent Extraction-LC-MS/MS

ASE 200, Thermo Scientific Dionex
- Oven temperature of 60 °C,
- methanol/acetone (9:1, v/v)
- 5 min heat-up time under a pressure of 1500 psi and two static cycles with a static time of 2 min.
- Flushing
- Purging using nitrogen for 3 min

O. Pardo et al., J. Chromatogr. A 1154 (2007) 287–294

Marine Algae Toxins

Methanol Extraction - LC-MSMS

- 2 g of sample + 9 mL of MeOH
- Homogenize with Vortex, 3 min
- Centrifuge, 2000g, 10 min
- Transfer to 20 mL flask

Repeat the extraction, homogenize 1 min in Turrax

- Combine the extracts
- Make up to 20 mL with methanol
- Filtration, 0.2µm filter
- LC-MS/MS

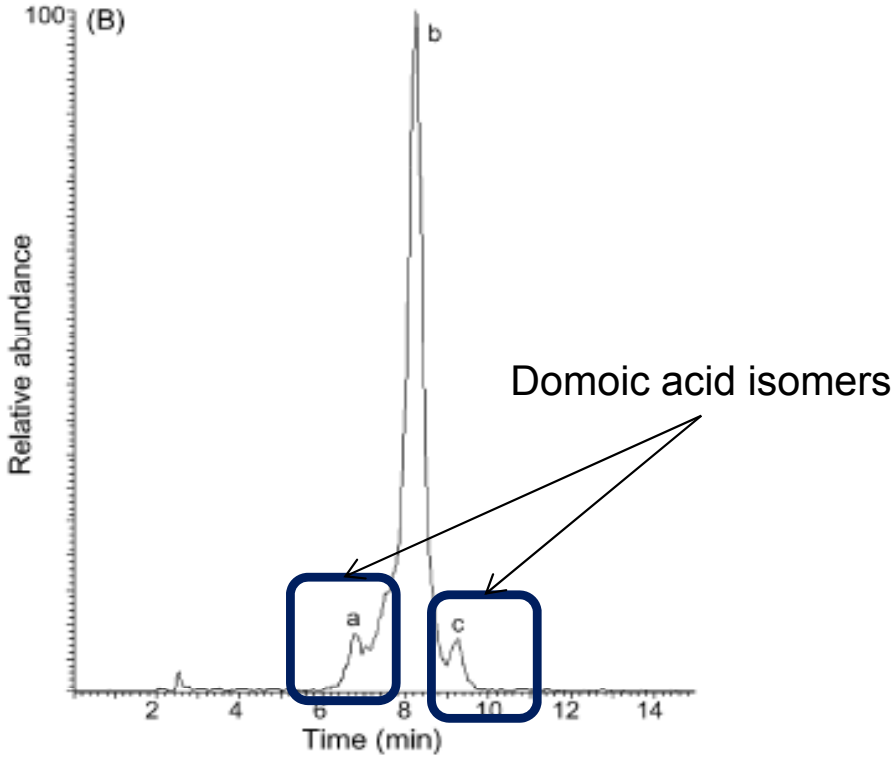
Hydrolysis for total content of OA/DTX

- Add 2.5M NaOH
- Homogenize, 0.5 min
- Heat to 76°C
- Neutralize with 2.5M HCl

EU Harmonized SOP
for the determination of lipophilic marine biotoxins in molluscs by LC-MS/MS, EURL-MB, Vigo, Spain

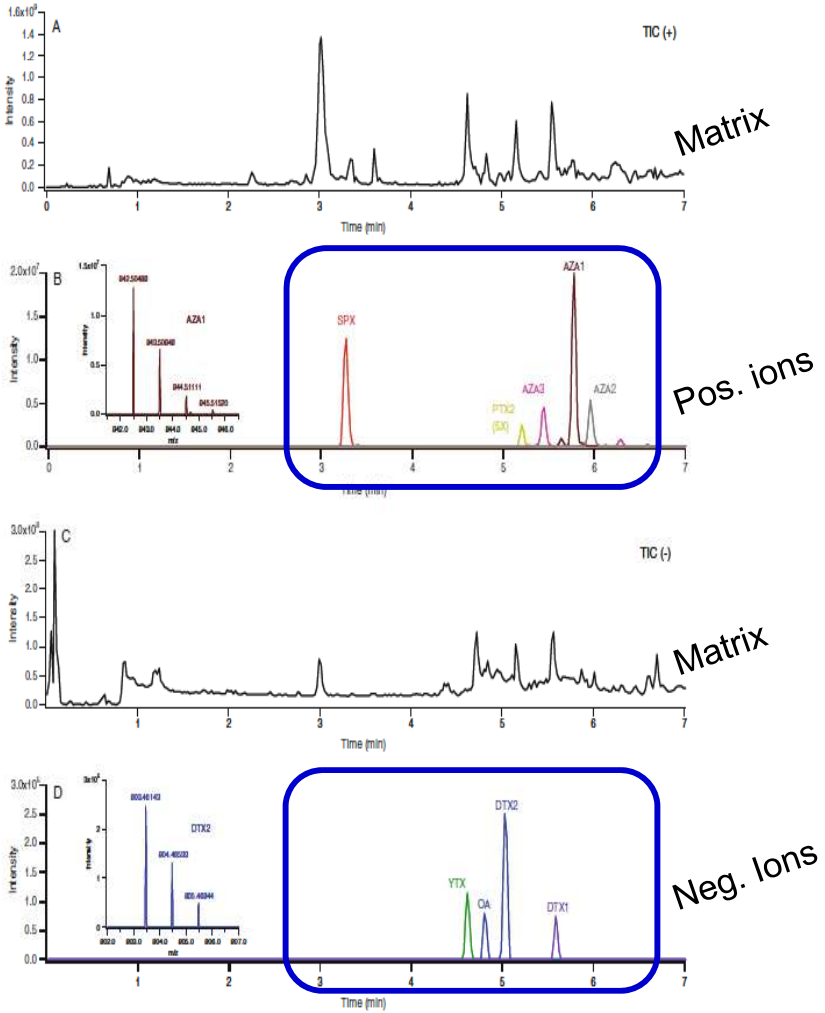
Marine Algae Toxins

TSQ MRM, 41 µg/g domoic acid (CRM)



All target toxins in one run: high **TSQ** matrix selectivity
LOD: 0.2 µg/kg (200 ppb)
Average recovery: 92%
RSD: 7%

Orbitrap Screening for multiple classes



Full Scan: **Targets + Unknowns, only 7 min**
Resolution: 20 000 – 50 000
LOD: 0.2 – 0.5 µg/kg

Blay, P., Hui , J.P.M. et al., 2011

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Analysis of Pesticides

Dried Herbal Product
10 g of Sample

↓

Extraction
Accelerated Solvent Extraction
EtOAc/cHexane, 120 °C, 100 bar

↓

Clean-up
Gel Chromatography
Polystyrene gel, EtAC/cHexane

↓

Concentration Step
RotaVap Evaporation

↓

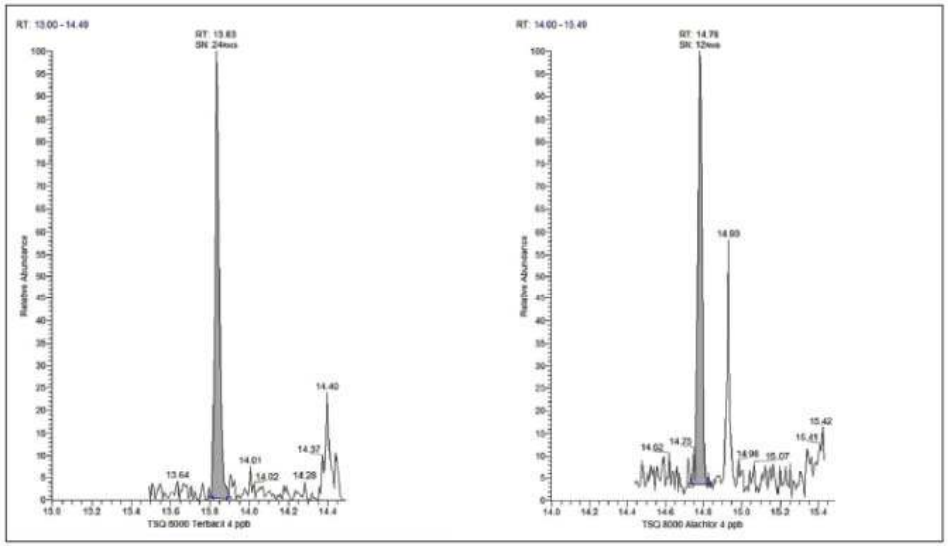
Analysis
GC-MS/MS
MRM method, 2 SRMs/analyte

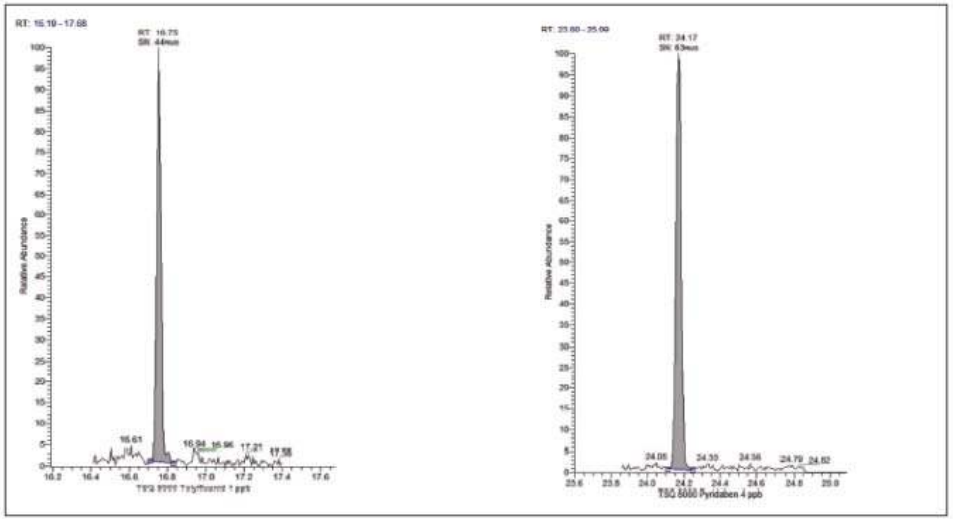
↓

Data Processing
Found/not-Found Reports
Opt. QC, recovery reporting

Courtesy N. Rückert, J.Kirchner, E.Häfner, Phytolab, Germany – EPRW 2012

Analysis of Pesticides





- MRL level 10 ppb
- Terbacil and Alaclor at 4 ppb
  - Terbazil (left, 161.1 > 88.0, CE 15 V) and Alaclor (right, 188.1 > 130.1, CE 25 V)

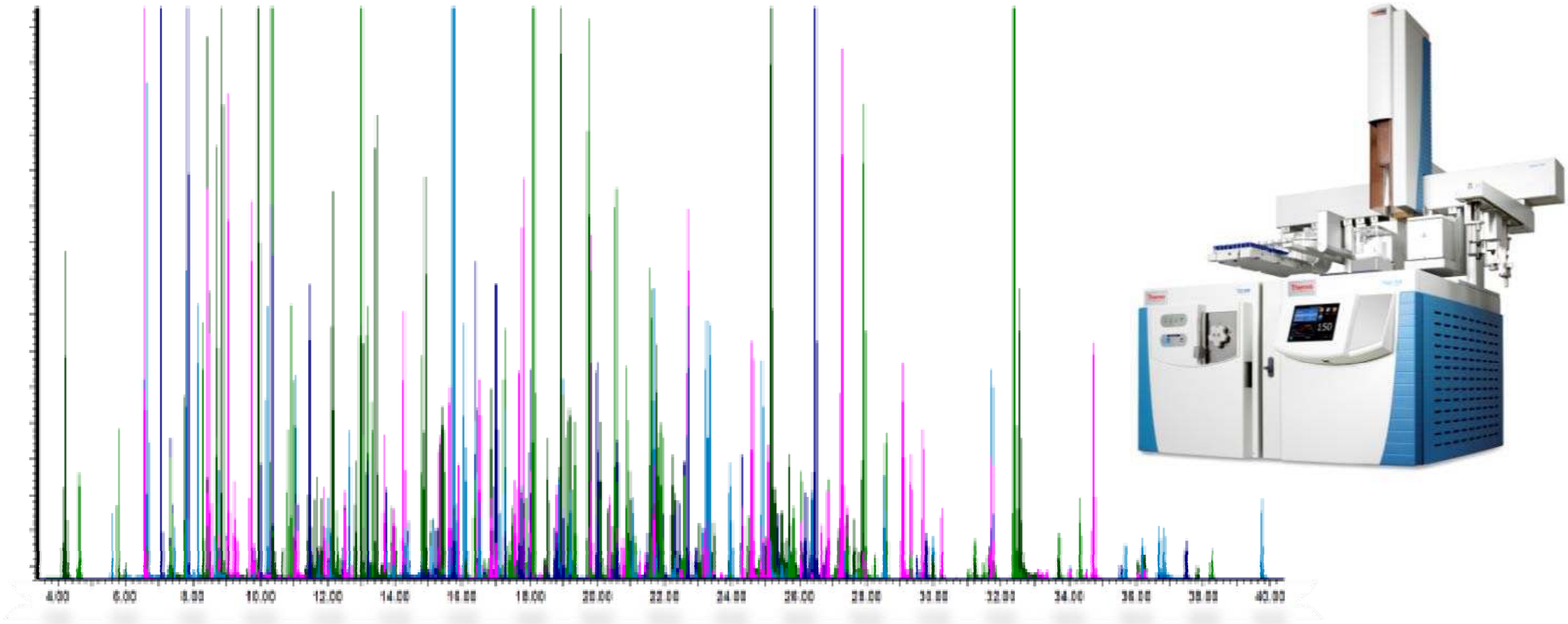
> 800 Pesticides

Only <u>one</u> GC-MS/MS method

- Tolylfluanid and Pyridaben at 4 ppb
  - Tolylfluanid (left, 238.1 > 137.1, CE 15 V) and Pyridaben (right, 309.1 > 147.1, CE 15 V)

Analysis of Pesticides - Multi-Component Methods

- Typical method for cereals covers 350 pesticides, MRM >700 transitions
- With TSQ 8000 GC-MS/MS multi-component analysis is made easy.

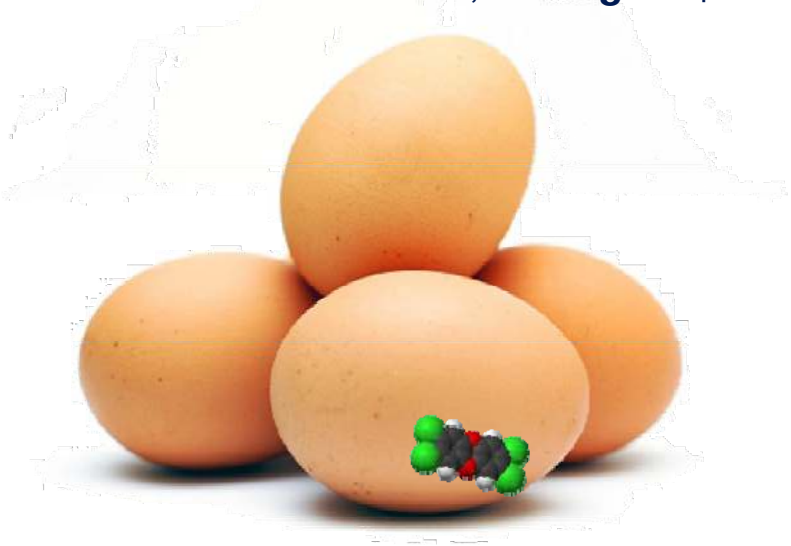


“Dioxin Incidents” – Serious Economic Impacts

- **Dioxin and PCB incidents …**

  … are expensive.

  - Egg production enterprise with $5M hens would **cost in excess of $30m**.
  - Broiler enterprise producing 3M broilers per week would **exceed $85m**.
  - Times Beach, Missouri, road dust covered by oil spread. The **cleanup cost $110m.**
  - The total costs of the Belgian food crisis are estimated **up to $1000m**.
  - EU farms closed, **damage > $350m**



Repeated “Dioxin” cases from only recent years:

- 1998 Milk from citrus pulp pellet feed
- 1999 Belgian PCB/dioxin in eggs, poultry
- 1999 Clay and zeolithes for feed
- 2000 Choline chloride
- 2002 “Carbosan Copper”
- 2004 Potato pulp
- 2005 Hydrochloric acid
- 2007 Indian Guar Gum thickener
- 2008 Irish Pork Meat, Italian Mozarella
- 2011 Contamin‘d feed - 4000 farms closed
- 2012 Organic eggs contaminated

The POPs Analysis Workflow – 1 Day to Results!

Soxhlett

ASE

PowerPrep

TSQ Quantum XLS Ultra
GC-MS/MS

DFS
GC-HRMS

Sample Preparation Clean-up

Food, Feed for PCDD/Fs, PCBs, OCPs, BFRs, Toxaphene, etc.

Add ^{13}C labelled ISTD and clean-up standard

Screening

Negative

(TEQ < ML)

Analysis Done

TEQ Report LIMS System

Positive
(TEQ > ML)

Confirmation

Negative

Analysis Done

Positive

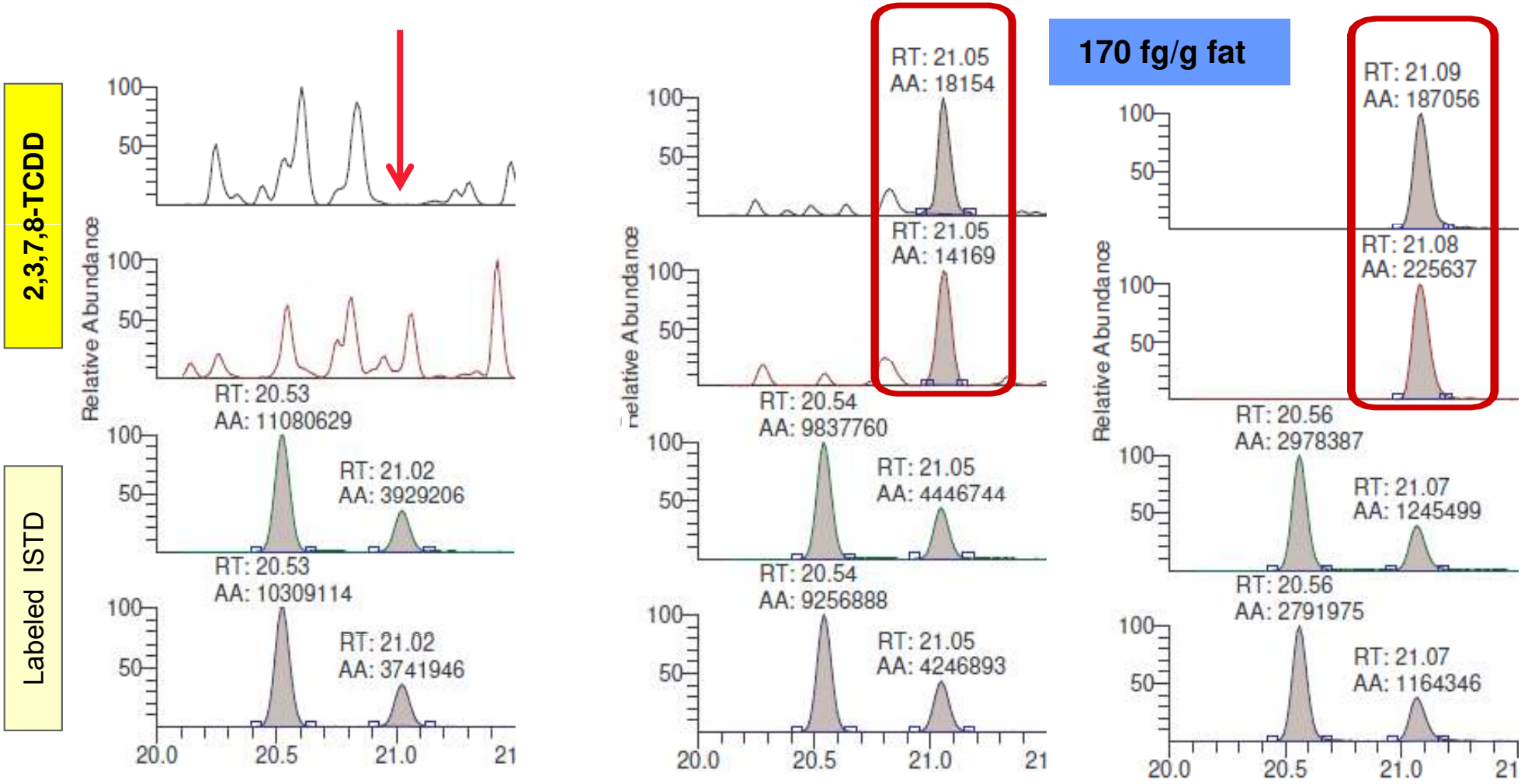
Final TEQ Calculation

Confirmation Report LIMS System

GC-MS/MS Screnning using the TSQ Quantum XLS

TCDD in Buffalo Milk Samples:

Blank (GC-MS/MS) | Buffalo Milk (GC-MS/MS) | Buffalo Milk (GC-HRMS)



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Complete Method Setup for dl-PCBs and PCDD/Fs

- For TSQ Quantum XLS Screening and DFS GC-HRMS Confirmation

Application Note: 10262

Analysis of PCBs in Food and Biological Samples Using GC Triple Quadrupole

Application Note: 51843

PCDD/F Screening at the Maximum Residue Level for Food Safety Analysis using Highly Selective Triple Quadrupole GC/MS/MS

Dirk Kruneweide, Hans-Joachim Huebschmann, Thermo Fisher Scientific, Bremen, Germany

Key Words
- EPA 1613A
- Food Safety Analysis
- Multiple Reaction Monitoring MRM
- PCB
- PCDD
- PCDF
- Screening

Abstract

This paper presents a trace level screening method for PCDD/Fs and PCBs at the relevant maximum residue level for foodstuff. The method described employs a triple quadrupole mass spectrometer equipped with hyperbolic quadrupole rods for increased selectivity. The described MRM method is using selected MS/MS transitions for polychlorinated dioxins/furans and PCBs from two different precursor ions and detecting individual product ions for each chlorination degree. The analytical strategy follows the well-established United States Environmental Protection Agency (US EPA) Method 1613A by using isotope dilution quantitation with ^{13}C labeled internal standards.

Introduction

Polychlorinated dioxins (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs) are amongst the biggest concerns related to food safety considerations. Considerable effort is taken worldwide to reduce global contamination with the effect of constantly decreasing levels found in feed and food. In contrast to lower general levels an increasing number of regional "accidents" in the food chain became public causing elevated levels of dioxins with worldwide impact (Irish pork, Italian mozzarella, and others).

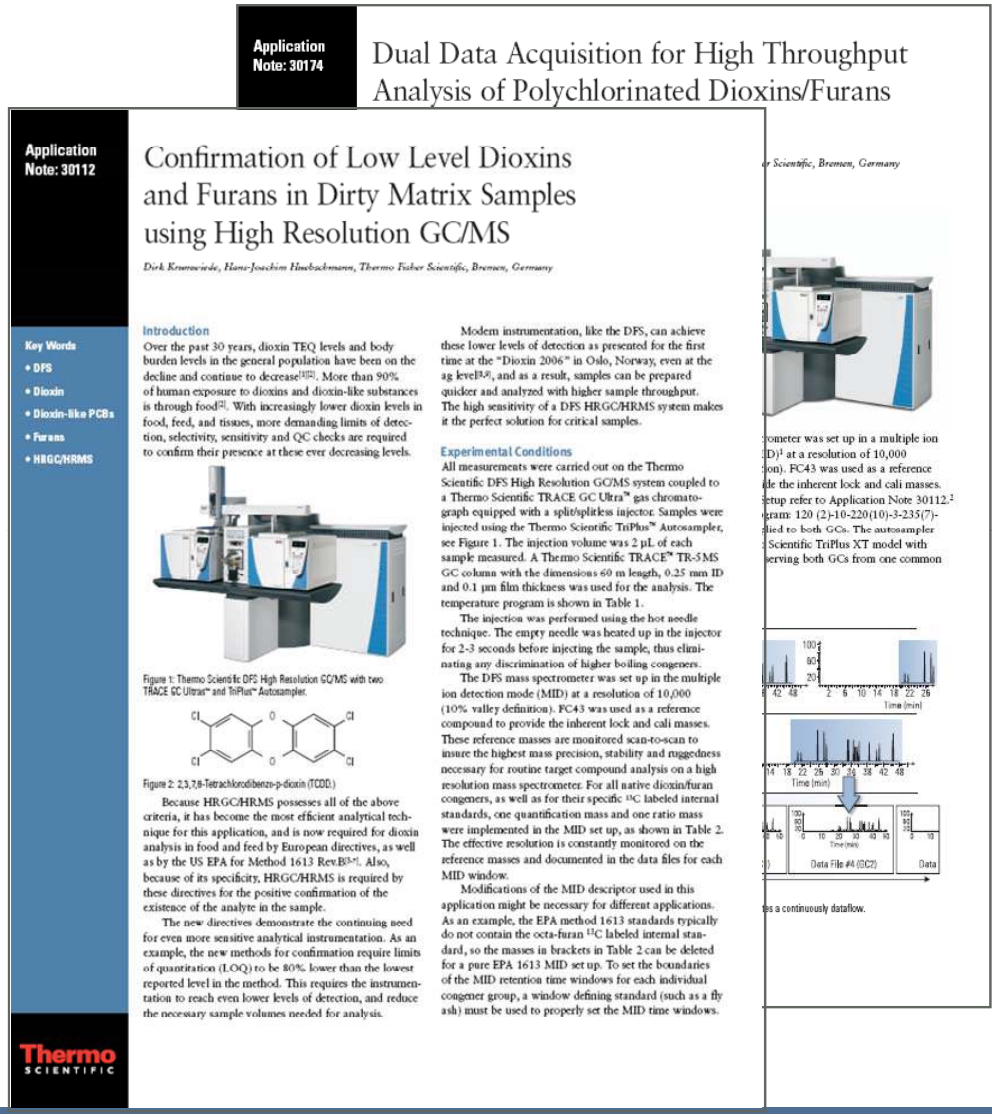
In food production and control the continued low level screening is required to monitor the absence and compliance of raw materials as well as food products with current directives. In the European directives the maximum levels for dioxin and dioxin-like PCB contaminants are regulated for foodstuff, as well as the methods of analysis for screening and confirmation.[1,2] Requirements on performance of analytical methods applied are regulated by providing identification points to analytical techniques and their combinations.[3] Here the GC/MS/MS technique provides the potential for an instrumental screening method earning five identifications point when measuring two precursor ions with one product ion each, see Table 1.

Technique	Number of Ions	Identification Points
GC/MS (EI or CI)	N	n
GC/MS (EI and CI)	2 (EI) + 2 (CI)	4
GC/MS (EI or CI) 2 derivatives	2 (Derivative A) + 2 (Derivative B)	4
GC/MS-MS	1 precursor and 2 product ions	4
GC/MS and HRMS	2 + 1	4
GC/MS/MS	2 precursor ions, each with 1 product ion	5
HRMS	N	2n

Table 1: Examples of the number of identification points earned for a range of GC/MS techniques (N,n = an integer)[3]

Sample screening requires the economical analysis of a large number of samples. While high resolution mass spectrometry is typically reserved for confirmation analysis of non compliant samples, alternative screening techniques as bioassays are discussed. Triple quadrupole GC/MS uses similar sample preparation steps and offers the potential to overcome known false positive results of bioassays with a highly sensitive and reliable target compound quantitation at the required low trace levels.

Triple quadrupole GC/MS/MS has turned out to become a standard trace analytical method for priority pollutants and pesticides in food and environmental analysis today. Using the highly selective precursor ion selection available with the Thermo Scientific TSQ Quantum XLS using high precision hyperbolic quadrupole technology an increased selectivity for low level contaminants in matrix samples became available.



Application Note: 30174

Dual Data Acquisition for High Throughput Analysis of Polychlorinated Dioxins/Furans

Application Note: 30112

Confirmation of Low Level Dioxins and Furans in Dirty Matrix Samples using High Resolution GC/MS

Dirk Kruneweide, Hans-Joachim Huebschmann, Thermo Fisher Scientific, Bremen, Germany

Key Words
- DFS
- Dioxin
- Dioxin-like PCBs
- Furans
- HRGC/HRMS

Introduction

Over the past 30 years, dioxin TEQ levels and body burden levels in the general population have been on the decline and continue to decrease[1][2]. More than 90% of human exposure to dioxins and dioxin-like substances is through food[2]. With increasingly lower dioxin levels in food, feed, and tissues, more demanding limits of detection, selectivity, sensitivity and QC checks are required to confirm their presence at these ever decreasing levels.

Figure 1: Thermo Scientific DFS High Resolution GC/MS with two TRACE GC Ultra™ and TriPlus™ Autosampler.

Figure 2: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD.)

Because HRGC/HRMS possesses all of the above criteria, it has become the most efficient analytical technique for this application, and is now required for dioxin analysis in food and feed by European directives, as well as by the US EPA for Method 1613 RevB[3-5]. Also, because of its specificity, HRGC/HRMS is required by these directives for the positive confirmation of the existence of the analyte in the sample.

The new directives demonstrate the continuing need for even more sensitive analytical instrumentation. As an example, the new methods for confirmation require limits of quantitation (LOQ) to be 80% lower than the lowest reported level in the method. This requires the instrumentation to reach even lower levels of detection, and reduce the necessary sample volumes needed for analysis.

Modern instrumentation, like the DFS, can achieve these lower levels of detection as presented for the first time at the "Dioxin 2006" in Oslo, Norway, even at the ag level[3,6], and as a result, samples can be prepared quicker and analyzed with higher sample throughput. The high sensitivity of a DFS HRGC/HRMS system makes it the perfect solution for critical samples.

Experimental Conditions

All measurements were carried out on the Thermo Scientific DFS High Resolution GC/MS system coupled to a Thermo Scientific TRACE GC Ultra™ gas chromatograph equipped with a split/splitless injector. Samples were injected using the Thermo Scientific TriPlus™ Autosampler, see Figure 1. The injection volume was 2 µL of each sample measured. A Thermo Scientific TRACE™ TR-5MS GC column with the dimensions 60 m length, 0.25 mm ID and 0.1 µm film thickness was used for the analysis. The temperature program is shown in Table 1.

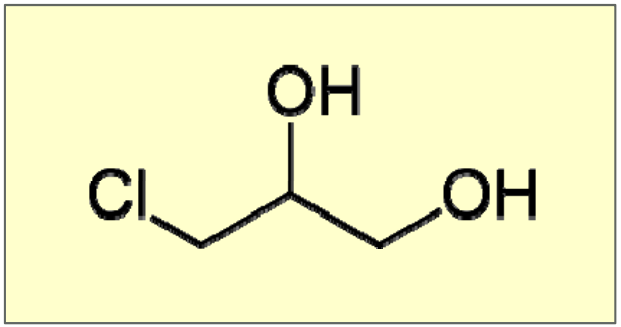
The injection was performed using the hot needle technique. The empty needle was heated up in the injector for 2-3 seconds before injecting the sample, thus eliminating any discrimination of higher boiling congeners.

The DFS mass spectrometer was set up in the multiple ion detection mode (MID) at a resolution of 10,000 (10% valley definition). FC43 was used as a reference compound to provide the inherent lock and cali masses. These reference masses are monitored scan-to-scan to insure the highest mass precision, stability and ruggedness necessary for routine target compound analysis on a high resolution mass spectrometer. For all native dioxin/furan congeners, as well as for their specific ^{13}C labeled internal standards, one quantification mass and one ratio mass were implemented in the MID set up, as shown in Table 2. The effective resolution is constantly monitored on the reference masses and documented in the data files for each MID window.

Modifications of the MID descriptor used in this application might be necessary for different applications. As an example, the EPA method 1613 standards typically do not contain the octa-furan ^{13}C labeled internal standard, so the masses in brackets in Table 2 can be deleted for a pure EPA 1613 MID set up. To set the boundaries of the MID retention time windows for each individual congener group, a window defining standard (such as a fly ash) must be used to properly set the MID time windows.

3-Chloropropane-1,2-diol (3-MCPD)

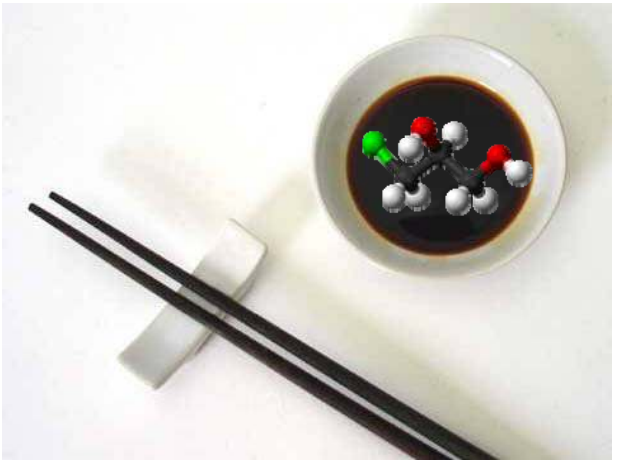
- Known as food processing contaminants since 1978
  - soy sauces and soups
  - bakery products, malt,
  - vegetable protein (HVP)
  - Edible oils, e.g. palm oil
    - 15–20 times higher in refined oils (deodorization)
  - grilled cheese and meat products, fish products
  - 3-MCPD and other CPs are carcinogenic (group 2B), and possibly genotoxic
- 3-MCPD is formed from glycerol, acylglycerols and chloride ions
  - major part is ester-linked with fatty acids
  - determined as free and bound 3-MCPD
- European Commission regulation
  - limit of 0.02 mg/kg for 3-MCPD (20 ppb)
  - in liquid soy sauce and HVP products



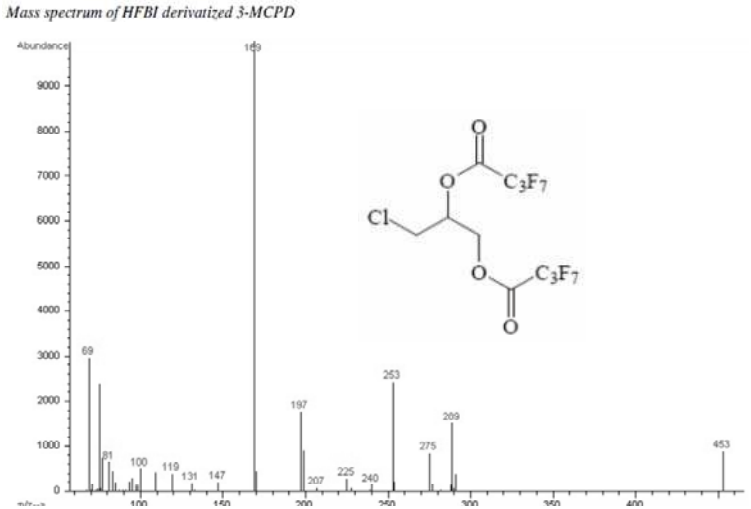
free 3-MCPD

3-Chloropropane-1,2-diol (3-MCPD)

- Sample preparation
  - Acidic hydrolysis with sulfuric acid/methanol
  - Derivatization with HFBA or phenylboronic acid
- Internal standard isotope-labeled 3-MCPD

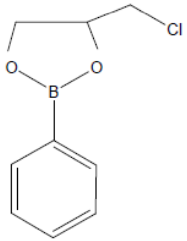


- GC/MS analysis
  - using ISQ single quadrupole systems
  - Trace GC using PTV injector and concurrent backflush
  - TG-5MS fused-silica column (30 m, 0.25 mm, 0.25 mm film)



Lim ,Y .G., Ch'ng, A. L. 2005

or



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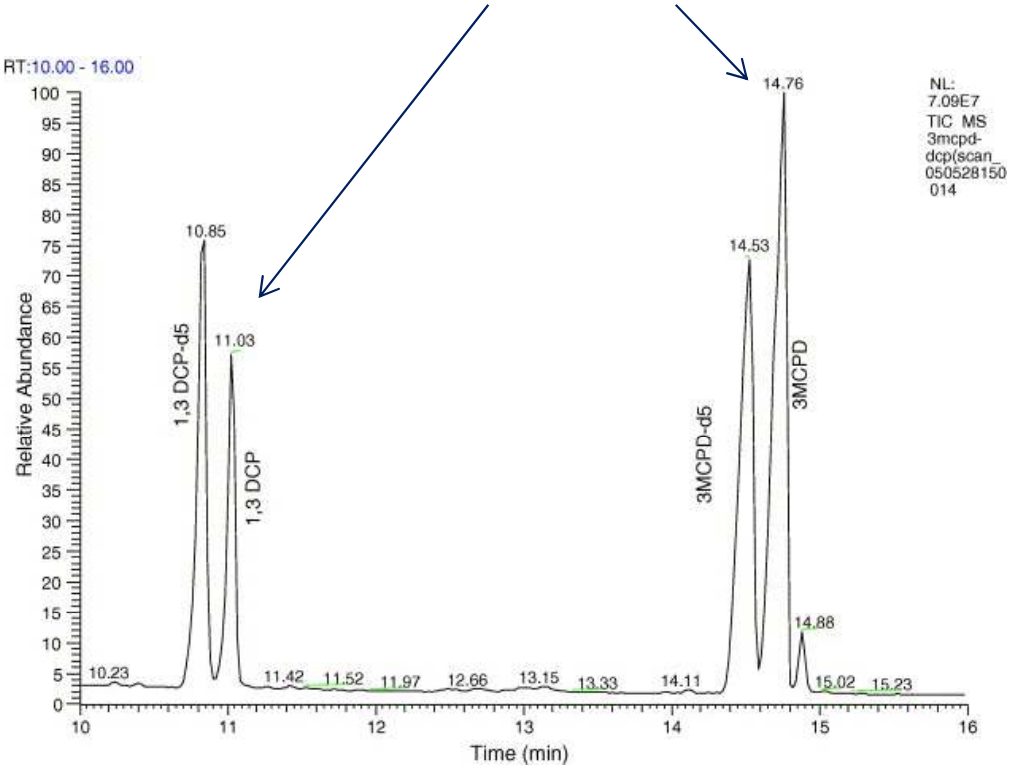
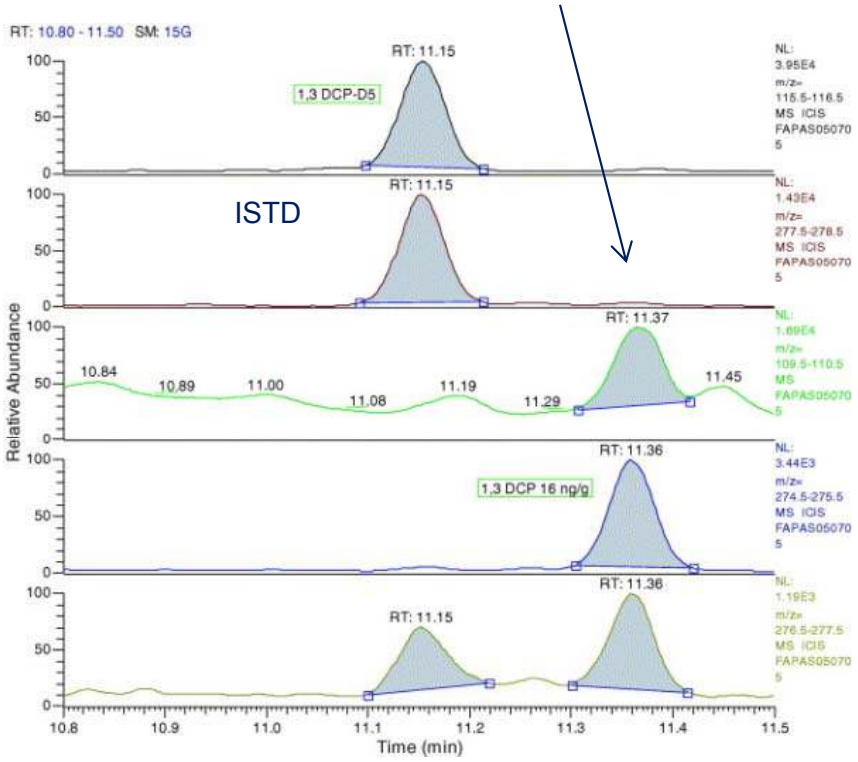
3-Chloropropane-1,2-diol (3-MCPD)

- Results on 3-MCPD - EU MRL 20 µg/kg (ppb) – GC/MS Analysis
  - Barbecue steaks found up to 365 µg/kg
  - Smoked sousages up to 103 µg/kg

Elution sequence 1,3-DCP, 3-MCPD



Soy Souce at 16 µg/kg 1,3-DCP



Abu-El-Haj, S., Bogusz, M.J., 2005

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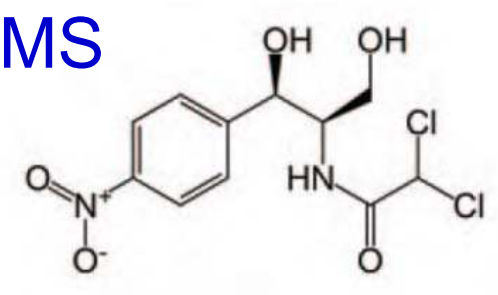
Veterinary Drug Residues

- Analysis of Chloramphenicol in Milk by LC-MS/MS
  - Simple sample preparation
  - Only the acetonitrile protein precipitation
- Validation
  - according to the European Commission Decision 2002/657/EC



0.5 g Milk + d5–CAP (0.3 ppb) as IS

↓

+ 0.75 mL CH_3CN, vortex 1 min, Centrifuge @ 14000 rpm for 10 min

↓

Take 0.7 mL Supernatant + 0.3 mL Water, store at 4°C for ≥ 1 hr

↓

Pipette 0.8 mL upper solution for LC-MS/MS Analysis

Chromatography Conditions

HPLC Module: Thermo Scientific Accela High Speed LC System

Column: Thermo Scientific Hypersil GOLD 50 mm×2.1 mm and 1.9 µm particle size

Column Temperature: Ambient

Mobile Phase: A: Methanol B: Water

Gradient:

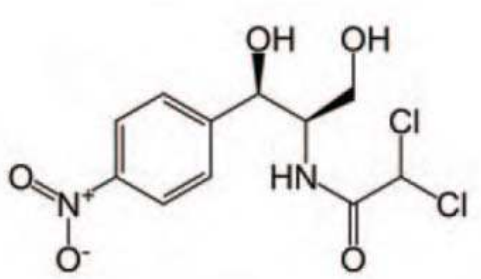
Time (min)	A%
0.0-0.6	5%
2.3	100%
2.35-3.0	5%

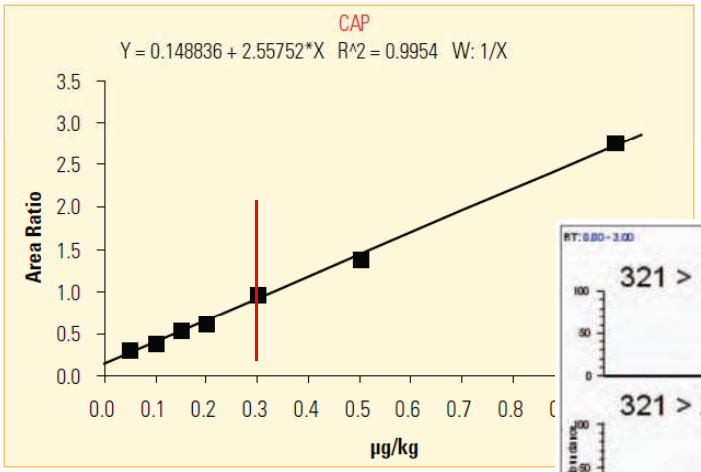
Flow Rate: 500 µL/min

Injection Volume: 20 µL (with loop)

Veterinary Drugs

- Thermo Scientific TSQ Quantum Access LC-MS/MS
  - ESI negative ion, MRM method
- Screening
  - LOQs < 0.050 µg/kg CAP (MRPL of 0.3 µg/kg)





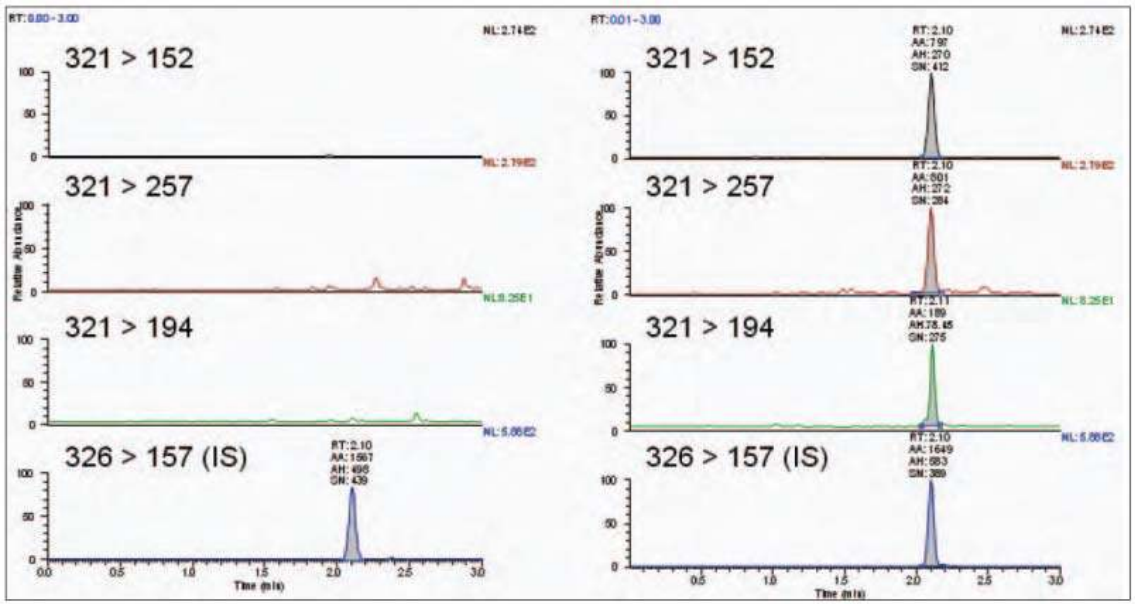


Figure 2: SRM chromatograms for milk blank and 0.050 µg/kg spiked milk samples

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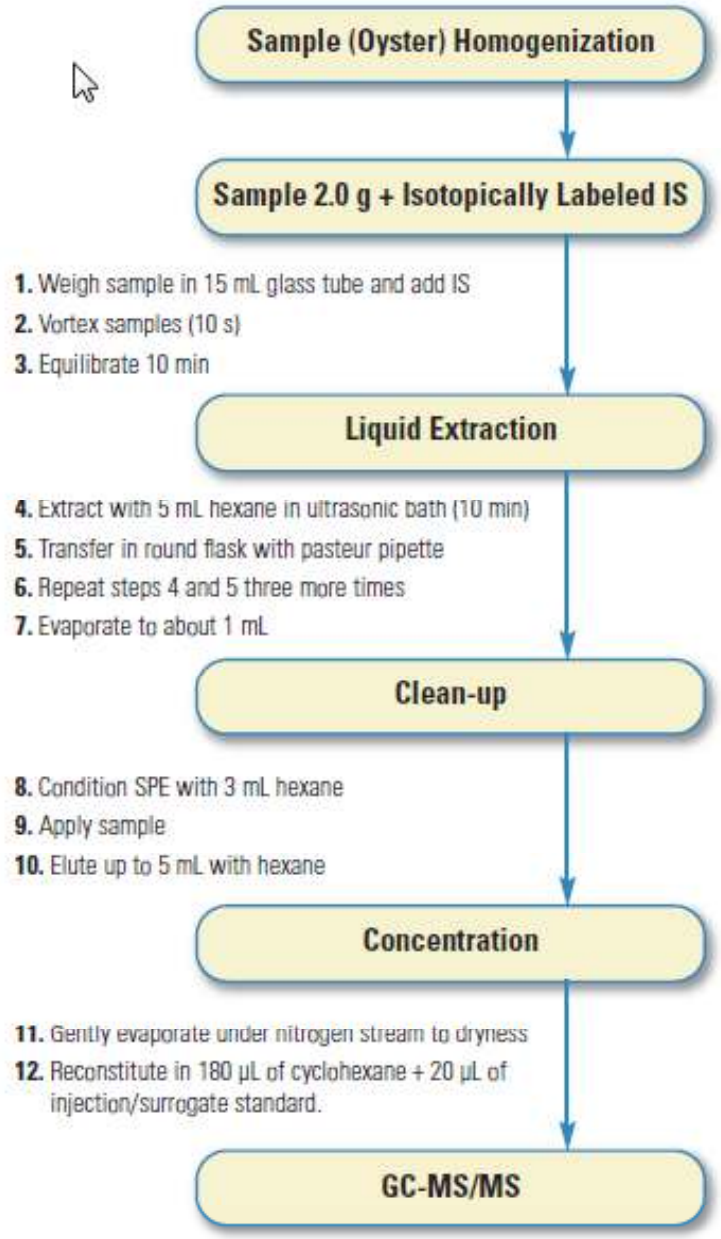
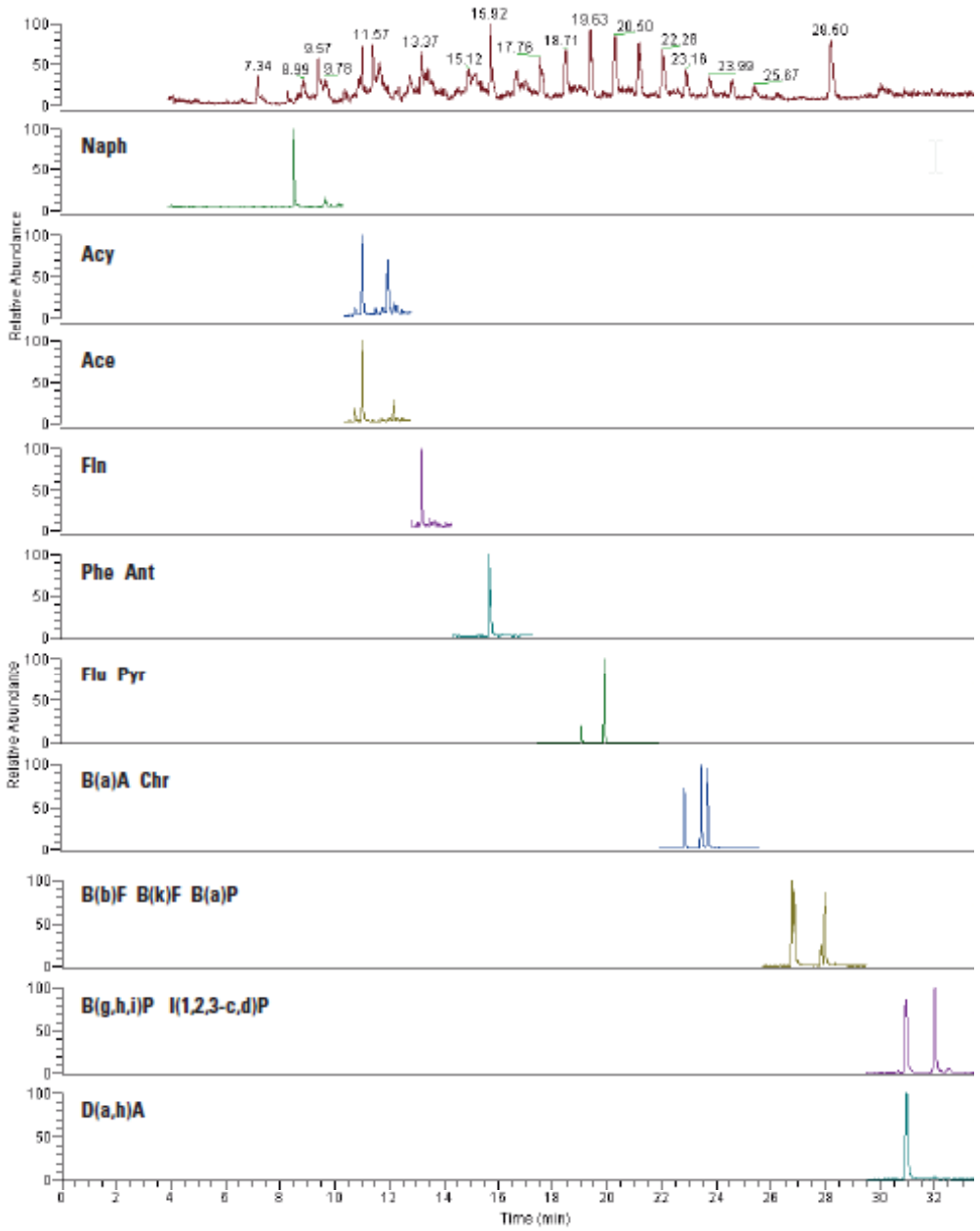
Polycyclic Aromatic Hydrocarbons (PAHs)

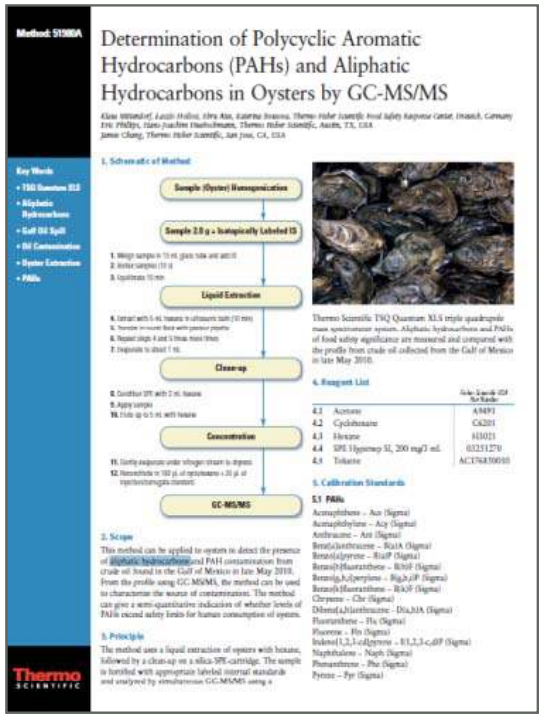


- FSRC Method
  - Developed for oil spill Gulf of Mexico 2010
  - Full scan + MRM method
- Matrix
  - All type of seafood, here oysters
- Aliphatic hydrocarbons
  - Pattern provides source of contamination

Polycyclic Aromatic Hydrocarbons (PAHs)



- Hydrocarbon pattern
  - From Full Scan data
- Complete 16 PAHs
  - Individual MRM quantitation
  - Here 10 ng/g in oyster matrix
  - Cylcle time 35 min only


Determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Aliphatic Hydrocarbons in Oysters by GC-MS/MS

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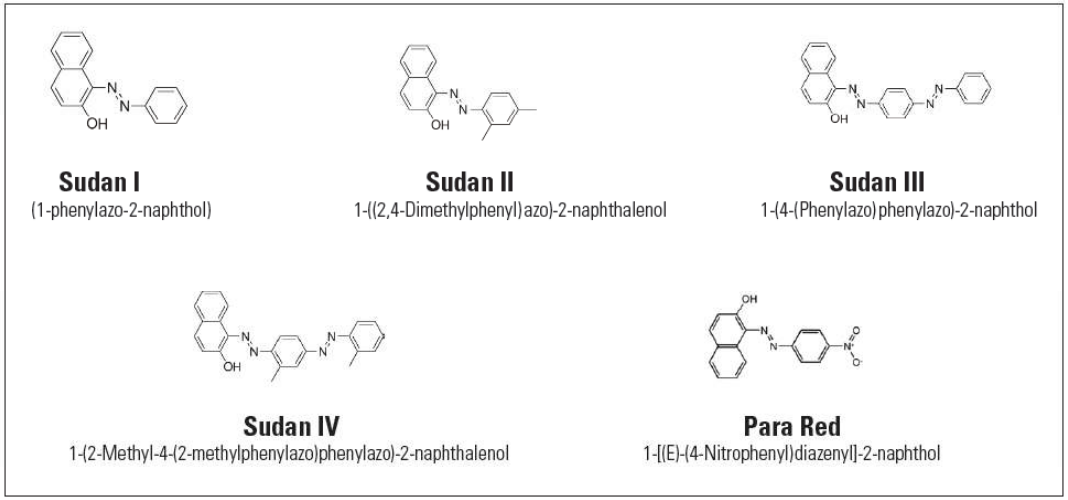
Sudan Dyes

- Sudan dyes are red dyes
  - Used for coloring solvents, oils, waxes, petrol, or as additives in shoe and floor polish.
  - Found in food products such as chili or chili-containing products.
  - Banned as food additives in the USA, the EU and many other countries
  - Links to cancer and other negative health effects.
- Most popular methods for analysis
  - LC-UV/vis and solid phase extraction (SPE), or using fast acetonitrile LLE
  - LC/MS using autoamted TurboFlow sample prep with Exactive MS



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Sudan Dyes

- Recovery 80-120%
- LOQ LC-HRMS (Orbitrap)
  - R 50 000
  - 5 ng/g Sundan I-IV,
  - 20 ng/g Para Red

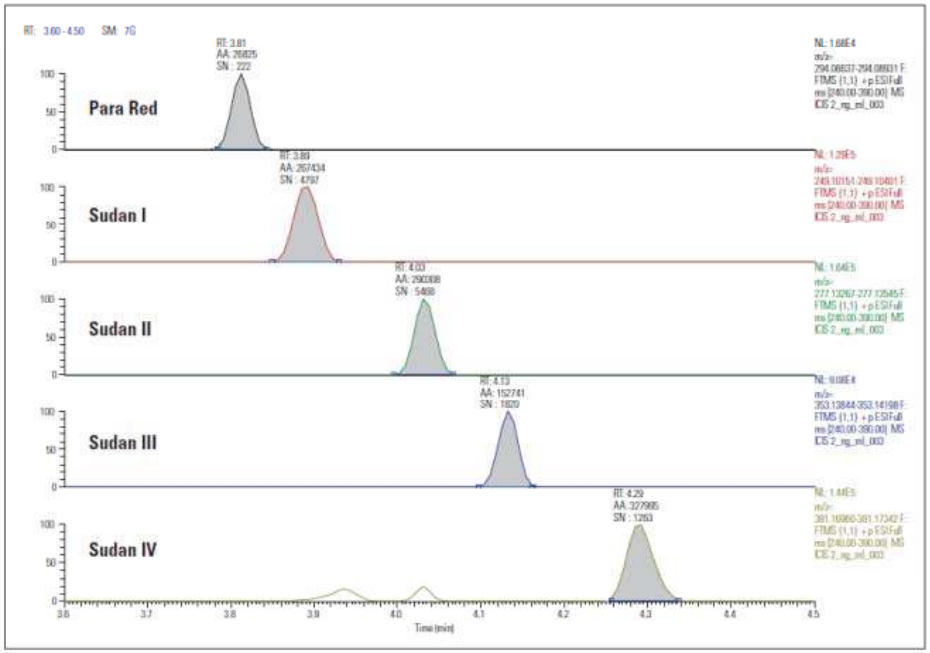

Figure 2. Representative chromatogram (20 ng/g in Hot Sauce II)

Thermo Scientific TurboFlow Method Parameters

Column:	TurboFlow XL C8 column 0.5 x 50 mm
Injection Volume:	25 µL
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN
Solvent C:	1:1 ACN: isopropanol

Mass Spectrometer Parameters

MS:	Thermo Scientific Exactive high performance benchtop Orbitrap™ MS
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Ionization Mode:	Positive
Scan Range:	*m/z* 240.0 to 390.0
Resolution:	50,000

	Formula	Exact Mass	$[M+H]^+$
Para Red	$C_{16}H_{11}N_3O_3$	293.080041	294.087841
Sudan I	$C_{16}H_{12}N_2O$	248.094963	249.102763
Sudan II	$C_{18}H_{16}N_2O$	276.126263	277.134063
Sudan III	$C_{22}H_{16}N_4O$	352.132411	353.140211
Sudan IV	$C_{24}H_{20}N_4O$	380.163711	381.171511

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Sudan Dyes

- LC-UV/vis method
  - Quick acetonitrile extraction
  - Automated 2D LC
  - UV 478 nm detection
  - MDL 3 ppb (ug/L) Sudan I-IV

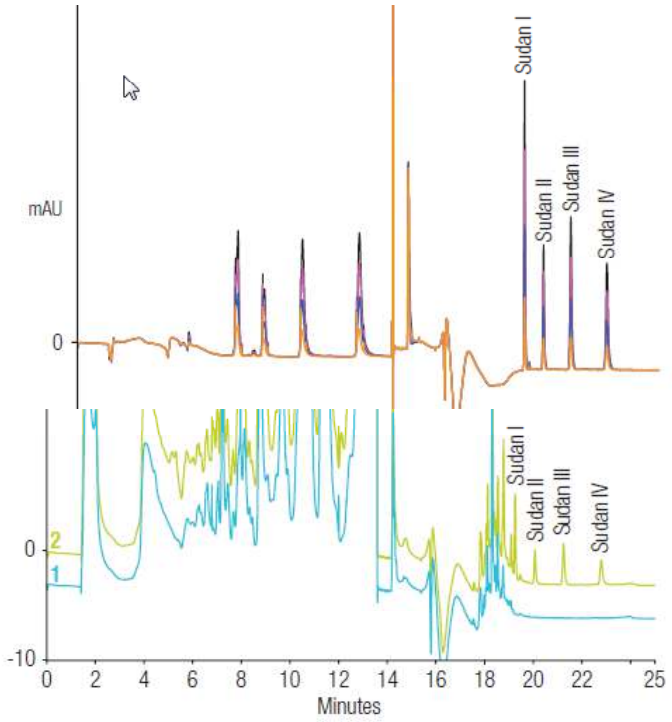

Figure 5. Overlay chromatograms of Panang curry paste and spiked Panang curry paste samples.

Sample Preparation

Weigh 10 g of a curry paste sample into a 50 mL glass bottle, add 20 mL of acetonitrile, and shake. Put the bottle in an ultrasonic bath for 10 min, then filter the sample using a 0.2 µm syringe filter before sample injection.

Chromatographic Conditions

First Dimension	
Column:	Thermo Scientific Acclaim™ PolarAdvantage II (PA2), 3 µm Analytical, 4.6 × 150 mm (P/N 063191)
Mobile Phase:	A: Water B: Acetonitrile C: 2-Propanol
Inj. Volume:	300 µL
Temperature:	30 °C
Detection:	UV, 478 nm
Second Dimension	
Column:	Acclaim Phenyl-1, 3 µm, Analytical, 4.6 × 150 mm (P/N 071969)
Mobile Phase:	A: Water B: Acetonitrile C: Methanol

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Sudan Dyes

Determination of Sudan Dyes I–IV in Curry Paste

Suparerk Tukkaorad and Jeffrey Rohrer[1]
[1]Thermo Fisher Scientific, Bangkok, Thailand; [2]Thermo Fisher Scientific, Sunnyvale, CA, USA

Application Note 1023

Key Words
Acclaim Phenyl-1 Column, Acclaim PA2 Column, 2D-LC, Food

Introduction

Sudan dyes are a class of synthetic dyes that are mainly used for industrial applications such as the coloring of plastic. These dyes are banned as a food-coloring agent because they are classified as carcinogens. For economical reasons, however, Sudan dyes are sometimes illegally used to color food to improve its appearance. Therefore, methods are needed to determine if food products have been adulterated with these dyes.

Typically, Sudan dyes are determined by reversed-phase chromatography with UV or mass spectrometry (MS) detection, but complex food samples usually require extensive off-line sample preparation, such as solvent extraction, solid-phase extraction (SPE), sample evaporation, and/or sample reconstitution.

Previously, Dionex™ (now part of Thermo Scientific™) Application Note (AN) 287 demonstrated that two-dimensional (2D) high-performance liquid chromatography (HPLC) combined with on-line SPE can successfully determine Sudan dyes I, II, III, and IV in chili oil.[1] The sample was first extracted off line with methylene chloride and acetonitrile. The extracted sample was then partially separated on the first dimension column, and the dyes were subsequently trapped using an on-line SPE cartridge between the two dimensions. The trapping required a third pump to dilute the mobile phase from the first dimension to ensure that the dyes were completely trapped on the SPE cartridge. The dyes were then sent to the second dimension for separation prior to MS detection.

The method shown here is a different 2D-HPLC approach for determining Sudan dyes I, II, III, and IV in curry paste that requires significantly less time, and thereby reduces the cost per analysis. After sample extraction using acetonitrile and subsequent filtration, the sample is injected into the first dimension for partial separation. By ensuring that the solvent strength of the sample entering the first dimension is low, sufficient sample can be injected to enable a sensitive method. Similarly, by ensuring that the mobile phase strengths of the fractions from the first dimension entering the second dimension are low enough, the Sudan dyes can be simply trapped on the second dimension prior to separation and UV detection.

This method requires neither an SPE column between the two dimensions nor the third pump to dilute the mobile phase from the first dimension. The same UV detector is used for both dimensions. To determine Sudan dyes in curry paste, only sample extraction and filtration are performed off line. The remaining steps are automated using a Thermo Scientific Dionex UltiMate™ 3000 x2 Dual Rapid Separation LC (RSLC) system controlled by Chromeleon™ Chromatography Data System (CDS) software.

Goal

To determine Sudan dyes I, II, III, and IV in curry paste by 2D-HPLC without off-line SPE sample pretreatment

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Application Note: 560

Analysis of Illegal Dyes in Food Matrices using Automated Online Sample Preparation with LC/MS

Yang Shi, Catherine Lafontaine, Matthew Berube, John Fink, François Espourteille
Thermo Fisher Scientific, Franklin, MA

Key Words
- Transcend TLX-1
- TurboFlow Technology
- Exactive
- Accucore HPLC Columns

Introduction

Sudan dyes are red dyes used for coloring solvents, oils, waxes, petrol, or as additives in shoe and floor polish. In addition, they have been found in a number of food products such as chili or chili-containing products. Sudan dyes are banned as food additives in the USA[1], the EU[2,3] and many other countries due to links to cancer and other negative health effects.

Liquid chromatography-ultraviolet-visible (LC–UV–vis) and liquid chromatography-mass spectrometry (LC/MS) are currently the most popular methods for analysis of Sudan dyes.[4] Traditional sample preparation methods, especially solid phase extraction (SPE), have also been widely used in the determination of Sudan dyes. However, these procedures can be labor-intensive, time-consuming and costly, resulting in low sample throughput when performed manually. Lower recoveries have also been noticed associated with SPE cleanup.[4] There is consensus that one of the major scientific challenges in the analysis of Sudan dyes is to achieve high sensitivity and selectivity while minimizing sample clean up.[5]

In this study we describe an easy, comprehensive LC method using a Thermo Scientific Transcend TLX-1 system powered by TurboFlow™ technology coupled to a Thermo Scientific Exactive MS to analyze five illegal dye residues in a variety of sauces.

Goal

Develop a rapid and sensitive automated online sample preparation LC-MS/MS method to detect and quantify multiple Sudan dyes in a variety of food matrices and also to shorten assay time and increase throughput.

Experimental

The Matrix Standard Curve
Five analytes, Sudan I, Sudan II, Sudan III, Sudan IV and Para Red (Figure 1) were obtained from Sigma-Aldrich (St. Louis, MO). A total of four different food products purchased from local grocery stores were used in this study: Chili Sauce I; Chili Sauce II; Hot Sauce I; Hot Sauce II.

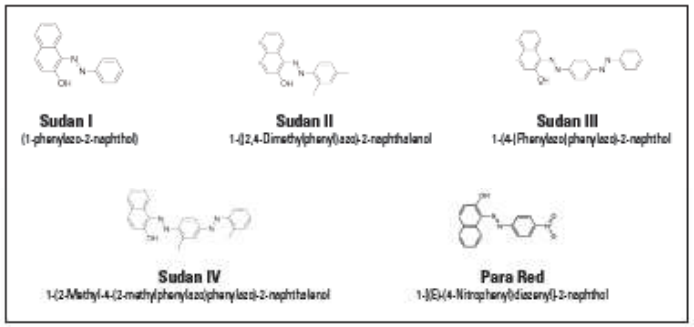

Figure 1. Chemical structure of test compounds

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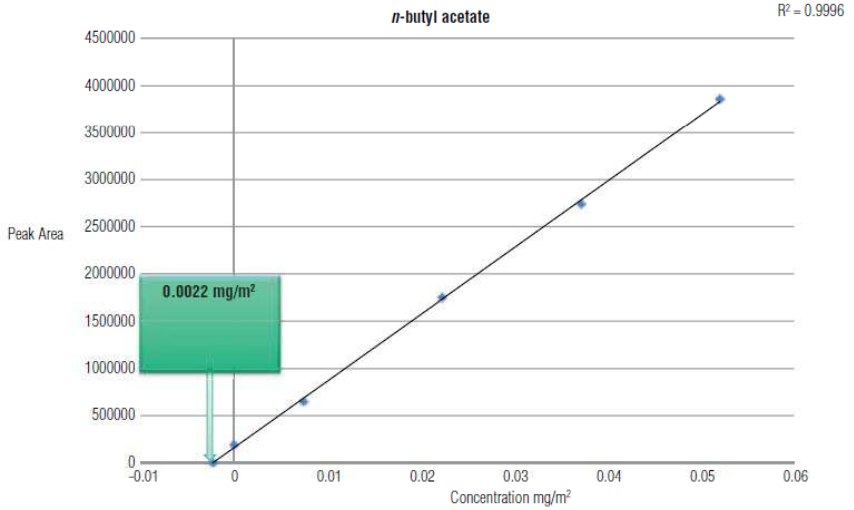
Volatile Organic Carbons (VOCs)

- Samples
  - Food packaging like paper board, multilayers, etc.
- Typical setup for VOC uses
  - Headspace or Purge&Trap sampling
  - Trace GOLD TG-624 column
  - GC/MS single quad detection
- TriPlus RSH with ISQ GC/MS
  - Automated standard dilutions
  - Automated standard addition
  - Automated staggered incubation
  - Automated analysis
  - Complete reporting
- LOQ below 0.01 mg/m²
  - For 23 commonly analyzed solvents

TriPlus RSH Method	
Sample Volume (µL)	1000
Incubation Time (min)	15
Incubation Temperature (°C)	80
Syringe Temperature (°C)	120



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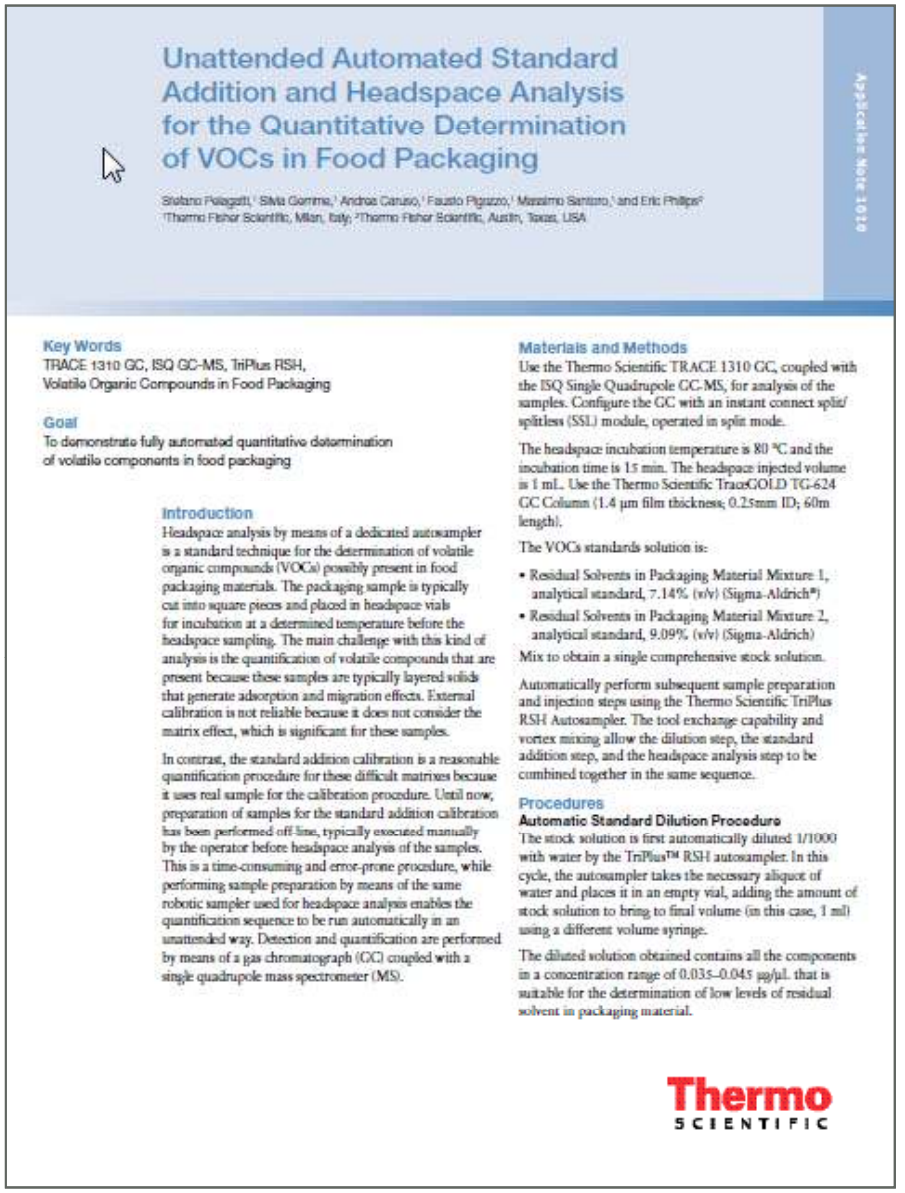
Volatile Organic Carbons (VOCs)

- ISQ with Trace GC 1300
- Triplus RSH autosampler
  - Headspace
  - SPME
  - Automated tool change to liquid inj.



Unattended Automated Standard Addition and Headspace Analysis for the Quantitative Determination of VOCs in Food Packaging

Application Note 1019

Stefano Pelagatti,[1] Silvia Gomma,[1] Andrea Caruso,[1] Fausto Pigozzo,[1] Massimo Santoro,[1] and Eric Phillips[2]
[1]Thermo Fisher Scientific, Milan, Italy; [2]Thermo Fisher Scientific, Austin, Texas, USA

Key Words
TRACE 1310 GC, ISQ GC-MS, TriPlus RSH, Volatile Organic Compounds in Food Packaging

Goal
To demonstrate fully automated quantitative determination of volatile components in food packaging

Introduction
Headspace analysis by means of a dedicated autosampler is a standard technique for the determination of volatile organic compounds (VOCs) possibly present in food packaging materials. The packaging sample is typically cut into square pieces and placed in headspace vials for incubation at a determined temperature before the headspace sampling. The main challenge with this kind of analysis is the quantification of volatile compounds that are present because these samples are typically layered solids that generate adsorption and migration effects. External calibration is not reliable because it does not consider the matrix effect, which is significant for these samples.

In contrast, the standard addition calibration is a reasonable quantification procedure for these difficult matrixes because it uses real sample for the calibration procedure. Until now, preparation of samples for the standard addition calibration has been performed off-line, typically executed manually by the operator before headspace analysis of the samples. This is a time-consuming and error-prone procedure, while performing sample preparation by means of the same robotic sampler used for headspace analysis enables the quantification sequence to be run automatically in an unattended way. Detection and quantification are performed by means of a gas chromatograph (GC) coupled with a single quadrupole mass spectrometer (MS).

Materials and Methods
Use the Thermo Scientific TRACE 1310 GC, coupled with the ISQ Single Quadrupole GC-MS, for analysis of the samples. Configure the GC with an instant connect split/splitless (SSL) module, operated in split mode.

The headspace incubation temperature is 80 °C and the incubation time is 15 min. The headspace injected volume is 1 mL. Use the Thermo Scientific TraceGOLD TG-624 GC Column (1.4 µm film thickness; 0.25mm ID; 60m length).

The VOCs standards solution is:

- Residual Solvents in Packaging Material Mixture 1, analytical standard, 7.14% (v/v) (Sigma-Aldrich®)
- Residual Solvents in Packaging Material Mixture 2, analytical standard, 9.09% (v/v) (Sigma-Aldrich)

Mix to obtain a single comprehensive stock solution.

Automatically perform subsequent sample preparation and injection steps using the Thermo Scientific TriPlus RSH Autosampler. The tool exchange capability and vortex mixing allow the dilution step, the standard addition step, and the headspace analysis step to be combined together in the same sequence.

Procedures
Automatic Standard Dilution Procedure
The stock solution is first automatically diluted 1/1000 with water by the TriPlus™ RSH autosampler. In this cycle, the autosampler takes the necessary aliquot of water and places it in an empty vial, adding the amount of stock solution to bring to final volume (in this case, 1 ml) using a different volume syringe.

The diluted solution obtained contains all the components in a concentration range of 0.035–0.045 µg/µl. that is suitable for the determination of low levels of residual solvent in packaging material.

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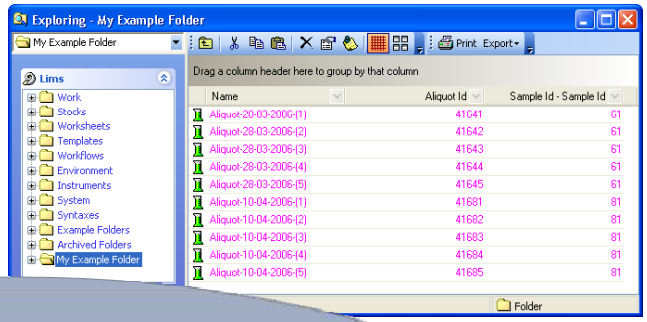
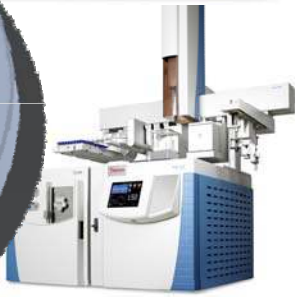
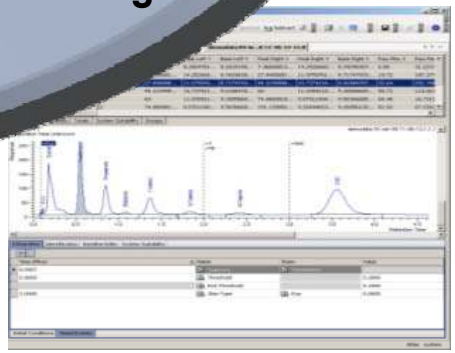
Selected Threats to Food Safety and Methods

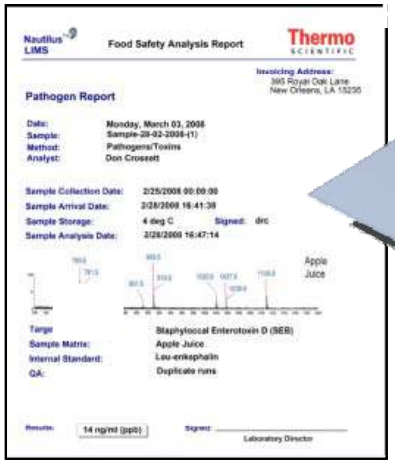
• Biological	
• Microorganisms/ Food-borne illness	Microbiology, Orbitrap HRAM
• Mycotoxins, Marine algae toxins	LC-MS/MS
• Residues and Trace Chemcials	
• Pesticides	GC-MS/MS, LC-MS/MS
• Persistant Organic Pollutants (POPs)	GC-MS/MS, GC-HRMS
• Veterinary Drugs	LC-MS/MS, Orbitrap HRAM
• Polyaromatic Hydrocarbons (PAHs)	GC-MS/MS
• Production/Packaging Related	
• Volatile Organic Carbons (VOCs)	GC-MS
• Plasticizer	GC-MS, LC-MS/MS
• MCPD	GC-MS
• Mineral Oil hydrocarbons (MOSH/MOAH)	LC-GC-FID
• Intentionally added compounds/fraud	
• Sudan dyes	LC-MS/MS, LC-UV

Where Does LIMS Fit into my Workflow? Everywhere!









Logging & Labelling

Method Worklists

Instrument Integration

Data Processing

QC / Review

Final Report, Invoice, ...

Sample Collection

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Thanks to the **Thermo** FSRC Food Response Center Team:

Ebru Ates

Katerina Bousova

Laszlo Hollosi

Thank you very much for your attention!

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