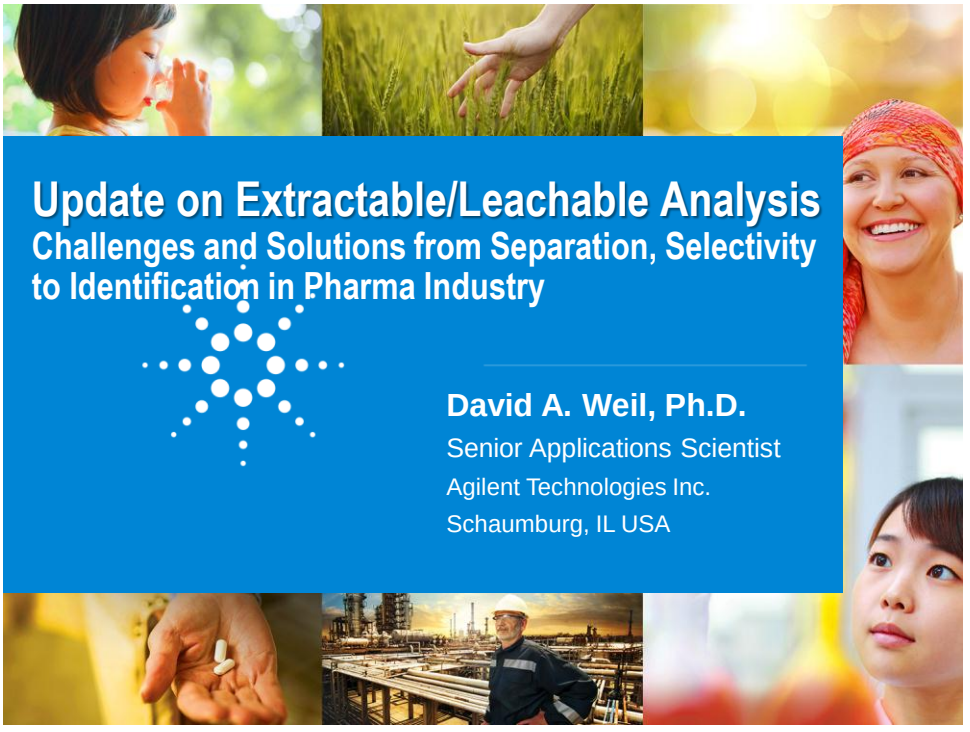

# Update on Extractable/Leachable Analysis

## Challenges and Solutions from Separation, Selectivity to Identification in Pharma Industry

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## Acknowledgements:

| | |
|---|---|
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| | |
|---|---|
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| **Dana Reed** | 3M CRL, St. Paul, MN |



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# Outline

- **Background:**
  - Recalls, Definitions, Regulations, Risk Assessment
- **Challenges of Extractable/Leachable Analysis**
- **Agilent GC/MS Solutions:**
  - GC/MSD and GC/QTOF Example
- **Agilent LCMS Solution: Accurate Mass, MS/MS**
  - Sensitivity with Jet Stream
  - Streamline MS/MS Databases Creation
- **Questions**



# Why Worry about Extractable/Leachable

## Merck recalls cholesterol drug Liptruzet due to packaging issues: Food and Drug recalls

Some of the outer laminate foil pouches may allow in air and moisture, according to the release, which "could potentially decrease the effectiveness or change the characteristics of the product." The likelihood of either of these outcomes is "remote," the company

## Ranbaxy recalling allergy drug in US over defective packaging

*(Ref: Business Standard, The Wall Street Journal, livemint.com)*
May 2nd, 2014

According to the FDA, Ranbaxy is recalling 29 790 packs of an allergy drug in the US, manufactured by its Ohms Laboratories unit, due to defective packaging. The agency said the voluntary Class II recall, which began in February, follows the discovery of "an unacceptable level of blister defects" affecting the loratadine and pseudoephedrine sulphate extended release tablets.

Johnson and Johnson recalls over 43 Over-the-counter (OTC) children's Medicines manufactured by McNeil Consumers Healthcare

**NeoProfen (ibuprofen lysine) Injection: Recall and Shortage - Risk of Particulate Matter**
[Posted 08/02/2010]

**Nutraloid Labs ejaculoid XXTREME and stimuloid II: Undeclared Drug Ingredient**

**Coumadin 1 mg Tablet Blister Packs: Recall**



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# FDA Drug Recalls Surges over 836 in 2014!

As FDA data show, the last two years have seen almost as many recalls (2,061) as the previous nine years combined (2,217)—and that's only counting the first seven months of 2014. *ref raps.org August 2014*

**Teva Parenteral Medicines Initiates Voluntary Nationwide Recall of Select Lots of Adrucil® (fluorouracil Injection, USP) 5 g/100 mL (50 mg/mL) Due to Particulate Matter**

**Mylan Initiates Voluntary Nationwide Recall of Select Lots of Injectable Products Due to the Presence of Particulate Matter**

**Heritage Pharmaceuticals Initiates a Nationwide Voluntary Recall of Colistimethate for Injection USP, 150 mg and Rifampin for Injection USP, 600 mg/vial Due to a Lack of Sterility Assurance**

**Hospira Issues A Voluntary Nationwide Recall Of One Lot Of Bupivacaine HCl Injection Due To Potential Iron Oxide Particulate In Glass Vials**

**Aventis Pharma Limited March 2015**
Defect with Epilim/Na valproate tablets: some batches have **an odor attributed to Foil Packaging**

**Reckitt Benckiser Healthcare** June 2014
Fybogel Hi-Fibre and Fybogel Orange potential risk of contamination **with metal particles**

**ViroPharma SPRL** July 2014
Buccolam Oromucosal Solutions
Recall **Chemical Contamination**



# Growing concern about impact of E/L impurities on final API and biologic products

Particulates, Mold, Moisture Stability

- 06/30/2014 Bristol-Myers Squibb Issues Voluntary Nationwide Recall of COUMADIN (Warfarin Sodium) for Injection Due to Presence of Particulate Matter
- 06/17/2014 Hospira Announces Voluntary Nationwide Recall of One Lot of 0.5% Marcaine™ (Bupivacaine HCl Injection, USP), 30 ML, Single-Dose, Preservative-Free Vial Due to Visible Particulates
- 06/02/2014 Alexion Initiates Voluntary Nationwide Recall of Certain Lots of Soliris® (eculizumab) Concentrated Solution for Intravenous Infusion Due to the Presence of Visible Particulate Matter in a Single Lot
- 03/05/2014 Baxter Initiates U.S. Voluntary Recall of One Lot of Peritoneal Dialysis Solution Due to Container-Closure Non-Integrity
- November 01, 2013 Perrigo Recalls Acetaminophen Infant Suspension Liquid, 160 mg/5 mL, Due to a Potential Defect with the Co-packaged Oral Syringe
- 05/16/2014 Labetalol Hydrochloride Injection 100 MG/20 ML (5MG/ML), 20 ML, Multidose Vial by Hospira: Recall - Visible Particulates
- 05/15/2014 Dobutamine Injection (250mg/20mL)/Hospira: Recall - Visible Particulates
- 05/14/2014 Hospira Announces Voluntary Nationwide Recall Of One Lot Of Dobutamine Injection, USP, 250 MG, 20 ML, Single-Dose Fliptop Vial, Due To Visible Particulates
- 04/21/2014 Hospira Announces Voluntary Nationwide Recall of One Lot of 0.25% Marcaine™ (Bupivacaine HCl Injection, USP), 10 ml, Single-Dose, Preservative-Free Vial Due to Visible Particulates
- 04/18/2014 Hospira Announces Voluntary Nationwide Recall of One Lot of 1% Lidocaine HCl INjection, USP, Due To Visible Particulates
- 04/18/2014 Cubist Pharmaceuticals Issues Voluntary Nationwide Recall of One Lot of CUBICIN (daptomycin for injection) 500 mg in 10 mL single use vials Due to Presence of Particulate Matter
- 04/17/2014 Hospira Announces Nationwide Voluntary Recall of Seven Lots of Propofol Injectable Emulsion, USP, Due to Visible Particulates



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# Federal Regulations on Extractable/Leachable

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

## FDA Cosmetic Act

Section 501 (a)(3) of the ACT states t a drug is deemed to be adulterated "if it container is composed, in whole or in part, of any poisonous or deleterious substances that may render the contents injurious to health. ..

Center for Drug Evaluation and Research
FDA

## Guidance for Industry

**Container Closure Systems for Packaging Human Drugs and Biologics**

**Mav 1999**

"Drug product containers and closures shall not be reactive, additive, or absorptive so as to later the safety, identity, strength, quality or purity of the drug beyond the official or establishment requirements"



emea
European Medicines Agency
*Inspections*

Dec 2005

# Guideline on Immediate Plastic Packaging Materials

- **Excludes** elastomers, natural and synthetic rubbers
- No Data needed if materials previously approved by EP or member state
- Sec 4: Extraction solvent used should have the same propensity to extract substances as the active substance dosage.
- Sec 5.1: Migration Studies Required: with specific objectives
- Sec 6: Toxicological Information and Documents



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# Pharma Industry Expert Working Groups

PQRI (Product Quality Research Institute) is a working group established to developed regulatory guidance for Extractable/Leachable analysis, which is also recognized by the FDA



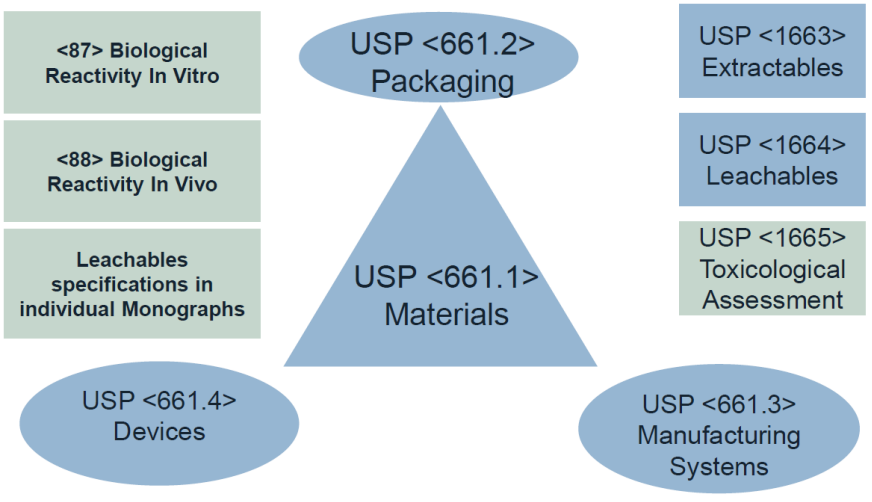
# Guidelines and Assessments for E/L in Construct

**USP (United States Pharmacopeia)**

- USP<1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems **Revised**
- USP<1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems **Revised**

- **EP (European Pharmacopeia)**
  - EP 3.1 Materials used for the manufacture of containers
  - EP 3.2 Containers for pharmaceutical use
- **ICH (International Conference of Harmonization)**
  - ICH Q1A, Q3A, Q3B, Q6A, Q7B Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substance
- **ISO (International Organization of Standardization)**
  - ISO10993 Biological Evaluation of Medical Devices
- **PQRI (Product Quality Research Institute)**

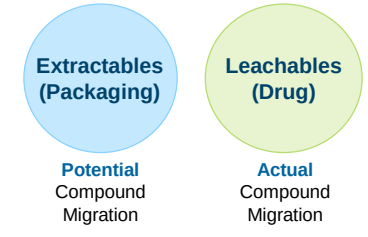
# New USP Chapters just released




**Ref: Denise R. Jenke, Daniel L. Norwood, and Desmond G Hunt**



# Definitions: Extractable, Leachable, Migrants




**Extractables**

Chemical compounds that can be extracted out of packaging component

- Analyze packaging component at
  - **High-temperatures**: to obtain the worst case leachable profile
  - **Solvent extraction**: polar and non-polar solvent to mimic similar properties as drug product

**Leachable**

- Chemical compounds from packaging component that leach into the drug product
- Analyze drug product at
  - Normal conditions
  - Stimulate extended storage conditions

**Migrants**

- Crossed a physical barrier primary packaging from secondary and tertiary packaging, accumulating in the drug product



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# Extractable and Leachable Applications

- **Pharma and BioPharma (Small Molecule/Biologics)**

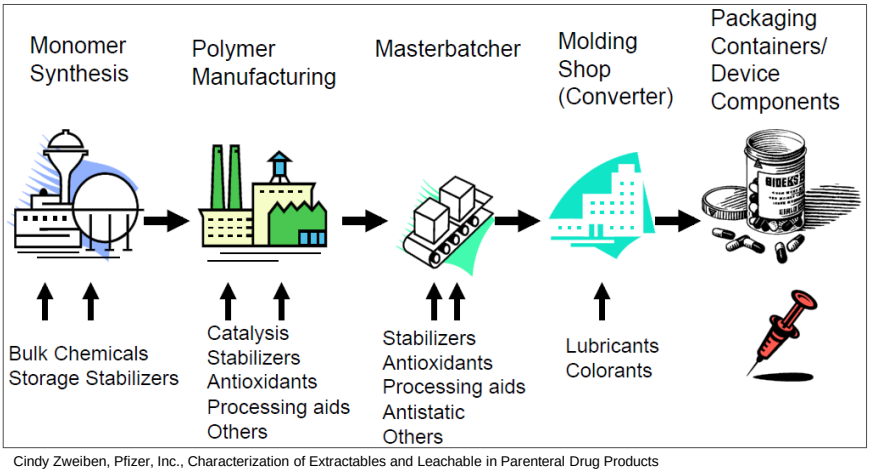
**Drug Delivery Technologies**


  - Single Dose Prefilled Syringes
  - Medical Device Products
  - Drug Packaging (Constructs, Films, Closures)
  - Orally inhaled and nasal drug products (OINDPs)
- **Food Packaging**
  - Similar Materials and Concerns (BPA, Phthalates, Colorants, Dyes



# Plastics Industry – Sources of Extractables

Sources of extractables are plastic and elastomeric components (plasticizers, etc), ink and adhesives (label), and degradation products (processing, storage, and sterilization)




Cindy Zweiben, Pfizer, Inc., Characterization of Extractables and Leachable in Parenteral Drug Products



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# Common Sources of Extractable and Leachable

**Plastic Components:** Polymers Additives, Phthalates, Lubricants, Fatty Acids, Nitrosamines

**Elastomers/Rubber:** Vulcanizing Agent, PAHs, Accelerators Antioxidants, Carbon Black,

**Inks/Labels:** Azo Dyes, Aromatic Amines

**Adhesives:** Antioxidants (AO), Catalyst Residues, Heavy Metals, Silicones, Surfactants

**Pigments:** Inorganic (TiO2, FeO's), Organic Pigments

**Cyclic Oligomers:** Polybutylene terephthalate (PBT) polyester Silicone Cyclic Oligomers



# Risk categories of pharmaceutical packaging

| | Likelihood of interaction between packaging component and dosage form | | |
|---|---|---|---|
| **Degree of concern associated with Route of Administration** | **High** | **Medium** | **Low** |
| **Highest** | Inhalation aerosols and solution<br>Injections and injectable suspensions | Sterile powders<br>Injection powders<br>Inhalation powders | |
| **High** | Ophthalmic solutions and suspensions<br>Transdermal ointments and patches<br>Nasal aerosols and sprays | | |
| **Low** | Topical solutions and suspensions<br>Topical and lingual aerosols<br>Oral solutions and suspensions | Topical powders<br>Oral powders | Oral tablets<br>Oral hard capsules<br>Oral soft gelatin capsules |

Adapted from Guidance for *Industry; Container Closure Systems for Packaging Human Drug and Biologics*, US Department of Health and Human Services, Food and Drug Administration, Rockville, MD, May 1999

- Interested in **high-risk categories** packaging components
- What contributes to the high-risk in pharmaceutical packaging?
  - Prefilled syringe containing an injectable drug suspension
    - Interacts with multiple components in the packaging material (plastic barrel, rubber plunger, metal needle) with direct delivery to the bloodstream



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## Biologics Typically Differ from Traditional Small Molecule Drugs for the following Reasons

- Large MW and complex structure (glycosylated, multiple domains)
- Abundance of both hydrophilic and hydrophobic sites may render biologic drugs more efficient extraction media
- Could be heterogeneous mixtures
- Produced by a complex manufacturing process employing living organisms

Ref: Ingrid Markovic, CBER Presentation USP/PQRI E/L Workshop April 2014



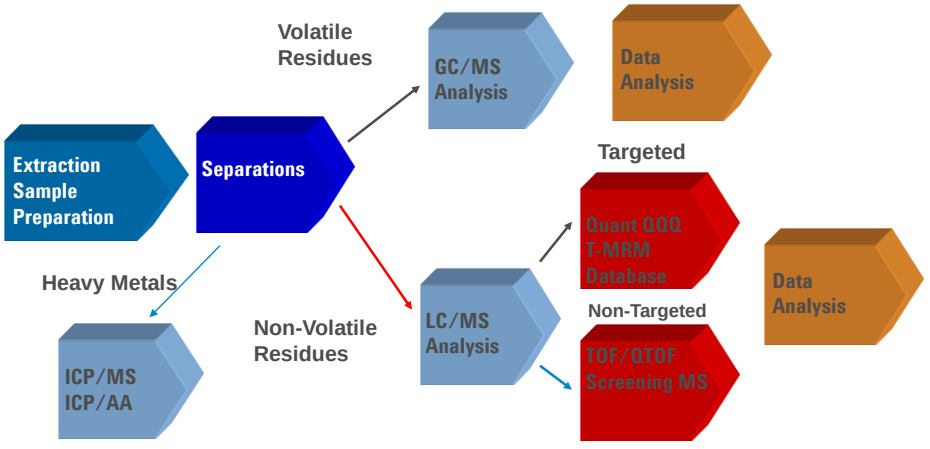
## Special Concerns about E/L Effect on Biologics

- **Leachable may affect protein products in the following ways**
  - Oxidation
  - Unfolding
  - Aggregation
  - Increase in particulates
  - Formation of clipped variants
  - Formation of Protein Adducts
  - Post translational events during fermentation (glycosylation)
  - Altered protein translation

Examples from Ref: Ingrid Markovic, CBER April 2014

# Extractable Leachable Analytical Workflow

**Objective:** To detect low level quantities organic or inorganic impurities that result from interaction of API or Biologic with Drug Delivery






# Simulated Extraction Chart

- Vigorous/exaggerated/-exhaustive conditions
- no material deformulation

➢„Hot“ extraction techniques but no sample dissolving solvents

- Solvents should be attributed to the expected universe of substances (cover wide range of polarity)
- Solvents should mimic drug product formulation

| | Thermal | n-Hexane | Iso-propanol | Isopropan-ol/Water | Aqueous pH 2.5 | Aqueous pH 9.5 |
|---|---|---|---|---|---|---|
| Headspace | X | --- | --- | --- | --- | --- |
| Reflux | --- | X | X | PC/PVC only | --- | --- |
| Soxhlet | --- | X | X | --- | --- | -- |
| Sealed Vessel | --- | --- | --- | 55°C/3d | (121°C/ 1hr)[1] | (121°C/ 1hr)[1] |
| Sonication | --- | --- | --- | --- | x | x |

[1]: autoclave conditions: (121°C/1hr)

Ref: T.Egert, A. Hendricker, C.Houston, 2011 Talk at Best Practices fro PODP



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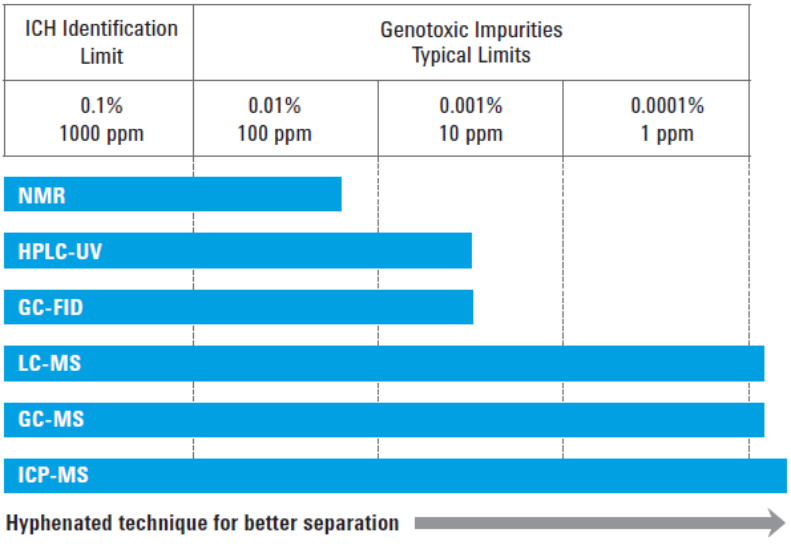
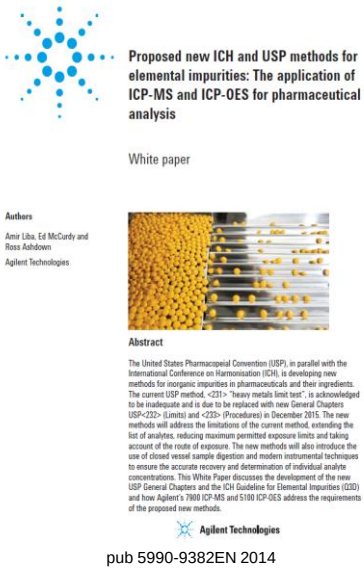
# Analytical Techniques for Impurity Analysis






# New Heavy Metal ICH and USP Methods/Regulations



**Proposed new ICH and USP methods for elemental impurities: The application of ICP-MS and ICP-OES for pharmaceutical analysis**

White paper

Authors

Amir Liba, Ed McCurdy and Ross Ashdown

Agilent Technologies

**Abstract**

The United States Pharmacopeial Convention (USP), in parallel with the International Conference on Harmonisation (ICH), is developing new methods for inorganic impurities in pharmaceuticals and their ingredients. The current USP method, <231> "heavy metals limit test", is acknowledged to be inadequate and is due to be replaced with new General Chapters USP<232> (Limits) and <233> (Procedures) in December 2015. The new methods will address the limitations of the current method, extending the list of analytes, reducing maximum permitted exposure limits and taking account of the route of exposure. The new methods will also introduce the use of closed vessel sample digestion and modern instrumental techniques to ensure the accurate recovery and determination of individual analyte concentrations. This White Paper discusses the development of the new USP General Chapters and the ICH Guideline for Elemental Impurities (Q3D) and how Agilent's 7900 ICP-MS and 5100 ICP-OES address the requirements of the proposed new methods.

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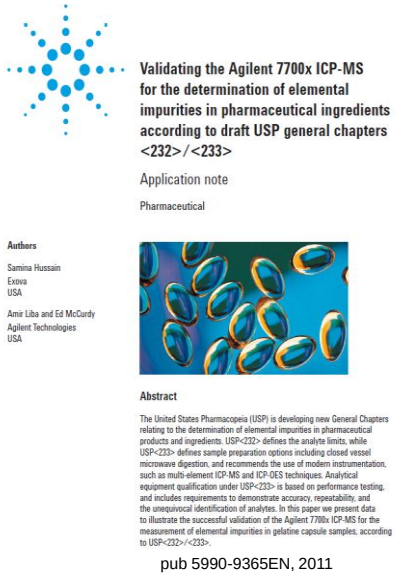
pub 5990-9382EN 2014



**Validating the Agilent 7700x ICP-MS for the determination of elemental impurities in pharmaceutical ingredients according to draft USP general chapters <232> / <233>**

Application note

Pharmaceutical

Authors

Samina Hussain
Exova
USA

Amir Liba and Ed McCurdy
Agilent Technologies
USA

**Abstract**

The United States Pharmacopeia (USP) is developing new General Chapters relating to the determination of elemental impurities in pharmaceutical products and ingredients. USP<232> defines the analyte limits, while USP<233> defines sample preparation options including closed vessel microwave digestion, and recommends the use of modern instrumentation, such as multi-element ICP-MS and ICP-OES techniques. Analytical equipment qualification under USP<233> is based on performance testing, and includes requirements to demonstrate accuracy, repeatability, and the unequivocal identification of analytes. In this paper we present data to illustrate the successful validation of the Agilent 7700x ICP-MS for the measurement of elemental impurities in gelatine capsule samples, according to USP<232>/<233>.

pub 5990-9365EN, 2011



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## Inorganic Extractable Impurities

**Inorganic Impurities** can result from the manufacturing process and are generally known, identified and include:

- Reagents, Ligands, Catalysts
- Heavy Metals
- Inorganic Salts
- Manufacturing Aids (charcoal, filters)

Agilent 7700 Series ICP-MS

**Determination of Toxic Elements in Traditional Chinese Medicine Using Inductively Coupled Plasma Mass Spectrometry**

This Application Note describes a method for the analysis of Be, Cr, Mn, Ni, Cu, Zn, As, Ag, Cd, Ba, Hg, Tl and Pb in Traditional Chinese Medicines using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The samples were dissolved by microwave digestion. To validate the method, two certified reference materials were digested and measured. The method detection limits for all target elements were between 0.1-7.2 ng·g$^{-1}$.



## Instrumentation for Extractable Leachable Analysis

7010
GC/QQQ

5975E
GC/MS

Hi-DEF Q-TOF
6500 series

7200
GC/QTOF

SFC/MSD/QTOF

QQQ
6400 Series

TOF
6200 series



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# Agilent GC and GC/MS

5977A with 7890B GC

7890B GC

5975T LTM SQ

Purge and Trap

7010 QQQ

7200 Q-TOF

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# GCMSD New Application Notes!

5991-5616EN

**Analysis of Extractable/Leachable Compounds From Plastic Intravenous Bag Sets Using GC/MSD Systems**

Application Note

Pharmaceuticals

**Abstract**

Two Agilent 5977A Series GC/MSD Systems were used for the analysis of extractable and leachable compounds in plastic IV bag sets. Two types of IV bags were investigated: 150-mL dextrose bag (expired) and 1-L sodium chloride bag (warmed). Potentially toxic additives, such as phthalate plasticizers, were shown to have migrated from the IV bag to its infusion solution using the complementation of headspace sampling and liquid injection techniques. High temperature analysis was accomplished using the 7697A Headspace and a 7890A GC coupled with a 5977A MSD. Solvent extracts were analyzed using the MMI 7890A GC coupled with a 5977A MSD. Single ion monitoring (SIM) was used to confirm compound migration.

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5991-5605EN

**Analysis of Extractable/Leachable Compounds from Transdermal Patches Using GC/MSD Systems**

Application Note

Pharmaceutical

**Abstract**

A lidocaine adhesive patch and film release liner were used to investigate extractable and leachable compounds in transdermal drug delivery systems using two Agilent 5977A Series GC/MSD Systems. Plastic and adhesive additives were identified in acetone, dichloromethane, and hexane extracts using the large volume liquid injection technique. Pharmaceutical ingredients were also identified using high temperature headspace and liquid sampling techniques.

**Introduction**

Particular interest has been given to extraction techniques in container closure systems (CCS) used in the pharmaceutical industry. Regulators have become increasingly aware of the need to understand whether chemical species can be extracted from the primary packaging material (package with direct contact to the drug product), as well as whether the extracted species (from the package) will appear as leachable species in the drug product. Extractables analysis involves extracting compound from the packaging material using elevated temperatures and solvents related to the packaging composition. Leachables analysis involves identifying compounds in the drug formulation that may have leached from the primary packaging material.

The major source of extractables and leachables are additives that provide physical and protective properties to packaging material, such as flexibility, rigidity, stability, and barrier. Extractables include plastic and elastomeric components, inks and adhesives from coating, and degradation products during processing, storage, and sterilization. Leachables are usually a subset of extractables, however new compounds can form from the interaction between drugs and packaging material.

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5991-5632EN

**Analysis of Extractable/Leachable Compounds from Generic Liquid Drug Formulations Using GC/MSD Systems**

Application Note

**Abstract**

Pharmaceutical liquid formulations are commonly stored in plastic containers at all risk categories. A pharmaceutical suspension was used as model for investigating compound migration from packaging material. Two Agilent 5977A Series GC/MSD Systems were used. Fatty acid plasticizers were identified using the 7697A Headspace Sampler and a 7890A GC coupled with a 5977A MSD. Phthalate plasticizers were found using the MMI 7890A GC coupled with a 5977A MSD. Single ion monitoring (SIM) confirmed the identification of these plasticizers.

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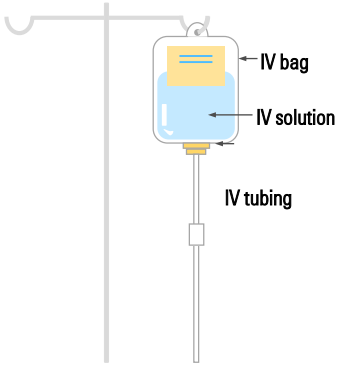
# Extractable/Leachable from Plastic IV Bag Sets

**Samples Investigated**
150-mL dextrose IV bag (expired 8 years)
1-L NaCl IV bag (2 months at 100°F)
Kolliphor + NaCl solution
IV tubing (expired 3 years)




## Background

- PVC IV bags are a source of DEHP, which can leach into drug solutions
- DEHP extracts faster in non-polar solvents or when liquid is warmed or agitated
- Kolliphor EL is an nonionic polyethoxylated castor oil solubilizer/emulsifier that can enhances leaching of DEHP from PVC bags

## Method

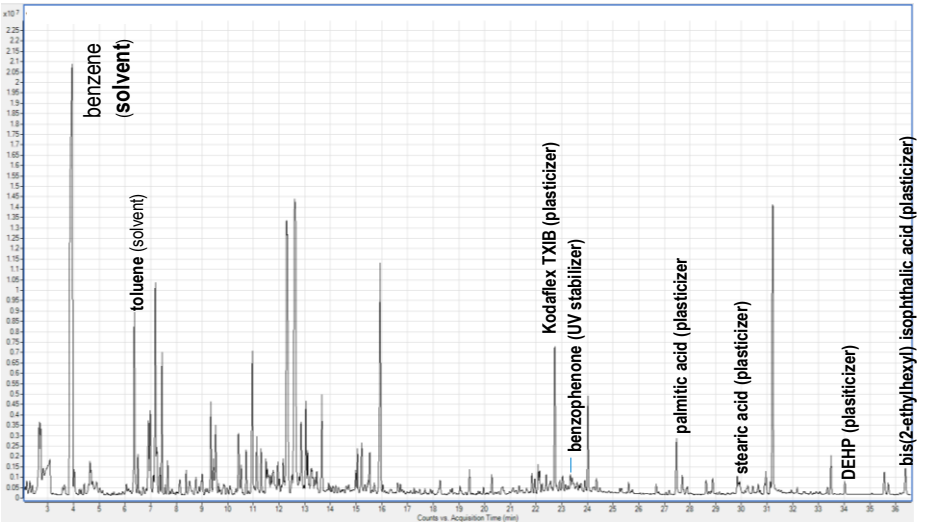
- Headspace temperatures: 85-275°C
- DCM and hexane extraction by sonication

## Summary

- DEHP and benzophenone was identified in IV tubing using HS
- DEHP, BHT (antioxidant), benzothiazole (rubber), acetophenone (ink, coating, adhesives) were identified in 150-mL IV bag by HS
- Phthalates, BHT, acetophenone, and Metilox (plasticizer) were identified in 1-L IV bag using HS
- DEHA and DEHP migration was observed in DCM and hexane extracts of dextrose IV bags using MMI and SIM analysis
- Phthalates and DEHA were observed in DCM and hexane extracts of NaCl and Kolliphor-NaCl IV bags



# IV tubing using the headspace GC/MS at 250 °C




5991-5616EN



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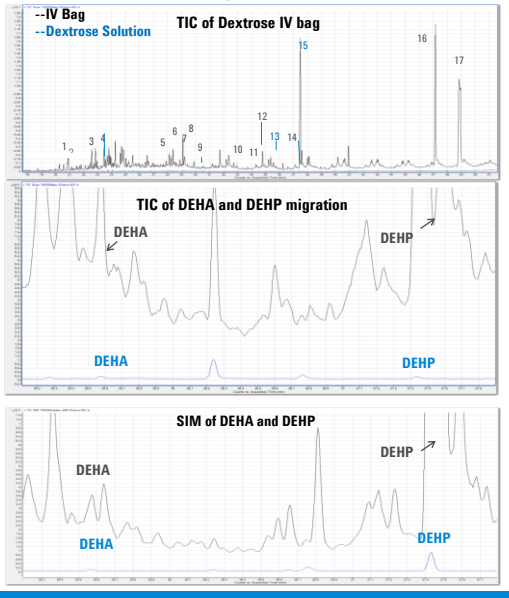
# Compound Migration due to Extended Storage Conditions



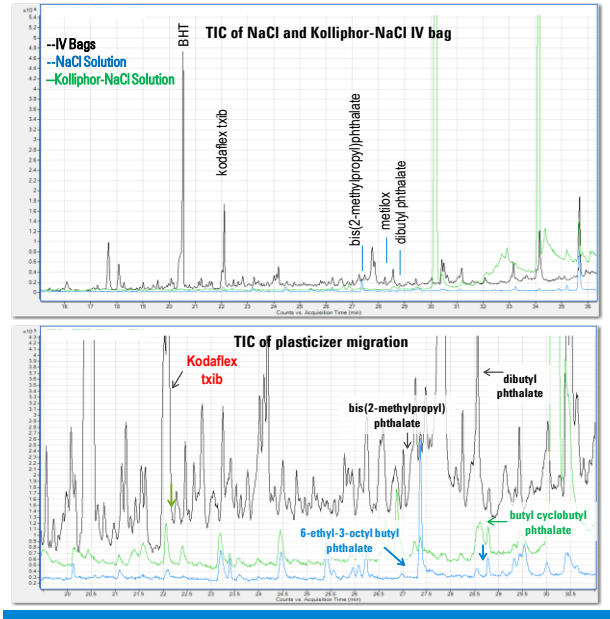

SIM analysis of DCM extracts from IV Bag and dextrose solution to confirm the identification and migration of DEHA and DEHP

Hexadecane (1)
Butylated Hydroxytoluene (BHT) (2) –antioxidant
Kodaflex TXIB (3) –plasticizer
Benzophenone (4) – UV stabilizer
Isobutyl nonyl phthalate (5)
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (6)
**2-Mercaptobenzothiazole (7) - Rubber**
Palmitic acid (8) – plasticizer
Isopropyl palmitate (9)
Palmitic acid, butyl ester (10)
2-Ethylhexyl trans-4-methoxycinnamate (11)
Benzyl butyl phthalate (12)
**DEHA (13) –plasticizer**
di(oct-3-yl) phthalate (14)
**DEHP (15) –plasticizer**
Irgafos 168 (16) –antioxidant **
Tris(2,4-di-tert-butylphenyl) phosphate (17)

5991-5616EN



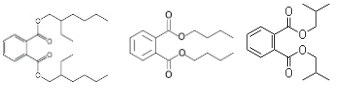
# Compound migration due to extended heating & Kolliphor EL




MMI 5977 GC/MS of DCM extracts of NaCl and Kolliphor-NaCl IV bags

Kolliphor enhanced leaching of Kodaflex TXIB plasticizer, which may suggest that alternative plasticizers was utilized

At 27 min, migration can be bis(2-methylpropyl) phthalate or 6-ethyl-3-octyl butyl phthalate. At 28.5 min, migration can be dibutyl phthalate or butyl cyclobutyl phthalate at 28.5min



6-ethyl-3-octyl butyl phthalate | dibutyl phthalate | bis(2-methylpropyl) phthalate

Kodaflex txib: 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
Metilox: benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy -, methyl ester



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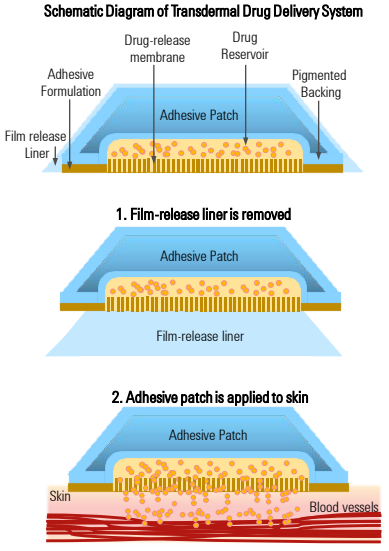
# Extractable/Leachable From Transdermal Patch




**Samples Investigated**

- Adhesive material contains 5% lidocaine applied to a nonwoven polyester (PE) felt backing
- Film-release liner is made of polyethylene terephthalate (PET)

**Method**

- Headspace temperatures: 100-275°C
- Acetone, DCM, and hexane extraction by sonication

**Summary**

- Identified (active and inactive) pharmaceutical ingredients
- Identified potentially toxic plasticizers
- Acetone extraction: terephthalates, phthalates, fatty acids
- Hexane extraction: phthalates and fatty acid
- **DCM** extraction: Diethyhexylphthalate and benzophenone
- **Headspace**: Fatty acid plasticizers

**Reference**

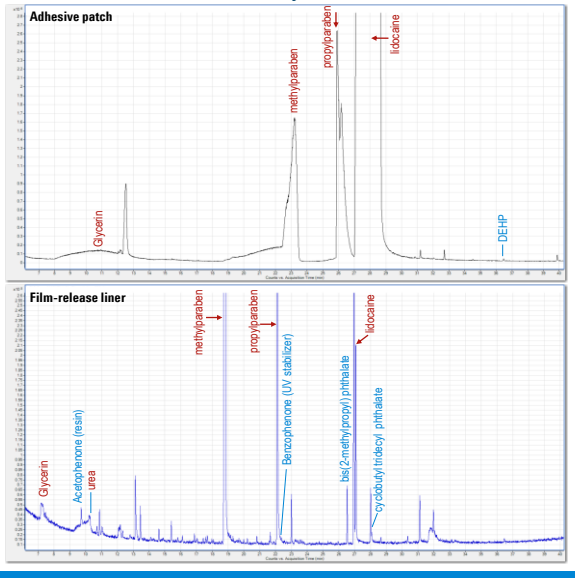
5991-5605EN Application Note consisted of:

- TIC and table listing extractables identified by HS and MMI
- Summary Table of extractables organized by sampling techniques and origin
- Detailed description of system parameters

5991-5605EN



# Analysis of Lidocaine Patch using MMI 5977 GC/MSD by DCM extraction




TIC is zoomed-in to emphasize low-level leachables

Active/Inactive ingredients produce distorted peaks due to high concentration

Phthalates and plasticizers may originate from the PE material in patch or from the PET in film

5991-5605EN



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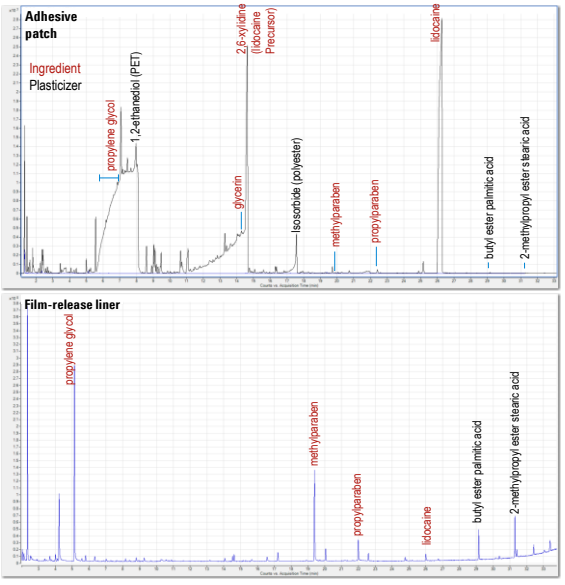
## Lidocaine Patch using Headspace 5977 GC/MSD 250°C




Propylene glycol and glycerin produces distorted peaks due to high concentration of the inactive ingredient

Lidocaine produce broad peaks due to high concentration of an active ingredient



## New 7200B GC/Quadrupole Time-of-Flight (QTOF)

- Both EI and CI sources are Standard
- Backflush ready GC
- Resolving Power: >12.5K at *m/z* 272
  13K to 15K typical
- Mass Accuracy: <3 ppm <2 ppm typical
- MS Sensitivity <250 fg of OFN derived statistically
- Dynamic Range: > 3 orders of magnitude
  - 4 orders with Dual Gain
- Extended Mass range: *m/z* 20-3000
- MS/MS Mass range: *m/z* 20-1050
- Spectral Rate: 1-50 Spectra/sec



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# Example: Fluorotelomer Alcohols in Biosolids

## Source unknown

- Possible intermediate degradation products of fluorinated polymers
- Oxidize to form fluorinated carboxylic acid, some of which are **toxic**
- Structure: $F_3C(CF_2)_{N-1}(CH_2)_MOH$ N:M FTOH is the shorthand notation

**Positive Chemical Ionization (PCI) 20% Methane**

| Acronym | Formula | Exact Mass + H | Observed Mass | Δ ppm |
|---|---|---|---|---|
| 4:2 FTOH | $C_6H_5F_9O$ | 265.0269 | 265.0270 | -0.38 |
| 6:2 FTOH | $C_8H_5F_{13}O$ | 365.0206 | 365.0206 | 0 |
| 8:2 FTOH | $C_{10}H_5F_{17}O$ | 465.0142 | 465.0140 | 0.43 |
| 10:2 FTOH | $C_{12}H_5F_{21}O$ | 565.0078 | 565.0078 | 0 |
| 7:2 sFTOH | $C_9H_5F_{15}O$ | 415.0174 | 415.0190 | -3.85 |
| 5:1 FTOH | $C_6H_3F_{11}O$ | 301.0081 | 301.0079 | 0.66 |
| 6:1 FTOH | $C_7H_3F_{13}O$ | 351.0049 | 351.0050 | -0.28 |
| 7:1 FTOH | $C_8H_3F_{15}O$ | 401.0017 | 401.0016 | 0.25 |
| 8:1 FTOH | $C_9H_3F_{17}O$ | 450.9985 | 450.9985 | 0 |
| 9:1 FTOH | $C_{10}H_3F_{19}O$ | 500.9953 | 500.9956 | -0.60 |
| 10:1 FTOH | $C_{11}H_3F_{21}O$ | 550.9921 | 550.9922 | -0.18 |
| 11:1 FTOH | $C_{12}H_3F_{23}O$ | 600.9889 | 600.9896 | -1.16 |
| MeFOSE | $C_{11}H_8F_{17}NO_3S$ | 558.0026 | 558.0042 | -2.87 |
| EtFOSE | $C_{12}H_{10}F_{17}NO_3S$ | 572.0183 | 572.0167 | 2.80 |



# Targeted Quantitation vs Non-Target Screening

## Targeted Quantitation

### LC/QQQ

**Benefits:**

- Most Sensitive Technique
- Good Quantitative Results

**Drawbacks:**

- Limited number of analytes
- Non-target compounds undetected

## Non-Target Screening

### LC/TOF or LC/Q-TOF

**Analysis Options:**

- MS only screening
- MS/MS with accurate mass library for confident identification

**Benefits:**

- Detect, Quantify, and Identify hundreds or thousands of compounds in a single injection
- Reanalyze old sample data any time *without reinjection* upon discovery of new compounds

**MS/MS using ALL Ions Approach**



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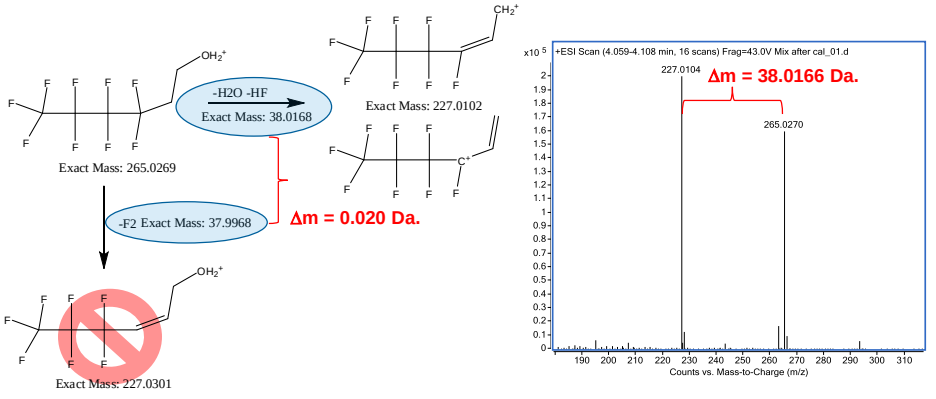
# Fluorotelomer Neutral Loss Mechanism Inferred

PCI, methane

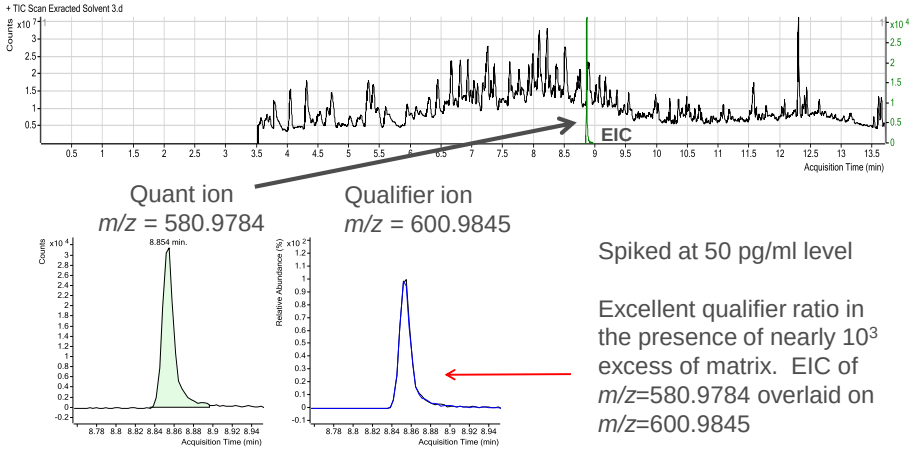



| Acronym | Observed Base Peak m/z | Molecular ion -$F_2$ m/z | Δppm | Molecular ion -$H_20$, -HF m/z | Δppm |
|---|---|---|---|---|---|
| 4:2 FTOH | 227.0104 | 227.0301 | 86.77 | 227.0102 | -0.88 |



# 11:1 FTOH Spiked in Biosolid Extract

PCI, methane




Spiked at 50 pg/ml level

Excellent qualifier ratio in the presence of nearly $10^3$ excess of matrix. EIC of *m/z*=580.9784 overlaid on *m/z*=600.9845

# The LCMS Match Game….

Choosing The Mass Spectrometer for the Task
Sensitivity, Selectivity, Identification..

**Unknown Identification**
**Accurate Mass MS, MSMS**
**MS/MS Sensitivity**

**6550 QTOF**

**6230 TOF**

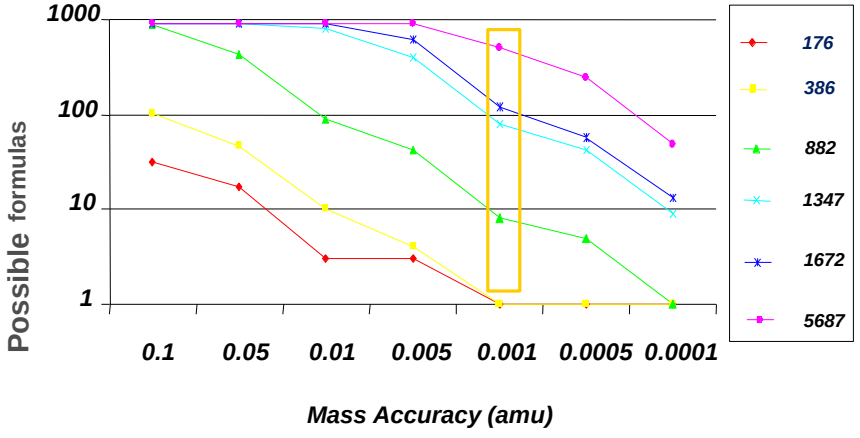
**Unknown Identification**
**Accurate Mass MS**

**6495 QQQ**

**Target Analysis**
**Enhanced Sensitivity by MS/MS**



# The Relationship of Mass Accuracy to the Number of Possible Molecular Formulas by Mass



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# Q(TOF) Innovations from Agilent

Ion Beam Compression Technology (resolution + mass accuracy)

Dual-stage ion mirror (resolution)

Longer flight tube (resolution)

INVAR flight tube (mass accuracy)

Orthogonal spray source (signal-to-noise)

ADC (dynamic range), w dual amplifier

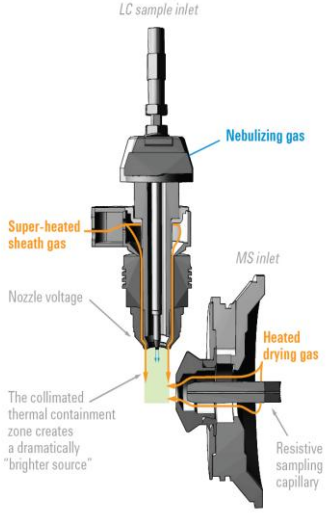
iFunnel technology

**4 GHz electronics (resolution, mass accuracy, sensitivity, dynamic range)**
**50 Spectra/sec**

Ion acceleration in hexapole collision cell (faster MS/MS spectra)



# How Does Agilent Jet Stream Technology Enhance ESI?




- Higher temperatures increase evaporation rate of mobile phase so formation of aerosol and evaporation of droplets will occur more quickly. Fewer large droplets are left in the spray.
- Concentric orientation of sheath gas with respect to spray helps reduce ion dispersion at normal LC flow rate.
- Introduces many more ions into the MS.
- Reduces number of neutral solvent clusters.

Result: stronger signals with lower relative standard deviation (RSDs) at the limit of detection.



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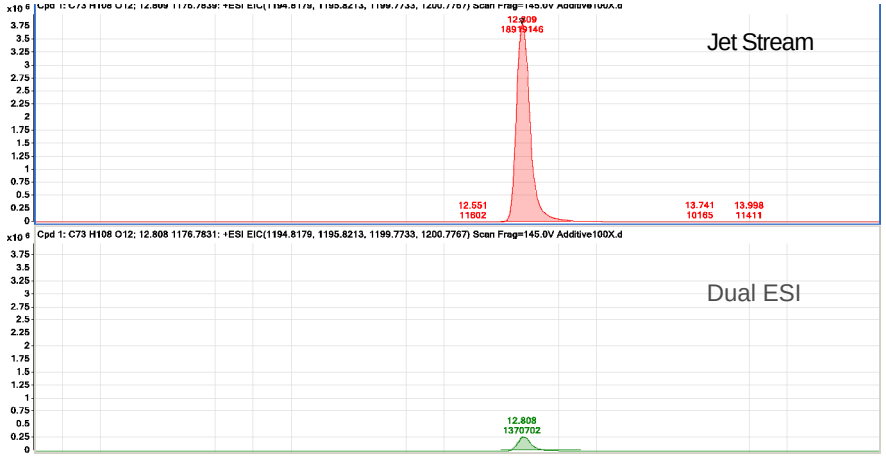
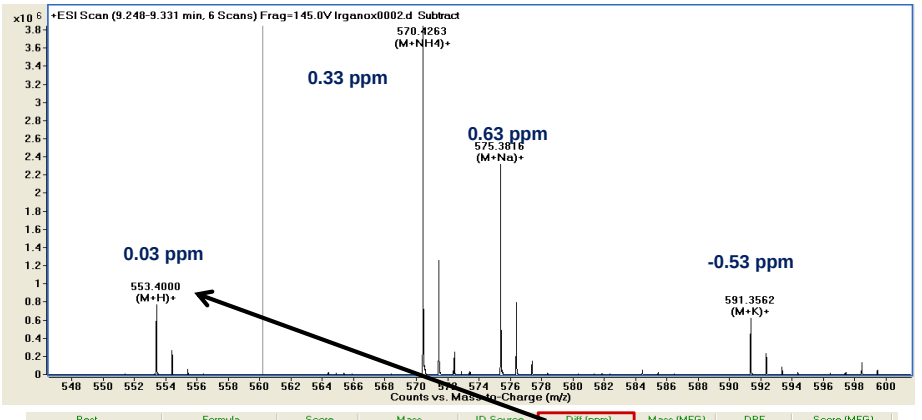
# Irganox 1010: Jet Stream versus Dual ESI

## > 10X Enhancement in Ionization






# Irganox 1024 Accurate Mass Measurement




| Best | Formula | Score | Mass | ID Source | Diff (ppm) | Mass (MFG) | DBE | Score (MFG) |
|---|---|---|---|---|---|---|---|---|
| | C34 H52 N2 O4 | 98.6 | 552.3927 | MFG | 0.03 | 552.3927 | 10 | 98.6 |

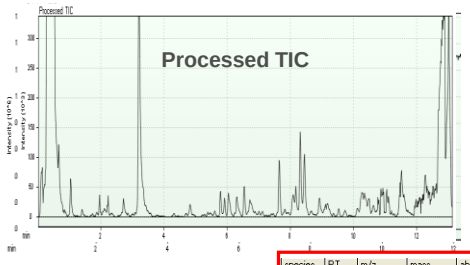
| Score (MFG, abund) | Score (MFG, mass) | Height | Species | Score (MFG, | m/z | Score (MFG) | Score (MFG, | Ion Formula |
|---|---|---|---|---|---|---|---|---|
| 95.25 | 100 | 779366.9 | (M+H)+ | 98.6 | 553.4 | 98.6 | 99.84 | C34 H53 N2 O4 |

| m/z | Height | m/z (Calc) | Height (Calc) | Diff (ppm) | Height % (Calc) | Height % |
|---|---|---|---|---|---|---|
| 553.4 | 779366.9 | 553.4 | 755457.4 | 0.05 | 100 | 100 |
| 554.4035 | 271310.4 | 554.4033 | 289083.9 | -0.34 | 38.3 | 34.8 |
| 555.4062 | 55121.2 | 555.4063 | 60007.5 | 0.27 | 7.9 | 7.1 |
| 556.4078 | 7772 | 556.4092 | 8860.9 | 2.6 | 1.2 | 1 |
| 557.4109 | 869.4 | 557.412 | 1030.2 | 1.96 | 0.1 | 0.1 |

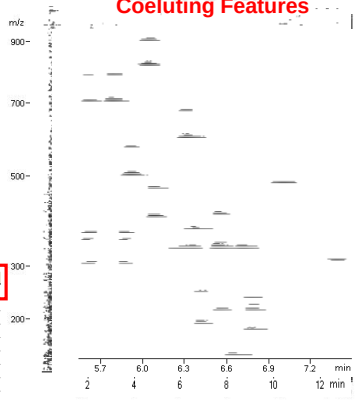
# Molecular Feature Extraction (MFE)

## Automated Data Reduction Software

### Finds Features in TOF/QTOF Data




3D Plot Before Coeluting Features

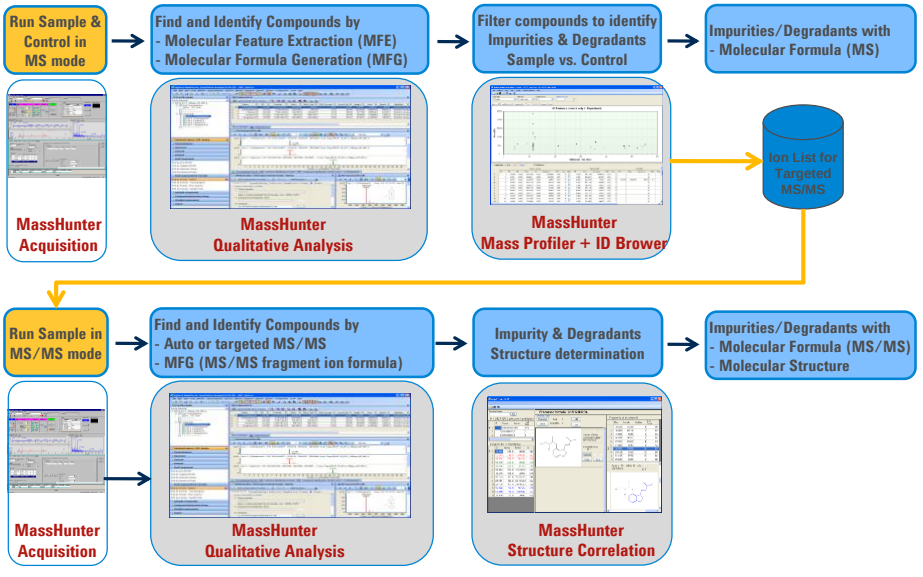



| species | RT | m/z | mass | abund |
|---|---|---|---|---|
| M | 8.162 | | 342.1467 | 130643 |
| M+H | 8.165 | 343.1547 | 342.1474 | 11889 |
| M+H+1 | 8.162 | 344.1581 | | 2290 |
| M+H+2 | 8.157 | 345.1748 | | 369 |
| | | | | |
| M+H4N | 8.164 | 360.1807 | 342.1469 | 8420 |
| M+H4N+1 | 8.156 | 361.1893 | | 1227 |
| | | | | |
| M+Na | 8.162 | 365.1359 | 342.1466 | 75678 |
| M+Na+1 | 8.163 | 366.1394 | | 15324 |
| M+Na+2 | 8.162 | 367.1429 | | 1901 |
| | | | | |
| 2M+Na | 8.164 | 707.2810 | 342.1459 | 4629 |
| 2M+Na+1 | 8.162 | 708.2860 | | 1808 |
| 2M+Na+2 | 8.173 | 709.2895 | | 336 |

**Data Reduced sum intensities of isotopes, adducts, clusters and multiply charges ions together.**



# Impurity ID Workflow: Untargeted Approach



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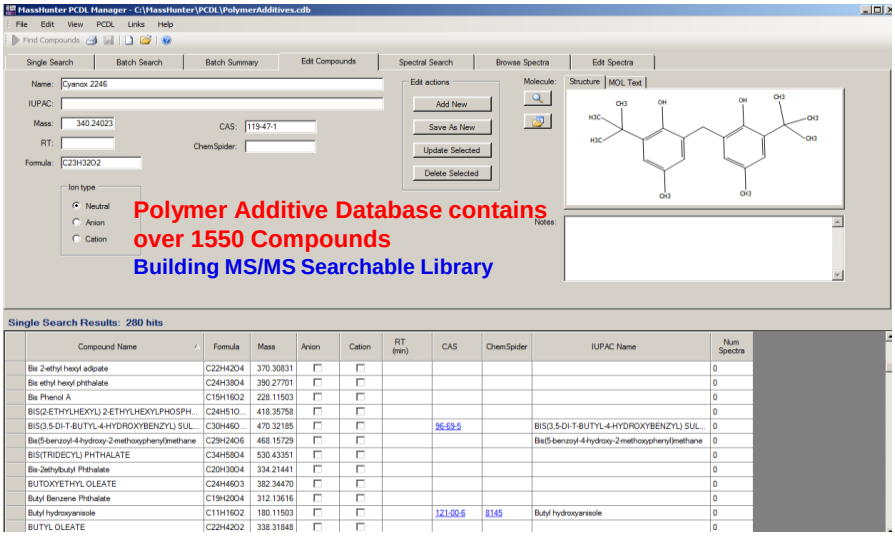
# Personal Compound Databases (PCDL)

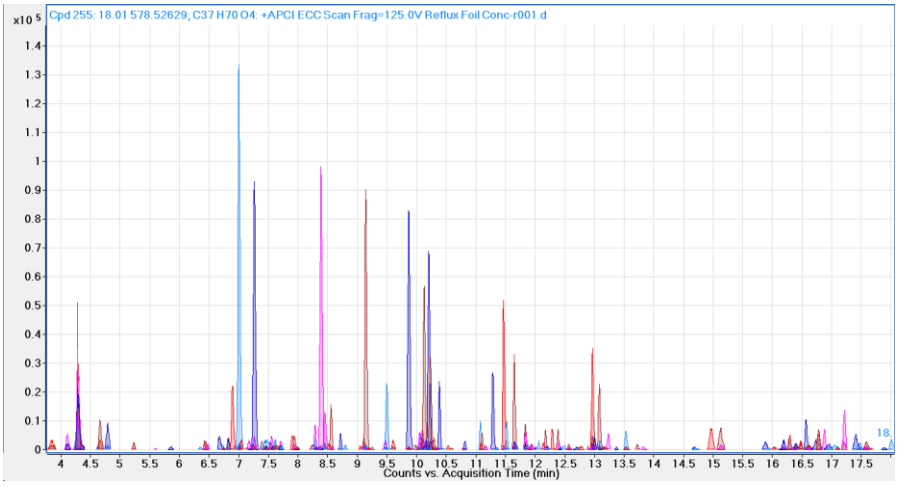



Accurate Mass and MS/MS Library Searchable Databases



# Analysis of IPA Reflux of Al Foil Closure

# IPA Extract from Foil – MFE APCI (+)






# Compounds Sorted by Abundance

| Cpd | RT | Name | Formula | Score | Mass | Score (MFG) | Diff (MFG, ppm) | Height | Vol | ID Source | ID Techniques Applied |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 8.38 | | C20 H32 O8 | 39.16 | 400.20937 | 78.31 | 0.86 | 15545 | 69788 | MFG | DBSearch-MFG |
| 6 | 7 | Cyclic TEDDEG | C24 H24 O10 | 87.98 | 472.13597 | 76.88 | 2.06 | 9111 | 53389 | DBSearch-MFG | DBSearch-MFG |
| 16 | 9.87 | | C23 H38 O8 | 97.71 | 442.25688 | 97.71 | -0.47 | 13733 | 52129 | MFG | MFG |
| 21 | 10.21 | | C24 H40 O8 | 99.66 | 456.27236 | 99.66 | -0.09 | 12219 | 44921 | MFG | MFG |
| 7 | 7.25 | Cyclic TEDDEG | C24 H24 O10 | 49.63 | 472.13691 | | | 7245 | 43422 | DBSearch | DBSearch-MFG |
| 22 | 10.23 | Proanthocyanidin A2 | C30 H24 O12 | 98.05 | 576.12632 | 97.07 | 0.79 | 6870 | 36652 | DBSearch-MFG | DBSearch-MFG |
| 11 | 9.14 | | C29 H36 N2 O8 | 99.41 | 540.24707 | 99.41 | 0.17 | 8423 | 32658 | MFG | MFG |
| 28 | 12.97 | nDOP; Chissocizer nDOP; N... | C24 H38 O4 | 49.8 | 390.27698 | | | 9417 | 29114 | DBSearch | DBSearch-MFG |
| 20 | 10.13 | | C30 H48 O12 | 98.24 | 600.31369 | 98.24 | 1.48 | 7319 | 26720 | MFG | MFG |
| 23 | 11.47 | Fungichromin | C35 H58 O12 | 96.21 | 670.39156 | 96.22 | 1.89 | 6324 | 20571 | DBSearch-MFG | DBSearch-MFG |
| 14 | 9.49 | | C22 H36 O8 | 49.75 | 428.24083 | 99.49 | 0.44 | 4146 | 13759 | MFG | DBSearch-MFG |
| 10 | 8.45 | Dimethyl sebacate | C12 H22 O4 | 81.92 | 230.15187 | 77.93 | -0.27 | 3612 | 12291 | DBSearch-MFG | DBSearch-MFG |
| 2 | 1.24 | 1-Heptene | C7 H14 | 85.24 | 98.10912 | | | 2059 | 11617 | DBSearch | DBSearch |
| 30 | 13.08 | 1β-hydroxyvitamin D3 / 1β-h... | C27 H44 O2 | 99.25 | 400.3343 | 99.45 | -0.42 | 4010 | 11452 | DBSearch-MFG | DBSearch-MFG |
| 35 | 18.25 | DG(P-14:0/18:1(9Z)) | C35 H66 O4 | 97.74 | 550.49534 | 98.11 | 1.4 | 2473 | 10883 | DBSearch-MFG | DBSearch-MFG |
| 8 | 7.61 | N,N'-Dicyclohexylurea | C13 H24 N2 O | 86.26 | 224.18864 | 86.26 | 0.98 | 1629 | 10464 | DBSearch-MFG | DBSearch-MFG |
| 13 | 9.46 | 2H-1-Benzopyran-3,6-dicarb... | C15 H22 O2 | 84.54 | 234.16187 | 85.17 | 0.49 | 2645 | 9443 | DBSearch-MFG | DBSearch-MFG |
| 33 | 15.14 | 3-Deoxyvitamin D3 | C27 H44 | 90.14 | 368.34432 | 90.14 | -0.05 | 1716 | 8464 | DBSearch-MFG | DBSearch-MFG |
| 29 | 13.01 | Dioctyl adipate | C22 H42 O4 | 80.66 | 370.30809 | 82.87 | 0.6 | 2403 | 8168 | DBSearch-MFG | DBSearch-MFG |
| 18 | 10.1 | Oxidized Latia luciferin | C13 H22 O | 87.27 | 194.16708 | 87.22 | -0.06 | 2185 | 7919 | DBSearch-MFG | DBSearch-MFG |
| 31 | 13.24 | Diamide L-200; Neutron-22;... | C22 H43 N O | 83.97 | 337.33437 | 84.5 | 0.28 | 2366 | 7686 | DBSearch-MFG | DBSearch-MFG |
| 26 | 11.94 | 1,2-Benzenedicarboxylic aci... | C20 H30 O4 | 84.23 | 334.21429 | 84.47 | 0.34 | 1774 | 7061 | DBSearch-MFG | DBSearch-MFG |
| 37 | 19.17 | | C37 H70 O4 | 93.8 | 578.52608 | 93.8 | 2.3 | 1334 | 6870 | MFG | MFG |
| 36 | 18.95 | Squalene | C30 H50 | 86.12 | 410.39047 | | | 1385 | 6790 | DBSearch | DBSearch |
| 15 | 9.6 | Phenol, 2,4-dimethyl-6-(2-m... | C15 H22 O | 84.01 | 218.16725 | 85.57 | -0.83 | 1675 | 6173 | DBSearch-MFG | DBSearch-MFG |
| 34 | 17.18 | | | | 522.46369 | | | 1076 | 5692 | | |
| 17 | 10.06 | MEHP | C16 H22 O4 | 85.19 | 278.15206 | 85.88 | -0.91 | 1922 | 5394 | DBSearch-MFG | DBSearch-MFG |
| 4 | 4.67 | | | | 158.10935 | | | 1113 | 5241 | | |
| 27 | 12.49 | Sansocizer DHP; Chissocize... | C22 H34 O4 | 81.17 | 362.24515 | 81.67 | 1.53 | 1233 | 5143 | DBSearch-MFG | DBSearch-MFG |
| 19 | 10.1 | | C13 H20 | 84.42 | 176.15638 | 84.42 | 0.67 | 1538 | 4839 | MFG | MFG |
| 12 | 9.46 | Irganox degradate | C17 H24 O3 | 83.24 | 276.17256 | 83.68 | -0.05 | 1793 | 4362 | DBSearch-MFG | DBSearch-MFG |
| 5 | 5.79 | | | | 114.10354 | | | 1256 | 4248 | | |
| 32 | 13.54 | previtamin D3 / precholecalci... | C27 H44 O | 86.93 | 384.33828 | 95.17 | 2.43 | 1172 | 3695 | DBSearch-MFG | DBSearch-MFG |
| 24 | 11.84 | 4-oxo-9Z,11Z,13E,15E-octad... | C18 H26 O3 | 84.86 | 290.18797 | 85.53 | 0.76 | 1574 | 3561 | DBSearch-MFG | DBSearch-MFG |
| 25 | 11.84 | Photoinitiator 32; Phenylglyo... | C10 H10 O3 | 84.74 | 178.06303 | 85.48 | -0.19 | 1445 | 3024 | DBSearch-MFG | DBSearch-MFG |



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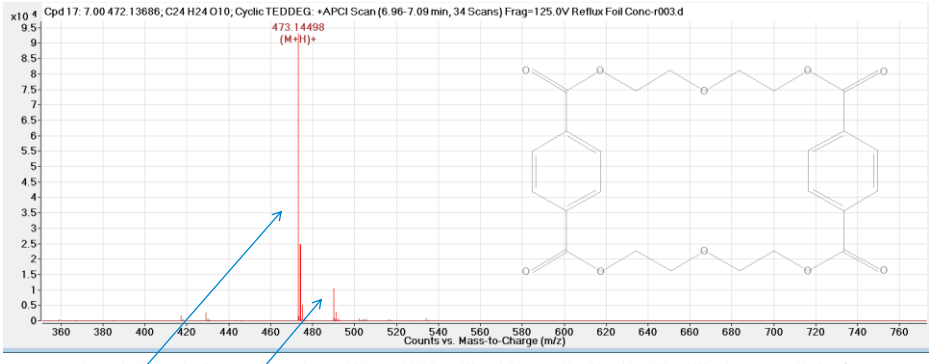
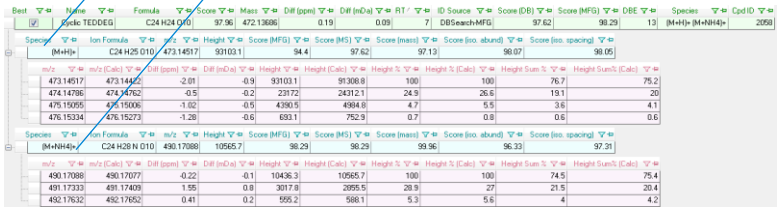
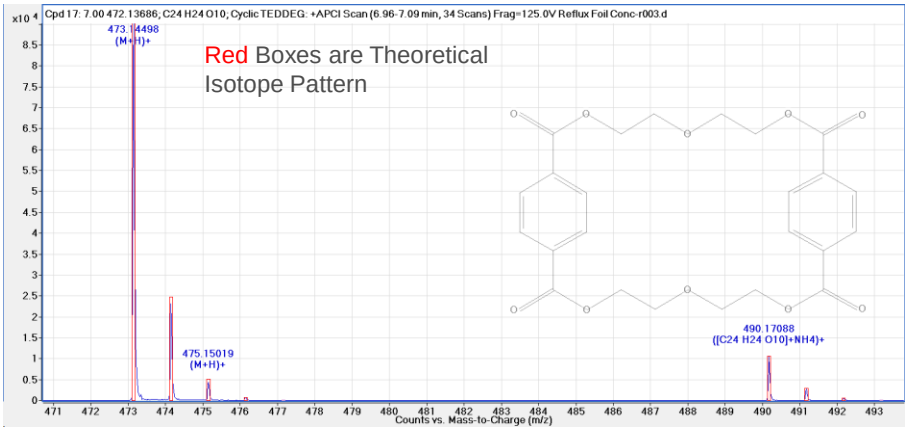
# Major Compound $C_{24}H_{24}O_{10}$ 0.19 ppm Error





# Isotope Pattern Matching



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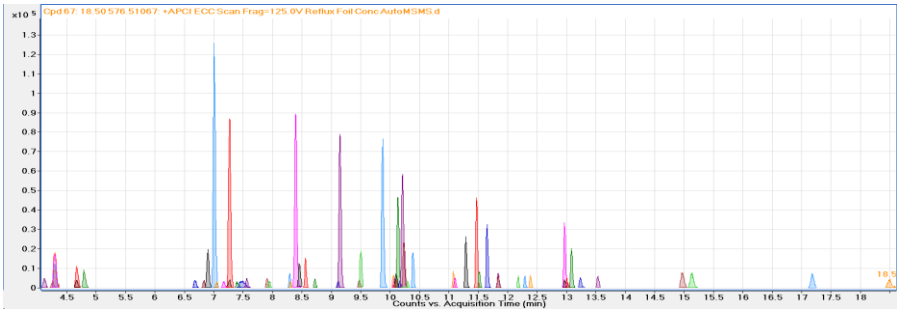
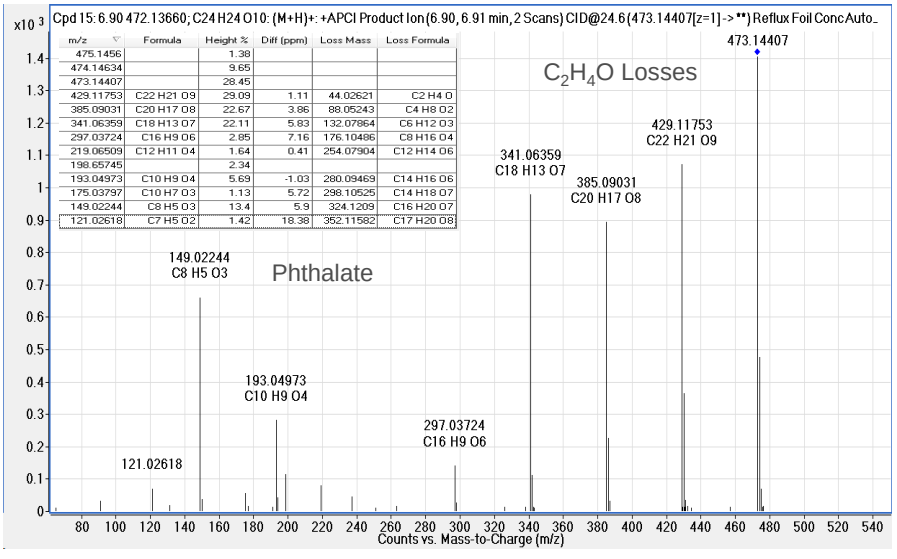
# APCI(+) Conc. Foil Extract AutoMSMS Results




| Cpd | RT | Name | Formula | Score | Mass | Score (MFG) | Diff (MFG, ppm) | Height | Vol | ID Source | ID Techniques Applied | MS/MS Count |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 7.01 | Cyclic TEDDEG | C24 H24 O10 | 96.76 | 472.13682 | 94.61 | 0.27 | 84382 | 313700 | DBSearch-MFG | DBSearch-MFG | 1 |
| 28 | 8.4 | | C20 H32 O8 | 99.02 | 400.21029 | 99.02 | -1.42 | 64359 | 230794 | MFG | MFG | 1 |
| 20 | 7.27 | Cyclic TEDDEG | C24 H24 O10 | 99.27 | 472.13743 | 99.33 | -1.03 | 60851 | 228367 | DBSearch-MFG | DBSearch-MFG | 1 |
| 33 | 9.15 | | C29 H36 N2 O8 | 98.72 | 540.24746 | 98.72 | -0.54 | 51346 | 191173 | MFG | MFG | 1 |
| 36 | 9.88 | | C23 H38 O8 | 98.97 | 442.25693 | 98.97 | -0.59 | 55434 | 186137 | MFG | MFG | 1 |
| 43 | 10.21 | | C24 H40 O8 | 99.03 | 456.27223 | 99.03 | 0.2 | 41187 | 153333 | MFG | MFG | 1 |
| 41 | 10.14 | | C30 H48 O12 | 99.34 | 600.31468 | 99.34 | -0.16 | 30774 | 114724 | MFG | MFG | 1 |
| 50 | 11.47 | | C35 H58 O12 | 98 | 670.39203 | 98 | 1.19 | 26249 | 99397 | MFG | MFG | 1 |
| 58 | 12.97 | nDOP; Chissocizer nDOP; N... | C24 H38 O4 | 99.69 | 390.27707 | 99.67 | -0.17 | 25983 | 76240 | DBSearch-MFG | DBSearch-MFG | 1 |
| 7 | 4.3 | | C9 H6 O3 | 88.2 | 162.03169 | 88.2 | 0.02 | 16623 | 68658 | MFG | MFG | 1 |
| 52 | 11.65 | | C36 H60 O12 | 98.12 | 684.40765 | 98.12 | 1.21 | 19955 | 65386 | MFG | MFG | 1 |
| 44 | 10.23 | | C30 H24 O12 | 49.38 | 576.12616 | 98.75 | 1.07 | 12262 | 62263 | MFG | DBSearch-MFG | 1 |
| 8 | 4.3 | | C12 H12 O5 | 99.29 | 236.06845 | 99.29 | 0.09 | 11680 | 61160 | MFG | MFG | 1 |
| 49 | 11.29 | | C34 H56 O12 | 96.19 | 656.37646 | 96.19 | 1.1 | 17114 | 54406 | MFG | MFG | 1 |
| 15 | 6.9 | Cyclic TEDDEG | C24 H24 O10 | 98.08 | 472.1366 | 97.74 | 0.75 | 13403 | 52203 | DBSearch-MFG | DBSearch-MFG | 1 |
| 35 | 9.5 | | C22 H36 O8 | 98.39 | 428.24087 | 98.39 | 0.35 | 13794 | 48643 | MFG | MFG | 1 |
| 61 | 13.09 | | C27 H44 O2 | 89.77 | 400.33376 | 89.77 | 0.93 | 14901 | 46976 | MFG | MFG | 1 |
| 46 | 10.39 | | C39 H52 N2 O12 | 96.31 | 740.35099 | 96.31 | 1.4 | 10786 | 39574 | MFG | MFG | 1 |
| 29 | 8.46 | Dimethyl sebacate | C12 H22 O4 | 87.51 | 230.15205 | 88.24 | 1.04 | 11301 | 33310 | DBSearch-MFG | DBSearch-MFG | 1 |



# MS/MS of $[M+H]^+$ $C_{24}H_{24}O_{10}$ RT 6.9 minutes



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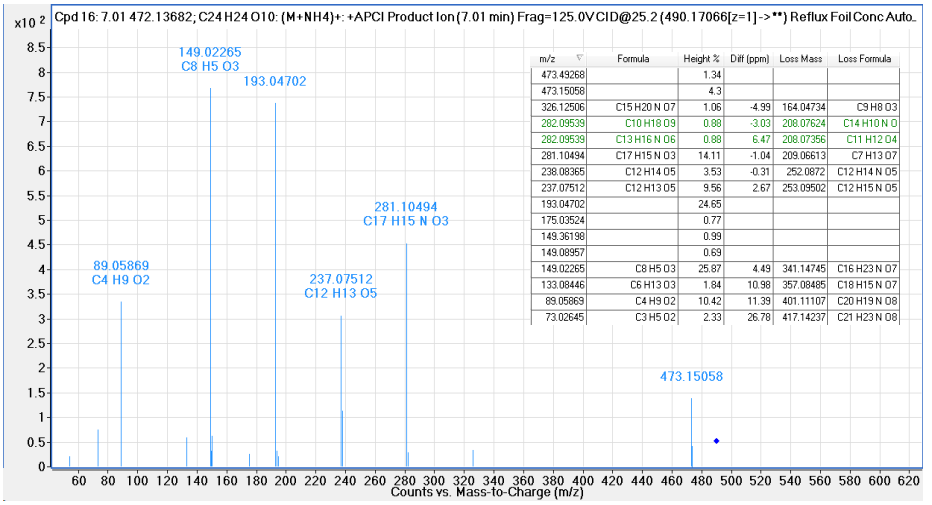
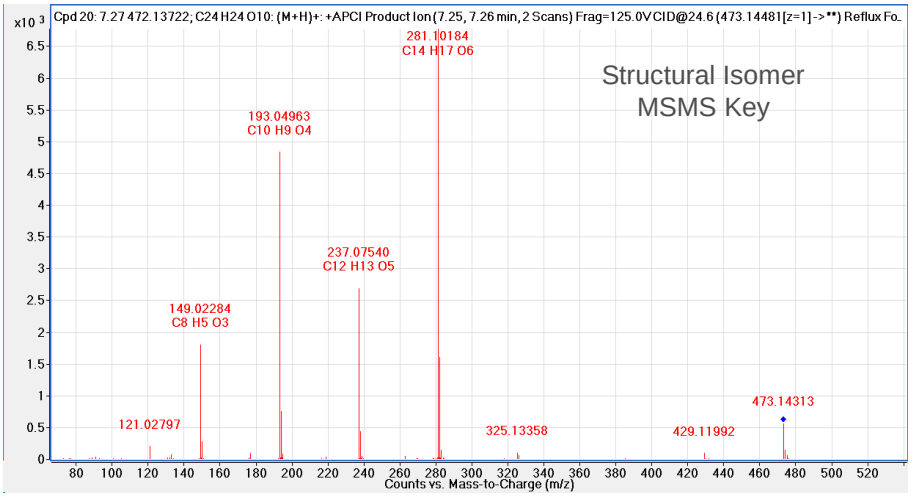
# MS/MS Data $[M+NH_4]^+$ peak for $C_{24}H_{24}O_{10}$ at RT 7.0




| m/z | Formula | Height % | Diff (ppm) | Loss Mass | Loss Formula |
|---|---|---|---|---|---|
| 473.49268 | | 1.34 | | | |
| 473.15058 | | 4.3 | | | |
| 326.12506 | C15 H20 N O7 | 1.06 | -4.99 | 164.04734 | C9 H8 O3 |
| 282.09539 | C10 H18 O9 | 0.88 | -3.03 | 208.07624 | C14 H10 N O |
| 282.09539 | C13 H16 N O6 | 0.88 | 6.47 | 208.07356 | C11 H12 O4 |
| 281.10494 | C17 H15 N O3 | 14.11 | -1.04 | 209.06613 | C7 H13 O7 |
| 238.08365 | C12 H14 O5 | 3.53 | -0.31 | 252.0872 | C12 H14 N O5 |
| 237.07512 | C12 H13 O5 | 9.56 | 2.67 | 253.09502 | C12 H15 N O5 |
| 193.04702 | | 24.65 | | | |
| 175.03524 | | 0.77 | | | |
| 149.36198 | | 0.99 | | | |
| 149.08957 | | 0.69 | | | |
| 149.02265 | C8 H5 O3 | 25.87 | 4.49 | 341.14745 | C16 H23 N O7 |
| 133.08446 | C6 H13 O3 | 1.84 | 10.98 | 357.08485 | C18 H15 N O7 |
| 89.05869 | C4 H9 O2 | 10.42 | 11.39 | 401.11107 | C20 H19 N O8 |
| 73.02645 | C3 H5 O2 | 2.33 | 26.78 | 417.14237 | C21 H23 N O8 |



# MS/MS of $[M+H]^+$ $C_{24}H_{24}O_{10}$ at RT 7.27 mins



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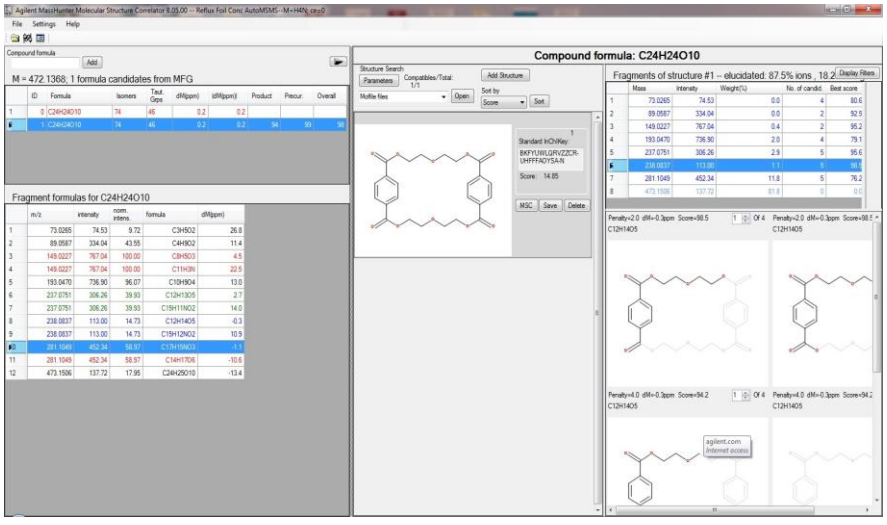
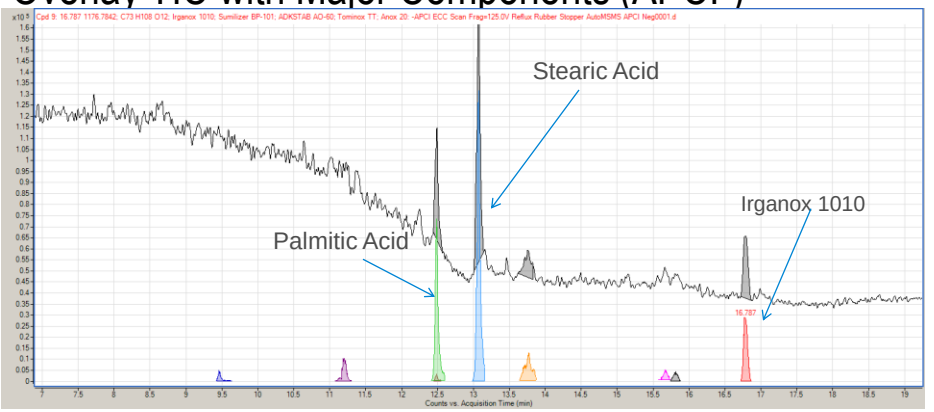
# Molecular Structure Correlator of $[M+H]^+$ $C_{24}H_{24}O_{10}$






# Analysis of Rubber Extract

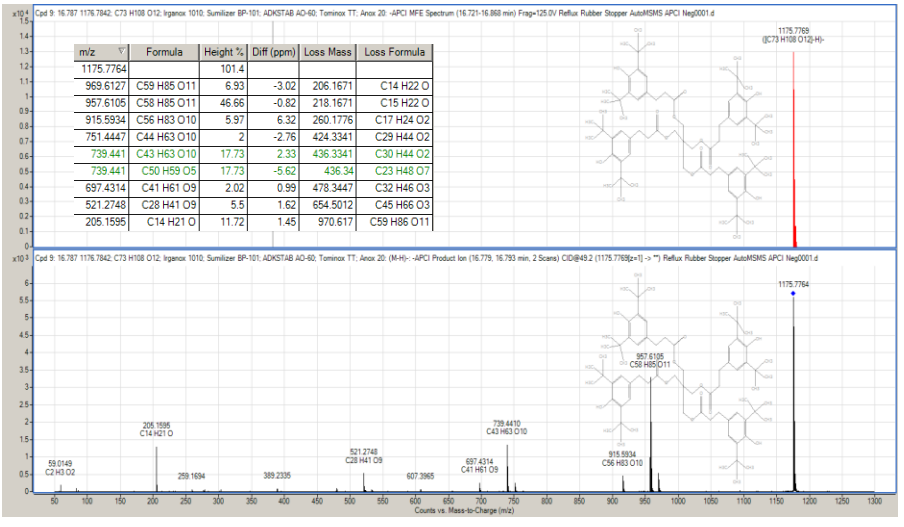
# Overlay TIC with Major Components (APCI-)




| Name | RT | Formula | Mass | Score | Diff (MFG, ppm) | Height | Score (MFG) |
|---|---|---|---|---|---|---|---|
| Octadecanoic acid; Stearic acid; ADEKA FATTY ACID SA-200; LUNAC S-30; F-3, VL | 13.07 | C18 H36 O2 | 284.2717 | 99.39 | -0.73 | 109508 | 99.46 |
| Palmitic acid; Hexadecanoic acid | 12.49 | C16 H32 O2 | 256.2404 | 99.76 | -0.68 | 61915 | 99.78 |
| Irganox 1010; Sumilizer BP-101; ADKSTAB AO-60; Tominox TT; Anox 20 | 16.787 | C73 H108 O12 | 1176.7842 | 99.16 | -0.09 | 12968 | 98.89 |
| 9,10-EPOXYSTEARIC ACID | 11.2 | C18 H34 O3 | 298.2508 | 85.92 | 0.06 | 9075 | 85.93 |
| | 13.763 | C40 H74 O9 | 698.534 | 98.11 | -0.95 | 8080 | 98.11 |
| 3,5-Di-tert-butylbenzoic acid | 9.464 | C15 H22 O2 | 234.1621 | 84.49 | -0.57 | 4110 | 84.51 |
| | 15.673 | C38 H72 O8 | 656.523 | 96.4 | -0.47 | 3394 | 96.4 |
| | 12.488 | C17 H34 O4 | 302.2454 | 83.29 | 1.17 | 2852 | 83.29 |
| | 15.812 | C42 H74 O P2 | 656.5226 | 68.93 | -1.75 | 2821 | 68.93 |



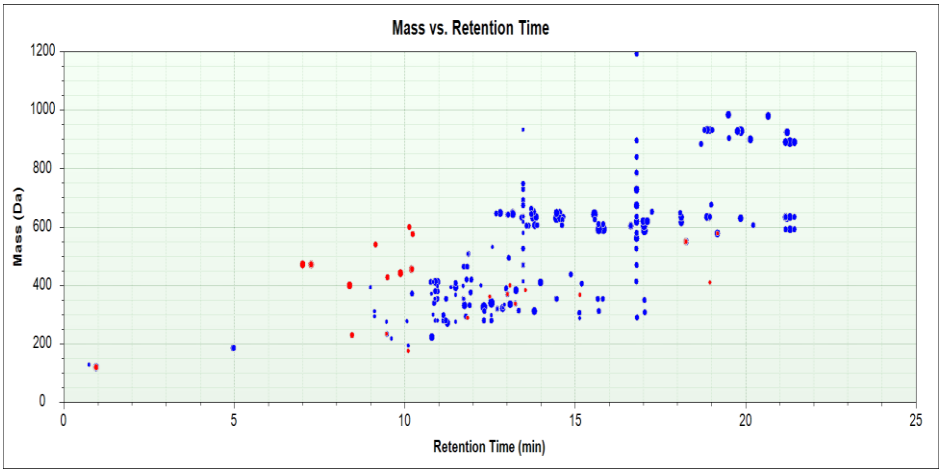
# MS and MS/MS of [M-H]- of Irganox 1010




| m/z | Formula | Height % | Diff (ppm) | Loss Mass | Loss Formula |
|---|---|---|---|---|---|
| 1175.7764 | | 101.4 | | | |
| 969.6127 | C59 H85 O11 | 6.93 | -3.02 | 206.1671 | C14 H22 O |
| 957.6105 | C58 H85 O11 | 46.66 | -0.82 | 218.1671 | C15 H22 O |
| 915.5934 | C56 H83 O10 | 5.97 | 6.32 | 260.1776 | C17 H24 O2 |
| 751.4447 | C44 H63 O10 | 2 | -2.76 | 424.3341 | C29 H44 O2 |
| 739.441 | C43 H63 O10 | 17.73 | 2.33 | 436.3341 | C30 H44 O2 |
| 739.441 | C50 H59 O5 | 17.73 | -5.62 | 436.34 | C23 H48 O7 |
| 697.4314 | C41 H61 O9 | 2.02 | 0.99 | 478.3447 | C32 H46 O3 |
| 521.2748 | C28 H41 O9 | 5.5 | 1.62 | 654.5012 | C45 H66 O3 |
| 205.1595 | C14 H21 O | 11.72 | 1.45 | 970.617 | C59 H86 O11 |

# Mass Profiler Foil versus Rubber

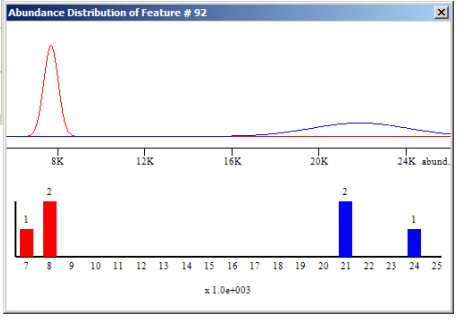
Differential Analysis Impurity Profiling






# Erucamide $C_{22}H_{43}NO$ MW 337.3344

1.5 fold change between Foil and Rubber



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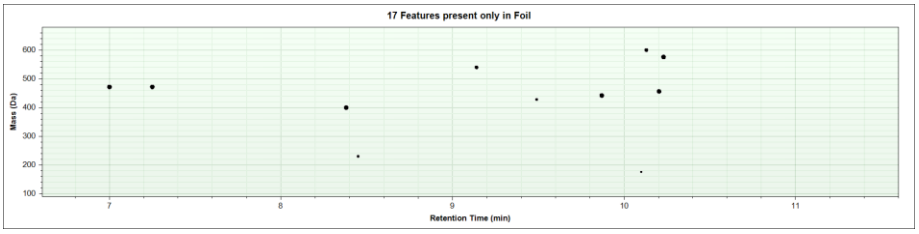
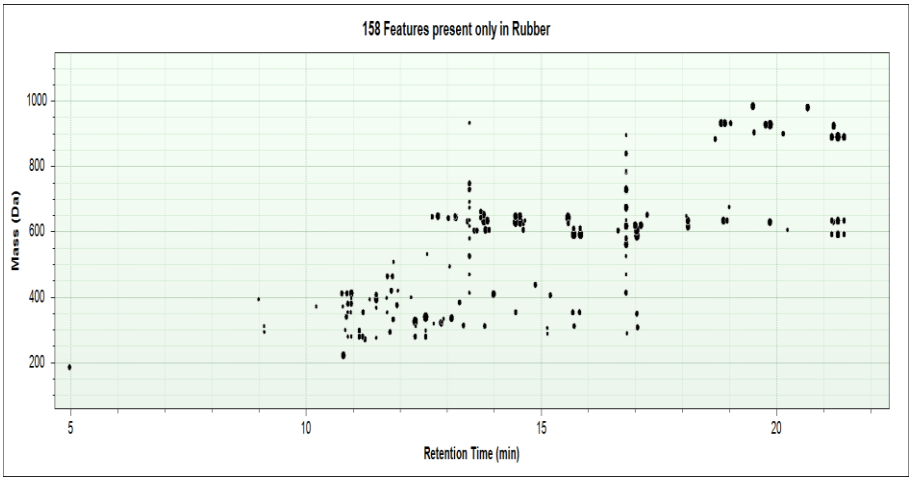
# 17 only in the Foil Extracts top formula hits




| Name | Formula | CAS | RT | SD | Mass | SD | Abundance | RSD |
|---|---|---|---|---|---|---|---|---|
| 2,13-Dimethyl-1,4,12,15-tetr... | C20 H32 O8 | | 8.380 | 0.000 | 400.2099 | 0.0002 | 35080 | 1.10 |
| | C23 H38 O8 | | 9.870 | 0.000 | 442.2568 | 0.0001 | 26551 | 1.10 |
| nDOP; Chissocizer nDOP; N... | C24 H38 O4 | 117-84-0 | 12.970 | 0.000 | 390.2770 | 0.0002 | 25613 | 0.14 |
| Cyclic TEDDEG | C24 H24 O10 | | 7.000 | 0.000 | 472.1367 | 0.0002 | 24075 | 1.10 |
| Cyclic TEDDEG | C24 H24 O10 | | 7.250 | 0.000 | 472.1367 | 0.0002 | 22803 | 1.10 |
| | C24 H40 O8 | | 10.203 | 0.006 | 456.2722 | 0.0003 | 22614 | 1.10 |
| | C30 H24 O12 | | 10.230 | 0.000 | 576.1265 | 0.0001 | 18445 | 1.10 |
| | C29 H36 N2 O8 | | 9.140 | 0.000 | 540.2468 | 0.0003 | 16163 | 1.10 |
| | C30 H48 O12 | | 10.130 | 0.000 | 600.3140 | 0.0003 | 13373 | 1.10 |
| | C13 H22 O | | 10.100 | 0.000 | 194.1670 | 0.0002 | 7889 | 0.02 |
| | C22 H36 O8 | | 9.490 | 0.000 | 428.2409 | 0.0003 | 6989 | 1.10 |
| Dimethyl sebacate | C12 H22 O4 | 106-79-6 | 8.450 | 0.000 | 230.1518 | 0.0003 | 6472 | 1.10 |
| | C27 H44 O2 | | 13.083 | 0.006 | 400.3344 | 0.0002 | 5482 | 1.10 |
| | C27 H44 | | 15.137 | 0.006 | 368.3443 | 0.0001 | 4062 | 1.10 |
| MEHP | C16 H22 O4 | 4376-20-9 | 10.060 | 0.000 | 278.1521 | 0.0005 | 3594 | 0.78 |
| Squalene | C30 H50 | 111-02-4 | 18.950 | 0.000 | 410.3904 | 0.0002 | 3205 | 1.10 |
| Sansocizer DHP; Chissocize... | C22 H34 O4 | 3648-21-3 | 12.493 | 0.006 | 362.2458 | 0.0008 | 2905 | 1.10 |



# Mass Profiler Unique in Rubber Extract



# Compounds in Rubber Extract (+)

| Name | Formula | CAS | RT | SD | Mass | SD | Abundance |
|---|---|---|---|---|---|---|---|
| 1-(Decanoyloxy)-3-(octanoyloxy)-2-propan... | C39 H70 O6 | | 13.777 | 0.006 | 634.5173 | 0.0002 | 249490 |
| 1,3-Bis(octanoyloxy)-2-propanyl (9E,12E)-... | C37 H66 O6 | | 17.023 | 0.006 | 606.4859 | 0.0001 | 237680 |
| 1-PALMITIN-3-LINOLEIN | C37 H68 O5 | | 15.687 | 0.006 | 592.5067 | 0.0001 | 216493 |
| 1-PALMITIN-3-LINOLEIN | C37 H68 O5 | | 15.830 | 0.000 | 592.5066 | 0.0002 | 158559 |
| | C21 H40 O3 | | 12.540 | 0.000 | 340.2982 | 0.0000 | 147920 |
| 1,2,4-Benzenetricarboxylic acid, 1,2,4-trid... | C39 H66 O6 | 4130-35-2 | 14.450 | 0.000 | 630.4857 | 0.0001 | 140291 |
| Hydroxyethyl oleate | C20 H38 O3 | 4500-01-0 | 12.317 | 0.006 | 326.2825 | 0.0001 | 127793 |
| | C37 H64 O5 | | 17.030 | 0.000 | 588.4749 | 0.0001 | 99203 |
| | C49 H103 N4 ... | | 21.300 | 0.000 | 890.7561 | 0.0002 | 90816 |
| | C57 H100 O9 | | 19.860 | 0.000 | 928.7352 | 0.0001 | 90258 |
| | C39 H64 O7 | | 15.563 | 0.006 | 644.4645 | 0.0003 | 87914 |
| 1,2,4-Benzenetricarboxylic acid, 1,2,4-trid... | C39 H66 O6 | 4130-35-2 | 14.550 | 0.000 | 630.4855 | 0.0002 | 87035 |
| | C59 H101 Br O... | | 19.493 | 0.006 | 984.6606 | 0.0004 | 74418 |
| | C39 H72 O5 | | 16.993 | 0.006 | 620.5372 | 0.0002 | 69718 |
| 1-(Decanoyloxy)-3-(octanoyloxy)-2-propan... | C39 H70 O6 | | 18.110 | 0.000 | 634.5164 | 0.0002 | 69460 |
| 1-(Decanoyloxy)-3-(octanoyloxy)-2-propan... | C39 H70 O6 | | 13.850 | 0.000 | 634.5168 | 0.0000 | 69423 |
| | C51 H105 N4 ... | | 18.897 | 0.046 | 932.7666 | 0.0002 | 59555 |
| | C50 H101 O12 P | | 21.210 | 0.010 | 924.7037 | 0.0001 | 59392 |
| | | | 0.938 | 0.008 | 120.1149 | 0.0001 | 57139 |
| | C32 H34 O9 | | 16.800 | 0.000 | 562.2196 | 0.0001 | 56015 |
| 2-Oxiraneoctanoic acid, 3-octyl-, octyl ester | C26 H50 O3 | 106-84-3 | 13.983 | 0.006 | 410.3758 | 0.0001 | 51641 |
| | C39 H72 O5 | | 17.113 | 0.006 | 620.5372 | 0.0001 | 51543 |



## Identification of Extractable Compounds from PolyProplyene Bottle

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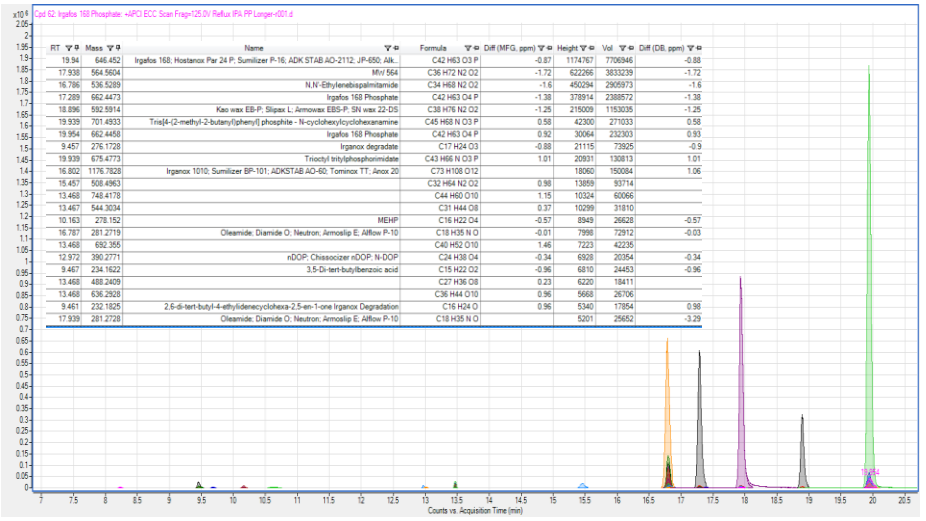
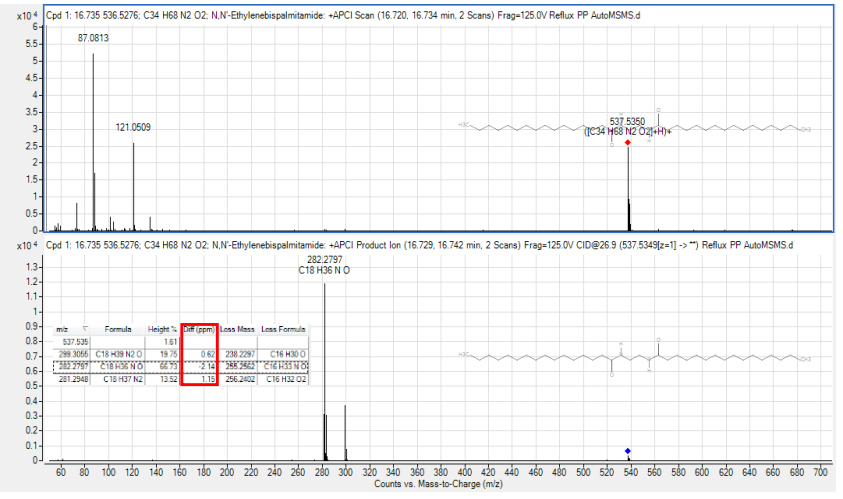
# Components using Data Mining (MFE)



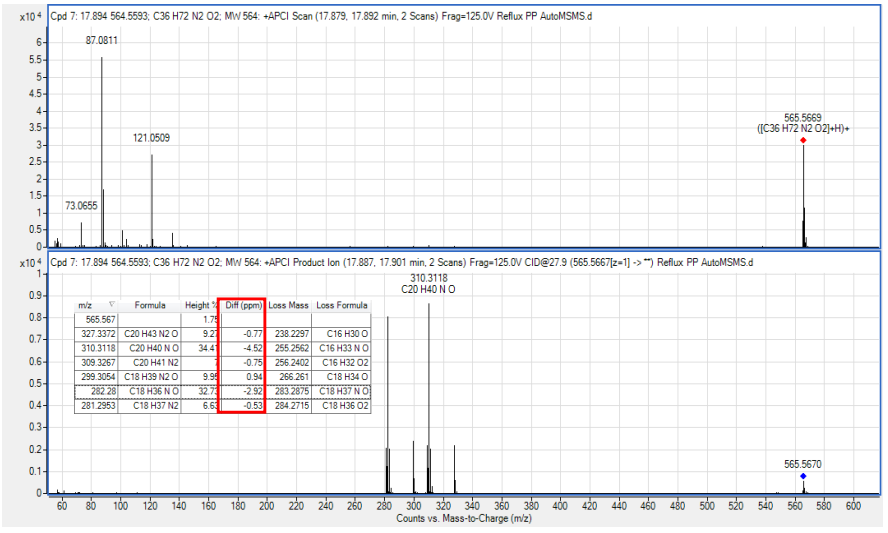

| RT | Mass | Name | Formula | Diff (MFG, ppm) | Height | Vol | Diff (DB, ppm) |
|---|---|---|---|---|---|---|---|
| 19.94 | 646.452 | Irgafos 168; Hostanox Par 24 P; Sumilizer P-16; ADK STAB AO-2112; JP-650; Alk.. | C42 H63 O3 P | -0.87 | 1174767 | 7706946 | -0.88 |
| 17.938 | 564.5604 | Mvl 564 | C36 H72 N2 O2 | -1.72 | 622266 | 3833239 | -1.72 |
| 16.786 | 536.5289 | N,N'-Ethylenebispalmitamide | C34 H68 N2 O2 | -1.6 | 450294 | 2965973 | -1.6 |
| 17.289 | 662.4473 | Irgafos 168 Phosphate | C42 H63 O4 P | -1.38 | 378914 | 2388572 | -1.38 |
| 18.896 | 592.5914 | Kao wax EB-P; Slipax L; Armowax EBS-P; SN wax 22-DS | C38 H76 N2 O2 | -1.25 | 215009 | 1153035 | -1.25 |
| 19.939 | 701.4933 | Tris[4-(2-methyl-2-butanyl)phenyl] phosphite - N-cyclohexylcyclohexanamine | C45 H68 N O3 P | 0.58 | 42300 | 271033 | 0.58 |
| 19.954 | 662.4458 | Irgafos 168 Phosphate | C42 H63 O4 P | 0.92 | 30064 | 232303 | 0.93 |
| 9.457 | 276.1728 | Irganox degradate | C17 H24 O3 | -0.88 | 21115 | 73925 | -0.9 |
| 19.939 | 675.4773 | Trioctyl trityliphosphorimidate | C43 H66 N O3 P | 1.01 | 20931 | 130813 | 1.01 |
| 16.802 | 1176.7828 | Irganox 1010; Sumilizer BP-101; ADKSTAB AO-60; Tominox TT; Anox 20 | C73 H108 O12 | | 18060 | 150084 | 1.06 |
| 15.457 | 508.4963 | | C32 H64 N2 O2 | 0.98 | 13859 | 93714 | |
| 13.468 | 748.4178 | | C44 H60 O10 | 1.15 | 10324 | 60066 | |
| 13.467 | 544.3034 | | C31 H44 O8 | 0.37 | 10299 | 31810 | |
| 10.163 | 278.152 | MEHP | C16 H22 O4 | -0.57 | 8949 | 26628 | -0.57 |
| 16.787 | 281.2719 | Oleamide; Diamide O; Neutron; Armoslip E; Alflow P-10 | C18 H35 N O | -0.01 | 7998 | 72912 | -0.03 |
| 13.468 | 692.366 | | C40 H52 O10 | 1.46 | 7223 | 42235 | |
| 12.972 | 390.2771 | nDOP; Chissocizer nDOP; N-DOP | C24 H38 O4 | -0.34 | 6928 | 20354 | -0.34 |
| 9.467 | 234.1622 | 3,5-Di-tert-butylbenzoic acid | C15 H22 O2 | -0.96 | 6810 | 24453 | -0.96 |
| 13.468 | 488.2409 | | C27 H36 O8 | 0.23 | 6220 | 18411 | |
| 13.468 | 636.2928 | | C36 H44 O10 | 0.96 | 5668 | 26706 | |
| 9.461 | 232.1825 | 2,6-di-tert-butyl-4-ethylidenecyclohexa-2,5-en-1-one Irganox Degradation | C16 H24 O | 0.96 | 5340 | 17854 | 0.98 |
| 17.939 | 281.2728 | Oleamide; Diamide O; Neutron; Armoslip E; Alflow P-10 | C18 H35 N O | | 5201 | 25652 | -3.29 |



# MS/MS *m/z* 537.5350 $[M+H]^+$ $C_{34}H_{68}N_2O_2$



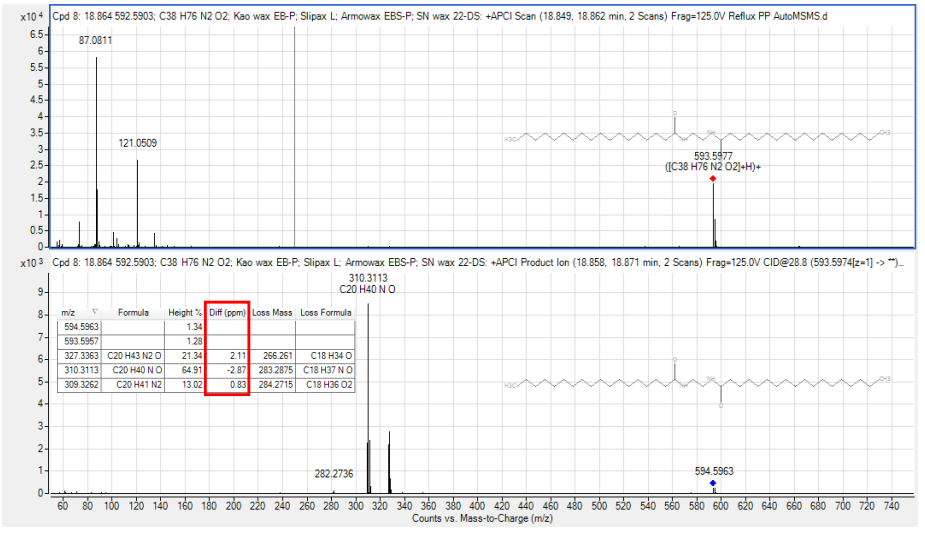
# MS/MS *m/z* 565.5669 $[M+H]^+$ $C_{36}H_{72}N_2O_2$




| m/z | Formula | Height % | Diff (ppm) | Loss Mass | Loss Formula |
|---|---|---|---|---|---|
| 565.567 | | 1.75 | | | |
| 327.3372 | C20 H43 N2 O | 9.23 | -0.77 | 238.2297 | C16 H30 O |
| 310.3118 | C20 H40 N O | 34.41 | -4.52 | 255.2562 | C16 H33 N O |
| 309.3267 | C20 H41 N2 | 3 | -0.75 | 256.2402 | C16 H32 O2 |
| 299.3054 | C18 H39 N2 O | 9.99 | 0.94 | 266.261 | C18 H34 O |
| 282.28 | C18 H36 N O | 32.73 | -2.92 | 283.2875 | C18 H37 N O |
| 281.2953 | C18 H37 N2 | 6.63 | -0.53 | 284.2715 | C18 H36 O2 |

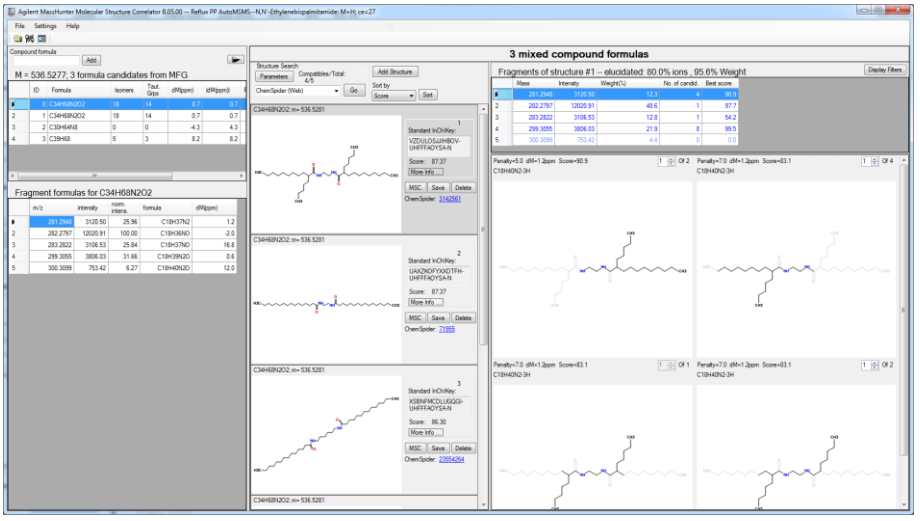


# MS/MS *m/z* 593.5977 $[M+H]^+$ $C_{38}H_{76}N_2O_2$




| m/z | Formula | Height % | Diff (ppm) | Loss Mass | Loss Formula |
|---|---|---|---|---|---|
| 594.5963 | | 1.34 | | | |
| 593.5957 | | 1.28 | | | |
| 327.3363 | C20 H43 N2 O | 21.34 | 2.11 | 266.261 | C18 H34 O |
| 310.3113 | C20 H40 N O | 64.91 | -2.87 | 283.2875 | C18 H37 N O |
| 309.3262 | C20 H41 N2 | 13.02 | 0.83 | 284.2715 | C18 H36 O2 |

# Molecular Structure Correlator $C_{34}H_{68}N_2O_2$

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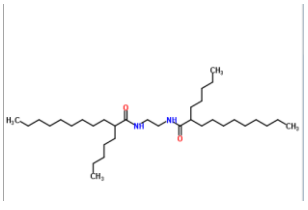
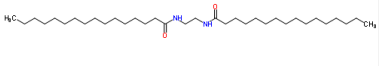
# Possible Structures for $C_{34}H_{68}N_2O_2$

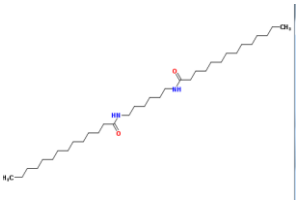
**N,N'-1,2-Ethanediylbis(2-pentylundecanamide)**



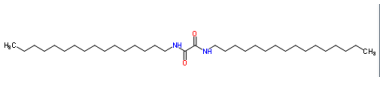
**N,N'-ethane-1,2-diyldihexadecanamide**



**N,N'-1,6-Hexanediylditetradecanamide**



**N,N'-Dihexadecyloxamide**



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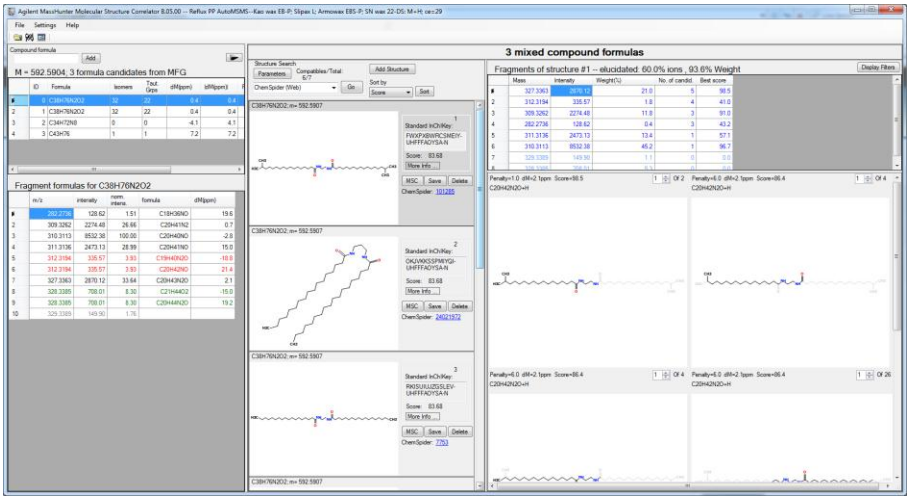
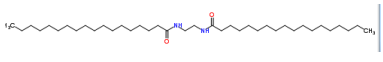
# Molecular Structure Correlator $C_{38}H_{76}N_2O_2$



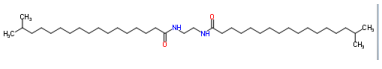



# Total of Six Possible Structures

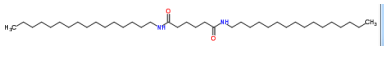
**N,N'-Ethylenebis(stearamide)**



**N,N'-1,2-Ethanediylbis(16-methylheptadecanamide)**



**N,N'-Dihexadecylhexanediamide**



# Internet Search Confirms Database Hit

**KAO WAX EB-P**

Kao Wax EB-P is an amide type of wax which has high melting point and special property showing the low viscosity in molten form. In molten form under high temperature, it's compatible well with resin or solvents.
Kao Wax EB-P is a lubricants and/or mould releasing agent for thermoplastic resin such as ABS resin, PS, PVC and so fort.

**Chemicals structure :**

$CH_3$-$(CH_2)_n$ CO-NH-$CH_2$-$CH_2$-NH-CO-$(CH_2)_n$-$CH_3$

N,N'-Ethylene-Bis- Stearamide

**Specification :**

| | | |
|---|---|---|
| Appearance | : | White powder |
| Color (Gardner) | : | 5 max |
| Acid value (mg KOH/g) | : | 10 max |
| Total Amine value (mg KOH/g) | : | 2.5 max |
| Melting point, °C | : | 141.5 - 146.5 |
| Volatile matter, % | : | 0.5 max |
| Particle size | : | 10 % max. on 16 mesh screen |

**Applications :**

Lubricant for ABS resin, polystyrene and copolymers, PVC, polyolefine, phenol resin, POM, EPS, PA

**Octadecanamide,N,N'-1,2-ethanediylbis- ( CAS:110-30-5 )**

Updatetime:Jun 04 2014
Purity:99 Min. Order:1/Kilogram
Supply Ability:100 Year/Metric Ton Formula:C38H76N2O2
Octadecanamide,N,N'-ethylenebis- (6Cl,7Cl,8Cl);1,2-Bis (octadecanamido)ethane;1,2-Ethylenebis(stearamide);Abril 10DS;Abril wax 10DS;Accurel SL 624;Acrawax C;Acrawax C-DF 1;Acrawax CT;Acrawax HMS;Advawax;Advawax 240;Advawa...

**Ethylene bis(stearamide) ( CAS:110-30-5 )**

Updatetime:Jun 04 2014
Formula:C38H76N2O2
Ethylene bis(stearamide)

**Ethylene Bis Stearamide ( CAS:110-30-5 )**

Updatetime:Jun 03 2014
Purity:98% Min. Order:25/Kilogram
Supply Ability:1-500 Year/Metric Ton Formula:C38H76N2O2
Ethylene Bis Stearamide CAS:110-30-5 EINECS NO.: 203-755-6 M.F.:C38H76N2O2 M.W.:593.0222

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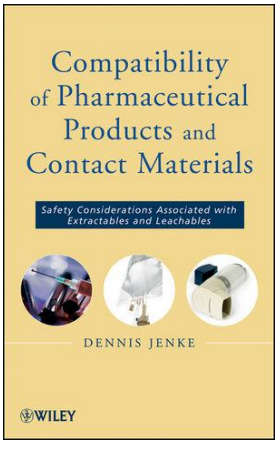
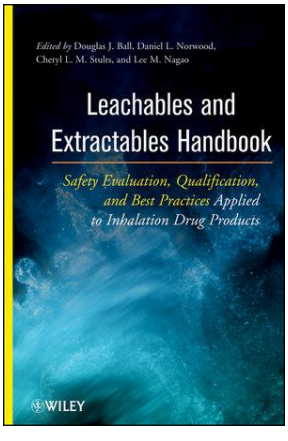
# What to learn More….




ISBN: 978-0-470-28176-5
June 2009

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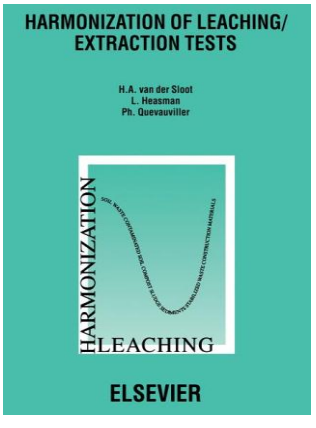




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# Thank you

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## More Rapid Extractable & Leachable Analyses with Advanced Mass Spectrometers

By Cynthia Challener, PhD

*Advances in technology are improving the sensitivity and accuracy of mass spectrometry increasing its use for the analysis of extractables and leachables.*

**Dennis Jenke**, a distinguished scientist in the Technology Resources Division of Baxter Healthcare Corporation, the coupling of high-efficiency chromatographic techniques with information-rich detectors is proving very useful for E&L analysis. In particular, he notes that the **coupling of ultra high-performance liquid chromatography (UHPLC) with high-resolution MS detectors** capable of providing **accurate mass information** enables the determination of the **empirical formulas** for otherwise unidentified E&Ls is a noteworthy recent advance.

With the sensitivity and accuracy of the instruments available today, this time can usually be significantly reduced because **structure elucidation is now possible without the need to perform laborious isolation work** for the individual unknowns," Rushing explains.

"Accurate mass is **not always able to give a single definitive empirical formula**. Oftentimes several viable candidates are identified and it is not possible, based on the accurate mass data alone, to establish which candidate formula is the correct one. There can be literally hundreds of compounds with a certain empirical formula but only a **few may be toxic**. It is **necessary to know the structure** in order to reach the ultimate end game for E&L, which is safety assessment. "Dennis Jenke"



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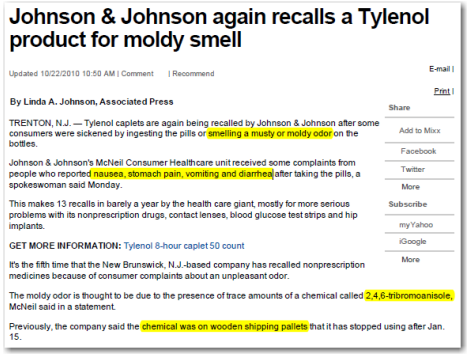
# Concerns Associated with Leachables from Packaging

Regulators in the pharmaceutical industry are becoming increasingly aware of the need to understand whether species that can be extracted from the primary packaging (direct contact packaging and container closures) will appear as leachable species across a range of dosage forms.



**Johnson & Johnson again recalls a Tylenol product for moldy smell**

Updated 10/22/2010 10:50 AM | Comment | Recommend

E-mail | Print |

By Linda A. Johnson, Associated Press

TRENTON, N.J. — Tylenol caplets are again being recalled by Johnson & Johnson after some consumers were sickened by ingesting the pills or smelling a musty or moldy odor on the bottles.

Johnson & Johnson's McNeil Consumer Healthcare unit received some complaints from people who reported nausea, stomach pain, vomiting and diarrhea after taking the pills, a spokeswoman said Monday.

This makes 13 recalls in barely a year by the health care giant, mostly for more serious problems with its nonprescription drugs, contact lenses, blood glucose test strips and hip implants.

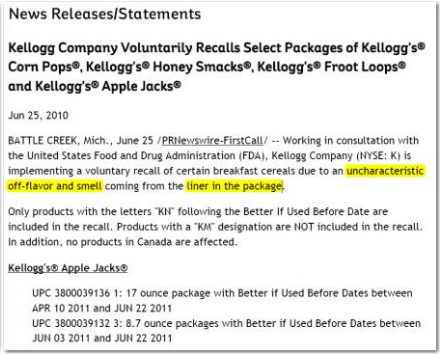
GET MORE INFORMATION: Tylenol 8-hour caplet 50 count

It's the fifth time that the New Brunswick, N.J.-based company has recalled nonprescription medicines because of consumer complaints about an unpleasant odor.

The moldy odor is thought to be due to the presence of trace amounts of a chemical called 2,4,6-tribromoanisole, McNeil said in a statement.

Previously, the company said the chemical was on wooden shipping pallets that it has stopped using after Jan. 15.

Share | Add to Mixx | Facebook | Twitter | More | Subscribe | myYahoo | iGoogle | More



News Releases/Statements

**Kellogg Company Voluntarily Recalls Select Packages of Kellogg's® Corn Pops®, Kellogg's® Honey Smacks®, Kellogg's® Froot Loops® and Kellogg's® Apple Jacks®**

Jun 25, 2010

BATTLE CREEK, Mich., June 25 /PRNewswire-FirstCall/ -- Working in consultation with the United States Food and Drug Administration (FDA), Kellogg Company (NYSE: K) is implementing a voluntary recall of certain breakfast cereals due to an uncharacteristic off-flavor and smell coming from the liner in the package.

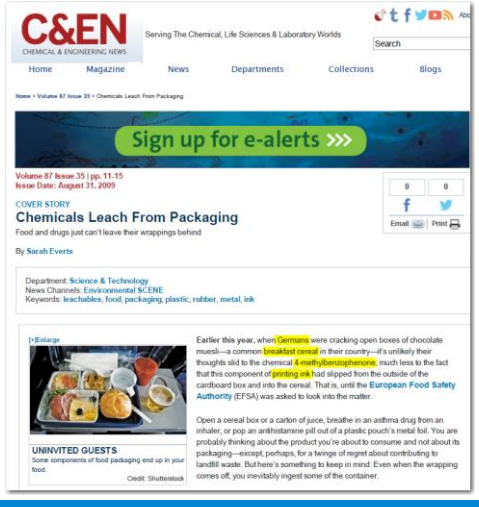
Only products with the letters "KN" following the Better If Used Before Date are included in the recall. Products with a "KM" designation are NOT included in the recall. In addition, no products in Canada are affected.

Kellogg's® Apple Jacks®

UPC 3800039136 1: 17 ounce package with Better if Used Before Dates between APR 10 2011 and JUN 22 2011
UPC 3800039132 3: 8.7 ounce packages with Better if Used Before Dates between JUN 03 2011 and JUN 22 2011



# Leachable examples from packaging component summarized in C&EN article, Issue Aug 31, 2009



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Volume 87 Issue 35 | pp. 11-15
Issue Date: August 31, 2009

COVER STORY

**Chemicals Leach From Packaging**

Food and drugs just can't leave their wrappings behind

By Sarah Everts

Department: Science & Technology
News Channels: Environmental SCENE
Keywords: leachables, food, packaging, plastic, rubber, metal, ink

UNINVITED GUESTS

Some components of food packaging end up in your food.

Credit: Shutterstock

Earlier this year, when Germans were cracking open boxes of chocolate muesli—a common breakfast cereal in their country—it's unlikely their thoughts slid to the chemical 4-methylbenzophenone, much less to the fact that this component of printing ink had slipped from the outside of the cardboard box and into the cereal. That is, until the European Food Safety Authority (EFSA) was asked to look into the matter.

Open a cereal box or a carton of juice, breathe in an asthma drug from an inhaler, or pop an antihistamine pill out of a plastic pouch's metal foil. You are probably thinking about the product you're about to consume and not about its packaging—except, perhaps, for a twinge of regret about contributing to landfill waste. But here's something to keep in mind: Even when the wrapping comes off, you inevitably ingest some of the container.

U.S. FDA 21 CFR 211.94(a) states "Drug product containers and closures shall not be reactive, additive, or absorptive so as to later the safety, identity, strength, quality or purity of the drug beyond the official or establishment requirements"

PQRI (Product Quality Research Institute) is a working group established to developed regulatory guidance for Extractable/Leachable analysis, which is also recognized by the FDA



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# Definitions:

## Extractable:

- Chemical compounds that can be removed from a primary container or component material into the drug or biologic by exertion of an artificial or exaggerated force, potential to migrate from polymeric construct into the drug product

  = What **can** come out

## Leachable:

- Chemical components that migrate from a container-closure system into the final packaged drug product under normal storage conditions. Direct contact with formulation under normal conditions
- Subset of Extractable

  =What **DOES** come our



# Classification of Extractable Substances

- **Passive Extractable**
  - "Extracted substance that was not chemically modified as a result of the drug product – container/closure interaction. This means that passive extracted substance is also an extractable substance by definition."
- **Reactive Extractable**
  - "An Extracted substance that was chemically modified as a results of the drug product – container/closure interaction. A reactive extracted substance is not an extractable substance but may be linked to one such as Irganox degradant." Reaction with peroxide e.g..

# Container Closure System: Drug Packaging

## What Drug Packing Should Never Do

- Contribute harmful components to the drug
- Increase toxicity of the product
- Add Genotoxic or Carcinogenic Components
- Impact the stability and Efficacy of Drug

- **Drug Manufacturing** may also contribute impurities not from packaging but from production and storage equipment such as filters, gaskets, tubing, storage bags, cleaning process



# Analytical Evaluation Threshold (µg/g)

**Device Component:**

$$AET = \frac{SCT \times D_t}{D_d \times m}$$

$D_d$ = Doses per day
$D_t$ = Total Labelled Doses
$m$ = Mass of Component

**Drug Delivery Product:**

$$AET = \frac{SCT \times D_t}{D_d}$$

**Final AET:**

$$AET_{fin} = AET_{Est}\{1 - (R_{f\%RSD}/100)\}$$

Extract Components Quantified Against a Single Authentic Reference Standard Are Quantified using Average response Factor ($R_{f\,av}$)



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# Risk Assessment Table by Dosage Forms

| Degree of concern associated with the route of administration | Likelihood of packaging component-dosage form interaction | | |
|---|---|---|---|
| | **HIGH** | **MEDIUM** | **LOW** |
| **HIGHEST** | Inhalation Aerosols and Solutions; Injections and Injectable Suspensions | Sterile Powders and Powders for Injections, Inhalation Powders | |
| **HIGH** | Ophthalmic Solutions and Suspensions, Transdermal Ointments and Patches, Nasal Aerosols and Sprays | | |
| **LOW** | Topical Solutions and Suspensions, Topical and Lingual Aerosols, Oral Solutions, and Suspensions | Topical Powders, Oral Powders | Oral Tablets and Oral (hard and soft Gelatin) Capsules |

^ *Figure 1: Risk Assessment Table for various dosage forms (adopted from ref. 19)*



# Toxicological Threshold for OINPD from PQRI Group

1) The Qualification Threshold (QT) is the level (5 µg/day) below which a given leachable is not considered for safety qualifications unless it presents a structure-activity (SAR) concern.

2) The Safety Concern Threshold (SCT) is the dose (0.15 µg/day) below which a leachable would present negligible concern for adverse carcinogenic and noncarcinogenic effects.

3) Known highly toxic substances such as PAHs, nitrosamines and 2-mercaptobenzothiazole) are considered to be "special case compounds" for orally inhaled and nasal drug applications and should be considered on a case-by-case basis.

4) By using one of these toxicological thresholds as well as dosing information, the Analytical Evaluation Threshold (AET) can be calculated. The AET is the level at or above which an OINDP pharmaceutical development team should identify and quantify a particular extractable and/or leachable and report it for potential toxicological assessment.

## Spectroscopy Organic Impurity Analysis Options

Agilent Cary 630 FTIR

Identifying and confirming the structure of an impurity or degradant

1260 Infinity Purification systems

Isolation of impurities

1260 Infinity Analytical SFC system

Detection of chiral impurities

7100 CE system

Analysis of polar impurities



## Container Closure System: Drug Packaging

- The sum of the packaging components that together contain and protect the dosage form. Includes both primary, secondary and tertiary packing components

### What Drug Packaging Should Do:

- Storage: adequately preserve the integrity of the drug
- Deliver the drug (Inhalers, prefilled syringes, ophthalmic, patches
- Protect the Drug during shelf life




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## Safety Thresholds from PQRI L/E Working Group

**Safety Concern Threshold: (SCT)** 0.15 $u$g per day, which is defined as the threshold below which an individual leachable would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects.

**Qualification Threshold: (QT)** 5 ug per day: Threshold below which a given leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure-activity relationship (SAR) concerns

**Analytical Evaluation Threshold: (AET)** is determined by consideration of the SCT and the specific drug product delivery configuration (number of doses in a Drug Product vs single dose

D. Norwood, L.M. Nagao, C.L.M.Stults; **J. Pharma Sci and Tech**., (2013) 67(5), 413-429



## Typical Extractable Leachable Project Workflow

## Extraction Method and Conditions Critical

Extraction conditions should mimic as close as possible drug product formulation and contact

- Simulate conditions of contact
- Simulate product conditions with extraction solution
- Simulate exposure using temperature and time
- Multiple solvents or extracting media with varying extracting power
- Multiple and complementary extraction techniques
- extraction conditions that allow equilibrium to be achieved.



## Extraction Conditions Critical

### Match conditions to product conditions:

- The chemical nature of the extracting media,
- The time and duration of the extraction process,
- The temperature at which the extraction is performed,
- The stoichiometry of the extraction process (extracted surface area per unit volume of extracting solution),
- The mechanism or process by which the extraction is accomplished

## Experimental Methods

Plastic IV bag System

- Headspace GC/MS
  - Packaging materials were cut into 1.0-$cm^2$ piece and placed into 10-mL headspace vial and purged with $N_2$ before analysis
  - Equilibration temperatures investigated
    - Plastics: 85, 100, 150, 200, 250 and 275 °C.
    - Full evaporation: 85 and 100 °C
- MMI GC/MS
  - Packaging material or liquid drug product were extracted with solvents by sonication for 5-8 hours
    - Solvent utilized: dichloromethane (DCM), hexane, ethanol, acetone
  - Organic extracts were transferred to a glass insert placed inside an autosampler vial for analysis



## Instrument Parameters

Two 5977 GC/MSD Systems

- Headspace GC/MS
  - 7697A Headspace Sampler and a 7890A GC coupled with a 5977A MSD
- MMI GC/MS
  - 7693A Automatic Liquid Sampler (ALS) and a 7890A GC coupled with a 5977A MSD
  - Equipped with a Multimode Inlet (MMI) operated in solvent vent mode

Headspace GC/MS

- Liner: 0.75-mm ultra-inert, straight tapered (p/n 5190-4048)
- Column: Agilent HP-5ms, UI, 30m x 0.25mm, 0.5µm (p/n 190915-133UI)

MMI GC/MS

- Liner
  - Drug suspension: 2mm ID ultra inert, dimpled (p/n 5190-4006)
  - Patch and IV bag: 4mm ID ultra inert (p/n 5190-3162)
- Column: Agilent HP-5ms UI, 30m x 0.25mm, 0.25µm (p/n 190915-433UI)

# Elemental/Inorganic Impurity Analysis ICP-MS

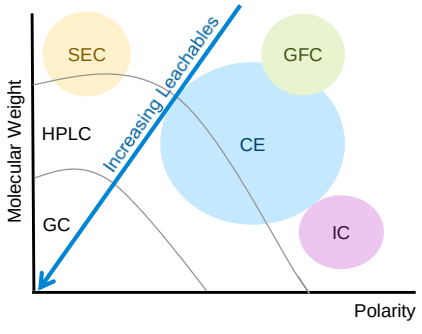
**Agilent 7700 Series ICP-MS**

- Reliable trace-level analysis of all **16 elements** whose limits are defined in USP<232>.
- **Low detection limits** ensure that all regulated elements can easily be determined at or below regulated levels, and even when large sample dilutions are required.
- Can also be used in combination with a **HPLC, GC, and CE**, providing several options for separation (or speciation) of the different chemical forms of the elements.
- ICP-MS **achieves low detection limits** for almost all elements, including those found in the more extensive analyte list proposed in the ICH Q3D, such as Au and Tl



# Analytical Technologies

**Potential Analytical Techniques Depending on Polarity and Molecular Weight**




CE: Capillary electrophoresis
GFC: Gel-filtration chromatography
SEC: Size-exclusion chromatography
IC: Ion chromatography

Most Agilent product lines can contribute
- GC/MS, GC–QQQ, GC-QTOF, HPLC, LC/MS, ICP/MS

No one method detects all extractables
- Headspace GC/MSD – volatile organic compound, high migration potential species (e.g. inks, adhesives, glue, processing solvent)
- GC-MS – semi-volatile organic compounds, residual monomers, antioxidant, plasticizers, antistatic agents, clarifying agents, preservatives, PAHs, slip agents
- **LC-UV-MS** – non-volatile organic compounds, large oligomers, large antioxidants, thermally labile compounds
- **Ion chromatography** – Bromide, chloride, fluoride in elastomers
- **ICP-MS** – metals from aluminum canister, glass
- **Chemiluminescence Detection** – nitrosamines

**Focus on the 5977 GC/MSD**
Headspace sampling
MMI – large volume liquid techniques



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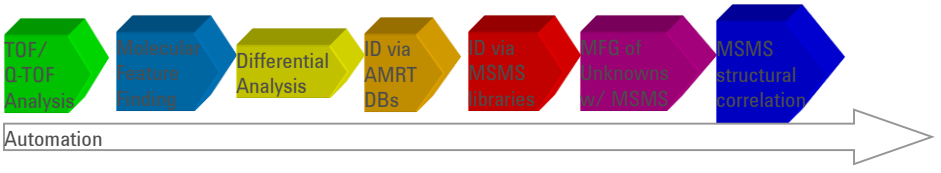
## QTOF Extractable Leachable Data Analysis Workflow

- Combination of UHPLC separation and accurate mass TOF technology
- Effective data mining algorithms to easily FIND compounds in a sample
- Optional SW to easily COMPARE samples or sample sets to identify differences
- AMRT DBs and MS/MS Lib Search to easily IDENTIFY targeted compounds
- Several algorithms to IDENTIFY unknown compounds (MFG, MSC)
- User Interface to easily NAVIGATE RESULTS
- Custom reporting to comprehensively REPORT results
- Full AUTOMATION of data acquisition, processing and reporting

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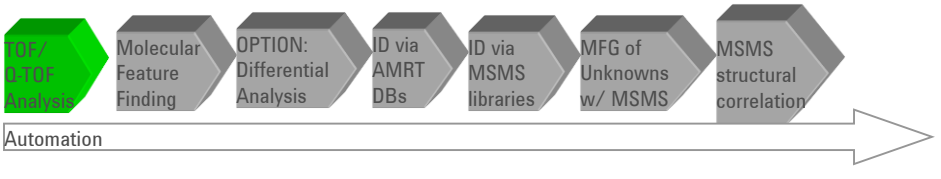
## TOF/Q-TOF Extractable/Leachable

### Acquisition via accurate mass LC/TOF and Q-TOF

- UHPLC for fast (throughput) highly efficient separation (minimize suppression)
- Accurate mass TOF and Q-TOF with:
  - High resolution (up to 40000 on 6540/6550)
  - Sub-ppm mass accuracy
  - Fast acquisition speed (20-50 Hz) to complement UHPLC
  - High dynamic range (10^5)
  - High sensitivity (low pg on-column)
- LC/TOF for screening and identification of TARGETED compounds
- LC/Q-TOF for confirmation of targeted and identification of UNKNOWN compounds

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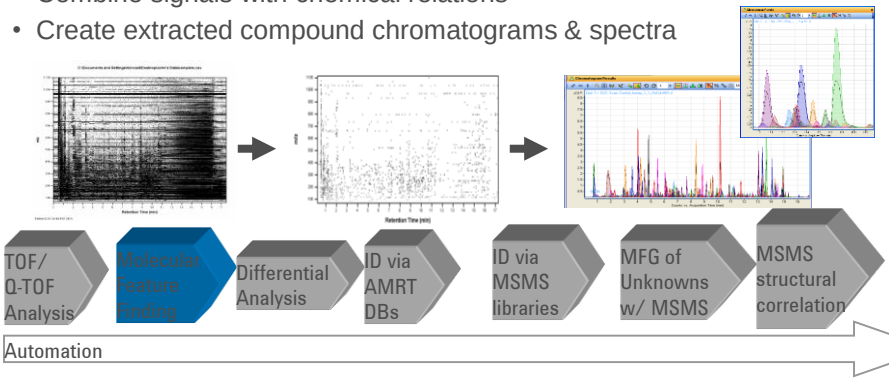
# Unsupervised Data Mining

**Molecular Feature Extractor (MFE)**

- Works on the 3-dimensional data set
- Remove noise
- Group covariant mass signals
- Combine signals with chemical relations
- Create extracted compound chromatograms & spectra

**=> It's crucial to find all detectable compounds in a sample efficiently**

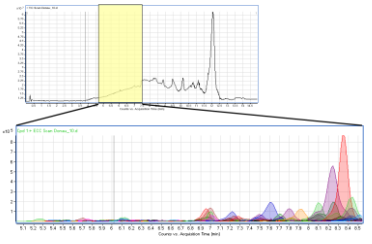


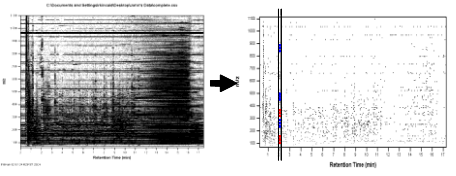



# Molecular Feature Extraction: Peak Finding Algorithm

Data is processed by proprietary feature finding algorithm

- Find chromatographic peaks
  - Find all ions that are related
  - Include any adducts, such as $Na^+$ or $K^+$
  - Include isotopes ($[M+H]^+$, $[M+H+1]^+$,…
  - Check for dimers
  - Create a compound chromatograms (ECC) and spectra
- Sum all ion signals into one value (Feature)
- Fully automated processing

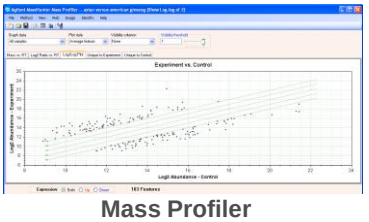




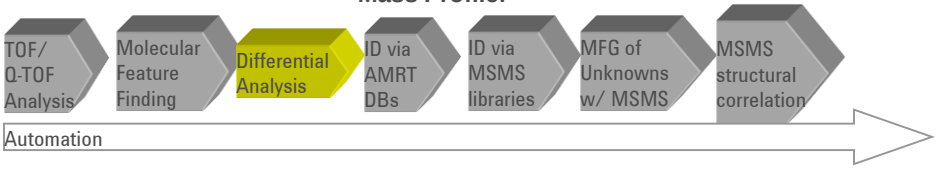
# Mass Profiler Software:

**Differential analysis**

- Some applications benefit from detecting the differences in compounds between samples, e.g.
  - Comparison of batches
  - Comparison of name brand and generic



Mass Profiler

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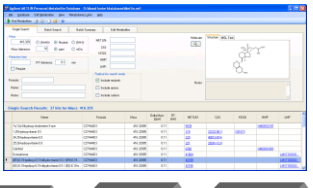
# Identification: Accurate Mass Databases

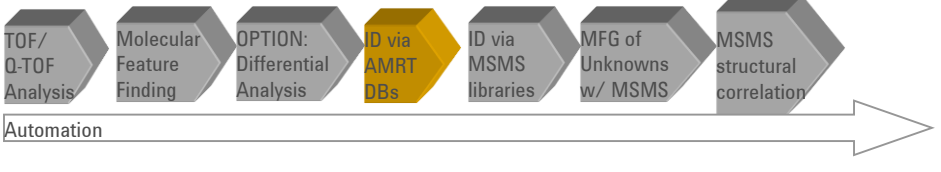
**Identification of targeted compounds via AM or AMRT databases**

- Personal Compound Databases (PCD)
  - PCD for extractable and leachable with several hundred compounds
- The addition of retention time into the database search (PCD) enables unambiguous identification of isobaric compounds



Compound List

| Name | Cpd | RT | Formula (DB) | Score (DB) | Diff (DB, ppm) | Mass (DB) |
|---|---|---|---|---|---|---|
| Cyromazine | 6 | 0.823 | C6 H10 N6 | 87.6 | -1.08 | 166.0967 |
| Cyromazine | 9 | 1.095 | C6 H10 N6 | 97.73 | -0.65 | 166.0967 |
| Cyprodinil | 138 | 12.245 | C14 H15 N3 | 99.32 | -1.26 | 225.1266 |
| Cyproconazole | 149 | 12.88 | C15 H18 Cl N3 O | 95.39 | -2.13 | 291.1138 |
| Cymiazole | 78 | 6.118 | C12 H14 N2 S | 98.04 | -1.6 | 218.0878 |

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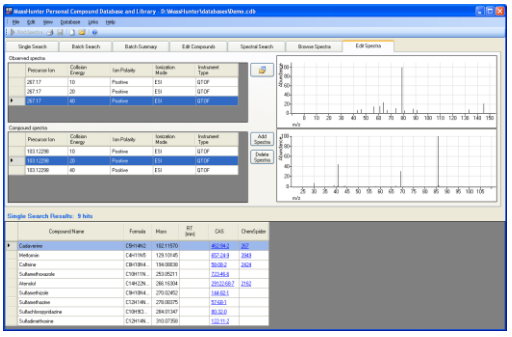
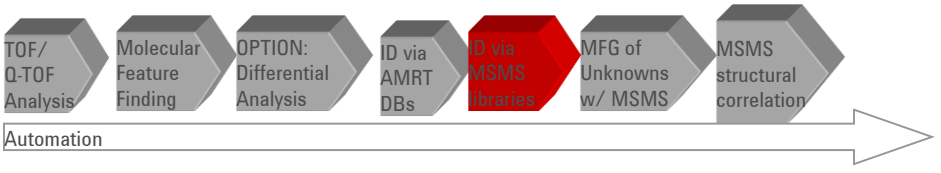
## Additional Confirmation using MS/MS Libraries

**LC/MS/MS library search**

- NIST LC/MS/MS library
- Agilent PCDL libraries
- Support of multiple CEs



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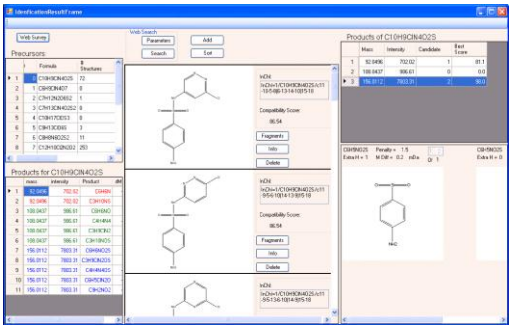
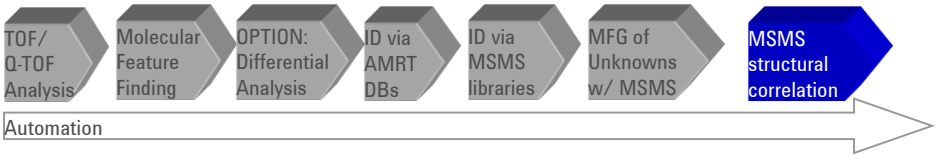
## Identification: Correlate MS/MS Spectra

**MS/MS Structural Correlation (MSC)**

- Algorithm to correlate "proposed structures" with accurate mass MS/MS fragment ion spectrum.
- Favor systematic bond dissociation approach over rule based fragmentation prediction approach.



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# MassHunter MS/MS Structural Correlation (MSC)

## Confirm proposed structures and aid true unknown analysis

Provides highest confidence in confirmation of proposed structures in minutes instead of hours

- Assigns fragment ions to substructures of the proposed parent structure
  - Metabolite ID
  - Metabolomics – compound identification
- Aids in the determination of true unknown compounds beyond Molecular Formula Generation via parallel MSC of all structures retrieved for a unique Molecular Formula