# Analysis of Extractables & Leachables in Pharmaceutical Products

## Regulatory & Analytical Aspects

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# Outline

## Section #1

- Introduction
- Defining extractables and leachables
- Guidelines
- Effects on biologic drug products
- The four essential steps of a study

## Section #2

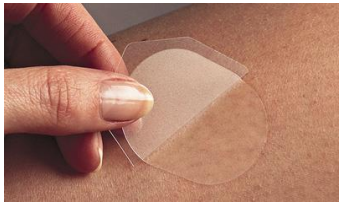
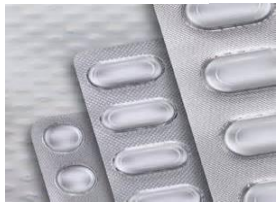
- Application Example: analysis of elemental E&Ls by ICP-MS
- Application Example: analysis of volatile E&Ls by GC-MS
- Application Example: analysis of non volatile E&Ls by LC-MS
- Appendix / References

# Introduction
# Drug Containers And Modern Drug Delivery Systems

*Drug containers and modern drug delivery systems meant to protect a drug from environmental contamination but they are actually themselves a source of contamination*

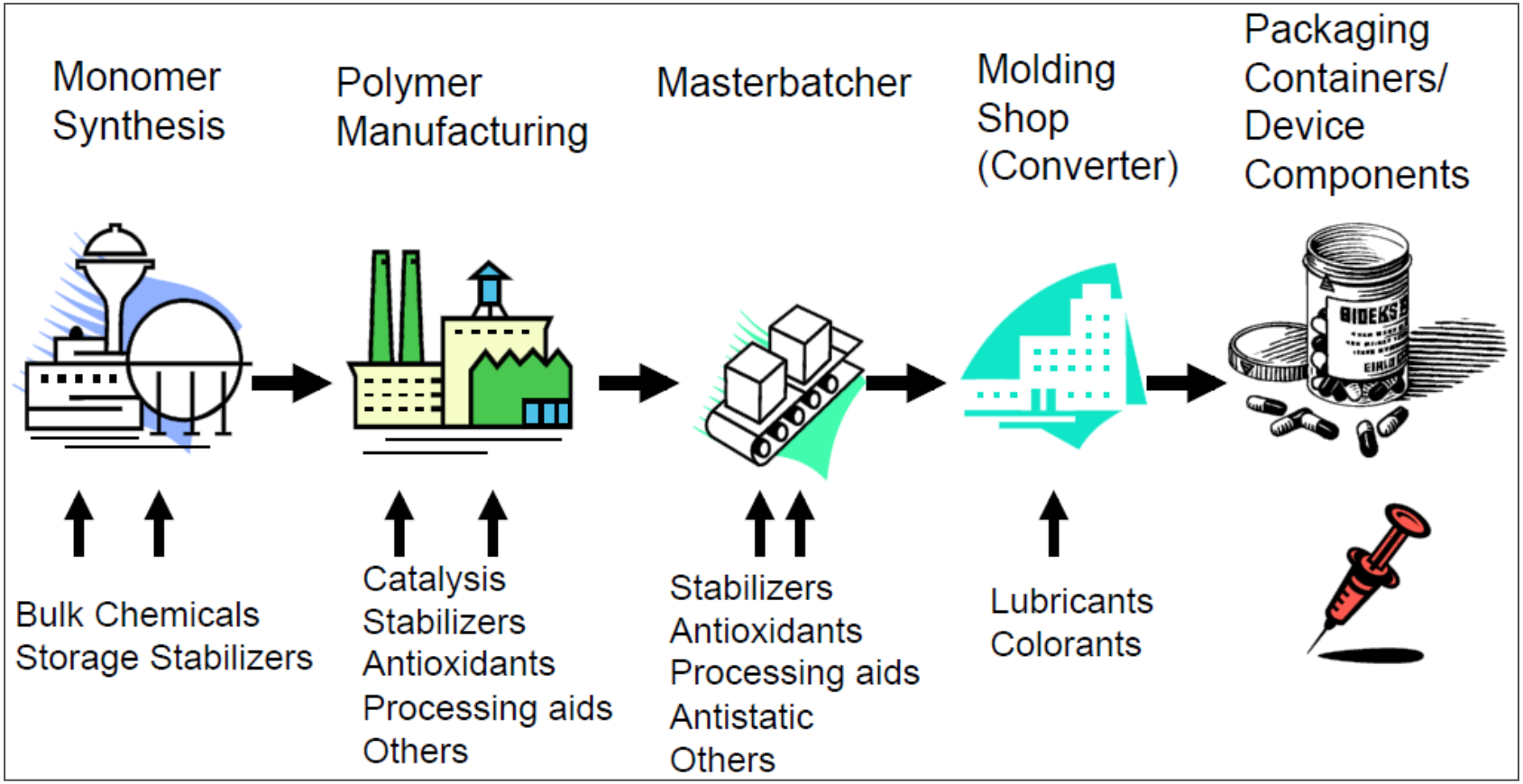




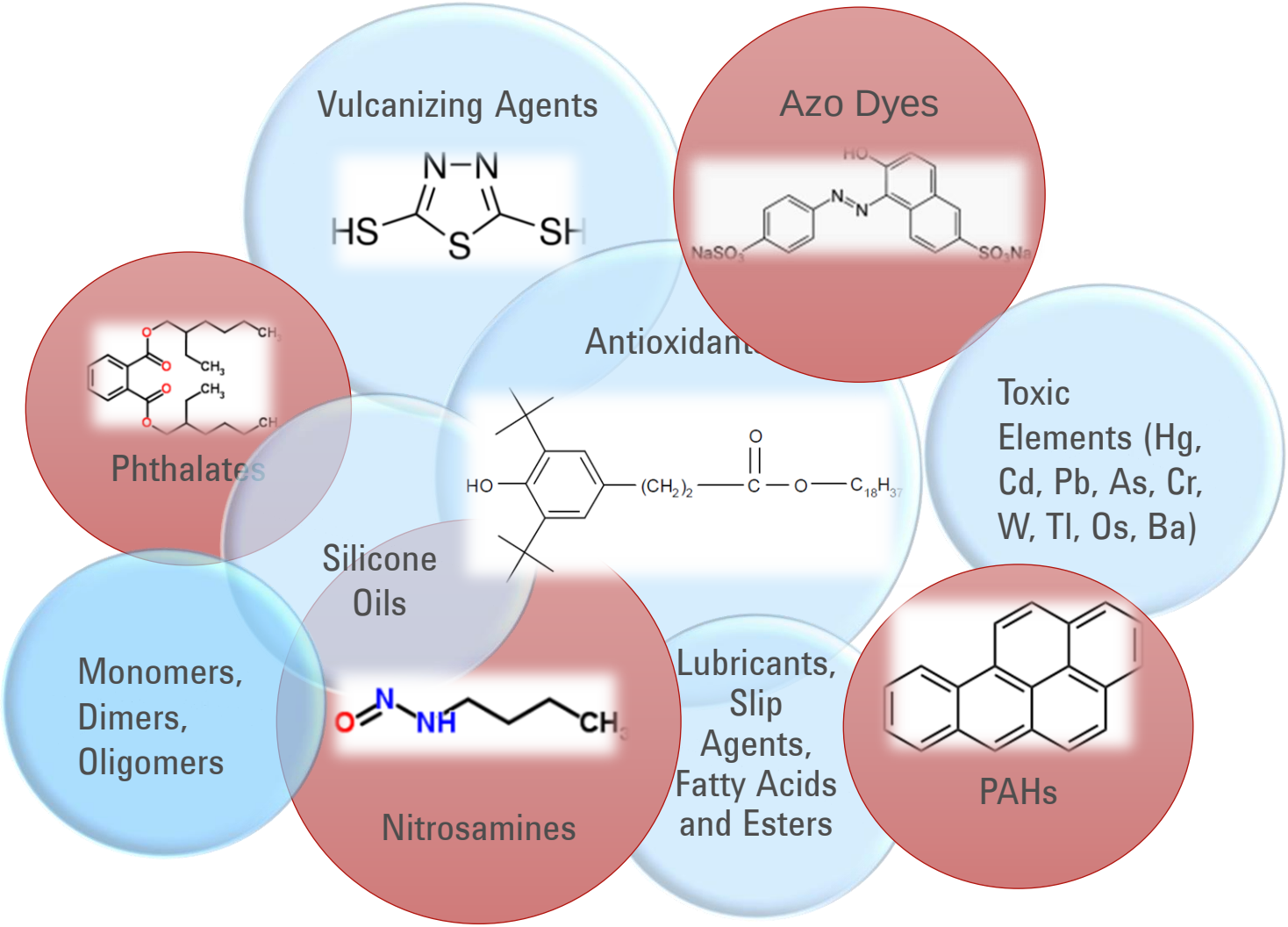

# Plastic Materials: Source of Contamination

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




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

# Compounds Identified as Extractables/Leachables

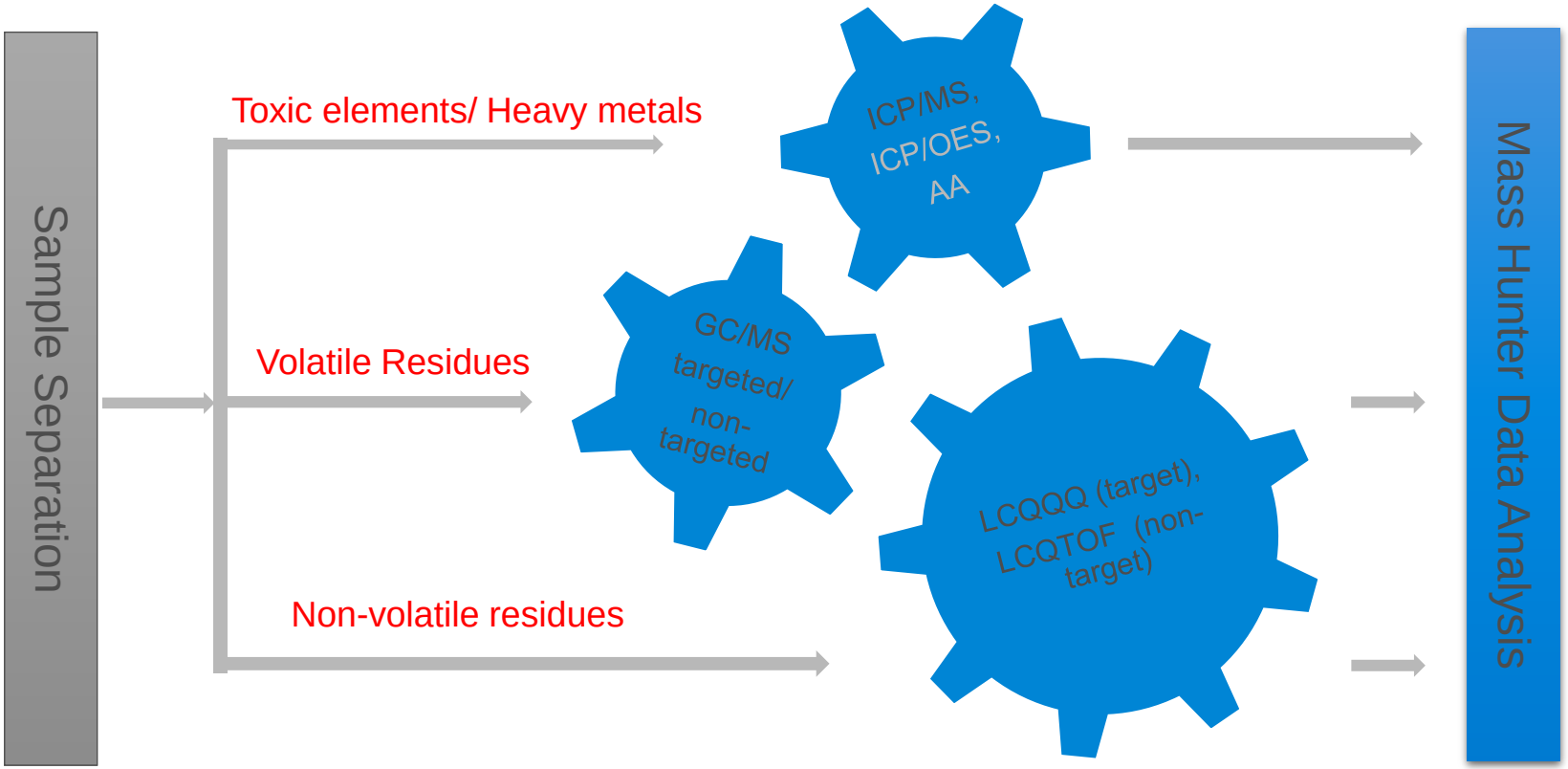



**Wide variety of Chemical Classes, Polarity, Molecular Weights, Properties**

# Various Analytical Technologies Required

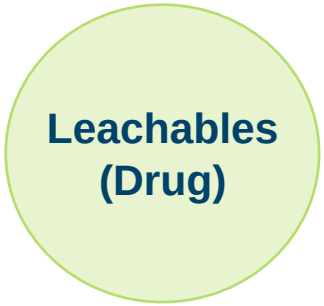
**Agilent delivers the most comprehensive analytical solutions portfolio**

**Objective:** To detect a wide class of known and unknown organic/inorganic compounds that maybe present in container closure systems at levels links to risk assessment threshold levels

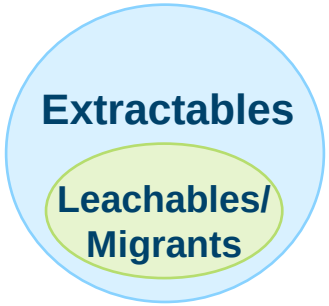


# Defining Extractables, Leachables, Migrants

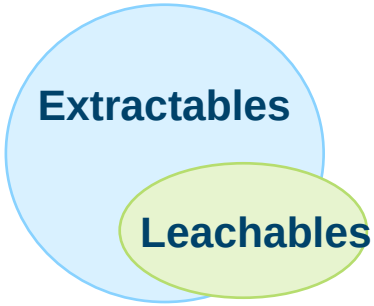

**Potential**
Compound
Migration




**Actual**
Compound
Migration




Leachables are often a **subset** of extractables




**New** Leachables may be identified which have been not observed as extractables

## Extractable

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
  - Simulate extended storage conditions

## Migrants

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

# FDA Regulation For Container Closure Systems (CCS)

**U.S. FDA 21 CFR 211.94(a) statement (April 2015)**

"(a) Drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug beyond the official or established requirements.

…(d) Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing, and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.94

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

# Why Worry about Extractables/Leachables ?

2014: FDA Drug Recalls Surges over 836 in 2014!

2014: FDA data shows 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*

August 2015: FDA warns against use of Becton-Dickinson (BD) 3 ml and 5 ml Syringes: http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm458955.htm

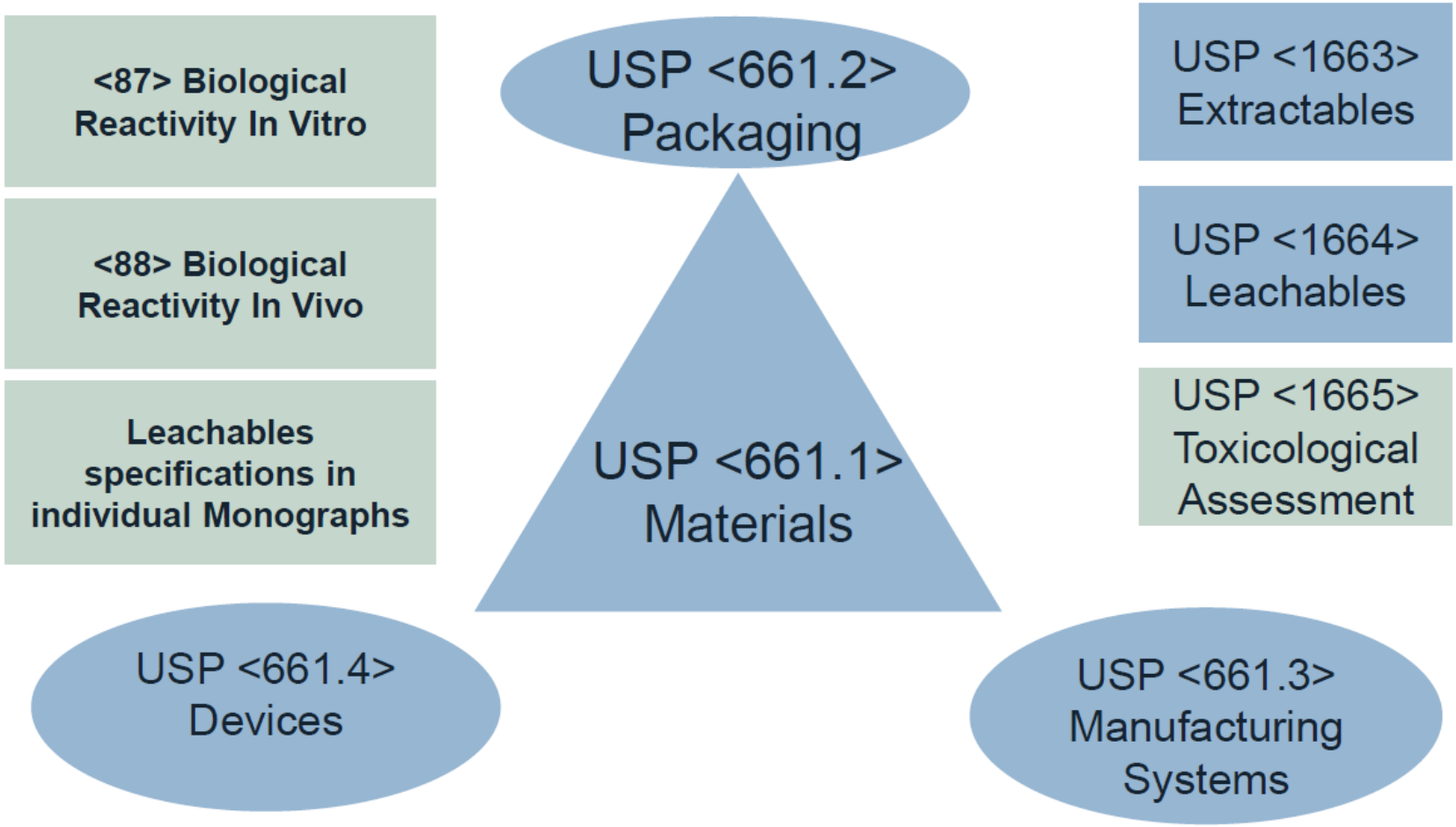
**Sodium Valproate Zentiva 500mg Gastro-resistant Tablets, PL 17780/0454, Zentiva Livery**

| Batch Number | Expiry date | Pack Size | First Distributed |
|---|---|---|---|
| J601 | Aug 2016 | 1 x 100 | 01 Oct 2014 |

We have been made aware of an unusual odour associated with the above batches of Epilim / Sodium Valproate Gastro-resistant tablets. The odour has variously been described as 'fishy', 'sweaty armpits' and 'cannabis'. An investigation by Sanofi has identified the root cause as the aluminium foil used in the packaging of affected batches. No impact on the quality or efficacy of the tablets has been detected. Sanofi has now ceased using this foil supplier.

https://assets.digital.cabinet-office.gov.uk/media/55191d6fe5274a142e000069/EL__15_A_02.pdf

# USP Chapters dealing with E&L

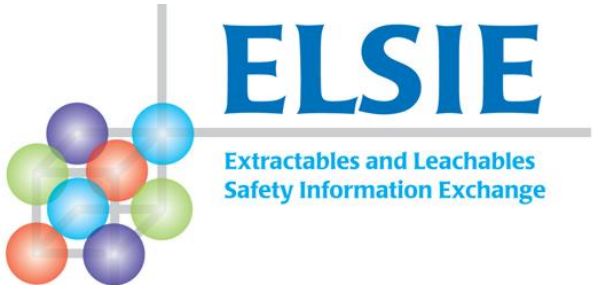



**Ref: Denise R. Jenke, Daniel L. Norwood, and Desmond G Hunt**

# Guidelines Delivered By 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



# PQRI guidance for OINDP

(Orally inhaled and nasal drug products)

**PQRI issued guidance for OINDP:**

"Safety thresholds and best practices for extractables and leachables in orally inhaled and nasal drug products (OINDP) also Applicable to parenteral and injectable products (PODP)

**PQRI established safety thresholds for leachables:**

- **Safety Concern Threshold (SCT)** ≤ 0.15 µg/day patient exposure which species represent no risk
- **Qualification Threshold (QT)** ≤ 5 µg/day patient exposure which a leachable is not considered for safety qualification. Lower threshold applies to PAH's, nitrosamines, and 2-mercaptobenzothiazole
- **Estimated Analytical Evaluation Threshold (AET) (µg/g**) = (SCT x total labeled doses) / (Doses per day x mass of component)

"Best practices" include controlled extraction studies and leachables studies.

# Inorganic Impurities

***New USP general chapters <232> and <233> for elemental impurities***

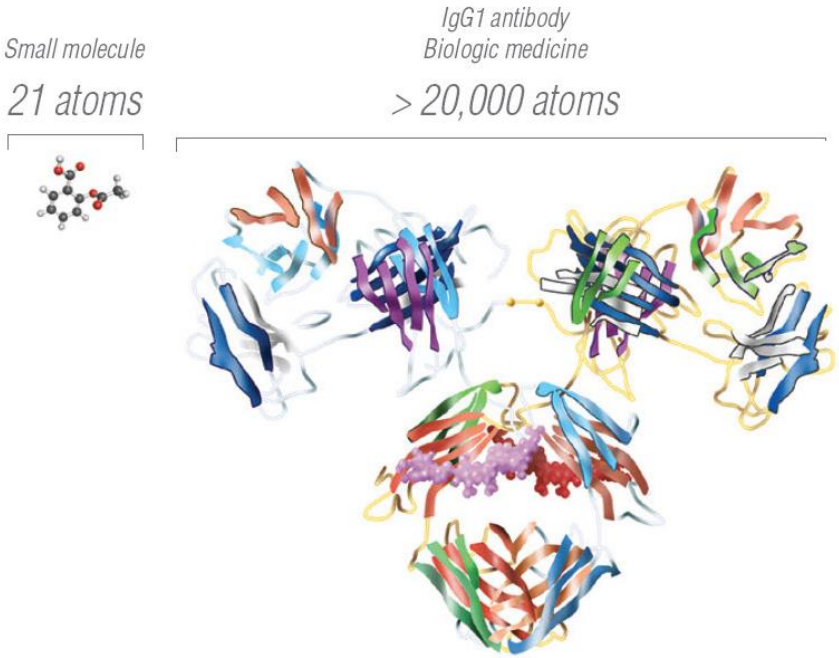
- Reagents, Ligands, Catalysts
- Manufacturing Aids
- Inks and Dyes

**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.

# Special Concerns About E&L Effects on Biologics

Even contaminations at trace levels with reactive E&L impurities can be deleterious for protein based drugs and will cause severe harm for the patient‘s health (immunogenic reaction)

E&L compound as Impurity



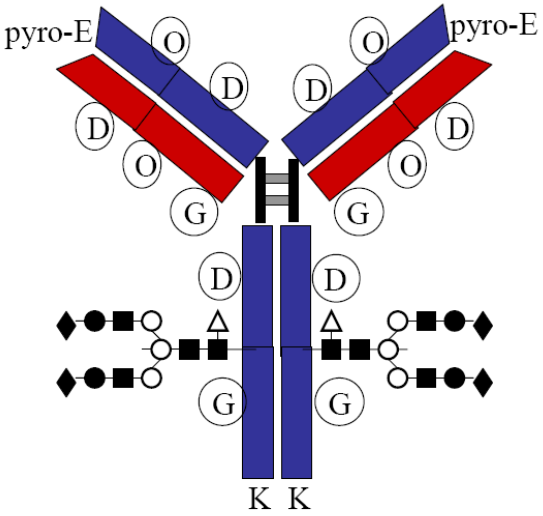

Contact materials are: plastics/elastomers, glass and stainless steel surfaces

# Sources of E&Ls as contaminants in biological drugs

- Plastics / Elastomers
  - Crosslinking agents, volatile organic E&Ls
- Glass surfaces
  - $Al^{3+}$; $Fe^{3+/2+}$; $Ca^{2+}$; $Ba^{2+}$; $Mn^{2+}$; $Zn^{2+}$
- Stainless steel surfaces
  - $W^{6+/4+}$; $Fe^{3+/2+}$; $Cr^{3+/2+}$; $Ni^{2+}$

- Sterile filtration processes are often a source of contamination
- Sterilization processes (steam autoclaving / gamma radiation) of drug containers will affect the concentration of leachables within the drug product
- Complexing agents (EDTA) facilitate migration of metal ions

# E&Ls may affect protein drug products by:




- Aggregation
- Increase in particulates*
- Oxidation
- Unfolding
- Formation of clipped variants
- Formation of Protein Adducts
- Post translational events during fermentation (glycosylation)
- Altered protein translation

* See also new USP monograph <787> PARTICULATE MATTER IN THERAPEUTIC INJECTIONS

Ref: Ingrid Markovic, CBER Presentation USP/PQRI E/L Workshop April 2014
https://www.usp.org/sites/default/files/usp_pdf/EN/meetings/09_markovich_presentation.pdf

# Critical Quality Attributes & Testing Methods for mAbs

Aggregate Analysis of Monoclonal Antibody

SEC
Aggregation

Disulfide Shuffling RP

Pyro-Glutamate IEC

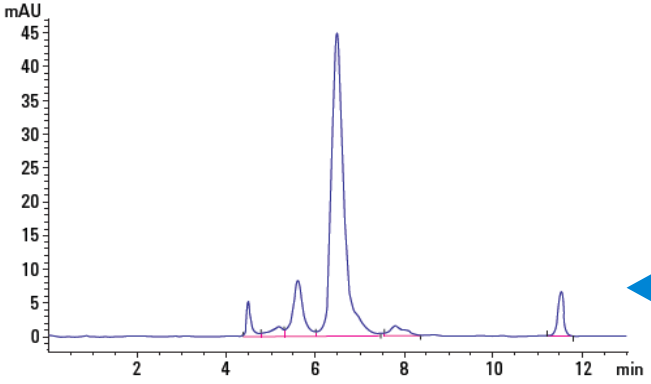
Deamidation /Oxidation IEC

Glycosylation (G0, G1, G2) HILIC

Fragmentation (Hinge) RP

Truncation (Lys 0, 1, 2) IEC

| | |
|---|---|
| Column: | Agilent AdvanceBio SEC 300Å, 2.7 µm, 7.8 x (p/n PL1180-5301) |
| Flow rate: | 1 mL/min |
| Mobile phase: | 150 mM phosphate buffer, pH 7.0 |
| Wavelength: | 220 nm |
| Temperature: | ambient |
| Injection volume: | 5 µL |
| Sample: | IgG |




# Key Principles of an E&L Study

1. Evaluating The Interactions Between Packaging Material And The Pharmaceutical Formulation And The Resulting Risks
2. Extractable Study: Applying Different Extraction Procedures And Different Analytical Technologies
3. Toxicological Assessment: Defining Threshold Levels For The Extracted Compounds
4. Leachable Study: Detection, Identification and Quantitation Of Leachables Within The Formulation

# Step 1: Evaluating Interactions And Risks

| | 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

- 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

# Step 2: Extraction Procedures

## Parenteral and Ophthalmic Drug Products (PODP)

Vigorous conditions
No sample dissolving solvents
No material deformation
Temp Hot extraction techniques

Solvents should cover a wide range of polarity

Solvents should mimic drug product formulation

| | Thermal | N-Hexane | Isopropanol | Isopropanol/ Water | Aqueous pH 2.5 | Aqueous pH 9.5 |
|---|---|---|---|---|---|---|
| Headspace | X | --- | --- | --- | --- | --- |
| Reflux | --- | X | X | PC/PVC only | --- | --- |
| Soxhlet | --- | X | X | --- | --- | --- |
| Sealed Vessel | --- | --- | --- | 55°C for 3d | 121°C for 1hr | 121°C for 1hr |
| Sonication | --- | --- | --- | --- | X | X |

Autoclave conditions: (121°C for 1hr)

Solvent Polarity/Drug Product Similarity

**Detection of additives in LDPE (Example)**

| Known Additives | Sonication pH 2.5 | Sonication pH 9.5 | Sealed Vessel IPA/Water |
|---|---|---|---|
| Irganox 1010 | --- | --- | X |
| BHT | --- | --- | X |
| Erucamide | X | X | X |

Sonication successfully detects erucamide, but no other anticipated additives

Solvents with different polarity provide better understanding of the material

PQRI: Threshold and Best Practices for Parenteral and Ophthalmic Drug Product (PODP)

# Step 2 : Threshold Levels and Actions

| *Extractable Level in Component* | *Assignment Category** |
|---|---|
| > 100 µg/g | Structure Confirmed |
| 20 – 100 µg/g | Confident |
| < 20 µg/g | Tentative |

**Assignment category:*

Structure confirmed: identification categories A, B (or C), and D (or E) (see Table 2) are positive.

Confident: sufficient data to preclude all but the most closely related structures.

Tentative: data is consistent with a class of molecule only.

| *Identification Category* | *Typical Identification Data* |
|---|---|
| A | Mass spectrometric fragmentation behavior |
| B | Confirmation of molecular weight |
| C | Confirmation of elemental composition |
| D | Mass spectrum matches automated library or literature spectrum |
| E | Mass spectrum and chromatographic retention index match authentic specimen |

Ref: ITFG/IPAC-RS Collaboration Response to FDA MDI Guidance on 2001

# Step 3: Toxicological Assessment & Thresholds (PQRI guidelines for OINDP)

- Based on Toxicological Thresholds of Concern **(TTC)** levels from to Kroes et al. (2004)
  http://foodcontactmaterials.com/links/ttc.pdf

- **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

# AET: How much sensitivity is required ?

- 0.05 µg of a genotoxic contaminant was extracted from a drug container
  - Weight ophtalmic solution container = 1 g

$$\text{AET} = \frac{0.05\ \mu g/container}{1g\ material/container} = 0.05\ \mu g/g \text{ container material}$$

- Safety Concern Threshold (SCT) for the contaminant = 0.15 µg /d
- Applied Dose = 3 opththalmic solution containers/day
- Content = 3 mL/container

- Analytical requirements to detect leachables within the formulation

$$\text{Leachables: AET} = \frac{0.15\ \mu g/day}{3\ doses/day}\ X\ 1\frac{\text{dose}}{\text{container}} = 0.05\mu g/\text{container}$$

$$\text{AET} = \frac{0.05\ \mu g/day}{3\ ml/container} = 0.017\ \mu g/mL$$

# Step #4 Leachables Study:

- Detection of leachables within the formulation
  - Different techniques of sample prep are required
    - Extraction procedures to reduce the matrix content
      - Liquid/Liquid extraction
      - SPE
      - Extraction of solids with different solvents
      - Headspace analysis
    - Recovery studies are required for quantitative analysis

## Threshold levels for leachables in drug products

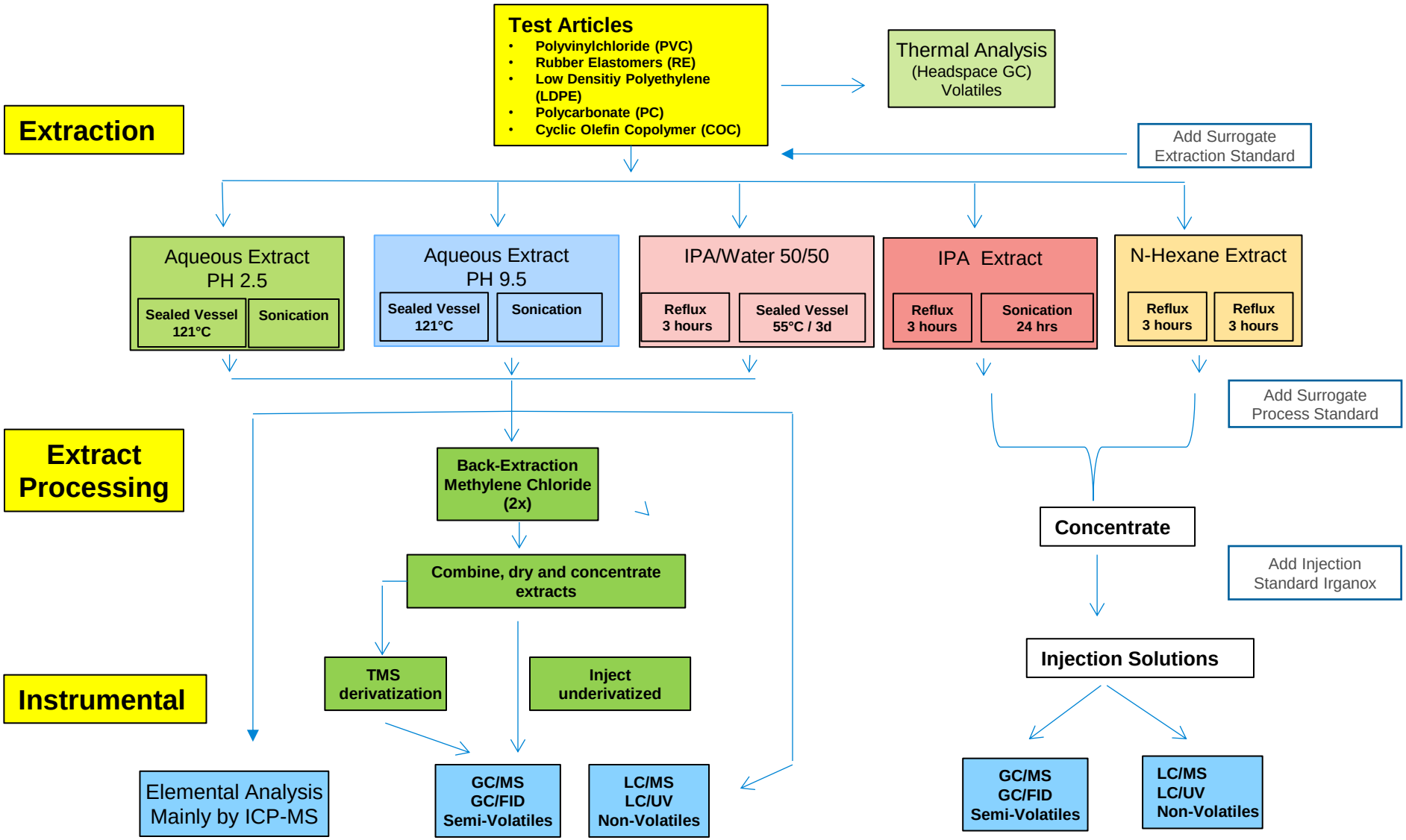
- **Reported** above 1 ppm (corresponding to 1µg /mL sample solution)
- **Identified** tentatively above 10 ppm
- **Structure confirmation** at 20 ppm

http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/UCM301045.pdf

# Section #2

- Application Examples: analysis of elemental E&Ls by ICP-MS
- Application Examples: analysis of volatile E&Ls by GC-MS
- Application Examples: analysis of non volatile E&Ls by LC-MS
- Appendix / References

# Analytical Workflows: Sample Preparation



# Analysis of Inorganic Extractables & Leachables by ICP - MS

Agilent 7800 / 7900 Series ICP-MS

**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.

Agilent Technologies

pub 5990-9382EN, 2014

# Published Application Notes

## Determination of Chromium in Gelatin Capsules using an Agilent 7700x ICP-MS

Application note

Pharmaceutical

**Authors**

Miao Jing, Yingping Ni, Yanping Wang and Zhixu Zhang

Agilent Technologies, China

### Introduction

Many medications that are administered orally are enclosed within capsules. Frequently both hard-shelled and soft-shelled capsules are made from edible animal protein (gelatin) which is prepared from various animal by-products such as bone and skin. The *Pharmacopoeia of the People's Republic of China* (2010 version) sets a clear standard for the grade of gelatin that can be used for drug capsule production and requires that pharmaceutical companies only purchase capsules from manufacturers that are licensed [1]. There have been recent reports that some companies in eastern China have been making and selling capsules made from cheaper industrial gelatin prepared from discarded leather [2]. Chromium, which is a known carcinogen, and can be toxic if ingested in large quantities, is used in the leather tanning process. Consequently, 20 to 90 times more Cr is typically found in the leather-derived gelatin than in pharmaceutical/edible grade gelatin. As a result, there is a need for a routine, highly sensitive method to

Agilent Technologies

pub 5991-1531EN, 2012

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

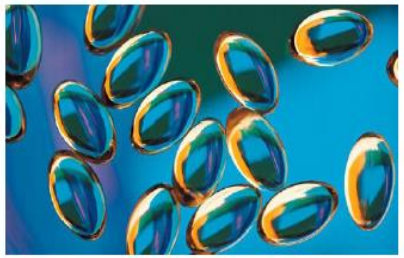
Application note

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, 2015

# Analysis of Volatile & Semi-Volatile E&Ls by GC-MS

5977B with 7890B GC

7697A Headspace Sampler

7200 Q-TOF

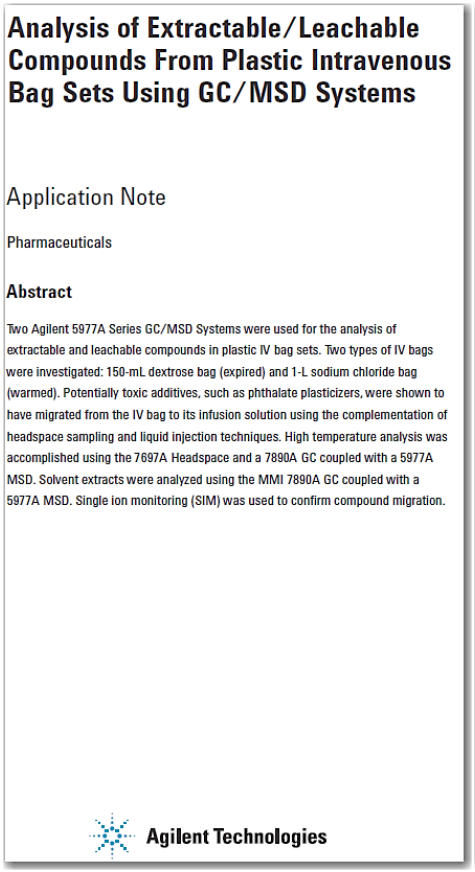
# Investigations of Pharmaceutical Products by

- Headspace-GC-MS
- MMI – GC-MS (Multi Mode Injection)

The following pharmaceutical products have been analyzed

- Intravenous (IV) Bag Set & PVC Tubings
- Transdermal Patch
- Liquid Drug Product
- Pressurized Metered-Dose Inhalers (pMDI)

# Please read the full story……



**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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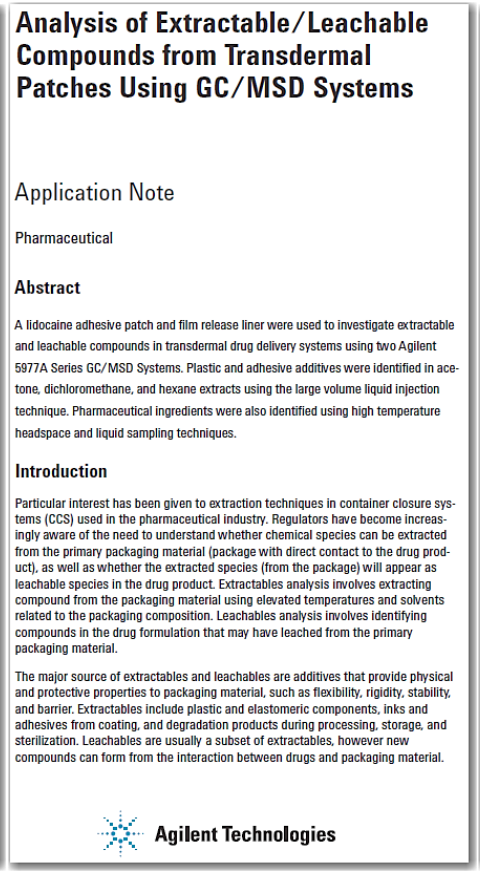
5991-5616EN



**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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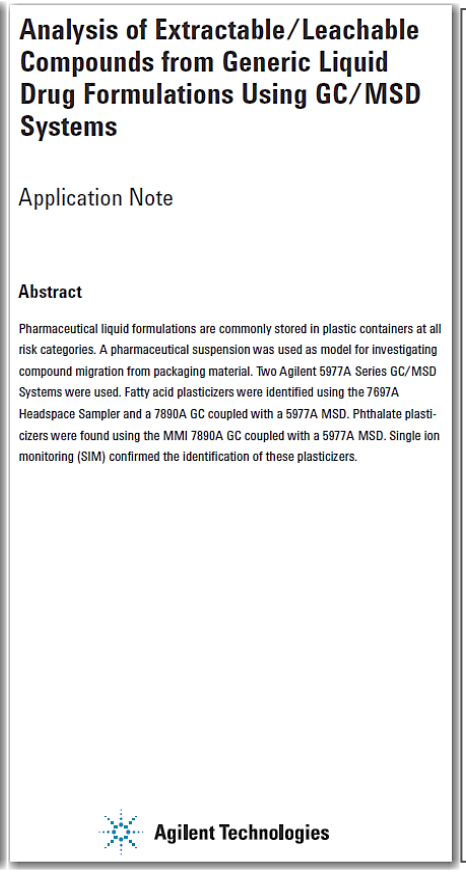
5991-5605EN



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

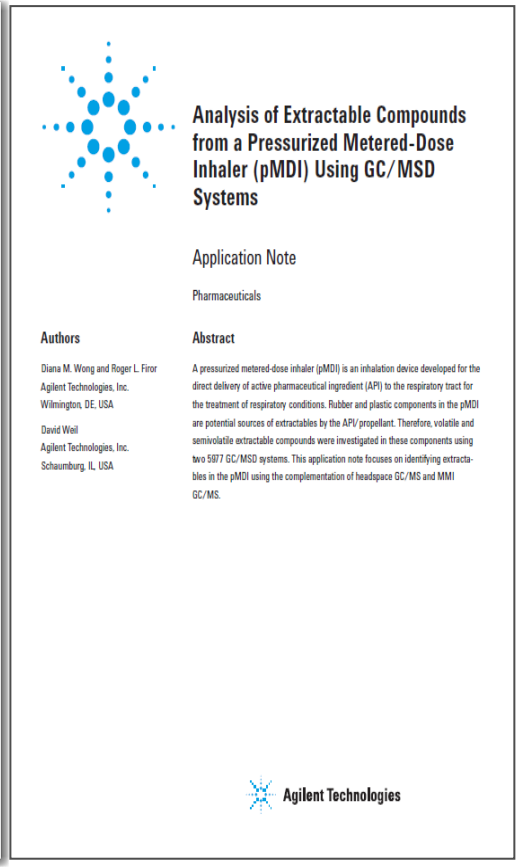
Application Note

**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.

Agilent Technologies

5991-5632EN



**Analysis of Extractable Compounds from a Pressurized Metered-Dose Inhaler (pMDI) Using GC/MSD Systems**

Application Note

Pharmaceuticals

**Authors**

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**Abstract**

A pressurized metered-dose inhaler (pMDI) is an inhalation device developed for the direct delivery of active pharmaceutical ingredient (API) to the respiratory tract for the treatment of respiratory conditions. Rubber and plastic components in the pMDI are potential sources of extractables by the API/propellant. Therefore, volatile and semivolatile extractable compounds were investigated in these components using two 5977 GC/MSD systems. This application note focuses on identifying extractables in the pMDI using the complementation of headspace GC/MS and MMI GC/MS.

Agilent Technologies

5991-6142EN

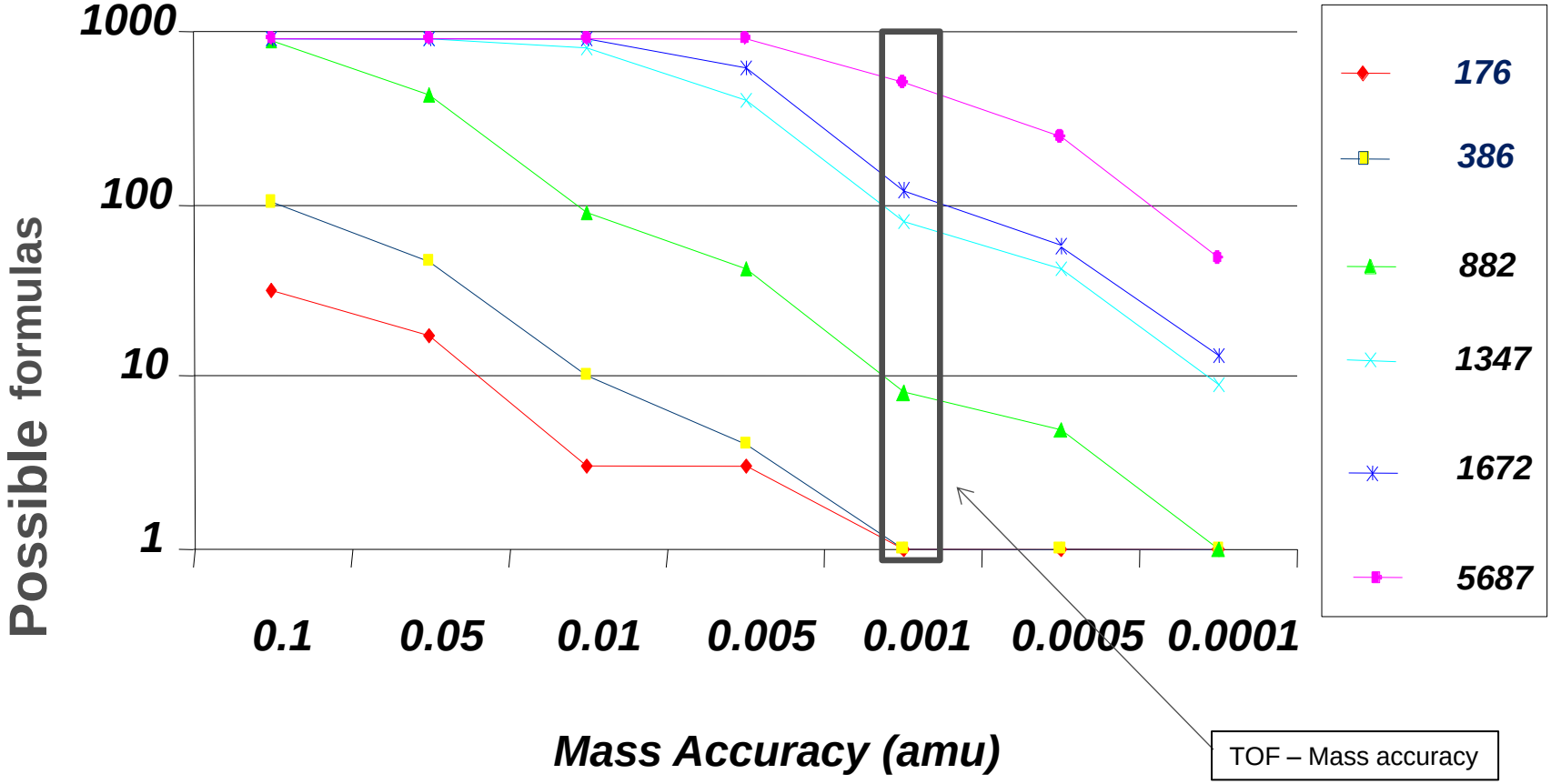
# Agilent Application Note

## In press…..Agilent Publication Number 5991-6688EN

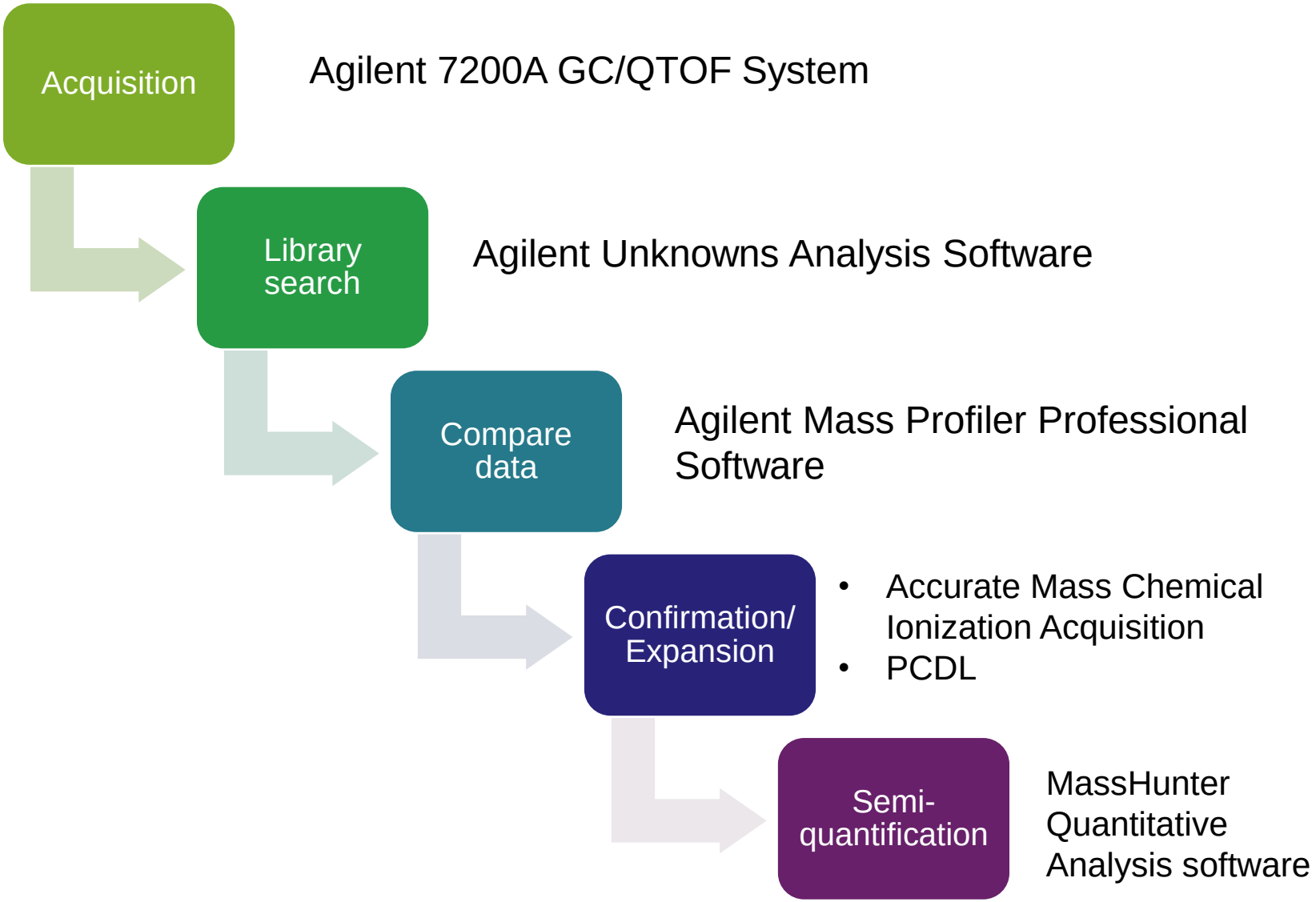
Differential Analysis in Screening Assays for an E&L Study Using an Agilent 7200 GC/Q-TOF System Combined with Data Mining Software

# Why TOF Technology?

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



# Analytical Workflow

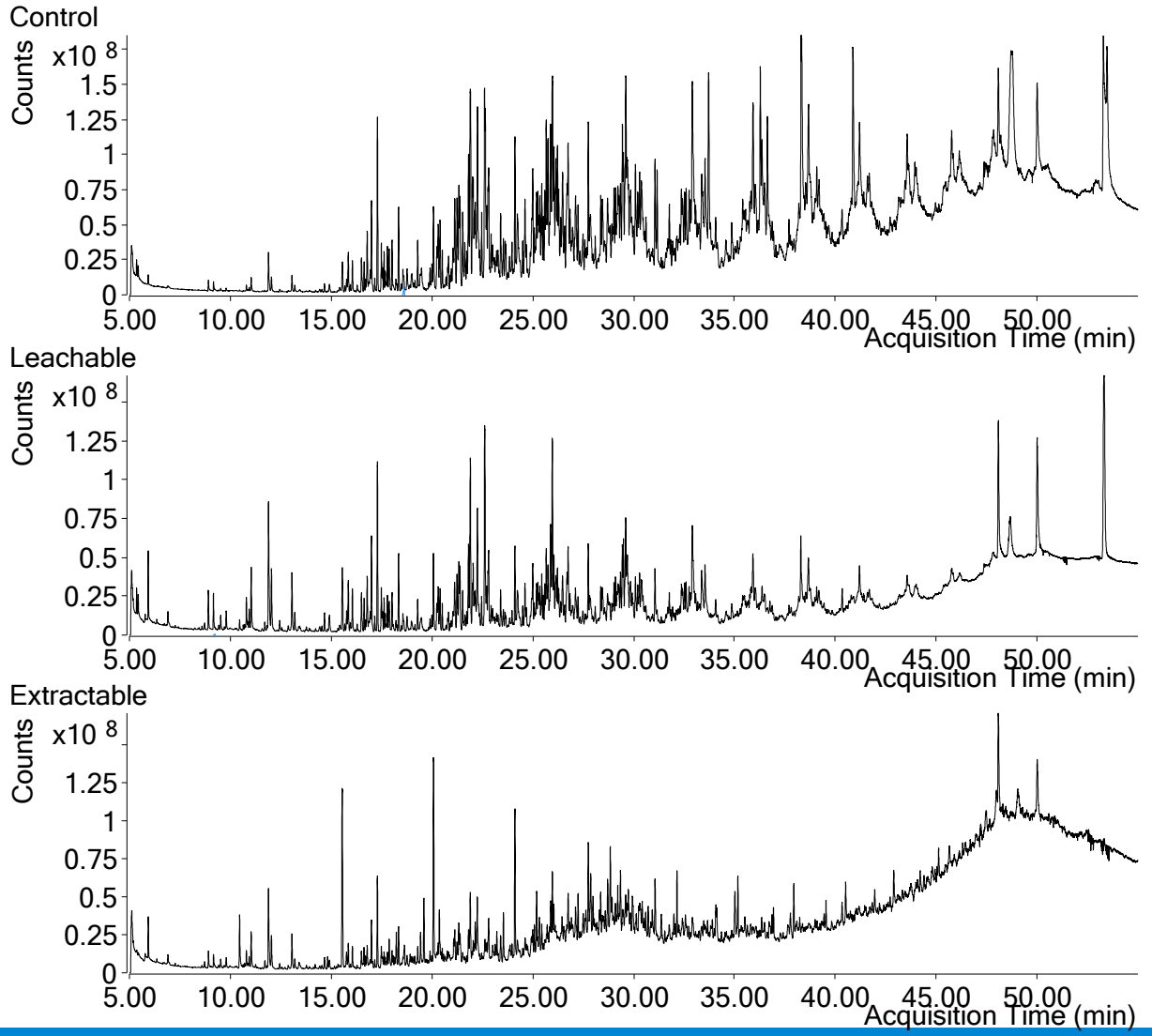


# TIC chromatograms in EI mode

Unheated Formulation
Leachable Sample

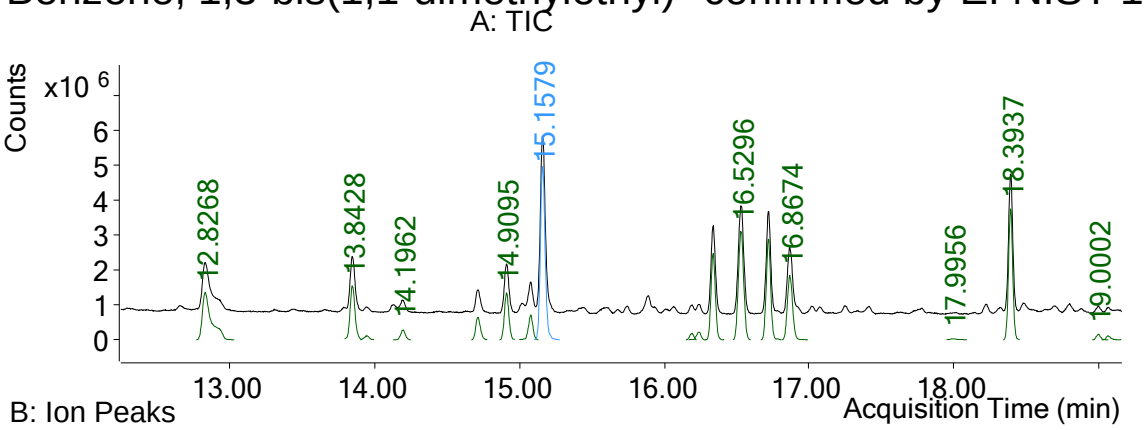
Heated Formulation
Leachable Sample

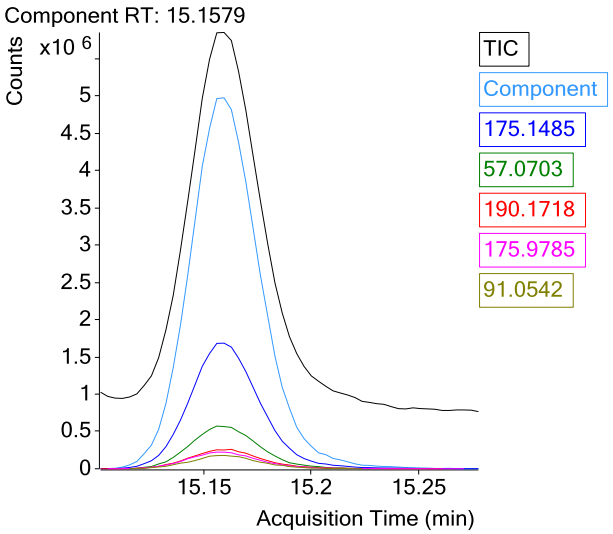
Container Extract
Extractable Sample

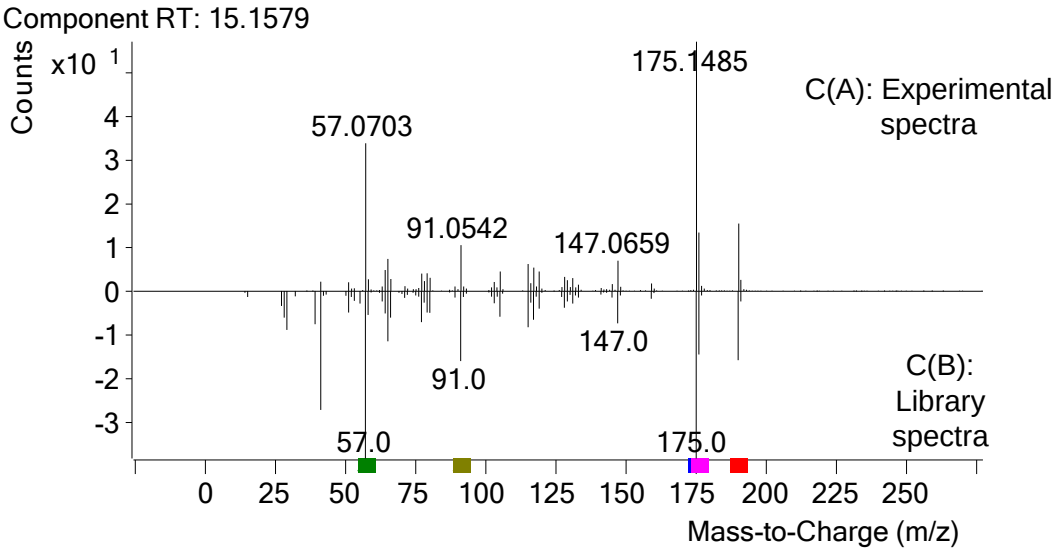


# Library search by *MassHunter Unknown Analysis Software*

Benzene, 1,3-bis(1,1-dimethylethyl)- confirmed by EI NIST 14.0



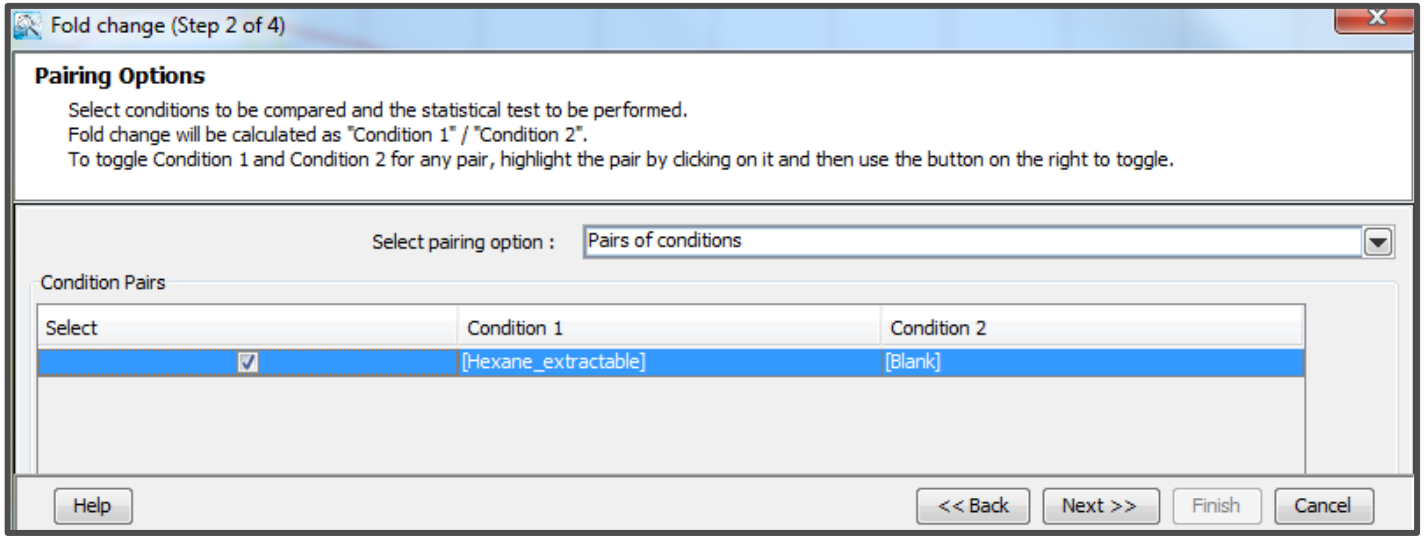




Di-Isobutylbenzene is potentially used for polymeric packaging

# Comparing Datasets to Detect Compounds

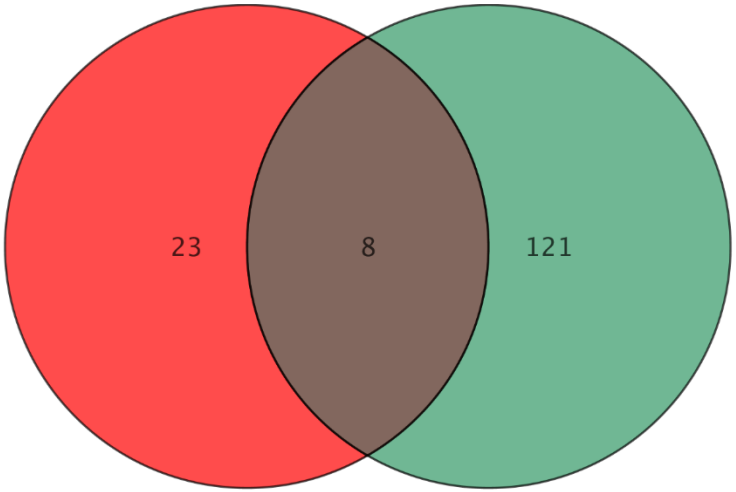
## Mass Profiler Professional Software




- Traditional blank subtraction could delete an extractable compound by mistake as it might be also present in the solvent, but in lower concentration
- A fold change analysis between the extractable and a blank sample helps to increase the confidence in results

# Results: Compounds Found in Different Samples

Leachables in Heated Formulation Total 31 compounds



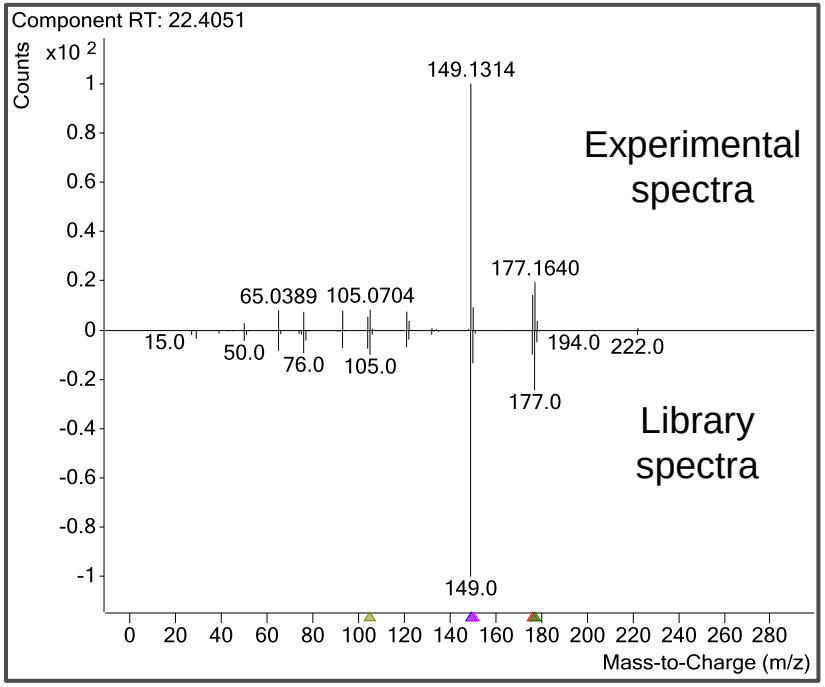

Extractable 129 compounds

- Benzene, 1,3-bis(1,1-dimethylethyl), and other compounds were also found in the non-heated leachable sample. The origin of these compounds are most likely from the container closure system.

| Retention Time | Extractable and leachable compounds | Fold Change in extractable |
|---|---|---|
| 8.75 | Octane, 3,5-dimethyl | UP |
| 15.16 | Benzene, 1,3-bis(1,1-dimethylethyl)- | UP |
| 15.75 | Dodecane, 4,6-dimethyl | UP |
| 16.19 | Tridecane | UP |
| 16.20 | Nonadecane | UP |
| 16.87 | Cyclohexasiloxane, dodecamethyl- | UP |
| 19.92 | Sulfurous acid, pentyl undecyl ester | UP |
| 20.53 | Cycloheptasiloxane, tetradecamethyl- | UP |

# Ambiguity of Results

NIST Library match of diethyl phthalate




Components

| Component RT | Compound Name | Match Factor | Best Hit | Formula | File Name |
|---|---|---|---|---|---|
| 16.2395 | Dodecane, 4,6-dimethyl- | 89.8 | ☑ | C14H30 | CE2c.D |
| 15.7427 | Dodecane, 4,6-dimethyl- | 85.3 | ☑ | C14H30 | CE2c.D |
| 14.7119 | Dodecane, 4,6-dimethyl- | 86.7 | ☑ | C14H30 | CE2c.D |
| 16.0637 | Dodecane, 4,6-dimethyl- | 91.0 | ☑ | C14H30 | CE2c.D |
| 13.8455 | Dodecane | 90.5 | ☑ | C12H26 | CE2c.D |
| 22.4051 | Diethyl Phthalate | | | C12H14O4 | CE2c.D |
| 28.0820 | Dibutyl phthalate | | | C16H22O4 | CE2c.D |
| 28.8504 | Dibutyl phthalate | | | C16H22O4 | CE2c.D |
| 8.8540 | Cyclotetrasiloxane, octamethyl- | | | C8H24O4... | CE2c.D |
| 12.8274 | Cyclopentasiloxane, decamethyl- | | | C10H30O... | CE2c.D |
| 23.8058 | Cyclooctasiloxane, hexadecamethyl | | | C16H48O... | CE2c.D |
| 16.8685 | Cyclohexasiloxane, dodecamethyl- | | | C12H36O... | CE2c.D |
| 25.4033 | Cyclohexane, undecyl- | | | C17H34 | CE2c.D |
| 23.5054 | Cyclohexane, decyl- | | | C16H32 | CE2c.D |
| 20.5230 | Cycloheptasiloxane, tetradecamethyl | | | C14H42O... | CE2c.D |
| 13.6585 | Cyclodecane, methyl- | | | C11H22 | CE2c.D |
| 26.8156 | Carbonic acid, octadecyl vinyl ester | | | C21H40O3 | CE2c.D |
| 18.5970 | Carbonic acid, eicosyl vinyl ester | 86.0 | ☑ | C23H44O3 | CE2c.D |



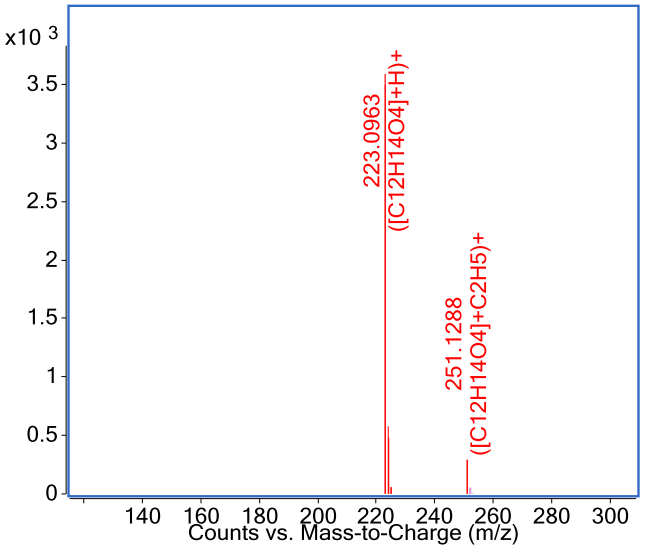
| Compound Name | Match Factor | CAS# | Formula |
|---|---|---|---|
| Diethyl Phthalate | 93.18 | 84-66-2 | C12H14O4 |
| Diethyl Phthalate | 92.71 | 84-66-2 | C12H14O4 |
| Diethyl Phthalate | 92.37 | 84-66-2 | C12H14O4 |
| Diethyl Phthalate | 91.17 | 84-66-2 | C12H14O4 |
| Diethyl Phthalate | 89.81 | 84-66-2 | C12H14O4 |
| Phthalic acid, ethyl isoporpyl ester | 81.29 | 1000314-99-6 | C13H16O4 |
| Phthalic acid, 2-chloropropyl ethyl ester | 80.23 | 1000356-82-2 | C13H15ClO4 |
| Phthalic acid, cyclobutyl ethyl ester | 79.43 | 1000315-41-1 | C14H16O4 |
| Phthalic acid, ethyl hex-2-yn-4-yl ester | 79.17 | 1000315-19-0 | C16H18O4 |
| Phthalic acid, ethyl pentyl ester | 78.97 | 1000308-93-6 | C15H20O4 |

Many other compounds also can give the same spectra with matching factor >80

# Eliminating Ambiguity by Chemical Ionization (CI)

| Extractables | Mass | Formula | PPM |
|---|---|---|---|
| .alpha.-Cubebene | 204.188 | C15 H24 | 3.52 |
| 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 278.152 | C16 H22 O4 | 4.18 |
| 1-Decanol, 2-hexyl- | 242.261 | C16 H34 O | 3.67 |
| 2-Methyltetracosane | 352.407 | C25 H52 | -0.6 |
| 9H-Fluorene, 9-methylene- | 178.078 | C14 H10 | 4.8 |
| Benzene, (1-butylheptyl)- | 232.219 | C17 H28 | 3.59 |
| Benzene, (1-butylhexyl)- | 218.203 | C16 H26 | 0.07 |
| Benzene, (1-butyloctyl)- | 246.235 | C18 H30 | 4.56 |
| Benzene, 1,2,4-trimethyl- | 120.094 | C9 H12 | 3.53 |
| Benzene, 1,3-bis(1,1-dimethylethyl)- | 190.172 | C14 H22 | 1.44 |
| Benzene, 1,3-dimethyl- | 106.078 | C8 H10 | 0.09 |
| Benzene, 1-ethyl-3,5-dimethyl- | 134.11 | C10 H14 | 3.55 |
| Benzophenone | 182.073 | C13 H10 O | 3.71 |
| Cyclopentasiloxane, decamethyl- | 370.094 | C10 H30 O5 Si5 | 2.85 |
| Diethyl Phthalate | 222.089 | C12 H14 O4 | 0.82 |
| Dodecane, 4,6-dimethyl-A | 198.235 | C14 H30 | 4.14 |
| Hexadecanal, 2-methyl- | 254.261 | C17 H34 O | 6.11 |
| Naphthalene, 1,6,7-trimethyl- | 170.11 | C13 H14 | 5.91 |
| Naphthalene, 2-methyl- | 142.078 | C11 H10 | 2.03 |
| (E)-Hex-3-enyl (E)-2-methylbut-2-enoate | 182.131 | C11 H18 O2 | 4.99 |

Diethyl Phthalate (RT 22.4 min)

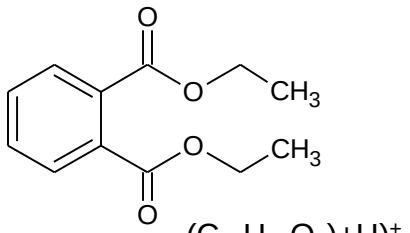


# Eliminating Ambiguity by Collision Induced Dissociation

## Fragments & accurate mass for unambiguous compound confirmation

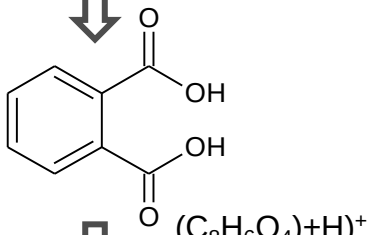
Diethyl phthalate

m/z 223.0937



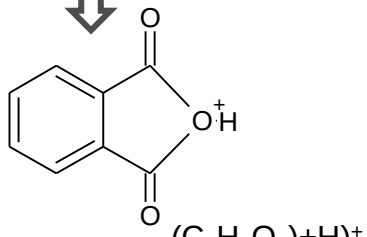
$(C_{12}H_{14}O_4)+H)^+$

m/z 167.0278

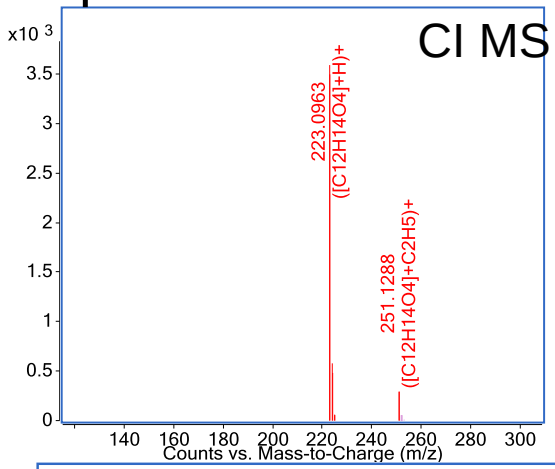


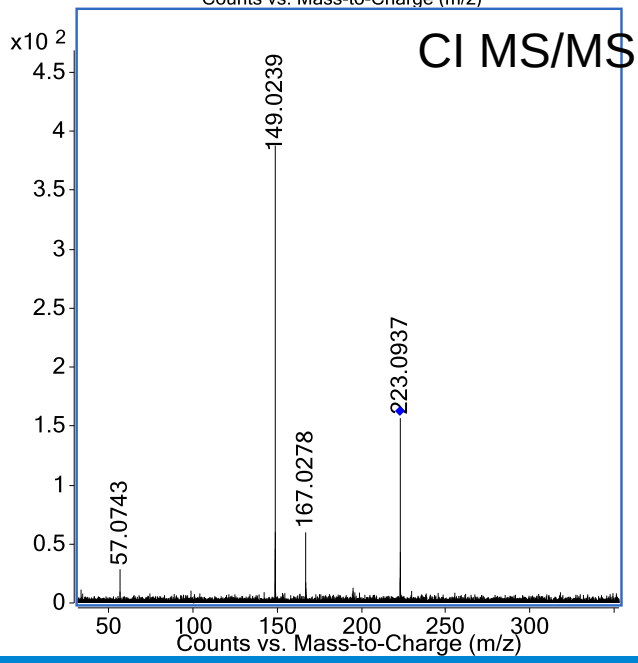
$(C_8H_6O_4)+H)^+$

m/z 149.0239



$(C_8H_4O_3)+H)^+$

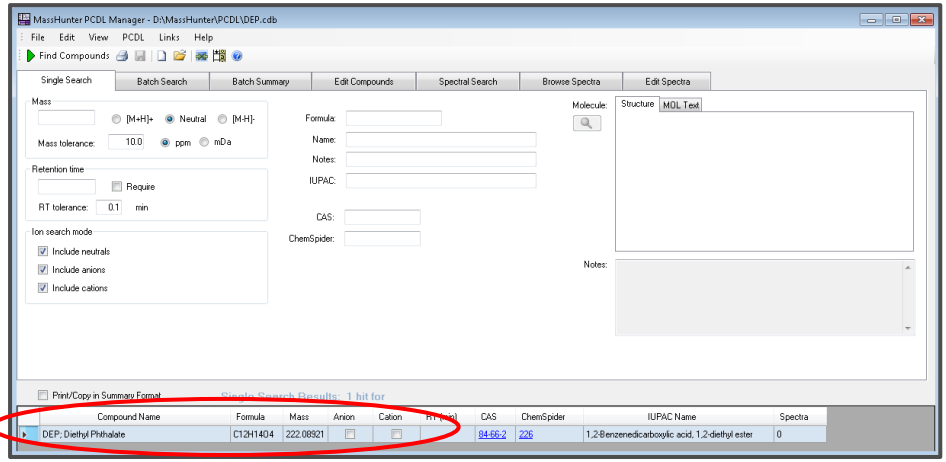


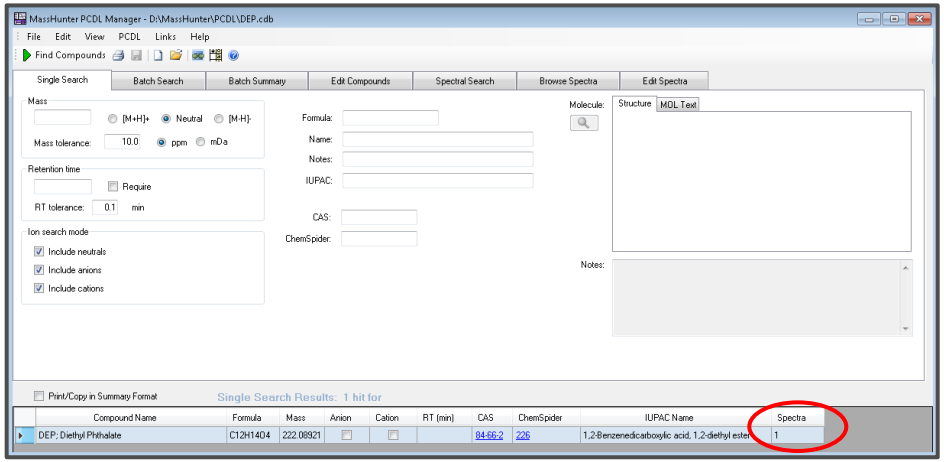

CI MS/MS spectra can be stored in PCDL software to built a custom library.

# Creation of libraries from CI-MS/MS data

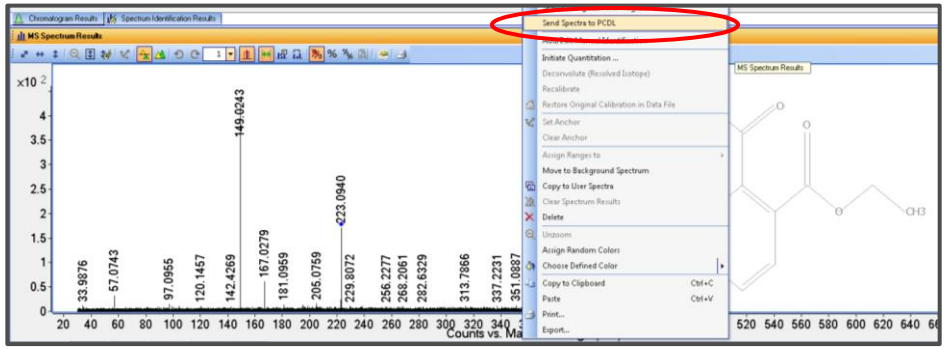
## Database creation



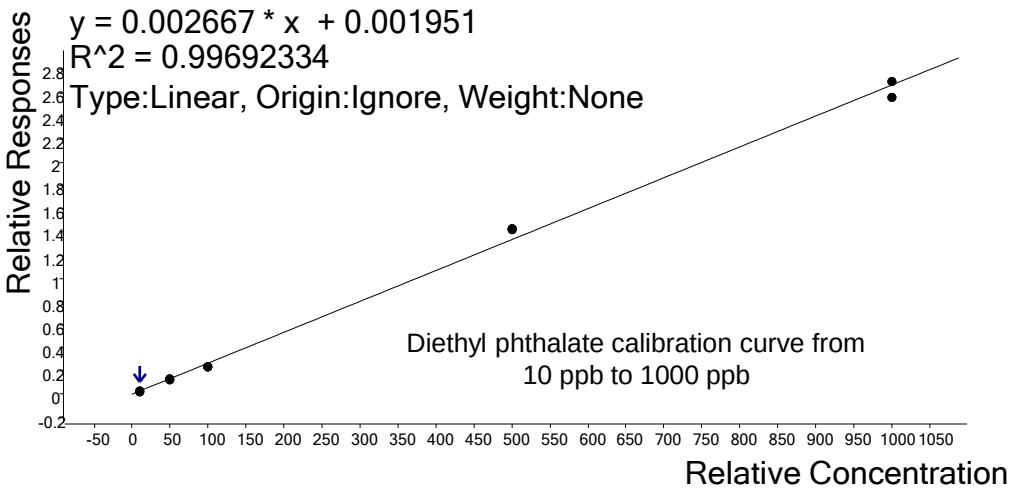

## Library creation




## Adding the spectra



# Semi-quantitative Determination of Impurities




| Retention Time | Leachables heated sample | Semi-quantitation estimation (ppb) ±30% |
|---|---|---|
| 6.03 | Cyclotrisiloxane, hexamethyl- | 88 |
| 9.06 | Nonane, 2,6-dimethyl- | 96 |
| 9.12 | Sulfurous acid, 2-ethyl hexyl undecyl ester | 200 |
| 11.42 | Octane 5 ethyl 2 methyl | 83 |
| 13.85 | Dodecane | 123 |
| 15.17 | Benzene, 1,3-bis(1,1-dimethylethyl)- | 383 |
| 16.54 | 3-Eicosene, (E)- | 128 |
| 16.79 | Tetradecane | 58 |
| 16.87 | Cyclohexasiloxane, dodecamethyl- | 127 |
| 18.40 | Heptadecane, 2,6,10,15-tetramethyl | 177 |
| 19.31 | Dodecane, 2,6,10-trimethyl | 44 |
| 19.92 | Sulfurous acid, pentyl undecyl ester | 80 |
| 20.75 | Phenol, 2,5-bis(1,1-dimethylethyl)- | 177 |
| 20.98 | 1-Decanol, 2-hexyl | 87 |
| 23.89 | Heptadecane, 2,6,10,15-tetramethyl | 66 |

- Quantification threshold: 5 ug/day
- Structure confirmed tentatively : 1ppm
- Structure elucidation: 20 ppm
- Semi quantitation: 0.1 ppm to 100 ppm

- The concentration of Benzene, 1,3-bis(1,1-dimethylethyl)- is ~ 0.4 ppm. Based on the daily dosage the consumption (of 9 mL solution/d) is below the quantitation threshold of 5 µg/day.

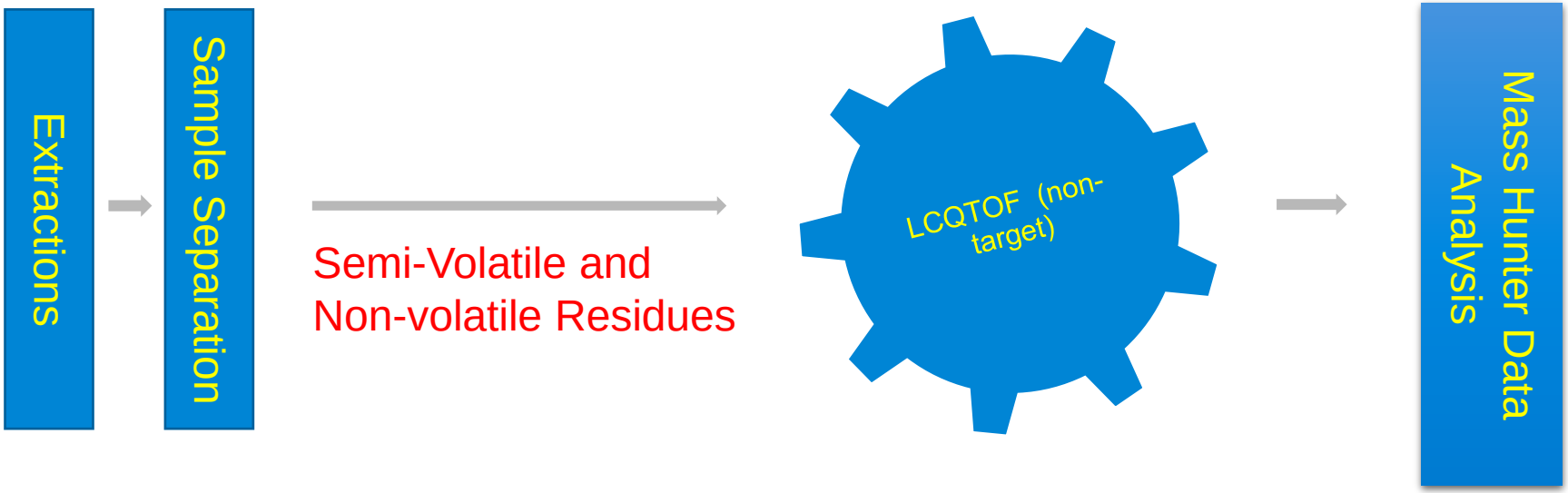
# Analysis of Non-Volatile E&Ls by LC-MS

## To be published soon……..

Detection and Identification of non volatile E&Ls in an ophthalmic solution by LC-QTOF-MS and MassHunter MassProfiler data mining software

1290 Infinity II UHPLC+ 6500 Series QTOF System

# Extractable/Leachable LC-QTOF Workflow




**Samples**
-Standards
-Extracts Drug Containers
-Extracts Drug Product

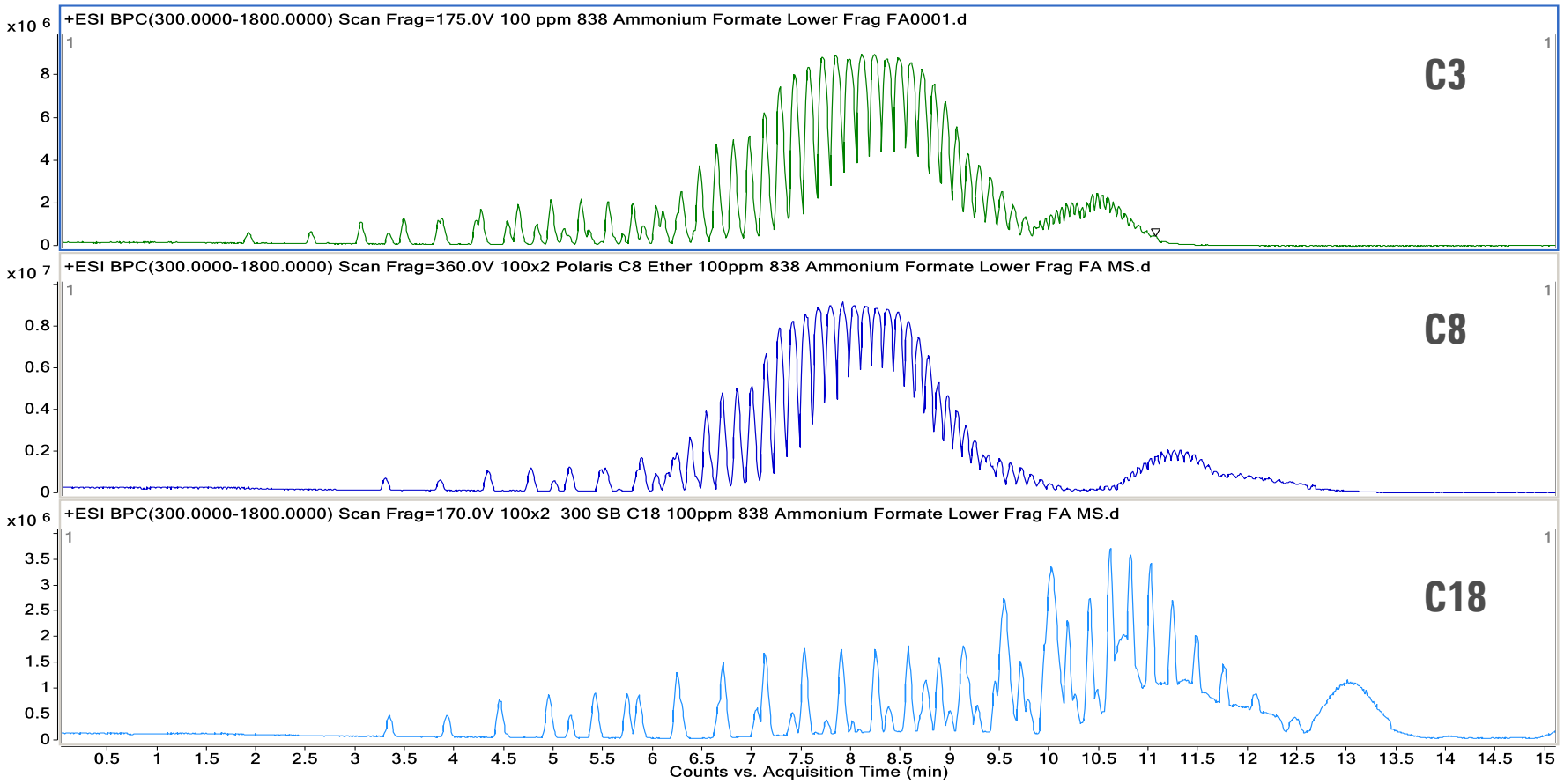
**Chromatography**
**Columns:** C18/C8/C3
**Organic Mobile Phase:** ACN, MeOH, ACN/IPA, MeOH/IPA
**Varied Buffers:** None, 0.1% Formic Acid, 2mM & 4mM $NH_4$Acetate

**Ionization Sources**
Jet Stream (ESI), APCI, Multimode

**MS-Instrument**
6530 / 6545 / 6550

# Comparing C3, C8, C18 Separations Using Same Buffers

## Base Peak Chromatograms

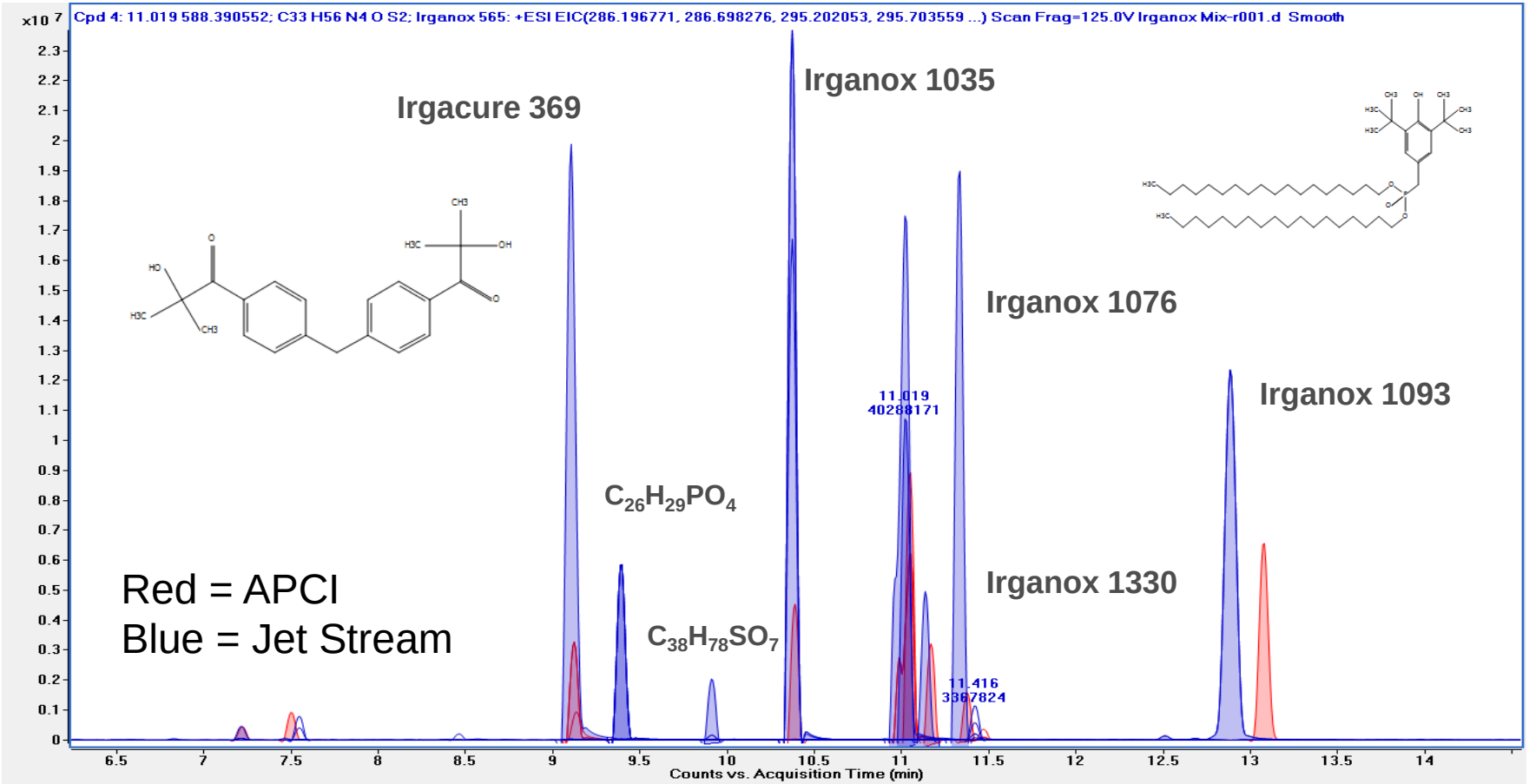



**Results: C3 Column Optimum for Higher Mass Extractables**

**Create orthogonality by using multiple chemistries**

# Ionization Modes: APCI vs Jet Stream ESI

## Examples: Irganox and Irgacure Mixture



# Method developoment for an ophthalmic drug product

## System Suitability Test Mix For Method Development

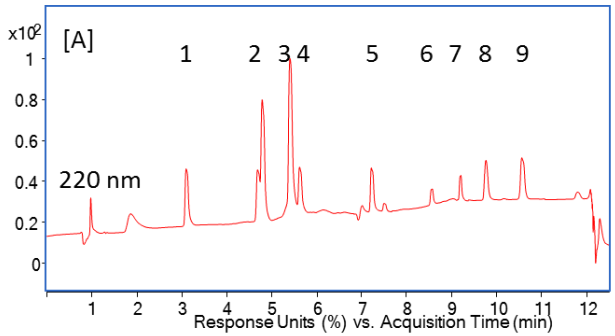
A list of plasticizers has been compiled after literature investigation.
The listed compounds have been selected according to their polarity.

| # | Analyte | CAS | Empirical Formula | Monoisotopic Mass |
|---|---|---|---|---|
| 1 | Ethyl paraben | 120-47-8 | $C_9H_{10}O_3$ | 166.063 |
| 2 | Irgacure 184 | 947-19-3 | $C_{13}H_{16}O_2$ | 204.115 |
| 3 | Irgacure 651 | 24650-2-8 | $C_{16}H_{16}O_3$ | 256.1099 |
| 4 | Dipropyl phthalate | 131-16-8 | $C_{14}H_{18}O_4$ | 250.1205 |
| 5 | 4-n-Octyl phenol | 1806-26-4 | $C_{14}H_{22}O$ | 206.1671 |
| 6 | Diethyl hexyl phthalate | 117-81-7 | $C_{24}H_{38}O_4$ | 390.277 |
| 7 | Irganox 1010 | 6683-19-8 | $C_{73}H_{108}O_{12}$ | 1176.7841 |
| 8 | Irganox 1076 | 2028-79-3 | $C_{35}H_{62}O_3$ | 530.4699 |
| 9 | Iragafos 168 | 31570-04-4 | $C_{42}H_{63}O_3P$ | 646.4515 |

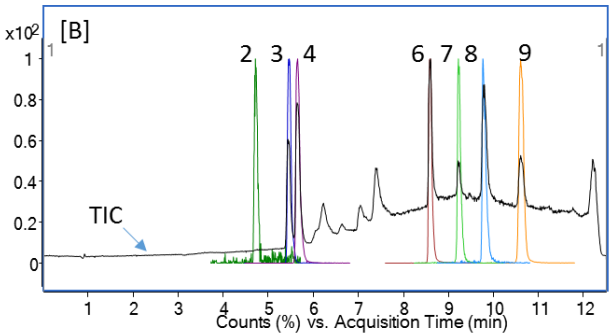
# Can I Detect All My Compounds ?

*Applying Positive & Negative ES Ionization Mode and UV Absorption*

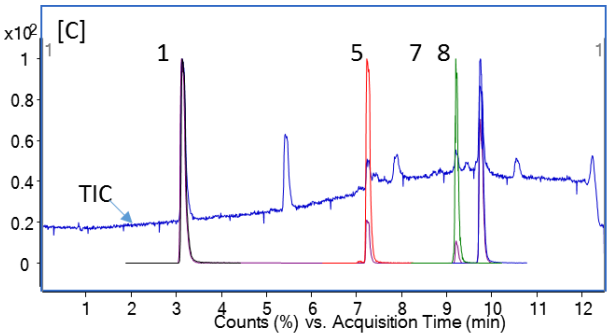
System suitability mix (at 50 ppb level)




UV absorption

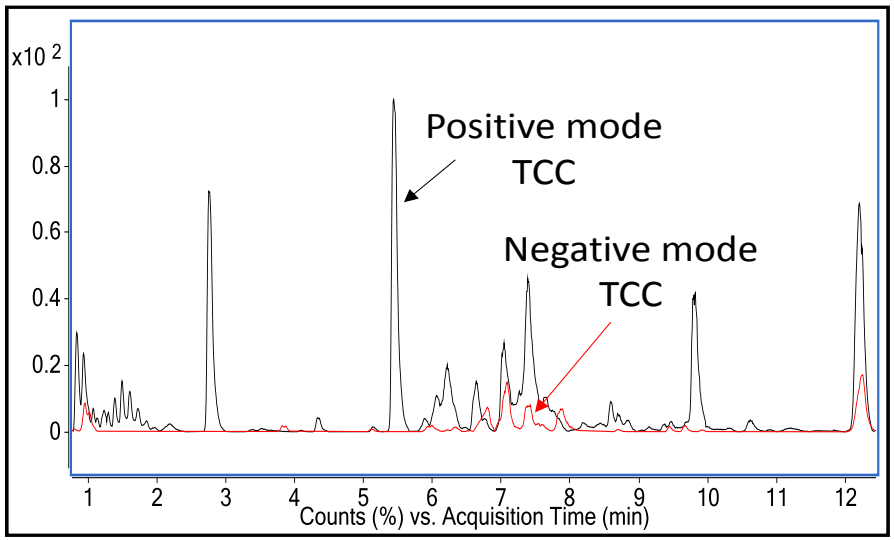



ESI pos; TIC & EIC




ESI neg; TIC & EIC

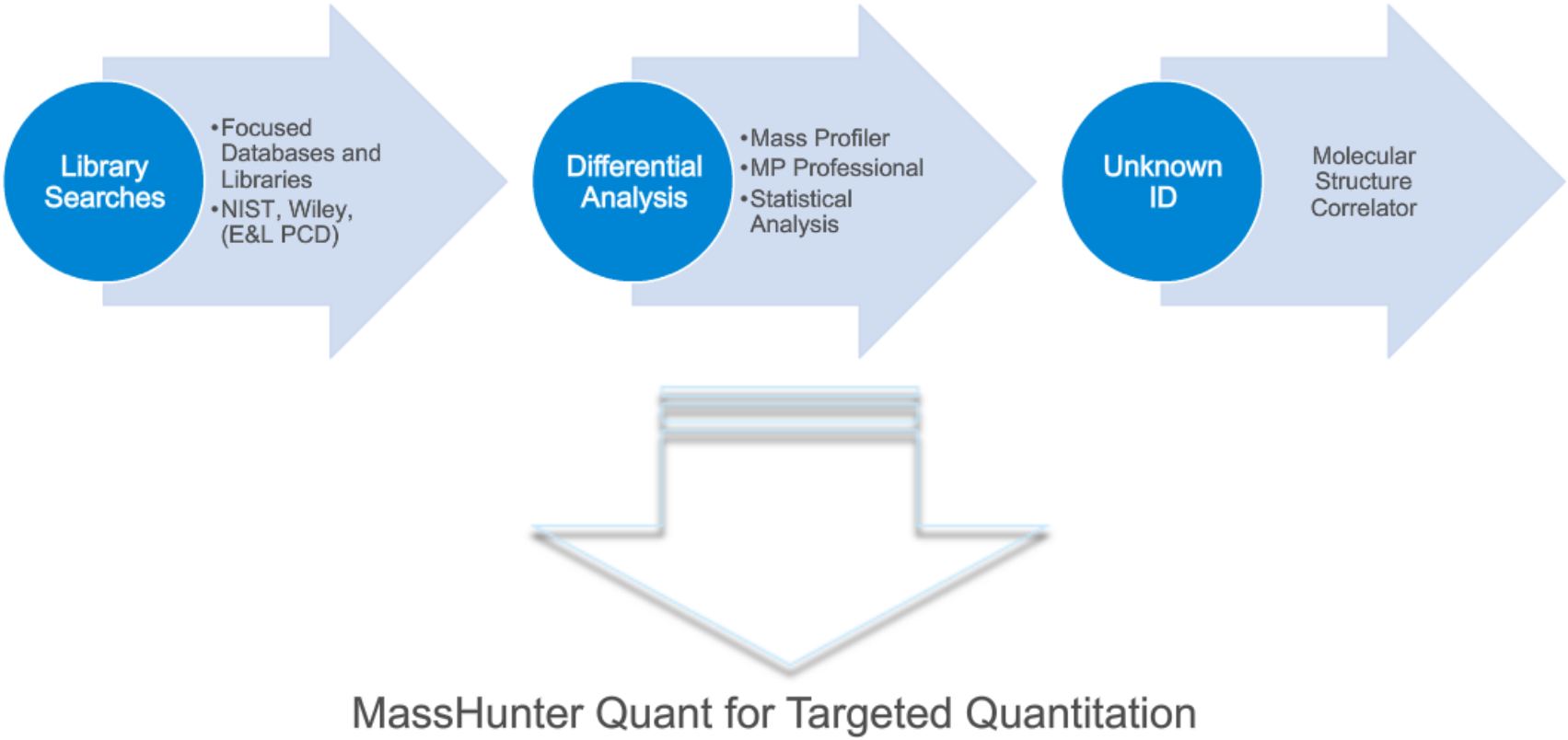
Ophthalmic bottle extract
Overlay of positive & negative TIC
Data acquisition in MS/MS mode



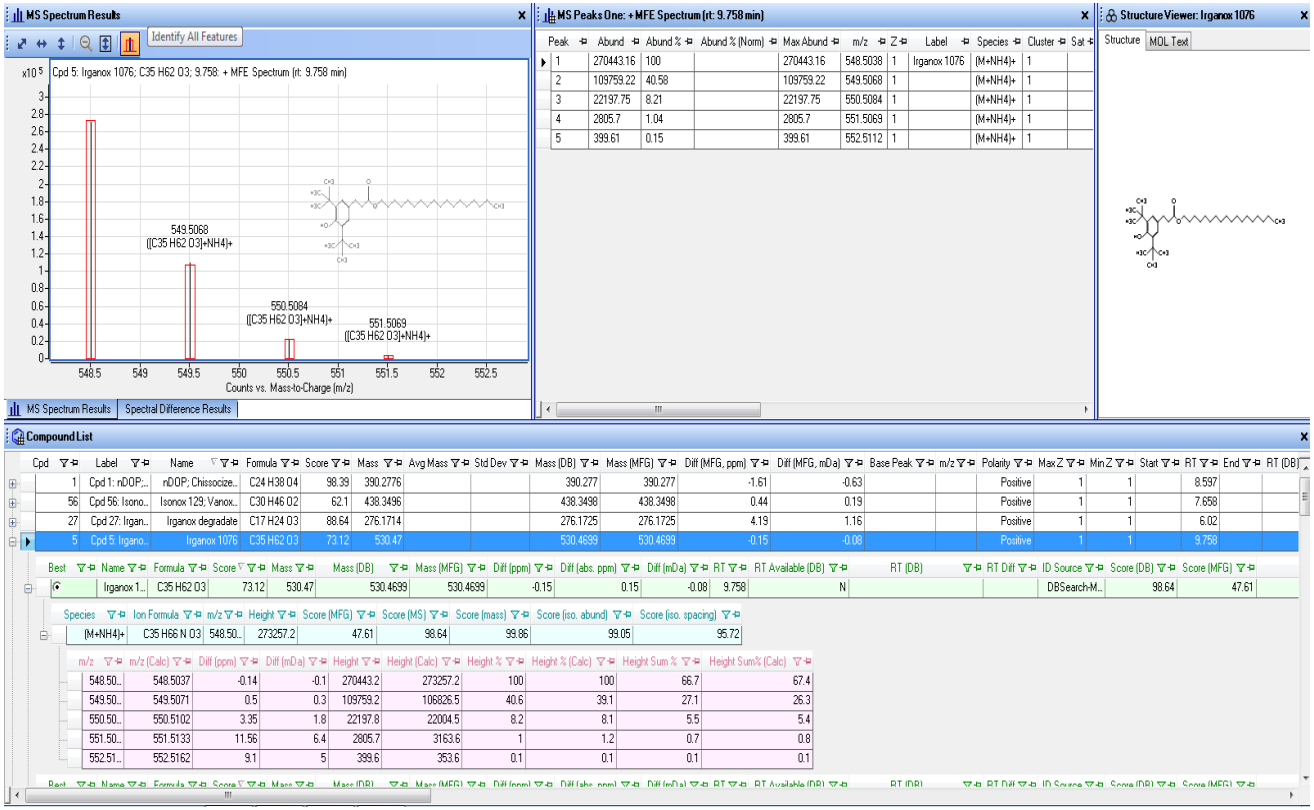
# MassHunter Software Tools

## Why intelligent data mining software matters….

Data mining is an essential step of the analytical workflow and as important as a successful chromatographic separation and detection of organic compounds



# Mass Profiler SW: Database Search & Formula Generation




Mass Profiler Software has been used to identify compounds by PCDL comparison
Unknown compounds have been identified by Molecular Structure Correlator Software

# MassHunter Mass Profiler Software (Rev. 7.0)

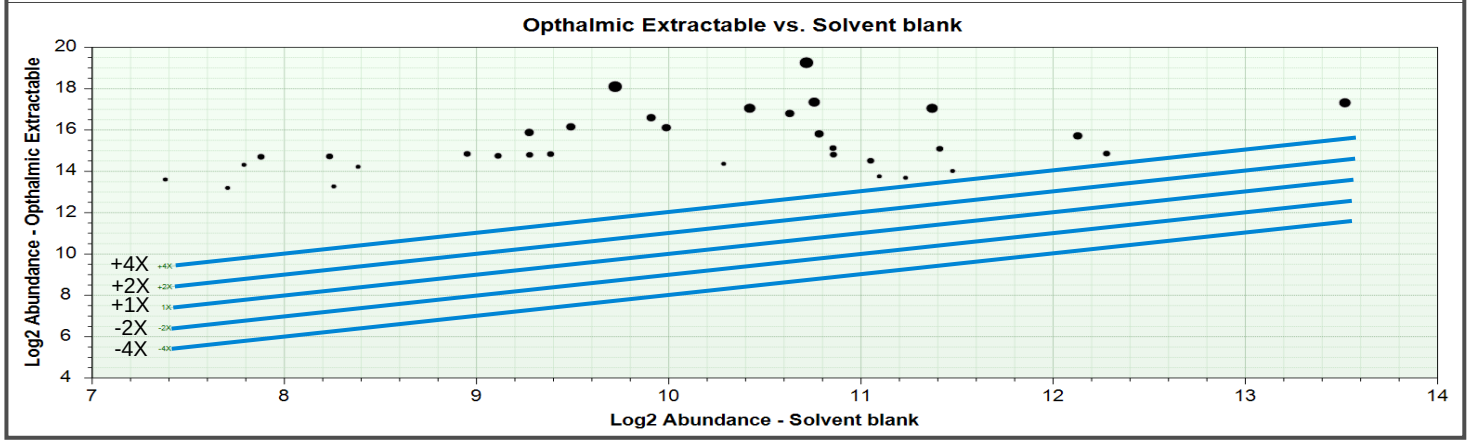
## Differential analysis between the sample and the solvent blank

52 features

| | | | | | Feature Summary | | | | | | | | | Opthalmic Extractable(3) | | | | | Solvent blank(3) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ID | Formula | Name | CAS | RT | SD | Mass | SD | Abundance | RSD | Freq. | Sat. | Mark | RT | Mass | Abundance | RSD | Freq. | RT | Mass | Abundance | RSD | Freq. |
| 1 | 30 | C23 H48 O6 | Tridecyl alcohol tetra(oxyethylene) ethanol | | 7.800 | 0.051 | 437.3669 | 0.0042 | 28469 | 1.22 | 4 | | | 7.775 | 437.3690 | 28469 | 0.03 | 3 | 7.877 | 437.3607 | 620 | 1.73 | |
| 2 | 3 | C12 H27 O3 P S2 | Thiophosphate | | 5.444 | 0.002 | 331.1433 | 0.0001 | 404558 | 0.00 | 3 | | | 5.444 | 331.1433 | 404558 | 0.01 | 3 | | | 0 | | |
| 3 | 18 | C18 H34 Na O3 | Sodium Ricinoleate | 5323-95-5 | 1.284 | 0.009 | 343.2210 | 0.0010 | 57651 | 0.04 | 6 | | | 1.291 | 343.2218 | 57651 | 0.00 | 3 | 1.277 | 343.2202 | 1761 | 0.06 | |
| 4 | 4 | C9 H8 O2 | PPD | 579-07-7 | 5.438 | 0.009 | 148.0517 | 0.0010 | 280418 | 0.08 | 6 | | | 5.444 | 148.0525 | 280418 | 0.01 | 3 | 5.431 | 148.0508 | 844 | 0.12 | |
| 5 | 37 | C28 H43 N | Irganox 5057 | 15721-78-5 | 7.765 | 0.030 | 393.3406 | 0.0025 | 21085 | 0.04 | 6 | | | 7.777 | 393.3426 | 21085 | 0.03 | 3 | 7.753 | 393.3387 | 1248 | 0.05 | |
| 6 | 40 | C18 H21 O3 P | Ethyl(2,4,6-trimethylbenzoyl)- phenylphosphinate | 84434-11-7 | 2.190 | 0.002 | 333.1490 | 0.0001 | 18460 | 0.02 | 3 | | | 2.190 | 333.1490 | 18460 | 0.03 | 3 | | | 0 | | |
| 7 | 2 | C18 H18 O5 | Ethanol, 2,2'-oxybis-, 1,1'-dibenzoate | 120-55-8 | 5.444 | 0.004 | 314.1174 | 0.0006 | 625441 | 0.11 | 6 | | | 5.444 | 314.1174 | 625441 | 0.00 | 3 | 5.443 | 314.1173 | 1682 | 0.15 | |
| 8 | 5 | C7 H12 N | Cyclohexane, isocyano- | 931-53-3 | 2.760 | 0.003 | 148.0529 | 0.0001 | 273503 | 0.01 | 3 | | | 2.760 | 148.0529 | 273503 | 0.01 | 3 | | | 0 | | |
| 9 | 9 | C6 H14 N3 O5 | | | 0.817 | 0.009 | 208.0948 | 0.0012 | 135663 | 0.12 | 6 | | | 0.822 | 208.0937 | 135663 | 0.00 | 3 | 0.811 | 208.0958 | 2647 | 0.17 | |
| 10 | 10 | C15 H31 N8 O4 | | | 1.382 | 0.008 | 387.2466 | 0.0007 | 114227 | 0.02 | 6 | | | 1.385 | 387.2471 | 114227 | 0.00 | 3 | 1.379 | 387.2461 | 1584 | 0.02 | |
| 11 | 11 | C8 H21 N4 O6 | | | 0.827 | 0.005 | 269.1459 | 0.0009 | 99120 | 0.15 | 6 | | | 0.830 | 269.1459 | 99120 | 0.02 | 3 | 0.824 | 269.1459 | 961 | 0.21 | |
| 12 | 12 | C36 H32 N3 O9 | | | 5.444 | 0.002 | 650.2138 | 0.0001 | 97335 | 0.00 | 3 | | | 5.444 | 650.2138 | 97335 | 0.00 | 3 | | | 0 | | |
| 13 | 13 | C21 H43 N8 O7 | | | 1.725 | 0.005 | 519.3239 | 0.0020 | 72831 | 0.07 | 6 | | | 1.726 | 519.3257 | 72831 | 0.01 | 3 | 1.723 | 519.3222 | 719 | 0.10 | |

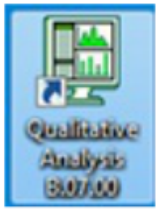
Plot mode: Avg. feature / Indiv. samples | Graph data: All samples | Colored by: Feature | Visibility criterion: None | Visibility threshold

Mass vs. RT | Log2 Ratio vs. RT | Log/Log Abund. | Unique to Opthalmic Extractable | Unique to Solvent blank

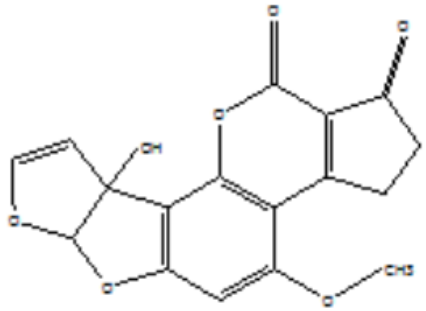



Mass Profiler Software supports the statistical comparison of data sets.
After applying a cut off filter of >3,000 and a >4-fold change (abundance by height) and abundance, **66 compounds** (positive and negative ionization modes) have been displayed for the bottle extract

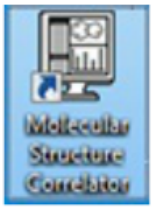
# Molecular Structure Correlator (MSC) Workflow

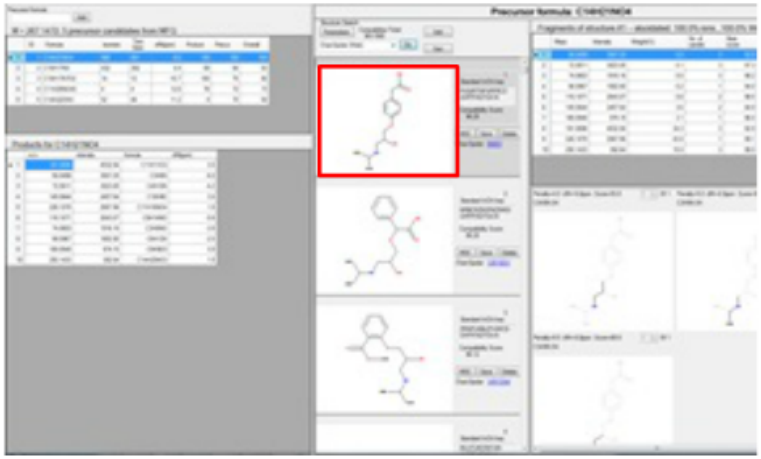



MS/MS spectra in
CEF file



Structures from PCD/L or
ChemSpider, PubChem, etc



Confirmation of structure based on LC or GC MS/MS data—**higher confidence identifications!**

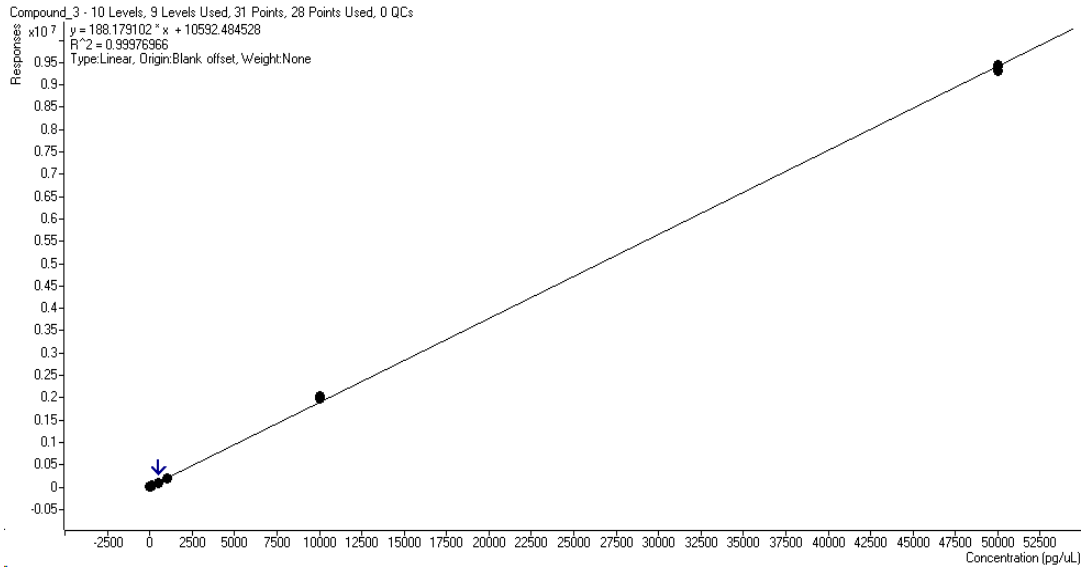
# Results: List of identified compounds by PCDL

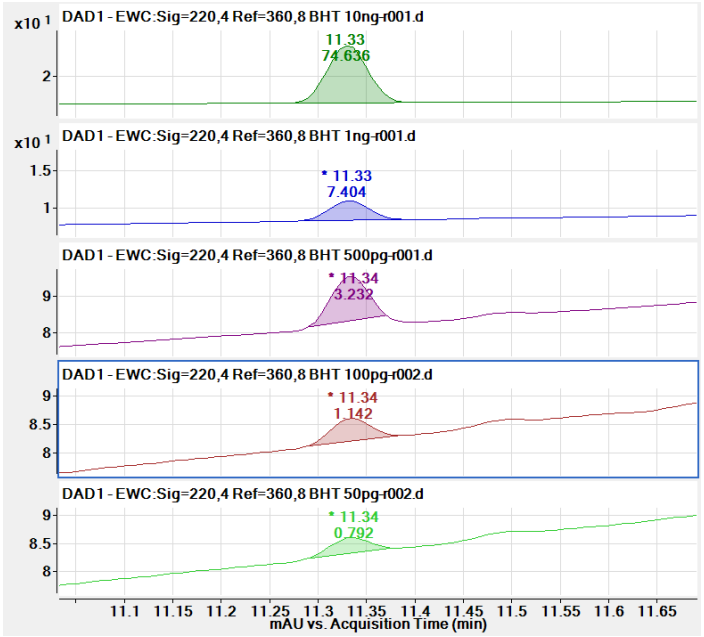
| Extractables identified by database |
| --- |
| diethylene glycol dibenzoate |
| tridecyl alcohol |
| sodium ricinoleate |
| irganox 5057 |
| ethyl(2,4,6-trimethylbenzoyl)-phenylphosphinate |
| isocyano cyclohexane |
| degradant of irganox |
| hexadecanoic palmitic acid |
| Dioctyl Adipate |
| Methyl-2-benzoylbenzoate |
| Irgacure 907 |
| Erucamide |
| Diisononyl phthalate |
| Dioctyl phthalate |
| Phthalic anhydride |
| Hexyl Amine |
| Ionox 100 |
| Glycerol dilaurate |
| Diisodecyl Phthalate |

| Leachables identified by database |
| --- |
| Diisononyl phthalate |
| Dioctyl phthalate |
| Phthalic anhydride |
| Methyl-2-benzoylbenzoate |
| Irgacure 907 |
| Hexyl Amine |
| Ionox 100 |
| Erucamide |
| Glycerol dilaurate |
| Diisodecyl Phthalate |
| Myristyl dimethylamine oxide |
| Acetic acid, propyl ester |

# Semi-quantitative compound determination

Required sensitivity for compound quantification




Quantitation of DEHP $C_{24}H_{38}O_4$
1 pg/µL to 50 ng/µL by Jet Stream ESI

UV Detection of BHT
(Butylated Hydroxy Toluene)
at 220 nm limit around 50 ppb

# Results : Semi-Quantification of identified E&Ls

| Leachables | ppm ±30% |
|---|---|
| Sansocizer DINP | 1.41 ±0.43 |
| N-DOP | 2.48 ± 0.74 |
| Phthalic anhydride | 0.14 ± 0.04 |
| Methyl-2-benzoylbenzoate | 0.11 ± 0.03 |
| Irgacure 907 | 0.02 ± 0.005 |
| Hexyl Amine | 0.04 ± 0.01 |
| Ionox 100 | 0.03 ± 0.01 |
| Erucamide | 1.68 ± 0.50 |
| Glycerol dilaurate | 0.08 ± 0.02 |
| 1,2-Benzenedicarboxylic acid, 1,2-bis(8-methylnonyl)ester | 0.16 ± 0.05 |
| Myristyl dimethylamine oxide | 0.0009 ± 0.0003 |
| Acetic acid, propyl ester | 0.10 ± 0.03 |


THANK
YOU

# Acknowledgements


| | |
|---|---|
| David Weil | Senior Applications Scientist |
| Syed Salman Lateef | Pharma Application Scientist |
| Diana Wong | GC/MS Applications Scientist |
| Roger Firor | Senior GC/MS Applications Scientist |
| Anthony Macherone | Senior GC/MS Applications Scientist |
| Amir Liba | US SPSD AE Manager |

# Appendix

- Posters published in 2015
- References

# Posters for ASMS 2015 on E/L

## Extractable/Leachable Compound Analysis in Pharmaceutical Packaging Using GC/MSD System

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ASMS 2015
Poster # ThP 197

Agilent Technologies

### Background

**Extractables** are chemical compounds that can be extracted out of packaging material when exposed to high-temperatures or appropriate solvents. Sources of extractables include additives, impurities, polymer component and degradation products during processing, storage, and sterilization.

**Leachables** are chemical compounds from packaging material that migrate into the drug product under typical process or accelerated storage conditions.

**Compound migration** involves the correlation of extractables to leachables.


Leachables are generally a **subset of extractables**

New leachables may be identified due to the extractable-drug product interaction

US FDA ranked drug delivery types in several risk categories

**Risk associated with various construct types**

| Concern based on Route of Administration | Likelihood of interaction between Packaging Component and Dosage Form | | |
|---|---|---|---|
| | High | Medium | Low |
| Highest | Inhalation aerosols/solution<br>Injection suspensions<br>Injectable suspensions | Sterile powders<br>Injection powders<br>Inhalation powders | |
| High | Ophthalmic solutions<br>Transdermal patches<br>Nasal aerosols/sprays | | |
| Low | Topical solutions/suspensions<br>Topical and lingual aerosols<br>Oral solutions/suspensions | Topical powders<br>Oral powders | Oral tablets<br>Oral hard capsules<br>Oral gel 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

**Industry guidelines and work-group assessments provide basis for the analysis of extractable/leachable testing in drug delivery and medical devices**

- USP<1663>" Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems (*Revised)
- US<1664>" Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems (*Revised)
- ISO10993 Biological Evaluation of Medical Devices
- Material Test: USP<661>, USP <661.1>, JP<61>, EP 3.2.2.1
- Elastomer Test: EP3.2.9, USP<381>, JP<59>, ISO 8871-1
- PQRI Parenteral and Ophthalmic Drug Product (PODP) and others

**Analytical Technologies**

The complementation of several analytical technologies is required to detect all extractables. Our investigation focuses on GC/MS to complement LC/MS and other instrumentations.

| Analytical Technology | Extractables detected |
|---|---|
| Headspace GC/MSD | volatile organic compound, high migration potential species (e.g. misc. adhesives, glue, processing solvent) |
| GC/MS | semi-volatile organic compounds, residual monomers, antioxidants, plasticizers, antistatic agents, clarifying agents, preservatives, PCAs, slip agents |
| LC-UV-MS | non-volatile organic compounds, large oligomers, large antioxidants, thermally labile compounds |
| Ion Chromatography | bromide, chloride, fluoride in elastomers |
| Chemiluminescence Detection | nitrosamines |

### Experimental

Two 5977 Series GC/MSD systems were utilized.

**Headspace Sampling GC/MS.** Packaging material were cut into 1.0-cm² pieces, placed into 10-mL headspace vial, and purged with $N_2$ before analysis. Equilibration temperatures investigated were 65, 100, 150, 200, 250, and 275 °C.

| Instrument | 7697A Headspace Sampler and 7890A GC coupled with 5977A MSD |
|---|---|
| MSD | SI mode, Scan: 15 – 700 amu typical, Gain=1 |
| Liner | 3.75-mm ultra inert, straight tapered (p/n 5190-4049) |
| Column | Agilent HP-5ms UI, 30m x 0.25mm, 0.5 µm (p/n 190915-433UI) |

**Multimode Inlet (MMI) GC/MS** configured in solvent vent mode. Packaging material or liquid drug product were extracted with solvent by sonication for 8-8 hrs. The solvents utilized were dichloromethane (DCM), hexane, ethanol, and acetone. Organic layer was analyzed.

| Instrument | 7693A Automatic Liquid Sampler and 7890A GC with 5977A MSD |
|---|---|
| MSD | SI mode, Scan: 20-700 amu typical, Gain=1 |
| Liner | 4-mm i.d. ultra inert (p/n 5190-3162) |
| Column | Agilent HP-5ms UI, 30m x 0.25mm, 0.25 µm (p/n 190915-433UI) |

**Compound Identification.** Compounds were identified using MSD Chemstation Data Analysis F.01.01, AMDIS 2.72, and MassHunter Unknowns B.07.00. Compounds with a mass spectral match ≥ 80 with the NIST14 Library were considered.

### References

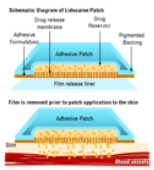
[1] D. Wong and R. Firor "Analysis of Extractable/Leachable Compounds From Plastic Intravenous Bag Sets Using GC/MSD Systems" Agilent Technologies Publication: 5991-5616

[2] D. Wong and R. Firor "Analysis of Extractable/Leachable Compounds From Transdermal Patches Using GC/MSD Systems" Agilent Technologies Publication: 5991-5605

[3] D. Wong and R. Firor "Analysis of Extractable/Leachable Compounds From Generic Liquid Drug Formulation Using GC/MSD Systems" Agilent Technologies Publication: 5991-5632

### Transdermal Patches

Transdermal drug delivery is a technology used to incorporate the active drug ingredient into the circulatory system through the skin. Patches are desirable because drug dosage can be controlled through the skin over a period of time. In this study, extractables were investigated in a lidocaine patch that was manufactured by a leading pharmaceutical company

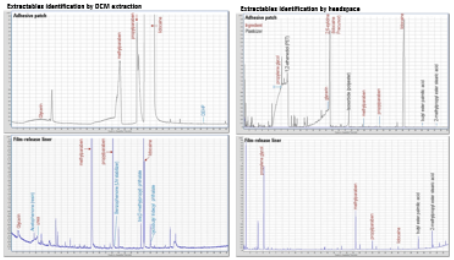



**MMI GC/MS**

Different plasticizers were identified by extraction with different solvents. DEHP and phthalates were examples of extractables characterized by dichloromethane (DCM) extraction. Terephthalates were identified using hexane extraction.

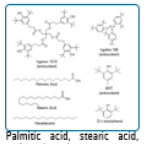
**Headspace GC/MS**

Plasticizers were identified using high-temperature headspace sampling. Poor chromatographic performance of lidocaine and inactive ingredient may be attributed to the concentration and column overload.




### Liquid Drug Formulation

In this study, leachables were investigated in a generic drug suspension provided for testing. Compounds of interest for investigation were the following:



Palmitic acid, stearic acid, and hexadecane were identified using MMI GC/MS

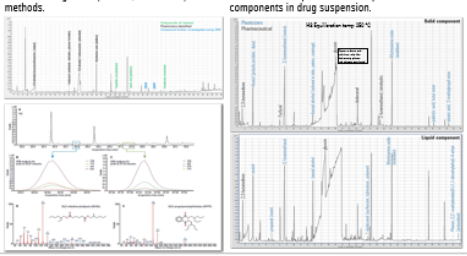
Irganox 1010 is a large molecule that is more suitable for LC/MS analysis

**MMI GC/MS**

DEHA and DPPP were identified from liquid extracts using SCAN(NIST14,AMDIS2.72)and SIM methods.

**Headspace GC/MS**

Plasticizers and pharmaceutical products identified in components in drug suspension.



### IV Bag System

PVC bags contain an average of 20-40% DEHP to impart flexibility. DEHP is a potentially toxic plasticizer with the potential to leach to contact solution when heated or agitated. The infusion form of the drug, cyclosporine contains a large amount of non-ionic surfactant, Kolliphor EL that can cause the stripping of DEHP from IV bags. In this study, extractables and leachables were investigated in an expired IV bag, heated IV bag, and IV solutions used in medical facilities.

**Expired IV Bag Set (MMI GC/MS)**

DEHA and DEHP were two compounds shown to have migrated due to extended storage and further investigated by SIM GC/MS

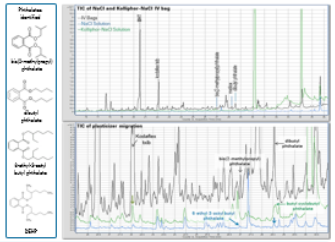

Extractables identified in expired IV bags: hexadecane (1), antioxidant butylated hydroxytoluene (2), Iodaflex TX12 (3), UV-stabilizer benzophenone (4), isobutyl nonyl phthalate (5), 7,9-di-tertbutyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (6), 2-Hydroxybenzenethanole, an accelerator for rubber vulcanization (7), Palmitic acid (8), isopropyl palmitate (9), Palmitic acid, butyl ester (10), 2-Ethylhexyl trans-4-methoxycinnamate (11), Benzyl butyl phthalate (12), DEHA (13), dioctyl-3-(4) phthalate (14), DEHP (15), antioxidant Irgafos 168 (16), Tris(2,4-di-tert-butylphenyl) phosphate (17)

**IV Tubing (Headspace GC/MS)**

DEHP and benzophenone were two potentially toxic additives identified.

**Heated IV Bag Set and Effects of Kolliphor EL (MMI GC/MS)**

Phthalate migration was observed due to extended heat. Kolliphor enhanced leaching of Kodaflex and phthalates with similar fragmentation pattern as DEHP. GC/QTOF analysis is necessary for confirmation.




### Conclusions

**GC/MS for extractables/leachables testing**

The complementation of headspace GC/MS and MMI GC/MS allowed for the comprehensive analysis of extractables and leachables in pharmaceutical packaging systems. The advantages of using headspace GC/MS is minimal sample preparation and a close controlled environment. MMI GC/MS in solvent vent mode allows for the identification of low level leachables by large volume injections.

**IV bags, Transdermal patches, and Drug formulation**

Plasticizers and potentially toxic additives (such as DEHP) were leachables identified in plastic medical devices. The limit of detection is one of the biggest topics in extractables/leachables analysis. The Product Quality Research Institute (PQRI) is a working group recognized by the FDA that provided definitions to identify the limit of quantification. The Safety Concern Threshold (SCT) of below 0.15 µg/day is a leachables threshold that would present negligible safety concern from possible carcinogenic to non-carcinogenic toxic effects. Although manufacturer stated that devices are DEHP-free and PVC-free, this suggest that levels of DEHP are below SCT or alternative plasticizers were utilized. GC/MS is the first step. Accurate mass is necessary for confirmation.

# Posters for ASMS 2015 on E/L

## Enhanced Detection of Oligomers, Polymers and Additives Using Ion Mobility Mass Spectrometry and new Data Mining and Differential Analysis Tools

David A. Weil [1] ; Caroline S. Chu [2]
[1] Agilent Technologies, Schaumburg, IL; [2] Agilent Technologies, Inc., Santa Clara, CA

ASMS 2015
ThP 630

Agilent Technologies

### Introduction

The analysis of polymeric/oligomer materials using mass spectrometry has been around since the early 80's using methods such as Field Desorption (FD), Laser Desorption (LD), Matrix-Assisted Laser Desorption Ionization (MALDI) and LC/MS interfaced with a various API methods (APCI, ESI, DESI, ASAP). The complex nature of these materials: (polydisperse, multiple monomers, end groups, degree of branching, MW ranges); challenges determining what stationary and mobile phase, gradients to use. In addition, the polymer mass spectral data with overlapping series of multiply charged species and adducts make data manual data analysis especially challenging. The low electric field ion mobility mass spectrometer (IMS) utilizing separation in the fourth dimension provided a means to simplify the analysis of several classes of structural polymers and also enhanced the detection polymer additives and dyes extracted from a complex mixture. In combination with a new 4D data mining algorithm and differential analysis software to find unique components present in 'GOOD' and 'BAD' groups of samples. Ion mobility provides a novel means to identify degradation, extractable, leachable impurities in pharmaceutical and consumer products.

### Structural Information from Drift

Ion mobility mass spectrometry provides insight into the confirmation of metabolites, proteins, glycan, lipids and carbohydrates. For polymers, the adducts used for ionization (H, $NH_4$, Na, Cs), chain length and degree of branching can lead to confirmation changes as reported by many research groups[1-4]. Star shaped poly(ethylene glycol) PEG polymers are the simplest class of branched polymers with a general structure consisting of several linear chains connected to a central core. These compounds have unique rheological properties that make them useful in drug delivery, thermoplastics and biologics. Polymer standards: hydroxyl terminated PEG 2K 4-arm and 10K 8-arm (Figure 1) and Phenyl T and MQ Siloxane resins were analyzed in this study. Confirmation changes were observed as a function of chain length and adducts for these standards.

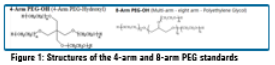
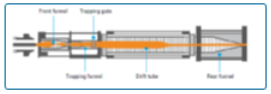


Figure 1: Structures of the 4-arm and 8-arm PEG standards

The extractable/leachable packaging materials extracts samples were prepared using THF solvent. The Beclomethasone dipropionate impurity profiling sample was obtained from a commercial source without additional sample preparation.

### Experimental Conditions

The Agilent 6560 uniform low field ion mobility mass spectrometer (drift ion schematic, Figure 2) interfaced with an Agilent 1290 high pressure liquid chromatograph was used for all experiments. Reverse phase separations were carried out using Agilent Stable.Bond.C8 and C18: 2.1 mm x 150 mm, 1.8 µm columns. The mobile phases of water and acetonitrile with 0.1% formic acid was used starting at 1.0% B and ramping to 99% B in 15.0 min with a 5 min final hold time. The flow rates were varied from 0.450 to 1.0 mL/min. The thermally assisted electrospray ionization source operated in positive-ion mode was used for all the experiments. For comparison, data was collected in both QTOF and IM modes.



### Polymer Trend Lines

A major objective in mass spectrometry of oligomeric and polymer materials is determining the chemical compositions of the monomers and end-groups, in addition obtaining information on chain length and molecular weight. Industrial grade materials are rarely "Pure" but typically complex mixtures with monomers, end groups, structures (linear, branched, cyclic). As John McLean's research group[5] and many others have shown that biomolecules of similar class with follow drift time trend lines. Polymers and oligomers of the same charge state, adduct, end-group composition also follow the trend lines and mass spectral data can be extracted along the trend lines and unique regions using IM Browser software as shown in Figure 3.

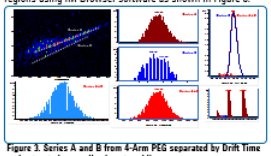


Figure 3. Series A and B from 4-Arm PEG separated by Drift Time and extracted manually along trend line.

### Structure/Confirmation Examples

Ion mobility drift heat map 8-arm PEG sample in Figure 4 clearly shows changes in drift time (bends) as a function of molecular weight and chain length. The low molecular weight singly charge linear PEG (shown in top spectrum) clearly separates from the higher molecular weight +3 and +4 charge states branched species (show on right).

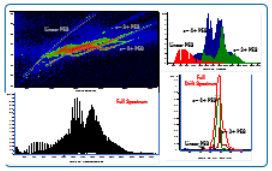


Figure 4: Drift Heat Map showing change in confirmation as a function of charge state and molecular weight 8 arm PEG sample

### Data Analysis 4D Feature Finding

Manual extraction of mass spectral data (with drift time) is useful but clearly is very time consuming, not automated, may lead to biased reporting and not applicable to applications like proteomics, metabolomics and impurity profiling. A new 4-dimenstional (4D) unsupervised data mining algorithm was developed that is based on the 3D Molecular Feature Extraction algorithm, which separates ions as a function of mass/charge, retention time, abundance and drift time[6]. Ions from the same isotope distribution are grouped together and while the adducts are grouped separately with drift time. More details about the algorithm is found in Poster MP168[7]. Results from LCMS separation of the 4 arm PEG sample is shown in Figure 5.

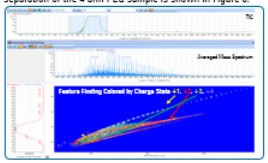
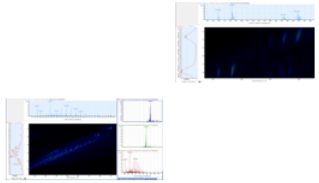


Figure 5: 4D MFE results for LCMS C8 Seperation of 4-arm 2K PEG standard

### Differential Analysis Mass Profiler

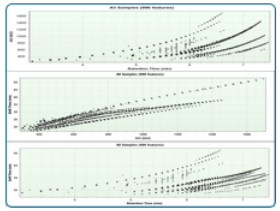
For Extractable Leachable experiments and many others the challenge is to reliably find differences between "Good" and "Bad" lots of sample, at low concentration, with minimum false positive and negative differences. The new 4D molecular feature finding software is integrated into a two sample group comparison with post-alignment filter settings including a feature quality score and an abundance ratio filter.

Either Add Silicone Example showing multiple drift times



### Graphical Display Drift Data

Ion Mobility data can be displayed using the Mass Profiler program by: Figure 7 **A)** m/z versus RT; **B)** Drift Time versus m/z and C) Drift Time versus RT. For 4-Arm PEG we clearly see the presence of minor impurities and drift time changes around RT 6.5 minutes and *m/z* 600-1000.



Figures 7A-D: Graphical Display 4-Arm PEG as function of Drift time, RT, m/z

### Results and Discussion

**Extractable Leachable Examples**

A major challenge in Extractable Leachable analysis is to identify the presence of low level potentially toxic polymer additives, impurities and dyes in the presence of oligomeric surfactants and polymers. Polymers of similar charge state, adducts, end-groups and composition will follow the same trend lines and mass spectral data can be extracted along those trend lines but additives will be single components and will have different drift times. Mass Profiler was used to identify unique dyes and additives extracted from two different packing materials. A custom accurate mass Extractable Leachable accurate mass database was used to identify copper phthalocyanine $C_{32}H_{16}N_8Cu$.

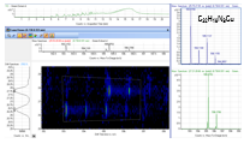


Figure 7: Separation of Possible Copper Phthalocyanine from other extractable using drift and m/z.

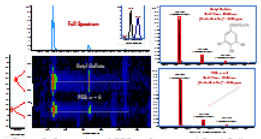


Figure 8: Separation of PEG surfactant and Octyl Gallate antioxidant extractable from expired Claritan medication.

**Impurity Profiling Example**

Impurity profiling experiments generally rely on extensive LC method development and/or 2D LC to optimize the separation of low concentration degradation products from the Active Pharmaceutical Ingredient (API). Using Ion Mobility mass spectrometry, two degradation products of Beclomethasone dipropionate ($C_{28}H_{37}ClO_7$), that overlap chromatographically and have overlapping isotope patterns were separated by drift time. Beclomethasone ($C_{22}H_{29}ClO_5$), and 21-Dehydro Beclomethasone ($C_{22}H_{27}ClO_5$) previously not detected using standards LCMS conditions Figure 9.

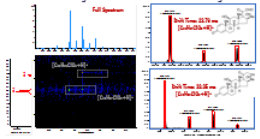


Figure 9: Separation of Beclomethasone ($C_{22}H_{29}ClO_5$), and 21-Dehydro Beclomethasone ($C_{22}H_{27}ClO_5$) by drift time

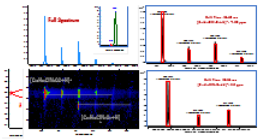


Figure 10: LC/MS Co-elution of degradation products of Claritan $C_{22}H_{32}N_2O_3Cl$ and $C_{22}H_{32}N_2O_3Cl$

### Conclusions

Ion Mobility in combination with high resolution accurate mass, and isotope fidelity using UHPLC separation and data mining with a new 4-D molecular feature extraction (MFE) provides enhanced detection and identification of polymers, oligomers and additives (dyes, antioxidants, pigments) from complex mixtures. Resolved overlapping isotopic patterns.

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3. Scott M. Grayson et.al. Macromolecules, 2011, 44, pp 6915–6918
4. Edwin De Pauw et. al.; Anal. Chem., 2014, 86 (19), pp 9693–9700
5. John A. McLean et. al.; Anal. Chem., 2014, 86, pp 2107–2116
6. Frank Kuhlmann et al. ASMS 2014 Poster ThP 547
7. Frank Kuhlmann et al. ASMS 2015 Poster MP 168
8. Srividya Kailasam et al. ASMS 2015 Poster ThP 193

# Poster for AAPS 2015 on E/L

AAPS 2015
Poster # R6198

## A Sensitive Quadrupole Time of Flight Mass Spectrometric Method for Detection and Accurate Identification of Extractables and Leachables

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Agilent Technologies

### Introduction

Drug substances and products can be contaminated by chemicals migrating from primary and secondary packaging materials. Due to the potential impact of these impurities on patient health, the US FDA has issued guidance to the industry on container closure systems for packaging human drugs and biologics. Profiling compounds that can be extracted from the packaging materials, or that have leached (E&L compounds) into a drug substance or product is a complex task due to:

- Wide range of materials used for the construction of primary and secondary containers
- Diversity of physico-chemical properties of the extracted and leached impurities
- Varying concentration of compounds in samples ranging from ng to µg amounts
- Challenges associated with the detection of these compounds in a wide range of different matrices

To overcome some of these analytical challenges, a sensitive high resolution LC/MS method operating in both positive and negative mode has been developed. In addition we have used a statistical approach to detecting and identifying E&L compounds. In this study, a generic method was developed for detecting a set of analytes belonging to different classes of compounds such as plasticizers, photo-initiators, stabilizers, antioxidants, and others. This method was then used for the analysis of E&L compounds in an ophthalmic drug preparation and data processed using a rigorous statistical approach.

**Workflow:**

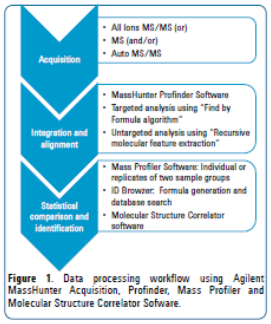


**Figure 1.** Data processing workflow using Agilent MassHunter Acquisition, Profinder, Mass Profiler and Molecular Structure Correlator Sofware.

### Experimental

**Methods**
Stock solutions of the standards; ethyl paraben(1), irgacure 184(2), irgacure 651(3), dipropyl phthalate(4), 4,n-octyl phenol(5), diethyl hexyl phthalate(6), irganox 1010(7), irganox 1076(8) and iragafos 168(9), were prepared in isopropanol. A mix of stock solutions diluted in water containing 10 mM ammonium acetate to produce an aqueous mixture containing 1 µg/mL of each analyte. The reverse phase UHPLC method and mass spectral parameters were optimized to achieve good chromatographic separation and sensitive analyte detection.

Extractable sample
An ophthalmic medicine bottle, purchase in Bangalore, was washed with water and filled with extraction solvent 1:1 methanol:water. This was incubated in an oven at 55 °C for 72 hours. An aliquot (200 µL) was taken for analysis. The blank used in this study was the extraction solvent.

Spiked sample for method development and system performance test:
10 µL of the standard spike solution (1 µg/mL) was added to 190 µL of the extracted sample.

Leachable stressed sample
The ophthalmic drug formulation along with its bottle, was heated as is at 60°C for 24 hrs and analyzed as is. The control in this study was the ophthalmic drug formulation bottle stored at recommended conditions (non-stressed).

Instrumentation:
Agilent 1290 Infinity Binary LC System and an Agilent Q-TOF G6530A System with a Dual Spray Jet Stream Source was used. The LC and MS conditions used in these analyses are shown in Table 1. The source conditions were optimized to enable sensitive detection of extractable and leachable compounds. The LC/MS analysis was performed in both positive and negative modes.

**Table 1:** LC and MS instrument parameters

| | | |
|---|---|---|
| **LC conditions** | | |
| Column | Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 X 100 mm, 1.8 µm (p/n 959758-306) | |
| Column temperature | 50 °C | |
| Mobile phase A | 100 mg/L Ammonium acetate in water | |
| Mobile phase B | Methanol | |
| Flow rate | 0.5 mL/min | |
| Max Light 60 mm flow cell | 220 nm | |
| Gradient | Time (min) | % Methanol |
| | 0 | 40 |
| | 8 | 100 |
| | 11 | 100 |
| | Stop | 11 min |
| | Post | 1.5 min |
| **MS conditions** | | |
| Ionization mode | Dual Spray AJS-ESI | |
| Drying Gas | 10 L/min @ 150 °C | |
| Nebulizer pressure | 30 Psi | |
| Sheath gas | 11 L/min @ 200 °C | |
| Capillary voltage | 3500 V | |
| Nozzle voltage | 300 V | |
| Fragmentor | 146 V | |

### Results and Discussion

**Analysis of Standards**
A spiked sample was used for method development and system suitability test. Figure 2 shows the extracted ion chromatogram from the spiked sample, as detected at UV 220 nm 2A, positive 2B and negative 2C MS ionization mode. Many standards were found in positive mode, although a few showed better response in negative mode. The LC analysis with UV detection was performed for performance evaluation, however the MS data are discussed due to requirement for analytical specificity and selectivity.

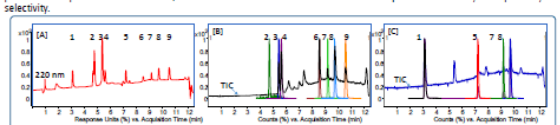


Figure 2. Performance suitability mix analysis using UV at 220 nm (A), accurate mass positive mode (B) and accurate mass negative mode (C). The standards numbering are listed in the method section.

**Extractables**
The chromatogram from the high resolution LC/MS extractable sample analysis is shown in Figure 3. The total compound chromatogram (TCC) of the sample in both positive and negative modes are also shown. The chemical features in the acquired data were extracted, aligned, and integrated using MassHunter Profinder Software (Figure 4). Any features that failed to integrate can be manually integrated. A total of 175 features from both positive and negative acquisition modes were processed in this manner.

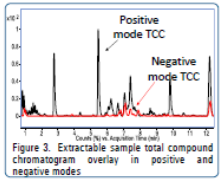


Figure 3. Extractable sample total compound chromatogram overlay in positive and negative modes

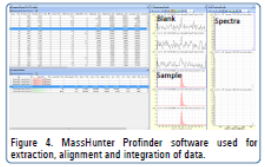


Figure 4. MassHunter Profinder software used for extraction, alignment and integration of data.

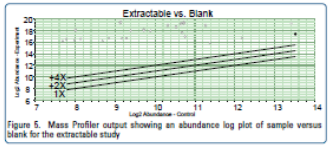


Figure 5. Mass Profiler output showing an abundance log plot of sample versus blank for the extractable study

Figure 5. Mass Profiler Software enabled the statistical comparison of data sets. A >4-fold change (abundance by height) and abundance cut off filter of >3,000, displays 66 features (positive and negative ionization modes) for extracted samples

### Results and Discussion

**Analysis of Leachables**
The control and heated ophthalmic formulation samples in leachable study were compared using Mass Profiler software. Statistical comparison using PCA plots is shown in Figure 6. The leachable stressed replicates (red dots) are found to cluster together and distinct from non-stressed replicates (blue dots). These results confirm the significant difference between the non-stressed and stressed leachable samples. Mass Profiler was also used to compare two different set of samples, the extractables and leachables to determine common and significantly different features (see Figure 7).

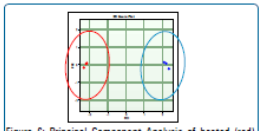

Figure 6: Principal Component Analysis of heated (red) and leachable non stressed study samples (blue).

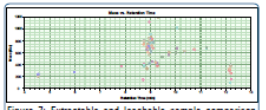

Figure 7: Extractable and leachable sample comparison to identify common compounds and their distribution. A total of 61 compounds were common to both sample types and >2 fold different with each other.

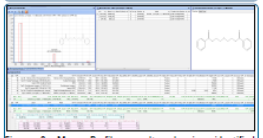

Figure 8: Mass Profiler results showing identified compounds for diethylene glycol dibenzoate.

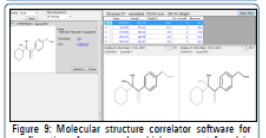

Figure 9: Molecular structure correlator software for confirmation of compounds which were not found in the library.

**Identification**
The ID Browser functionality within Mass Profiler helped identity compounds using high resolution accurate mass database searching and formula generation from a set of 1,560 compounds (Figure 8). Some of the compounds identified were, tridecyl alcohol (oxyethylene) ethanol, sodium ricinoleate, irganox 5057, ethyl(2,4,6-trimethylbenzoyl)- phenylphosphinate, isocyano cyclohexane, degradant of irganox, and hexadecanoic palmitic acid. Library LC/MS-MS spectra matching provided additional compound confirmation. However standards may not available for many extractable and leachable compounds to built a custom library. Therefore, Agilent Molecular Structure Correlator (MSC) software was used to aid their identification. MSC software proposes possible compound matches by correlating accurate mass/formula of MS/MS fragments with possible in silico fragment ions from proposed structure. Some of the compounds matched with MSC software in leachable sample were: Methyl 2-benzoylbenzoate, Irgacure 907, Erucamide, dioctyl adipate, dihydroehtylfuranone. MSC helps to provide tentative estimation of the compound identity better than a database search.

### Conclusions

- The analysis of a standard mix of nine plasticizers showed that the use high resolution accurate mass in both positive and negative ionization are required to ensure comprehensive LC/MS analysis of diverse classes of extractable and leachable compounds.
- Integration and alignment verification of samples was performed using Agilent MassHunter Profinder Software, and subsequently samples were compared using Agilent MassHunter Mass Profiler Software.
- The Mass Profiler database search using PCDL and molecular formula generation feature was used to identify compounds. Additional, data analysis of high resolution MS/MS was performed using Molecular Structure Correlator software to confirm the identification of compounds.
- The results of this study show that 66 compounds, including an antioxidant additive, for example, irganox and skin irritants such as ricinoleate were significantly present at significant concentration in the extractable samples.
- This high resolution and software processing methodology can further be extended to quantification and can also be used for the analysis of samples from drug container closure systems for vendor qualification studies.

# Poster for E&L Conference London November 2014

## Orthogonal Analysis for Extractables and Leachables Using Accurate Mass LC/Q-TOF and GC/Q-TOF Systems

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E&L Europe 2015

Agilent Technologies

### Introduction

Drug containers or drug delivery systems, meant to protect a drug from environmental contamination, may themselves become a source of contamination. Impurities extracted from the drug container closure system *when exposed to an appropriate solvent using exaggerated time and temperature conditions* are called extractables. Leachables are typically a subset of extractables, that migrate into a drug formulation from any product contact material *as a result of direct contact under normal process condition or accelerated storage conditions (BioProcess International Dec 2007).* The analysis of extractables and leachables (E&Ls) are often incomplete when only a single analytical technique is used because of diverse physico-chemical properties of many compound classes. Therefore, orthogonal analytical techniques are required to ensure comprehensive detection. Quadrupole time of flight mass spectrometers (Q-TOF) are suitable for E&Ls study because of their high resolution accurate mass and MS/MS capabilities. In addition, data mining and statistical analysis tools greatly facilitate sample analysis. Data mining software for processing and visualizing data which have been acquired on multiple analytical platforms is used to identify and statistically compare target compound profiles between samples.

In this study, both LC/Q-TOF and GC/Q-TOF systems were used for the analysis of an ophthalmic formulation and its container closure system. The LC/Q-TOF system was operated in both positive and negative ionization modes. A targeted database consisting of known compounds were created using Agilent Personal Compound Database software to process the LC/Q-TOF data. The MS/MS results were processed using Molecular Structure Correlator software for confirmation of the E&Ls. The GC/Q-TOF system was operated in both electron impact (EI), and chemical ionization (CI) modes. The EI spectra were matched with a NIST library while the CI spectra were searched against custom target databases. The statistical software tools, Mass Profiler (MP) for LC-MS and Mass Profiler Professional (MPP) for GC-MS, was used to find compounds which differed between samples. The extent of overlap and abundance differences of compounds between the extractables study and leachables study were determined using these informatics tools.

### Experimental

**Sample preparation for LC/Q-TOF and GC/Q-TOF analysis**

Extractable sample

An empty ophthalmic medicine bottle was filled with extraction solvent 1:1 methanol : water and incubated in an oven at 55 °C for 72 hours for the LC/Q-TOF analysis while it was filled with n-hexane and sonicated for 1.5 hrs for the GC/Q-TOF analysis. The extraction solvents were used as blanks.

Leachable sample

The ophthalmic drug formulation along with its container closure system was heated at 80°C for 24 hrs and analyzed, as is for LC/Q-TOF analysis. 5 µL of the sample was directly injected. The leachable sample were prepared for GC/Q-TOF analysis by liquid-liquid extraction with n-hexane. The control used was the ophthalmic drug formulation bottle stored at recommended conditions. 2 µL sample was directly injected.

Instrumentation:

Agilent 1290 Infinity Binary LC System and an Agilent Q-TOF G6530A System with a Dual Spray Jet Stream Source was used for LC/Q-TOF analysis while Agilent 7200A in EI and CI mode was used for GC/Q-TOF analysis. The LC/MS and GC/MS conditions used in this experiment are shown in Table 1.

Data analysis of CI and CI-MS/MS: Compounds with adducts of [M+H]+, [M+CH5]+ and [M+C2H5]+ were extracted.

**Table 1.** LC/MS and GC/MS experimental conditions used in this experiment.

| LC conditions | | GC conditions | |
|---|---|---|---|
| Column | Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 X 100 mm, 1.8 µm (p/n 959750-306) | Column, flow | Agilent DB-5ms, 30 m x 250 µm, 0.25 µm (p/n 122-5532) at constant flow of 1.2 mL/min |
| Column temperature | 50 °C | Injection port | Multimode Inlet |
| Mobile phase A | 100 mg/L Ammonium acetate in water | Mode | Splitless |
| Mobile phase B | Methanol | Septum purge flow | 3 mL/min |
| Flow rate | 0.5 mL/min | Inlet Program | 70°C (0.2 min) to 320°C (7 min at 900°C/min) |
| Max Light 80 nm flow cell | 220 nm | Liner | Ultra Inert Splitless, single taper, glass wool (p/n 5190-2293) |
| Gradient | Time (min) / % Methanol: 0 / 40; 8 / 100; 11 / 100; Stop 11 min; Post 1.5 min | Carrier gas | Helium |
| | | Purge flow to split vent | 60 mL/min at 2.73 min |
| | | Oven Program | 50°C (2 minutes) to 320°C (7 minutes) at 6°C/min. Equilibration time = 1 minute. Run time: 56 minutes. |
| | | Injection volume | 2 µL |
| MS conditions | | MS conditions | |
| Ionization mode | Dual Spray AJS ESI | MS Source | 300°C |
| Drying Gas | 10 L/min @ 150 °C | EI emission | 35 µA |
| Nebulizer pressure | 30 Psi | EI electron energy | 70 eV |
| Sheath gas | 11 L/min @ 200 °C | CI gas/mode | Methane/positive |
| Capillary voltage | 3500 V | Acquisition rate | 5.00 spectra/sec in 4 GHz high resolution mode |
| Nozzle voltage | 200 V | Acquisition time | 200 ms/spectrum |
| | | CI emission | 240 µA |

### Workflow

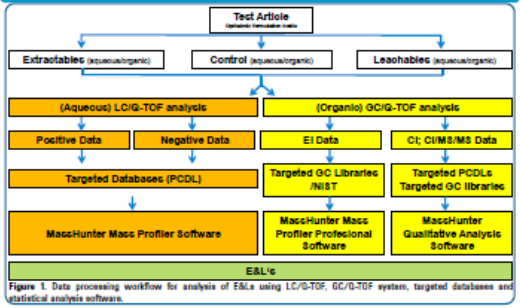


**Figure 1.** Data processing workflow for analysis of E&Ls using LC/Q-TOF, GC/Q-TOF system, targeted databases and statistical analysis software.

### Results and Discussion

**LC/Q-TOF Analysis**

The total ion chromatogram of extractable sample in both positive and negative modes are shown in Figure 2. The chemical features in the acquired data were extracted, aligned, and integrated using MassHunter Profinder Software. Features that failed to integrate accurately can be detected visually and integrated. A total of 175 features from both positive and negative acquisition modes were extracted from the samples. The data is exported to Mass Profiler Software.

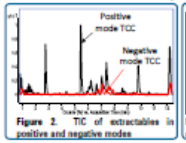


**Figure 2.** TIC of extractables in positive and negative modes

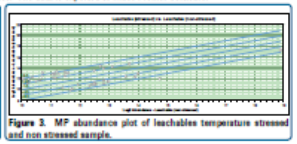

**Figure 3.** MP abundance plot of leachables temperature stressed and non stressed sample.

Mass Profiler Software helps to compare compound similarities and differences within a single sample group or between two samples or sample groups. Leachables sample comparison (temperature stressed vs non stressed) is shown in Figure 3. A total of 83 features were found in the leachables positive mode analysis. A 2-fold change (abundance by height) revealed 49 features to be UP fold changed in stressed sample. Further investigation can be made to study degradation compounds and chemical reactions happening due to the process of heat stress on the DOWN fold changed compounds.

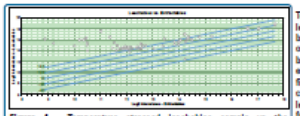

**Figure 4.** Temperature stressed leachables sample vs the extractables sample in positive mode

To find a subset of extractable compounds as leachables in the formulation, common compounds between leachables and extractables were filtered out. A total of 57 features were found common between temperature stressed leachables and extractables. Their abundance log2 plot is shown in figure 4. The results show that many common compounds were found in higher abundance in leachables compared to extractables. This suggests further optimization in extractable solvent such as the use of low and high pH solvents.

**Identification:** The ID Browser functionality within Mass Profiler helped confirm the identity of compounds by database searching and formula generation (Figure 5). A customized database of 1,800 compounds was used for the database search. LC/MS/MS spectra matching provide additional confirmation. However standards may not be available to create E&L spectral libraries. Therefore, Agilent Molecular Structure Correlator software was used (Figure 6). MSC software proposes possible compound matches by correlating accurate mass/formula of MS/MS fragments with possible in silico fragment ions of proposed structure. Some of the compounds identified through accurate mass database are listed in table 2. The compounds marked as underlined are common between extractables and leachables

**Figure 5.** Mass Profiler results showing database identified compounds for diethylene glycol dibenzoate.

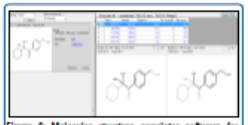

**Figure 6.** Molecular structure correlator software for confirmation of Irgacure 907.

**Table 2.** List of most abundant 8 extractables and leachables identified from LC/MS data using database search.

| Extractables | Leachables |
|---|---|
| Methyl-2-benzoylbenzoate | Acetic acid propyl ester |
| Erucamide | Irganox 1010 |
| Dioctyl Adipate | Myristal dimethylamine oxide |
| Diisononyl phthalate | Diisononyl phthalate |
| Dioctyl phthalate | Dioctyl phthalate |
| Phthalic anhydride | Phthalic anhydride |
| Hexyl Amine | Methyl-2-benzoylbenzoate |
| Glycerol dilaurate | Irgacure 907 |

Some of the compounds matched and confirmed with MSC software in the leachable sample were: methyl 2-benzoylbenzoate, Irgacure 907, Erucamide, dioctyl adipate, dihydrohydrofuranone.

**GC/Q-TOF Analysis**

Data analysis of EI runs: The EI runs were processed using MassHunter Unknowns Analysis software, mass spectra of all compounds were matched with the NIST 14 library with matching factor >80 (Figure 7).

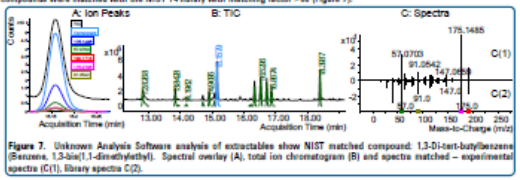


**Figure 7.** Unknown Analysis Software analysis of extractables show NIST matched compound: 1,3-Di-tert-butylbenzene (Benzene, 1,3-bis(1,1-dimethylethyl). Spectral overlay (A), total ion chromatogram (B) and spectra matched – experimental spectra (C(1), library spectra C(2).

### Results and Discussion

Statistical comparison of compound profiles using MPP: Compounds with acceptable spectral matches were exported to Agilent MPP software for differential analysis. The samples were adjusted to the intensity of the internal standard (triphenyl phosphate). Instead of simple blank subtraction, a fold change was used to prevent deleting compounds from samples like phthalates found in blanks. The compounds found greater than 2.0 fold from blank samples were added to the differential list. The leachable sample subjected to high temperature was compared with its control (Figure 8A) and with extractable samples (Figure 8B). Table 3 shows 8 compounds to be common between extractables and leachables. The table shows 1,3-Di-tert-butylbenzene (Benzene, 1,3-bis(1,1-dimethylethyl) was found in both extractable and leachables suggesting that its origin is the plastic container and leached into formulation at accelerated temperatures. A fold change analysis of stressed leachable vs extractable shows that the common compound were present in higher concentration in extractables with >2 fold abundance compared to leachables.

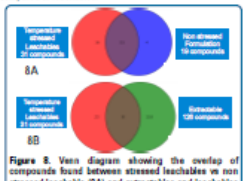


**Figure 8.** Venn diagram showing the overlap of compounds found between stressed leachables vs non stressed leachable (8A) and extractables and leachables (8B)

**Table 3.** A selected list of common compounds and their fold change.

| Common compounds | Fold Change in Leachable |
|---|---|
| Nonane, 2,6-dimethyl- | No Change |
| Dodecane | No Change |
| Benzene, 1,3-bis(1,1-dimethylethyl)- | No Change |
| 1-Docosene, (E)- | No Change |
| Tetradecane | No Change |
| Cyclohexasiloxane, dodecamethyl- | No Change |
| **Common compounds** | **Fold Change in extractable** |
| Octane, 3,5-dimethyl | UP |
| Benzene, 1,3-bis(1,1-dimethylethyl)- | UP |
| Dodecane, 4,6-dimethyl | UP |
| Tridecane | UP |
| Nonadecane | UP |
| Cyclohexasiloxane, dodecamethyl- | UP |
| Sulfurous acid, pentyl octadecyl ester | UP |
| Cycloheptasiloxane, tetradecamethyl- | UP |

**GC/Q-TOF CI and CI/MS/MS analysis**

The EI spectral match results sometimes require confirmation since different compounds could give the same spectral match. The CI data was used to confirm EI results. Here, the CI data was searched against a custom database made from the EI mode results. Table 4 shows the list of compounds ionized by CI confirming the results in EI mode.

**Table 4.** Extractables detected by CI ionization and searched against custom database made from EI mode results

| Extractables | Mass | Formula | PPM |
|---|---|---|---|
| alpha.-Cubebene | 204.188 | C15 H24 | 3.52 |
| 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 278.163 | C16 H22 O4 | 4.18 |
| 1-Decanol, 2-hexyl- | 242.261 | C16 H34 O | 3.67 |
| 2-Methyltetracosane | 352.407 | C25 H52 | -0.6 |
| 9H-Fluorene, 9-methylene- | 178.078 | C14 H10 | 4.8 |
| Benzene, 1,3-bis(1-methylethyl)- | 162.219 | C12 H18 | 3.59 |
| Benzene, (1-butylhexyl)- | 218.303 | C16 H26 | 0.07 |
| Benzene, (1-butyloctyl)- | 246.335 | C18 H30 | 4.56 |
| Benzene, 1,2,4-trimethyl- | 120.064 | C9 H12 | 3.53 |
| Benzene, 1,3-bis(1,1-dimethylethyl)- | 190.172 | C14 H22 | 1.44 |

| Extractables | Mass | Formula | PPM |
|---|---|---|---|
| Benzene, 1,3-dimethyl- | 106.078 | C8 H10 | 0.09 |
| Benzene, 1-ethyl-3,5-dimethyl- | 134.11 | C10 H14 | 3.55 |
| Benzophenone | 182.073 | C13 H10 O | 3.71 |
| Cyclopentasiloxane, decamethyl- | 370.094 | C10 H30 O5 Si5 | 2.85 |
| Diethyl Phthalate | 222.089 | C12 H14 O4 | 0.82 |
| Dodecane, 4,6-dimethyl-A | 198.235 | C14 H30 | 4.14 |
| Hexadecanal, 2-methyl- | 254.261 | C17 H34 O | 6.11 |
| Naphthalene, 1,6,7-trimethyl- | 170.11 | C13 H14 | 5.91 |
| Naphthalene, 2-methyl- | 142.078 | C11 H10 | 2.03 |
| (Z)-Hex-2-enyl (E)-2-methylbut-2-enoate | 182.131 | C11 H18 O2 | 4.09 |

Structure elucidation is a requirement if leachables exceed 20 ppm, here CI/MS/MS plays an important role. The CI/MS/MS analysis of diethyl phthalate is shown in Figure 9. The CI/MS/MS spectra was interpreted using ACD software (ACD Labs, Toronto) and shows the characteristic m/z 149 fragment.

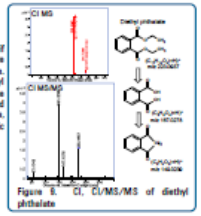


**Figure 9.** CI, CI/MS/MS of diethyl phthalate

### Conclusions

- This study shows the use of orthogonal techniques of LC/Q-TOF and GC/Q-TOF for the analysis of extractables and leachables.
- A customized accurate mass database was created using Agilent MassHunter PCDL software and used to search LC/Q-TOF data. CI accurate mass data was searched against customized EI results database to confirm some of the compounds detected by EI.
- The LC/MS/MS spectral information was used to confirm E&Ls using Molecular Structural Correlator Software.
- Statistical comparison of compound profiles between samples were successfully performed using Mass Profiler (MP)/Mass Profiler Professional (MPP) software. Unique as well common compounds between sample sets were determined visually by Venn diagrams (MPP software). This allowed the origin of a particular leachable to be determined from either a formulation or from an extractable sample.
- PCDL software allows customized targeted libraries to be created and used for future studies.
- The differential analysis and library search of leachable samples identified: acetic acid propyl ester, irganox 1010, myristal dimethylamine oxide, diisononyl phthalate, dioctyl phthalate, phthalic anhydride, methyl-2-benzoylbenzoate, irgacure 907. Among many other compounds, GC/MS analysis identified the differential presence of eye/skin irritant compounds - ethyl 4-ethoxybenzoate in heat stressed formulation.

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# Abbreviations

| | |
|---|---|
| AET = | Analytical Evaluation Threshold |
| SCT = | Safety Concern Threshold |
| TDI = | Total Daily Intake |
| TTC= | Threshold of Toxicological Concern |
| DP = | Drug Product |
| OINDP = | Orally Inhaled and Nasal Drug Product |
| MDI = | Metered Dose Inhaler |
| QT = | Qualification Threshold |
| SAR = | Structure-Activity-Relationship |

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