USP Updates on Extractables and Leachables (E&L)

Ravi Kiran Kaja, Ph.D.,
Senior Principal Scientist,
Complex Generics,
General Chapters – USP

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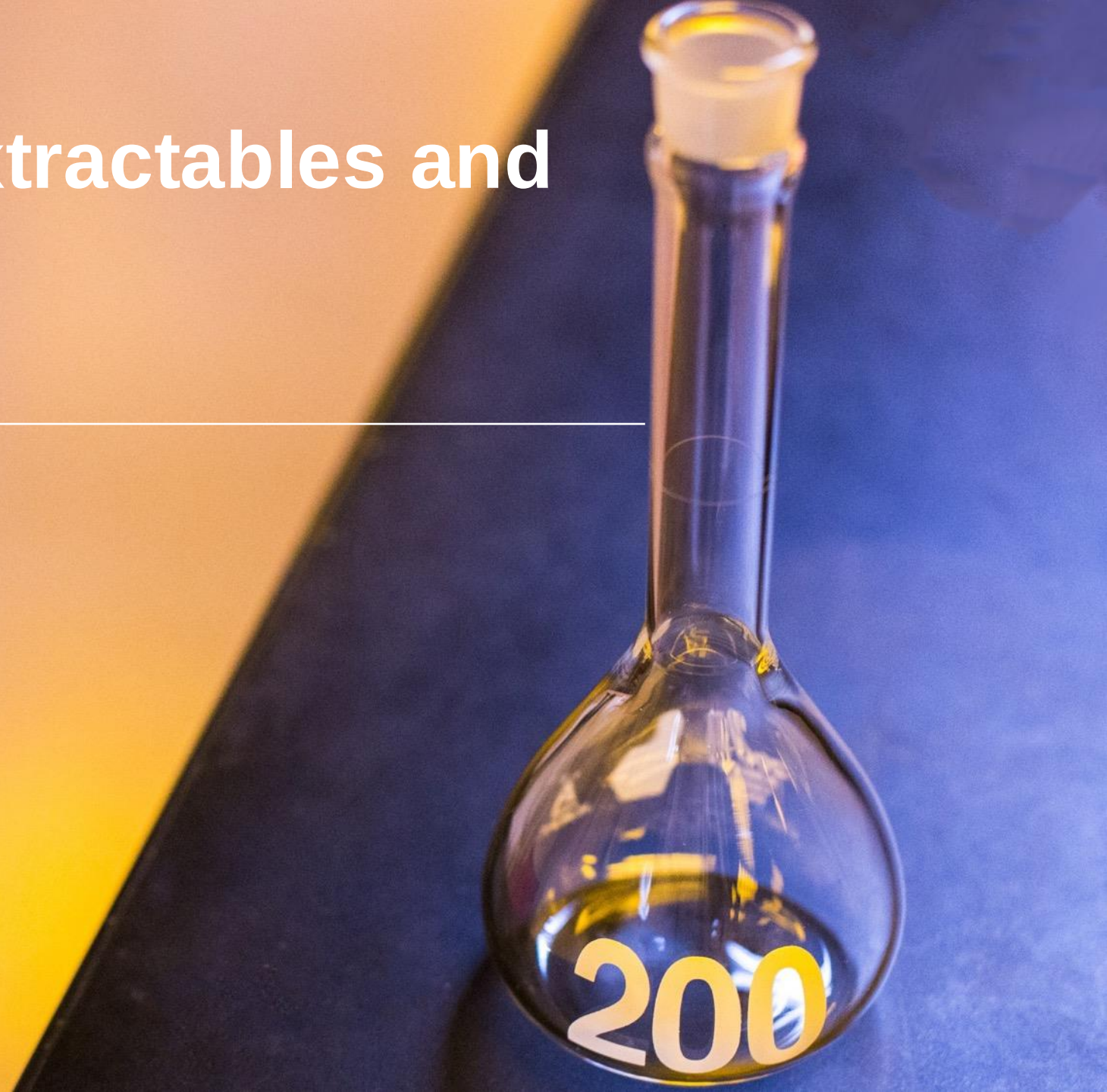
USA



Background

USP E&L System Suitability Standards Proposal:

- Headspace GC-MS
- GC-MS
- LC-MS, APCI with positive and negative ionization
- LC-MS, ESI with positive and negative ionization

Recap: USP interest on E&Ls?

- **Qualitative Survey (2021)**
  - Small survey (14, global)
- **Quantitative Survey (2023)**
  - Large study (356, global)
- **Email communication to the USP staff**
  - **Feedback:**
    - USP should prioritize the E&Ls topic due to growth and uncertainty.
    - USP should offer E&L reference standards and/or mixtures, including system suitability standards.
    - USP should offer a digital library to help identify relevant E&Ls.
    - USP should provide more guidance on extractable and next steps after they have been identified, including providing AET limits.
    - USP offering training and educational courses related to E&Ls.

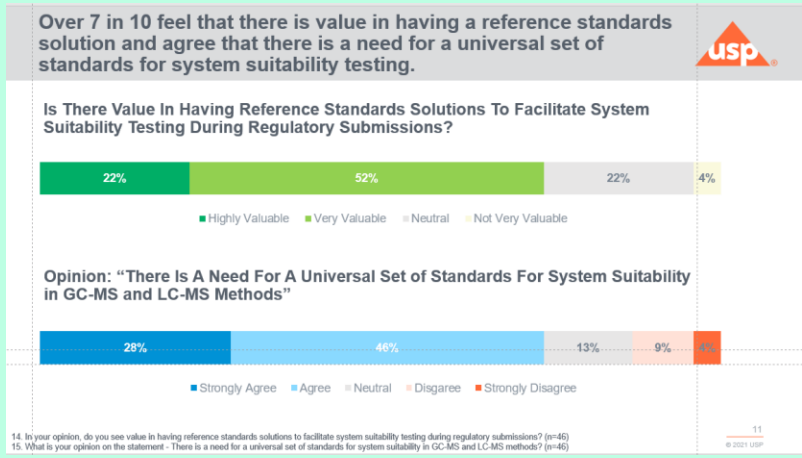
Respondents see value in USP developing extractables system suitability standards (62% rate it highly/very valuable); supported by similar scores for system suitability standards in the E&L 2023 survey

2024 Survey: Do You See Value In USP Developing Extractables System Suitability Standards?

E&L Stimuli Article Feedback Survey 2023

Opinions on system suitability standards tested in previous E&L study

- Over 7 in 10 feel that there is value in having a reference standards solution to facilitate system suitability testing during regulatory submissions
- Over 7 in 10 agree that there is a need for a universal set of standards for system suitability testing.



System suitability standards tested in 2023

Q13. In your opinion, do you see value in USP developing extractables system suitability standards? Base, n =181

Background: Stimuli article published USP-PF 49(4), July 2023

STIMULI TO THE REVISION PROCESS

Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

Proposals for the Development, Composition, and Routine Use of System Suitability Standard Mixtures in Support of Chromatographic Screening for Organic Extractables and Leachables[a]

Dennis Jenke[b], Jean-Marie Beusen[c], Philippe Verlinde[c], Jan Baeten[c], Piet Christiaens[c], Desmond Hunt[d], and Prabhakar Gruddanti[d]

Correspondence should be addressed to: Prabhakar Gruddanti, Science-General Chapters, US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790; tel: 1.240.221.4743; email: prabhakar.gruddanti@usp.org.

ABSTRACT

Leachables present in packaged drug products or released from medical devices can adversely affect patient health and safety. Thus, packaged drug products are screened for unspecified leachables, and packaging system or medical device extracts are screened for unspecified extractables as potential leachables, a process known as non-targeted analysis (NTA). Screening methods for organic extractables and leachables typically employ chromatography to separate analytes and information-rich detectors [such as mass spectrometry (MS)] to detect, identify, and quantify them.

Chromatographic screening methods are generally qualified to establish that they are suitable for their intended use. When the qualified methods are implemented during extractables and leachables studies, system suitability testing is conducted during each chromatographic run to ensure that the method performs properly at the time of use.

System suitability testing in extractables and leachables screening requires a standard mixture of relevant compounds that themselves are extractables and leachables. To facilitate consistent analytical performance across laboratories and to standardize system suitability testing, a standardized system suitability mixture (meaning a mixture with specified constituents), used by all practitioners, is necessary.

Based on several scientific and practical considerations, USP is developing a set of system suitability reference standards for the most commonly employed hyphenated chromatographic screening methods, such as gas and liquid chromatography with mass spectrometric detection (GC/MS and LC/MS). In this article, the USP approach to reference mixture development is discussed; the compositions of the reference standard mixtures are disclosed, discussed, and justified; and typical chromatograms are provided. USP is seeking feedback from stakeholders on the proposed mixtures. The article also discusses other opportunities for development of reference standards and reference standard mixtures to support extractables and leachables testing (e.g., calibration mixtures, individual reference standards for "hard-to-find" extractables, and leachables, etc.).

Major Comments on the Stimuli Article:

- **Relevance of proposed standards:**
  - Concerns for the relevance of some of the proposed standards, ex., Toluene is not an extractable
- **Compounds selection:**
  - Selection of compounds should be material (packaging or manufacturing component) specific
- **Round-robin testing:**
  - Majority mentioned to perform a round-robin testing at multiple labs for a wider acceptability
  - Results presented from only one lab
- **Cost-effectiveness of standards:**
  - Use of vast number of standards for “routine use” may not be cost effective or scientifically justifiable
- **Evaluation of non-MS and other methods:**
  - GC-FID, CAD, use of Acetonitrile and Methanol, multiple columns, etc.,
- **Flexibility in method parameters:**
  - Flexibility regarding the instruments specified, like using other similar instruments from other vendors
- **Premixed standards:**
  - Lot of interest for pre-mixed standards than individual
- **Clarity on linking to documentary standards:**
  - Will this be added to any current USP chapters?

Headspace GC-MS Method Parameters

Instrument: SHIMADZU -TQ8050NX triple quad mass spectrometer with AOC-20S autosampler

Column: DB-624 60-m x 0.25-mm x 1.4 µm (Agilent part number 122-1364)

Instrument Operating Parameters:

Headspace Parameters: Vial Temp: 75°C, Sample Line Temp: 85°C Transfer Line Temp: 95°C
Vial Equilibration Time: 20 min, GC Cycle Time: 65 mins

GC Parameters: Carrier Gas and Flow: He in constant flow 1.5mL/min, Total Flow: 19.5mL/min,
Total Program Time: 45 mins

Injection Mode: Interface Temp: 250 °C Ion source Temperature: 250 °C
Oven Program: Initial 3 min at 45 °C, to 90 °C (4 min) at 8°C/min
to 200 °C (3 min) at 5 °C/min to 220 °C (5.37 min) at 10 °C/min

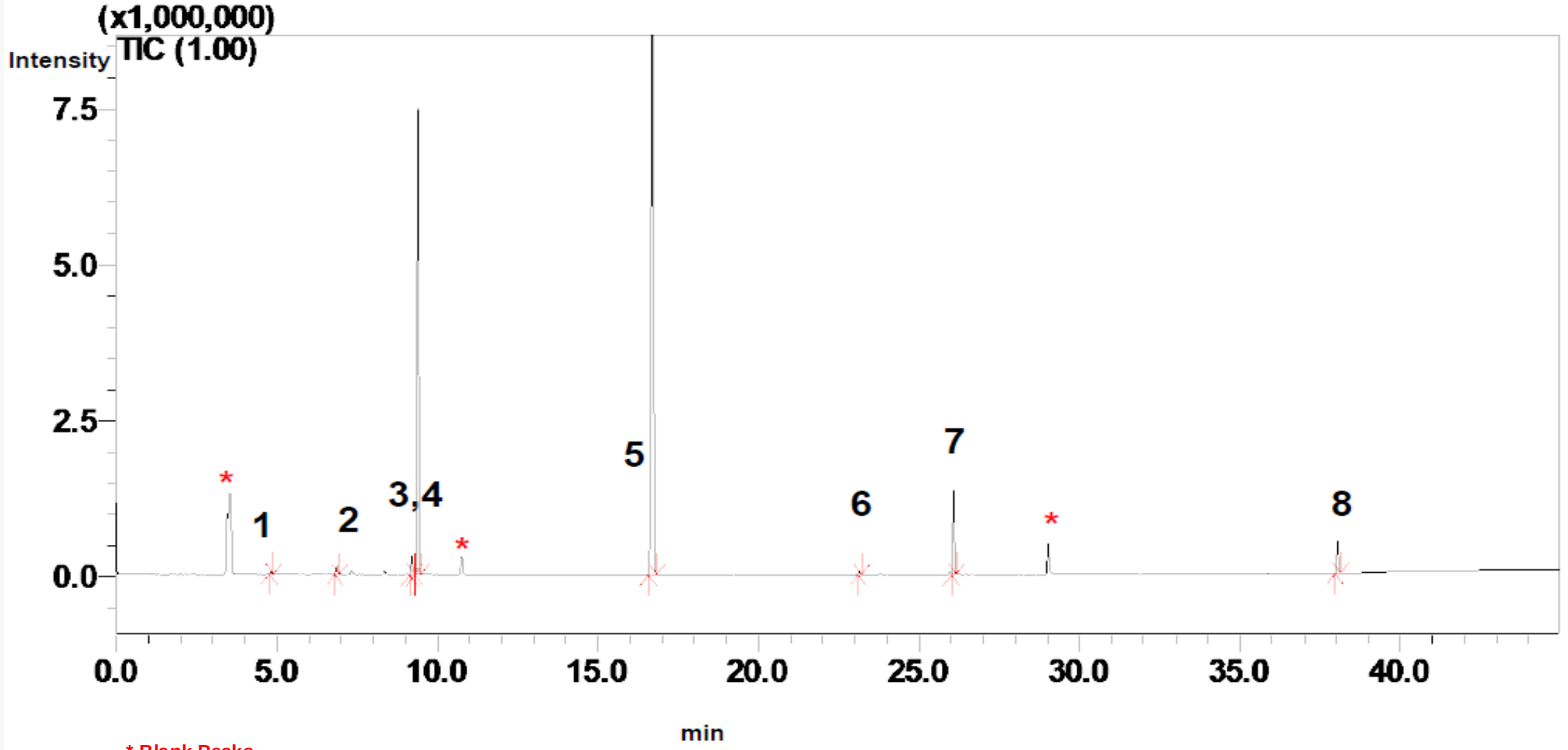
MS Parameters: Ionization Mode: Electron Impact
Acquisition Mode: Full Scan
Mass Range (m/z): 35 – 300

Revised List of Compounds [Procedure-1: Head Space GC/MS]

Composition of the HS-GC/MS Suitability Mixture: Solvent = UPW

Compound	CAS Number	Peak Number	Retention Time (min)	Concentration (mg/L)
Acetaldehyde (New)	75-07-0	1	4.81	1
Dimethoxymethane	109-87-5	2	6.87	0.5
Trimethlysilanol (New)	1066-40-6	3	9.21	1
Methyl cyclopentane (New)	96-37-7	4	9.39	1
Hexamethyl cyclotrisiloxane (New)	541-05-9	5	16.70	1
Cyclohexanone	108-94-1	6	23.15	1
2-Octanone (New)	111-13-7	7	26.08	1
n-Tetradecane	629-59-4	8	38.05	0.05

Headspace GC-MS Chromatogram



* Blank Peaks

GC-MS Direct Injection Method Parameters

GC Parameters:
Instrument: SHIMADZU -TQ8050NX triple quad mass spectrometer with AOC-20S autosampler.
Column: HP-5MS Ultra Inert 30-m × 0.25-mm × 0.25-µm
(Agilent part number 19091S-433UI)
Carrier gas: Helium
Constant flow: 1 ml/min
Injection mode: Splitless
Inlet temperature: 270 °
Injection volume:1 µL

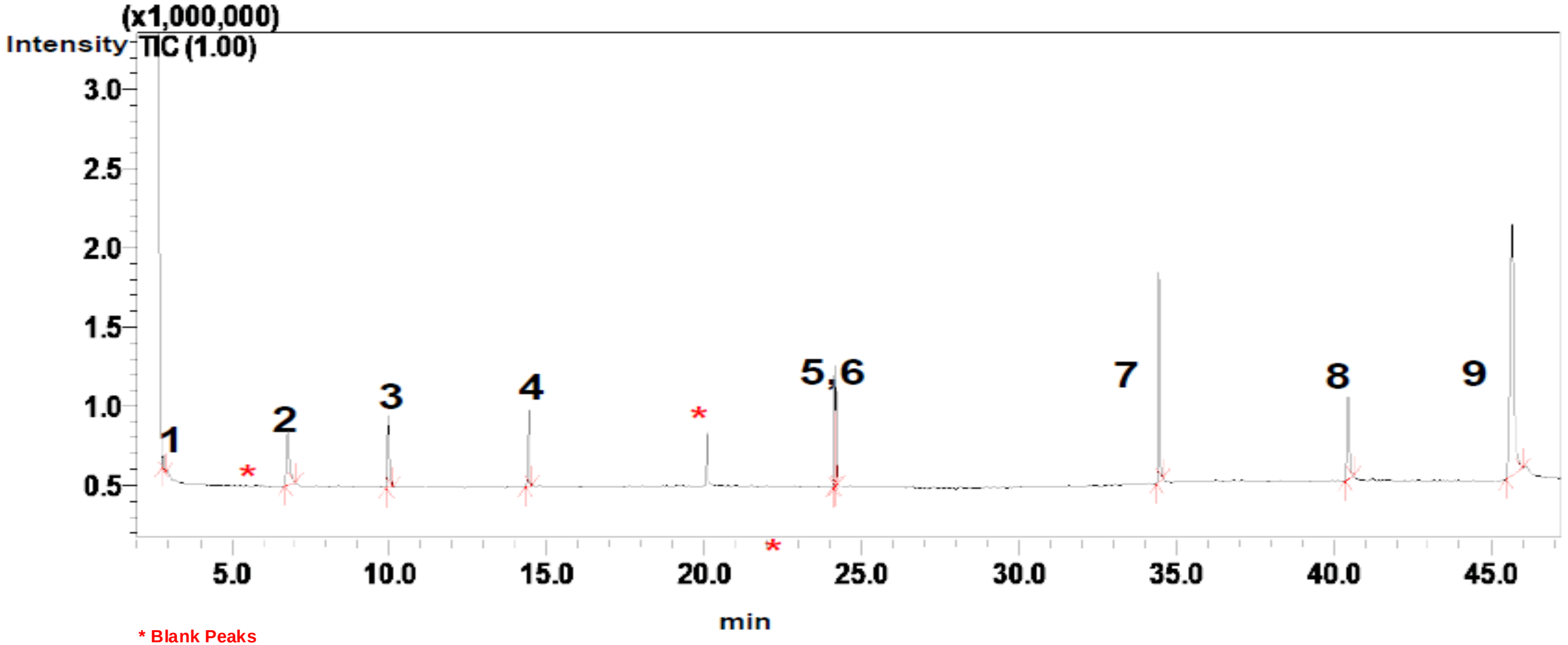
Column oven conditions	-	Rate°/min	Value°	Hold time (min)
	Initial	-	50	4
	Ramp-1	8	300	12

MS parameters:
Ionization mode: Electron impact
Acquisition mode: Full scan
Mass range (m/z): 35-700
Ion source temperature: 250°
Interface temperature:250°

Procedure-2: GC-MS (Direct) Compound Selection

Composition of the GC/MS Suitability Mixture: Solvent = DCM				
Compound	**CAS-number**	**Peak Number**	**Retention Time (min)**	**Concentration (mg/L)**
Methyl methacrylate (New)	80-62-6	1	2.84	10
Cyclohexanone	108-94-1	2	6.78	20
2-Ethyl-1-hexanol	150-13-0	3	9.97	10
Caprolactam	150-60-2	4	14.45	20
n-Nonadecane	629-92-5	5	24.14	5
2-Heptadecanone	2922-51-2	6	24.19	5
n-Nonacosane (New)	630-03-5	7	34.44	10
Irgafos 168	31570-04-4	8	40.45	10
18-Pentatriacontanone	504-53-0	9	45.66	50

GC-MS Chromatogram



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LC/MS-APCI Method Parameters

Instrument: Shimadzu / LCMS triple quad-8045

Column: Waters Acquity CSH-C18 100-mm × 3.0-mm × 1.7-µm (Waters part number 186005301)

Colum temperature: 40°

Autosampler temperature 5°

Injection volume: 5 µL

Acquisition mode: Full Scan APCI +/APCI-

Mass range:100-1500

MS conditions:

Nebulizing gas flow: 3 L/min

Interface temp: 350°

DL temperature:250°

Heat block temperature: 250°

Drying gas flow : 5 L/min

Time (min)	Flow rate (mL/min)	UPW	Methanol
0	0.5	80	20
7	0.5	0	100
25	0.5	0	100
26	0.5	80	20
29	0.5	80	20

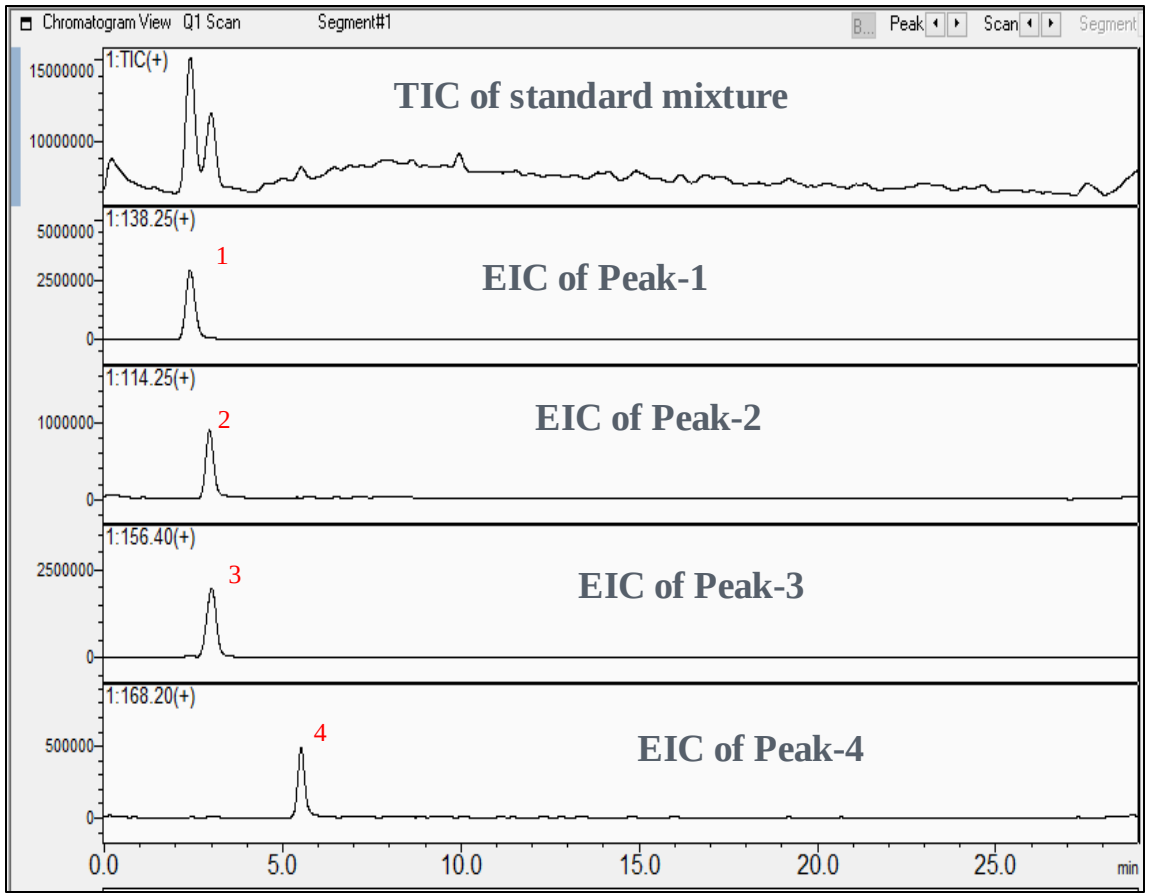
Procedure-3: LC/MS-APCI Compound Selection

Composition of the LC/MS-APCI Suitability Mixture: Solvent = MeOH

Compound	CAS-number	Peak Number	Retention time (min)	Mode	Concentration (mg/L)
4-Aminobenzoic acid	150-13-0	1	2.4	+/-	10
Caprolactam	150-60-2	2	2.9	+	1
*N,N-Diethyl cyclohexylamine	91-65-6	3	3.04	+	10
2-Mercaptobenzothiazole	149-30-4	4	5	+/-	1
Propyl paraben	94-13-3	5	6.3	+/-	1
Hostanox 03	32509-66-3	6	8.5	-	1
Erucamide	112-84-5	7	9.9	+	1
Irgafos 168	31570-04-4	8	14.1	+/-	1
1,3 Distearin (New)	504-40-5	9	16.2	+	1
Irganox PS802	693-36-7	10	24.6	+	1

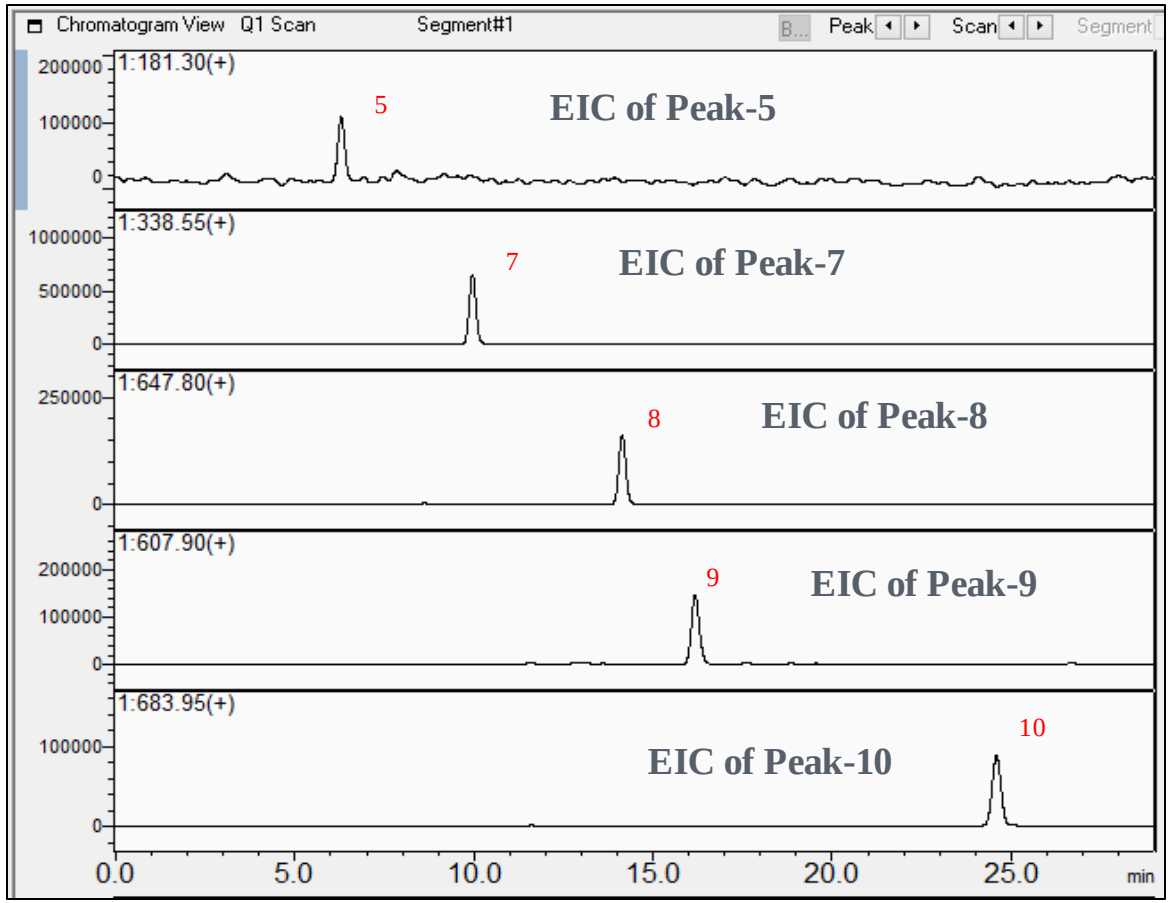
LC/MS-APCI Chromatogram

Total Ion Chromatogram (TIC) and Extracted Ion Chromatograms(EIC) of standard mixture in +Ve mode



Intensity

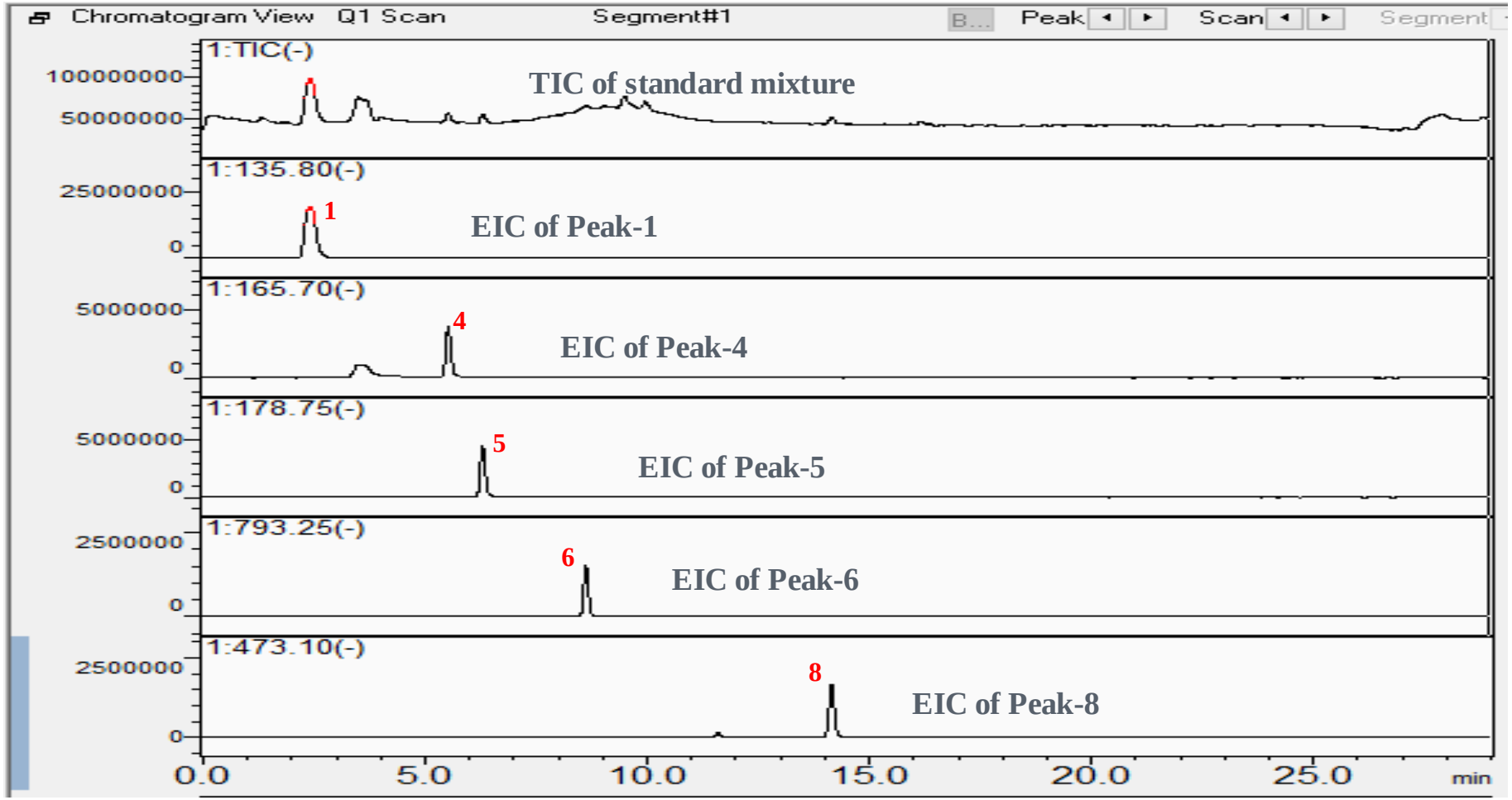
Time in minutes



Time in minutes

LC/MS-APCI Chromatogram

Total Ion Chromatogram (TIC) and Extracted Ion Chromatograms(EIC) of standard mixture in -Ve mode



Note: Peak-1, 4, 5, 6 and 8 are expected in both +Ve and –Ve modes.

LC/MS-ESI Method Parameters

Instrument: Shimadzu / LCMS triple quad-8045

Column: Waters Acquity HSS-C18 100-mm × 2.1-mm × 1.8-µm (Waters part number 186003533)

Colum temperature: 40°

Autosampler temperature 5°

Injection volume: 5 µL

Acquisition mode: Full Scan ESI+/ESI-

Mass range:100-1500

MS conditions:

Nebulizing gas flow: 3 L/min

Heating gas flow: 10 L/min

Interface temp: 300°

DL temperature:250°

Heat block temperature: 400°

Drying gas flow : 10 L/min

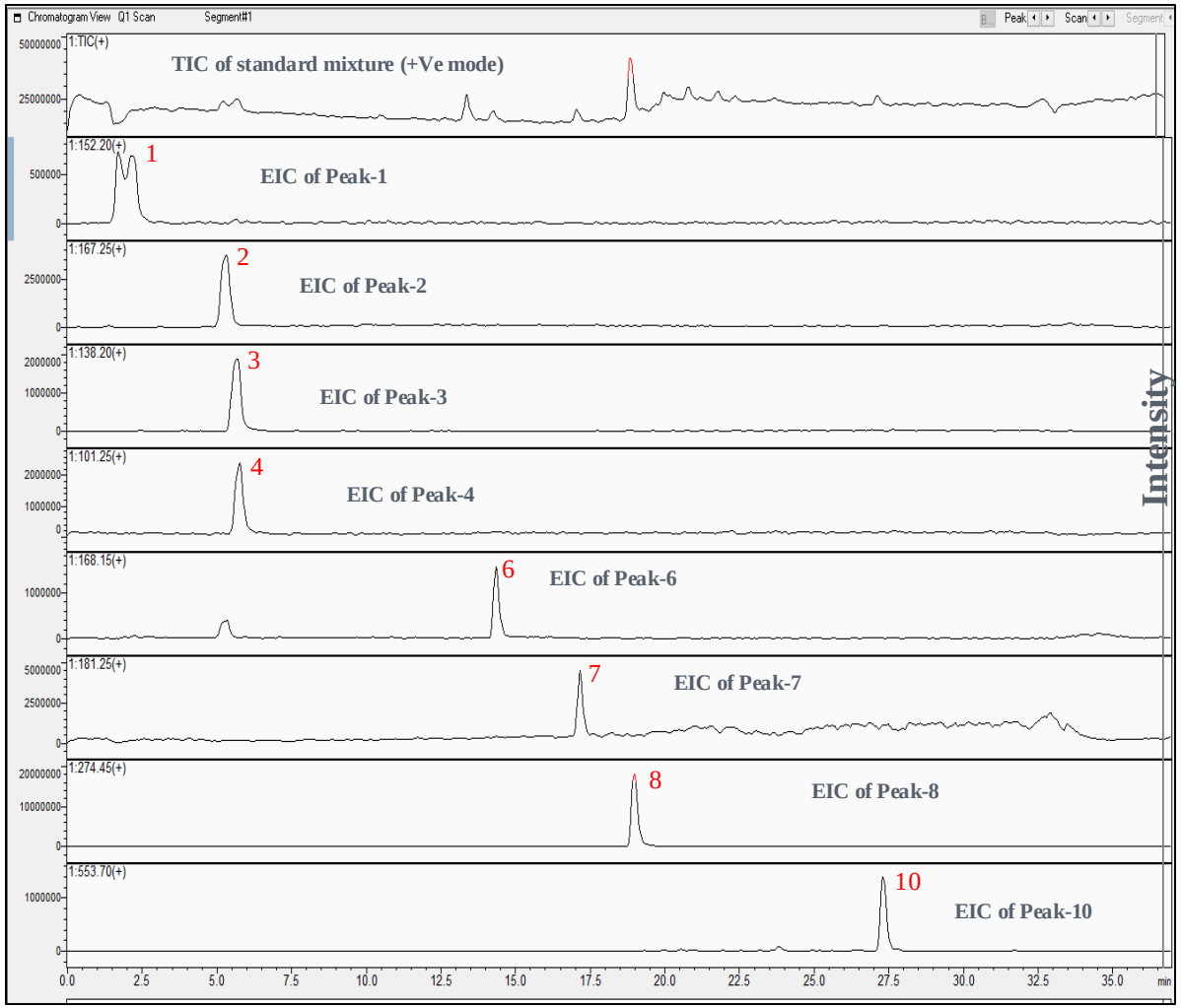
Time (min)	Flow rate (mL/min)	0.05% formic acid +UPW	0.05% formic acid+ Methanol
0	0.25	98	2
15.5	0.25	35	65
17.5	0.25	2	98
18	0.25	0	100
19.5	0.25	0	100
29.5	0.25	0	100
32.0	0.25	98	2
37.0	0.25	98	2

Procedure-4: LC/MS-ESI Compound Selection

Composition of the LC/MS-ESI Suitability Mixture: Solvent = UPW/MeOH 98/2 v/v					
Compound	CAS-number	Peak Number	Retention time (min)*	Mode	Concentration (mg/L)
Guanine	73-40-5	1	1.7	+/-	1
L-Phenylalanine-15N	29700-34-3	2	5.3	+/-	0.1-1
4-Aminobenzoic acid	150-13-0	3	5.6	+	1
*Nitrosopyrrolidine (New)	930-55-2	4	5.7	+	1
Adipic acid	124-04-9	5	7.3	-	0.1-1
2-Mercaptobenzothiazole	149-30-4	6	14.3	+/-	0.1-1
Propyl paraben	94-13-3	7	17.1	+/-	0.1-1
*N-Lauryl diethanolamine (New)	1541-67-9	8	18.9	+	0.1-1
Stearic Acid (New)	2975-39-3	9	22.5	-	0.1-2
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1076)	2082-79-3	10	27.3	+	0.1-1

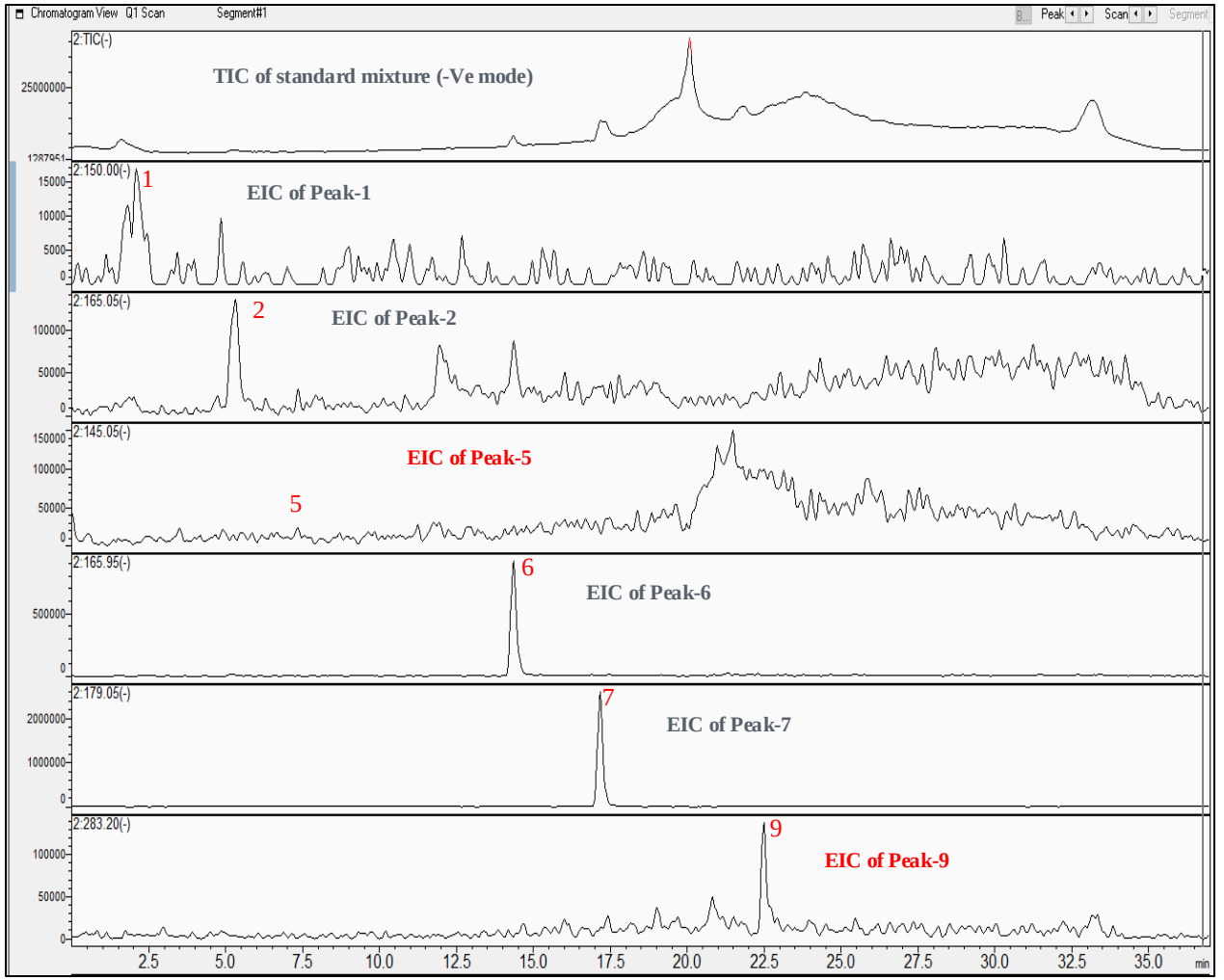
LC/MS-ESI Chromatogram

Positive



Time in minutes

Negative



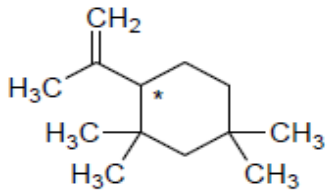
Time in minutes

Next Steps

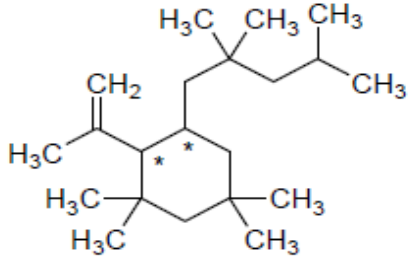
- **Round Robin Study:**
  - 10-12 Labs shown interest to participate for the study
  - Create a flexible approach [Not to mention instrument Make/Model/column/Methods etc.,]
  - Appropriateness of compounds in the mixtures
  - Adequacy of the selection of standards
  - Instrument sensitivity
  - Column efficiency (critical pair, anchor compounds, etc.,)
  - Orthogonal approach
  - Method Parameters like Specificity, Precision, Accuracy and Linearity (Acceptance Criteria's will be proposed based on the round robin study)
  - Timelines may vary depending upon the completion of round robin study [Target: July-Aug 2024]
- **Why this proposal?**
  - Intended only for the system suitability
  - To make sure the Instrument is working appropriately prior to the sample analysis
  - Not intended for either Extractable or Leachable analysis
  - Revised Stimuli Article to clarify and achieve consensus

Rubber Oligomers

Rubber Oligomers



C13 oligomer



C21 oligomer

- Oligomers: $C_{13}H_{23}Br$/ $C_{13}H_{23}Cl$ and $C_{21}H_{39}Br$/ $C_{21}H_{39}Cl$
  - Halogenated Cyclic Aliphatic Hydrocarbons (allyl halides)
  - Alkylating Agents
  - One double bond
  - Structure Activity Relationship: Human carcinogenicity is plausible
  - High concern

- Often arises due to Polymerization, the rubber curing at high temperatures and an interaction of elastomeric material with drug product.
- The butyl and halo butyl rubbers are among the most common elastomers used as components for injectable and other delivery systems
- Difficult synthesize/currently the low availability of both rubber oligomer physical standards and spectra in most commercial mass spectrometry libraries makes analysis challenging
- Known to draw concern from regulatory authorities about an effective leachable assessment for drug products
- USP is launching a new line of E&L Rubber Oligomer Analytical Reference Materials (ARMs) soon

Goal: MOC based E&L Standards

- **Packaging or Manufacturing component based standard proposals:**
  - Currently, Lack of guidance for selection of Reference Standards for E&L Study
  - Goal is to design reference standards mixtures suitable for different material of construction (MOC) based on a priori knowledge
  - Based on the specific packaging & manufacturing components (HDPE bottles, rubber stoppers, IV bags, and laminated pouch's & Filters, Gaskets, printed label, tubing,)
  - These Standards can be used either for Extractable study, Method Suitability and Leachable study purposes

Targeted

  - Necessary to use authentic standard to measure concentration
  - Method becomes highly accurate and more sensitive, selective and precise than screening…**validation**

USP General Chapter Updates:

- **USP <1663> and <1664>**
  - Conducted a survey to identify stakeholder needs (Apr 2024)
  - Intended to receive inputs and revise chapters
  - Total 190 respondents

- **New Chapter Proposals:**
  - **To address special considerations for Leachable assessment in various drug products**
  - **USP Subcommittee is currently working on**
    - <1664.2> Leachable Chapter for Parenteral Drug Products [Tentative Timeline: FY 25 Q2]
    - <1664.3> Leachable Chapter for Ophthalmic Drug Products [Tentative Timeline: FY 25 Q3]
    - <1664.4> Leachable Chapter for Topical and Transdermal Drug Products [Tentative Timeline: FY 25 Q4]
    - <1664.5> Leachable Chapter for Oral Dosage forms [Tentative Timeline: FY 25 Q3]

USP E&L Contacts

- Dr. Ravikiran Kaja, Ph.D,
  - Senior Principal Scientist, Complex Generics, General Chapters
  - Email: **Ravikiran.Kaja@usp.org**
- Dr. G. Prabhakar Reddy, Ph.D.,
  - Director-Pharmaceutical Sciences, General Chapters
  - Email: **Prabhakar.Gruddanti@usp.org**

Thank You