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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8040 NX, AOC™ -20i and TD-30R

Extractable Study of Pharmaceutical Packaging and Delivery System Used for Ophthalmic Drug Product

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User Benefits

- ASSP™ and simultaneous Scan/MRM analysis facilitates accurate qualitative identification and quantitation at trace levels
- UFsweeper™ allows quicker ion transmission in the collision cell suppressing crosstalk & enables faster MRM analysis
- Thermal Desorption (TD) System offers a direct sampling feature that significantly reduces extraction time and prevents the loss of VOCs

■ Introduction

Overview : Extractable and leachable (E & L) studies are becoming increasingly important in the pharmaceutical industry as it is mandatory requirement from FDA during filing of the drug product. The purpose of E & L studies is to identify and evaluate possible toxicological risks. E & L studies in the regulatory references aim to identify traces of potential chemical substances. These substances may be harmful to patients due to their toxicity or may impact the activity of the drug product. Hence, to ensure the safety and efficacy of the drug throughout its shelf-life period, E & L studies play an important role. Impact of Extractables on efficacy and safety of the drug product is presented in Figure 1.

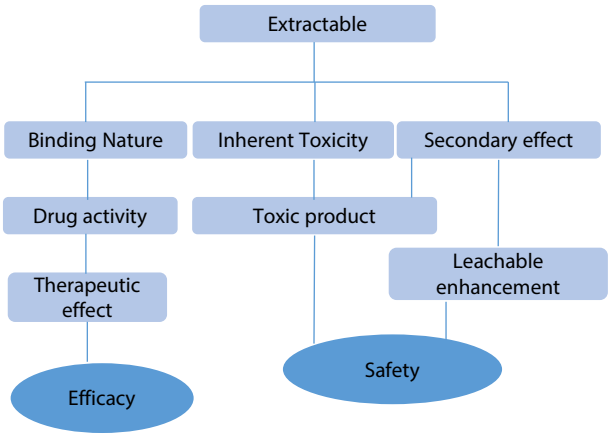

Figure 1: Impact of extractables

What are Extractables and Leachables ?

Extractables are organic and inorganic chemical entities that are released from a pharmaceutical packaging / delivery system, packaging component, or packaging material of construction into an extraction solvent under laboratory conditions.[1]

Leachables are foreign organic and inorganic chemical entities that are present in a packaged drug product because they have leached into the packaged drug product from a packaging/delivery system, packaging component, or packaging material of construction under normal conditions of storage and use or during accelerated drug product stability studies [1].

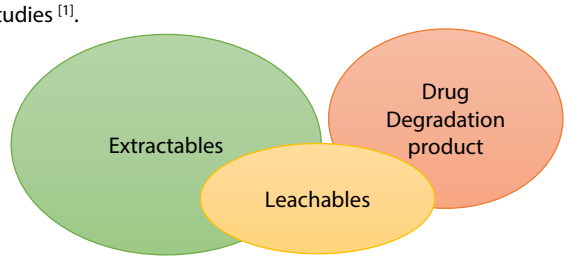

Figure 2: Extractables, Leachables & Drug degradation product

Figure 2 depicts hypothetical relation between Extractables, Leachables and Drug Degradation products.

Sources of extractables:

Extractables are derived from a variety of sources and exhibit extensive chemical diversity. Few of the primary sources are listed below.

i. Chemical additives in individual polymeric packaging material
ii. Chemical entities that are present in the packaging components
iii. Monomers and higher molecular mass oligomers derived from incomplete polymerization
iv. Migrants from secondary and tertiary packaging materials such as inks, label adhesives etc.
v. Surface residues on metal canisters and containers
vi. Chemical substances on the surface of components fabrication machinery such as anti-static and anti-slip agents.

Safety thresholds:

Product Quality Research Institute (PQRI) has specified the control thresholds like Safety Concern Threshold (SCT) and Analytical Evaluation threshold (AET) to guide the direction for initial assessment.

SCT is the threshold below which a leachable has a dose so low that it presents negligible safety concerns from carcinogenic and non-carcinogenic toxic effects. In PQRI, it is specified as 1.5 µg/day for Parenteral and Ophthalmic Drug Products (PODP).

AET is the threshold at or above which a leachable should be characterized and reported for toxicological assessment.

The AET can be mathematically derived from the SCT based on the factor that includes the dosing parameters of the drug product.

Before designing the extractable or leachable study, calculation of AET is a must. To calculate the AET, information of drug product such as maximum daily dose (MDD), Pack size, number of dose per container closure pack (CCP), root of administration and respective SCT etc. Moxifloxacin ophthalmic solution available in lab was considered for AET calculation. Some of the parameters which were considered for this calculation are mentioned in Table 1.

Table 1: Product information for AET calculation

Parameter	Value
Product under consideration	Moxifloxacin Eye drop IP
Route of administration	Eye
pack size (mL)	5
MDD (mL/day)	0.5
Material of construction (MOC) of CCP	Plastic
SCT for ophthalmic product	1.5 µg/day

Typical images of CCP:

Figure 3: Typical images of CCP

Calculation of AET:

$$\text{AET (µg/Container)} = \frac{\text{SCT (µg/Day)}}{\text{MDD (mL/Day)}} \times \text{No. of doses per CCP}$$

$$= \frac{1.5 \text{ (µg/Day)}}{0.5 \text{ (mL/Day)}} \times 10 \text{ Doses per CCP}$$

$$= 30 \text{ (µg/CCP)}$$

For liquid drug product:

$$\text{AET (µg/mL)} = \text{AET (µg/CCP)} \times \text{Uncertainty factor} = \frac{\text{AET (µg/CCP)}}{\text{Pack size (mL)}} \times 0.5$$

$$= 3 \text{ (µg/mL)} = \textbf{3 ppm}$$

As per above calculation, if the CCP under study is supposed to use for the storage of the Moxifloxacin with above doses form, then it should not leach any chemical above 3 ppm. And if any chemical entity observed above this threshold, then toxicological assessment of that entity is required.

Note: Uncertainty factor is generally applied to overcome the risk associated due to extraction error and to evaluate the toxic entities below its accepted level.

■ Experimental design:

There are many ways by which extractable study can be designed like generating extract by maceration (solvent soaking), by reflux/Soxhlet/sealed vessel/sonication or solvent extraction (manually and automated) etc.[1] However, one can perform this study by direct heating the CCP using the technique such as TD System.

Physical as well as chemical properties of the sample should be considered while designing the extraction experiment. Polarity, pH and chemical components of the drug product plays an important role in the extraction process. Extractable study is performed at accelerated temperature conditions considering worst case scenario for storage of the drug product.

Here, we conducted extractable study at variable pH and by refluxing the CCP with solvents of different polarities. Also, TD System was utilized for the assessment of extractable study.
Empty CCP designed for the storage of Ophthalmic drug product were purchased from the local resource.

Using these CCP's, multiple experiments were performed and acquired data were evaluated to identify the potential extractables. Below are the detailed explanation for Experiment A, B, C, D, E etc. Experiment A is performed by incubating CCP in aqueous solutions having different pH values and experiment B, C, D & E were performed by refluxing the CCP with organic solvents of different polarities.

Experiment-A: This experiment was performed by incubating the CCP filled with aqueous solutions (volume capacity is 5 ml X 2 CCP) of different pH like acidic (2.5 pH-Expt-A1), neutral (7.0 pH-Expt-A2) and basic (10.5 pH Expt-A3).
Acidic aqueous medium Expt-A1 was prepared by adjusting pH of water at about 2.5 with 10 % phosphoric acid. Mixed well and sonicated for 10 min.
Neutral aqueous medium Expt-A2 was prepared by adjusting pH of water at about 7.0 with 0.5 % diluted sodium hydroxide solution. Mixed well and sonicated for 10 min.
Basic aqueous medium Expt-A3 was prepared by adjusting pH of water at about 10.5 with 5 % Sodium hydroxide solution . Mixed well and sonicated for 10 min.

These solutions were filled in CCP (Two CCP per pH solution) and were kept for incubation at 65 ˚C for 72 hrs in the incubation chamber in inverted position so that the tip and nozzle will interact with the medium inside and monitored twice in a day during incubation for any leakage. Optimum extraction is ensured by intermittent shaking.
After incubation, all the CCP's were cooled at room temperature and extracts were collected in a volumetric flasks separately.
(Expt-A1) 10 mL of acidic pH extract was transferred in a separating funnel. Added 10 mL of ethyl acetate. Shaked well and allowed to separate. Ethyl acetate layer was used for analysis after drying over sodium sulphate.

Similarly, sample solutions for neutral (Expt-A2) and basic pH medium (Expt-A3) were prepared and used for analysis.

Experiment-B: CCP was cut down in the small pieces and refluxed with 10 mL ethanol at 75 ˚C for 2 hrs. Then this refluxed solution was cooled at room temperature, transferred all solution from reflux flask in a test tube and evaporated till dryness using nitrogen evaporator. Contents left in the tube were re-constituted with 5 mL of ethyl acetate, sonicated, filtered through with 0.2 µ syringe filter and used for analysis.

Experiment-C, D, E: These experiments were performed in the same way as like experiment-B but with different reflux solvents and temperatures. Such as,
experiment-C with n-Hexane at 65 ˚C
experiment D with Isopropanol at 80 ˚C
experiment E with Dichloromethane at 38 ˚C.

Experiment-F: In this experiment, small pieces of CCP were inserted in the TD sample tube (approx. 0.5 g) and analysed using thermal desorption system.

Entire experimental design flowchart is depicted in Figure 4 on page number 3.

All above experiments were designed to extract maximum extractables present in the CCP as mentioned below at extreme conditions like different solvent polarities and pH's without degrading the material of construction thereby ensuring maximum extraction efficiency.

Experiment A- Acidic, neutral and basic extractables
Experiment B- Polar organic extractables
Experiment C- Nonpolar organic extractables
Experiment D & E- Mid-polar organic extractables.

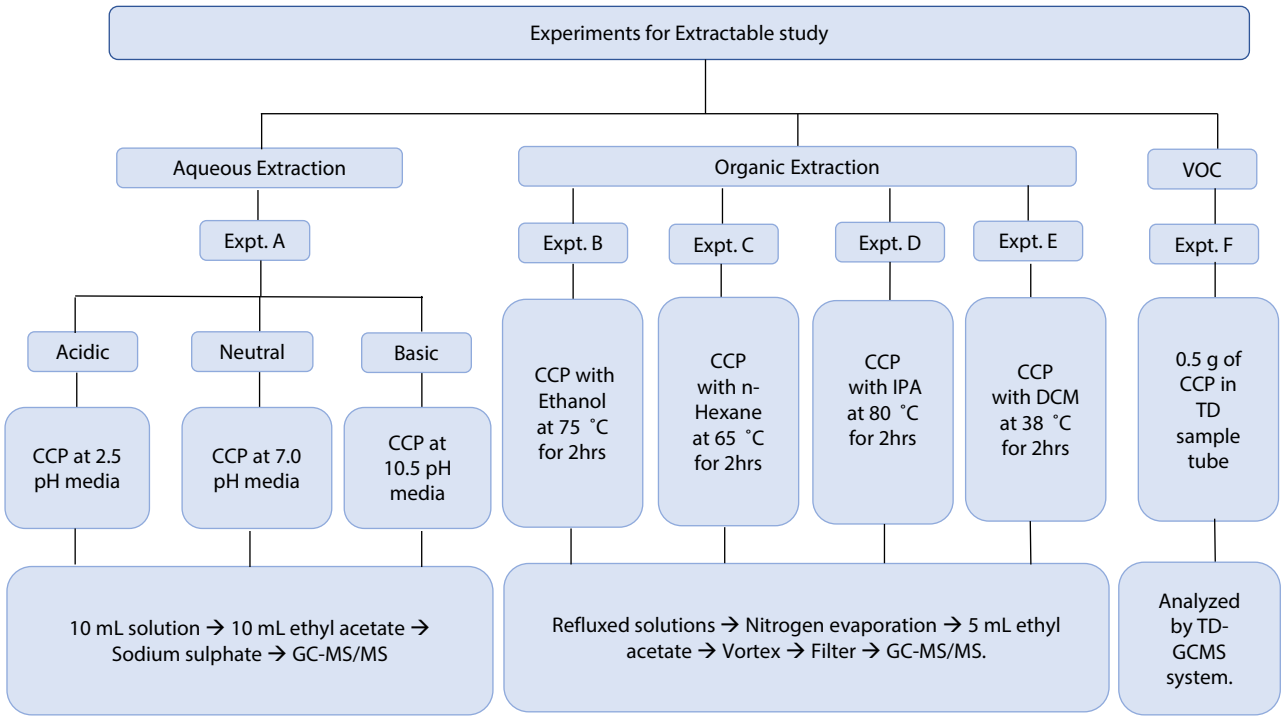

Figure 4 : Detailed layout of the experiments designed for sample preparation

Two technologies viz. GC-MS/MS and TD were used in this study for better limit of quantitation covering seventeen commonly identified extractables, sixteen polyaromatic hydrocarbons (PAHs) and volatiles/semi-volatiles.

Three methods were developed on GC-MS/MS.
Method-I → Seventeen commonly identified extractables
Method-II → Sixteen polyaromatic hydrocarbons (PAHs) For identification and semi-quantitation of unknown substances, Method-1 was taken into consideration.
Method-III → TD technique for volatiles/semi-volatiles extractables

■ Methods of analysis:

- **Method-I:**

In this method, seventeen standards were analyzed by GCMS-TQ8040 NX with AOC-20i autosampler. For the method suitability, few important parameters like system suitability, linearity and LOQ precision were performed.
Instrument Parameters are as mentioned below.

Instrument used	:	**GCMS-TQ8040 NX with AOC-20i**
Column	:	SH-I-5Sil MS 0.25 mm I.D. X 30 m d.f=0.25 µm (P/N: 221-75954-30)
Injection Mode	:	Split
Split ratio	:	10
Injection Temp.	:	250 ˚C
Flow control mode	:	Linear velocity
Linear velocity	:	36.5 cm/sec
Carrier gas	:	Helium
Purge Flow	:	3 mL/min
Total Flow	:	20 mL/min
Temp. Program	:	40 ˚C (1 min), 10 ˚C/min to 160 ˚C (7 min), 25 ˚C/min to 280 ˚C (5.20 min)
Ionization Mode	:	Electron Impact
Ion source Temp.	:	250 ˚C
Interface Temp.	:	280 ˚C
Injection volume	:	2 µL

Continued…

Acq. Mode	:	SCAN/MRM
Scan Range	:	40 m/z to 500 m/z
Detector Voltage	:	Optimized by adjusting the intensity of m/z 314 = 50000 in autotuning

MRM Transitions				
Compound name	**Target MRM**	**CE-1**	**Ref MRM**	**CE-2**
Isobutyl Benzene	134.00>91.10	22	92.00>65.10	26
Decamethyl Cyclopentyl siloxane	267.00>250.90	22	355.00>73.10	26
1,1'-Biphenyl, 2-fluoro-	172.00>170.00	26	172.00>146.20	22
Dimethyl phthalate	163.00>77.10	26	163.00>92.10	28
Tetradecamethyl Cycloheptasiloxane	147.00>73.10	18	281.00>73.10	32
3-tert-Butyl-4-hydroxyanisole (BHA)	165.00>137.10	8	180.00>165.10	10
Butylated Hydroxytoluene (BHT)	205.00>57.10	16	220.00>205.00	12
2-6-Di-tert-butyl-4-ethyl phenol (BHEB)	234.00>219.20	14	234.00>57.10	26
Diethyl Phthalate	149.00>65.10	22	149.00>93.10	14
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	219.00>191.10	10	219.00>57.10	22
Di-isobutyl phthalate	149.00>65.10	24	149.00>93.00	18
Diamyl phthalate	149.00>65.10	26	149.00>93.10	18
Benzyl butyl phthalate	149.00>65.10	26	149.00>93.10	16
Dicyclohexyl phthalate	149.00>65.10	26	167.00>149.00	8
Diphenyl phthalate	225.00>77.10	26	77.00>51.10	16
Dinonyl Phthalate	149.00>65.10	24	149.00>93.00	22
Di-n-octyl phthalate	149.00>65.10	26	149.00>93.10	20

Standard preparation:
Standard chemicals which are listed above, were procured and appropriately diluted with ethyl acetate to achieve the desired concentration as mentioned in Table 2.
Linearity of the all above listed compounds was also performed in the scan mode from 1 ppm to 15 ppm and used for semi-quantitation of unknown extractables.

Table 2 : Results of standard acquisition for method-I

Sr. No.	Compound Name	RT (min)	Linearity range (ppb)	LOQ (ppb)	Correlation coefficient (r^2)	LOQ precision (%RSD)	S/N ratio at LOQ
1	Isobutyl Benzene	7.3	5-150	5	0.996	5.1	25
2	Decamethyl Cyclopenta siloxane	9.2	5-150	5	0.998	0.8	79
3	1,1'-Biphenyl, 2-fluoro-	12.8	25-200	25	0.996	3.3	16
4	Dimethyl phthalate	13.9	50-250	50	0.995	10.6	33
5	Tetradecamethyl Cyclohepta siloxane	14.0	5-150	5	0.999	11.5	23
6	3-tert-Butyl-4-hydroxyanisole (BHA)	14.5	25-200	25	0.999	2.1	30
7	Butylated Hydroxytoluene (BHT)	14.7	5-200	5	0.995	8.6	21
8	2-6-Di-tert-butyl-4-ethyl phenol (BHEB)	15.7	5-200	5	0.994	6.0	52
9	Diethyl Phthalate	16.5	25-250	25	0.991	6.6	114
10	3,5-di-tert-Butyl-4- hydroxybenzaldehyde	21.3	10-200	10	0.997	1.8	51
11	Di-isobutyl phthalate	22.6	10-250	10	0.997	6.6	178
12	Diamyl phthalate	24.6	5-200	5	0.994	1.4	43
13	Benzyl butyl phthalate	25.6	25-250	25	1.000	5.1	54
14	Dicyclohexyl phthalate	26.5	5-250	5	0.986	0.8	60
15	Diphenyl phthalate	26.7	10-200	10	0.992	6.3	28
16	Dinonyl Phthalate	27.2	5-250	5	0.991	12.7	11
17	Di-n-octyl phthalate	27.8	10-250	10	0.987	3.9	37

Some compounds, such as 2-fluoro-1,1'-biphenyl, dimethyl phthalate, diethyl phthalate, and diphenyl phthalate, exhibit tailing during elution due to column chemistry. Despite having broad peaks, they are included in the reporting, as the method is not specific to them, but they are considered for screening purpose.

Representative data such as calibration curve (a), overlayed chromatograms of linearity standards (b) and chromatogram at LOQ level (c) is depicted in Figure 5 to 11.

Isobutyl benzene

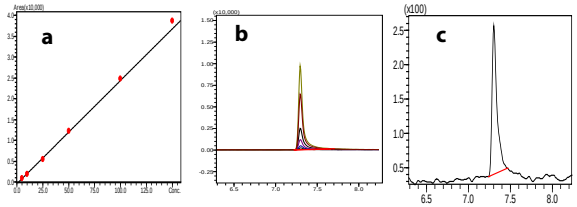

Figure 5: Data for Isobutyl benzene

Decamethyl cyclopenta siloxane

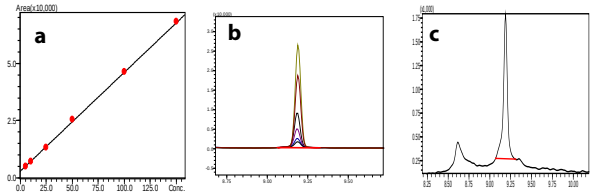

Figure 6: Data for Decamethyl cyclopentasiloxane

Tetradecamethyl cyclopenta siloxane

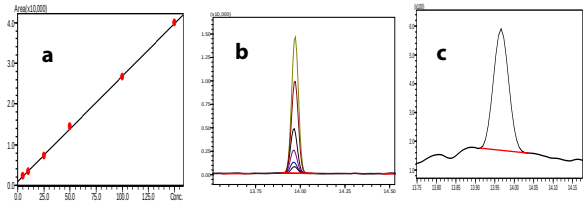

Figure 7: Data for Tetradecamethyl cyclopentasiloxane

Butylated Hydroxy Toluene

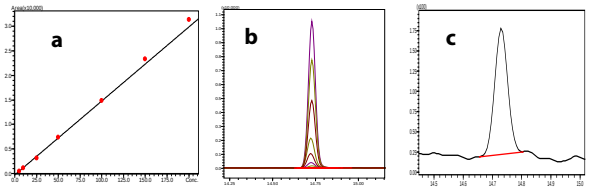

Figure 8: Data for Butylated Hydroxytoluene

2-6-Di-tert-butyl-4-ethyl phenol

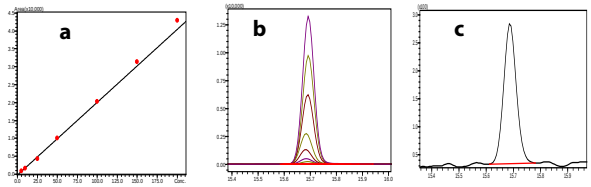

Figure 9: Data for 2-6-Di-tert-butyl-4-ethyl phenol

Diamyl Phthalate

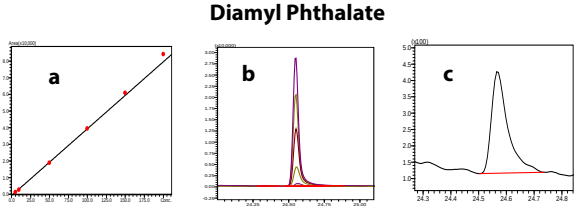

Figure 10: Data for Diamyl phthalate

Dicyclohexyl phthalate

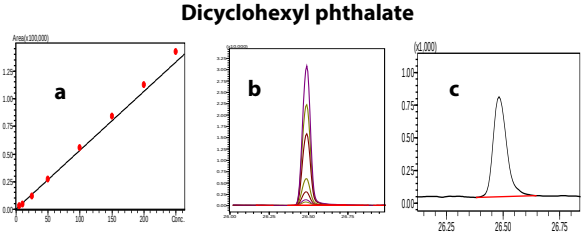

Figure 11: Data for Dicyclohexyl phthalate

- **Method-II:**

In this method, sixteen PAH standards were analyzed by GCMS-TQ8040 NX with AOC-20i autosampler. For the method suitability, few important parameters like system suitability, linearity and LOQ precision were performed.
Instrument Parameters are as mentioned below.

Instrument used	:	**GCMS-TQ8040 NX with AOC-20i**
Column	:	SH-I-PAH 0.25 mm I.D. X 30m d.f=0.1 µm (P/N: 227-36074-01)
Injection Mode	:	Split
Split Ratio	:	10
Injection Temp.	:	320 ˚C
Flow Control Mode	:	Linear velocity
Linear Velocity	:	50 cm/sec
Carrier Gas	:	Helium
Purge Flow	:	3 mL/min
Total Flow	:	24 mL/min
Temp. Program	:	50 ˚C (2 min), 12 ˚C/min to 270 ˚C (2 min), 30 ˚C/min to 300 ˚C (6.67 min)
Ionization Mode	:	Electron Impact
Ion Source Temp.	:	250 ˚C
Interface Temp.	:	300 ˚C
Acq. Mode	:	MRM
Detector Voltage	:	Optimized by adjusting the intensity of m/z 314 = 50000 in autotuning
Injection Volume	:	2 µL

MRM Transitions				
Compound	**Target MRM**	**CE-1**	**Ref MRM**	**CE-2**
Naphthalene	128.10>128.10	5	128.10>102.10	25
Acenaphthylene	152.10>152.10	5	152.10>126.10	25
Acenaphthene	153.10>153.10	5	153.10>151.10	25
Fluorene	166.10>166.10	7	166.10>164.10	37
Phenanthrene	178.10>178.10	5	178.10>152.10	25
Anthracene	178.10>178.10	5	178.10>152.10	23
Fluoranthene	202.10>202.10	5	202.10>200.10	29
Pyrene	202.10>202.10	5	202.10>200.10	33
Benz[a]anthracene	228.10>228.10	7	228.10>226.10	27
Chrysene	228.10>228.10	7	228.10>226.10	31
Benzo[b]fluoranthene	252.10>252.10	7	252.10>250.00	31
Benzo[k]fluoranthene	252.10>252.10	11	252.10>250.10	31
Benzo[a]pyrene	252.10>252.10	7	252.10>250.00	33
Indeno[1,2,3cd]pyrene	276.10>276.00	19	276.10>274.00	35
Dibenz(a,h)anthracene	278.10>278.10	13	278.10>275.90	35
Benzo[g,h,i]Perylene	276.10>276.10	7	276.10>274.00	37

PAHs are very critical for fragmentation due to stable molecular properties. Pseudo-MRM transitions were used as quantifier transitions which eliminates noise and enhances sensitivity. Also, these Quantifier MRMs are confirmed by the qualifier MRMs at the same retention time. Indeno[1,2,3cd]pyrene and Dibenz[a,h]anthracene were eluted at same. Hence, specific parent ions in MRM transitions were selected for quantification of these PAHs.

Standard preparation:
PAH standard mixture containing 16 PAH were appropriately diluted with Ethyl acetate to achieve the concentration for linearity levels. Linearity was analyzed from 1 ppb to 15 ppb as such concentration. For LOQ precision, 1 ppb standard was injected in six replicates and %RSD of area was evaluated.

Results observed in the data acquisition with standard solutions are as reported in the below Table 3.

Table 3 : Results of standard acquisition for method-II

Sr. No.	Compound Name	RT (min)	Corre. Coeff. (r^2)	LOQ precision (%RSD)	S/N ratio at LOQ
1	Naphthalene	7.9	0.998	4.4	66
2	Acenaphthylene	11.3	0.999	7.1	23
3	Acenaphthene	11.6	0.999	13.0	17
4	Fluorene	12.7	0.998	7.4	10
5	Phenanthrene	14.9	0.997	12.4	13
6	Anthracene	15.0	0.999	7.0	12
7	Fluoranthene	17.4	0.998	3.5	50
8	Pyrene	18.0	0.998	2.8	42
9	Benz[a]anthracene	20.6	0.999	3.7	12
10	Chrysene	20.7	0.999	7.9	17
11	Benzo[b]fluoranthene	23.3	0.999	4.9	11
12	Benzo[k]fluoranthene	23.4	0.999	7.3	10
13	Benzo[a]pyrene	24.2	0.999	4.7	11
14	Indeno[1,2,3-cd]pyrene	27.1	0.997	4.1	13
15	Dibenz(a,h)anthracene	27.1	0.997	7.5	16
16	Benzo[g,h,i]Perylene	28.2	0.998	6.6	13

Figure 12 to 15 depicts the calibration curve (a), overlay of linearity standards (b), LOQ level chromatograms (c) for the representative compounds.

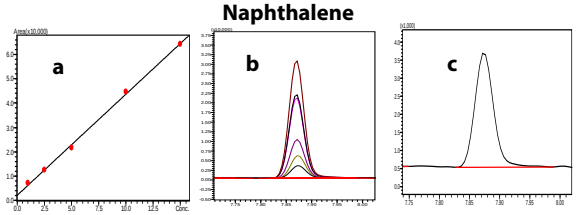

Figure 12: Data for Naphthalene

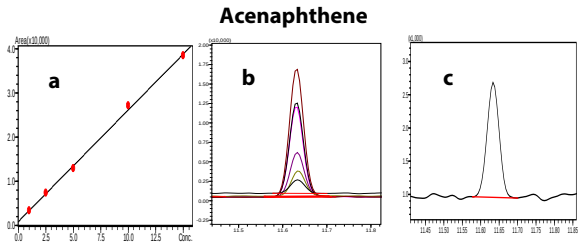

Figure 13: Data for Acenaphthene

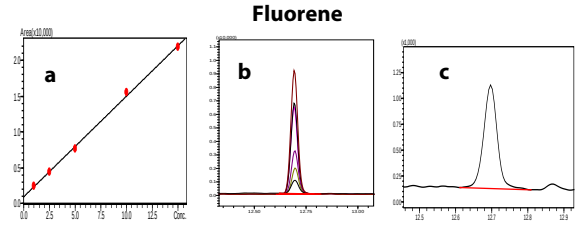

Figure 14: Data for Fluorene

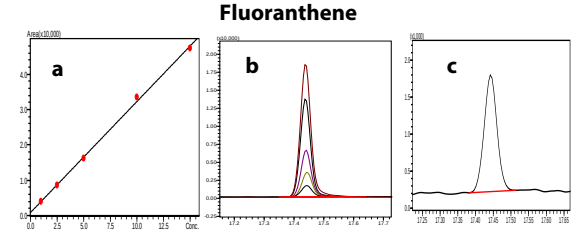

Figure 15: Data for Fluoranthene

- **Method-III:**

Thermal Desorption system allows user to load the sample directly in the sample tube which decreases extraction time. VOCs can be easily analyzed on TD as those can be lost during solvent extraction method.

Further, Shimadzu's TD-30R provides lowest trap cooling specification range i.e Room temperature -50 ˚C to 80 ˚C which doesn't allow very volatile compounds to vent out from the trap and benefits for trace level quantification of those compounds which are very volatile in the nature. Again, restore functionality in the TD-30R enables access to store the precious standards or samples by restoring those in the same tube. This Function benefits user in the extractable study to re-use the targeted compounds which are rare in the availability and costly due to certified references. Typical image of the Shimadzu's TD-30R is given below in Figure 16.

In this method, bottle was cut down in the long narrow pieces (approx. 5 cm X 0.1 cm) and inserted in the TD-sample tube and further analyzed by TD System configured with GCMS instrument. Five target standards were selected for quantification purpose which are benzene, toluene, ethyl benzene and o-Xylene & p-Xylene.
For the method suitability, few important parameters like system suitability, linearity and LOQ precision were performed.

Instrument Parameters was as mentioned below.

Instrument used	:	**GCMS-QP2020 NX with TD-30R**
Column	:	SH-I-5Sil MS 0.25 mm I.D. X 30 m d.f=0.25 µm (P/N: 221-75954-30)
Injection Mode	:	Split
Split Ratio	:	10
Flow Control Mode	:	Linear velocity
Linear Velocity	:	44 cm/sec
Carrier Gas	:	Helium
Total Flow	:	19.5 mL/min
Temp. Program	:	30 ˚C (2 min), 7 ˚C/min to 100 ˚C (5 min), 20 ˚C/min to 280 ˚C (4 min)
Ionization Mode	:	Electron Impact
Ion Source Temp.	:	200 ˚C
Interface Temp.	:	220 ˚C
Detector Voltage	:	Optimized by adjusting the intensity of m/z 314 = 160000 in autotuning
Acquisition Mode	:	Scan
Scan Range	:	35 m/z to 500 m/z
TD parameters		
Tube Desorb	:	300 ˚C for 10 min at 50 mL/min
Trap Cooling	:	-20 ˚C
Trap Desorb	:	280 ˚C for 5 min
Joint Temperature	:	250 ˚C
Valve Temperature	:	250 ˚C
Transfer Line Temp.	:	250 ˚C

Extracted ions details			
Compound name	Quantifier	Qualifier-1	Qualifier-2
Benzene	78	77	52
Toluene	91	92	65
Ethyl Benzene	91	106	55
O-Xylene	91	106	105
P-Xylene	91	106	105

Data was acquired in scan mode and processed with total ion chromatogram (TIC). Using the area observed for TIC, calibration curve was evaluated. Semi-quantification was performed based on the information on the calibration curve..
For the method suitability, few important parameters like system suitability, linearity and LOQ precision were performed.

Figure 16 : Shimadzu GC-MS/MS instrument with Thermal Desorption System

Standard preparation:
Standards of Benzene, Ethyl Benzene, Toluene, O-xylene and p-xylene were appropriately diluted with Methanol to achieve the concentration for linearity stock solutions as mentioned in the Table 4 . Linearity was analyzed from 0.2 ng to 20 ng. This is as such concentration in the TD sample tube which will become 0.4 ppb to 40 ppb with respect to sample concentration as 0.5 g. For LOQ precision, 0.2 ng standard was injected in six replicates and %RSD of area was evaluated.

Results observed in the data acquisition with standard solutions are reported in the below Table 4.

Table 4 : Results of standard acquisition for method-3

Sr. No.	Compound Name	RT (min)	Regre. Coeff. (r^2)	LOQ precision (%RSD)	S/N ratio at LOQ
1	Benzene	2.6	0.999	5.9	40
2	Ethyl Benzene	4.4	0.999	13.3	32
3	Toluene	6.5	0.999	13.1	25
4	o-Xylene	7.0	0.999	8.5	19
5	p-Xylene	7.3	0.999	7.7	30

Figure 17 to 21 depicts the calibration curve (a), overlay of linearity standards (b), LOQ level chromatograms (c) for the representative compounds.

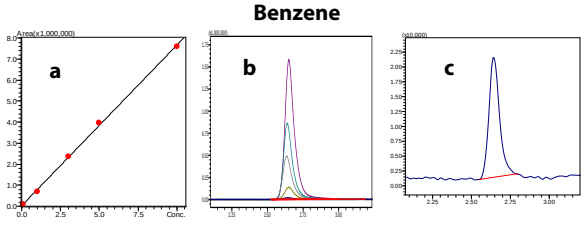

Figure 17: Data for Benzene

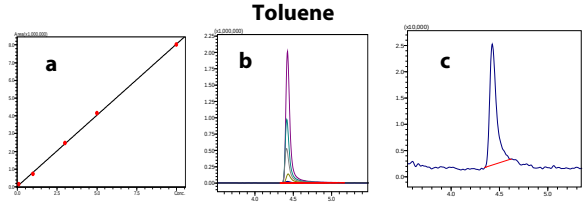

Figure 18: Data for Toluene

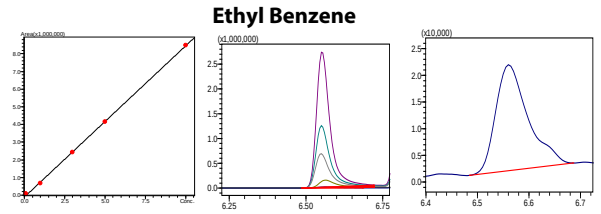

Figure 19 : Data for Ethyl benzene

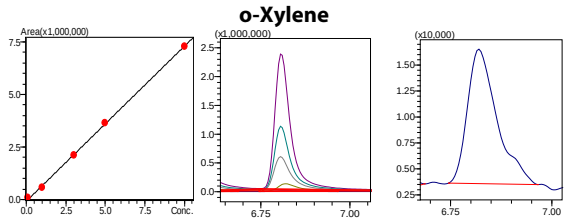

Figure 20: Data for o-Xylene

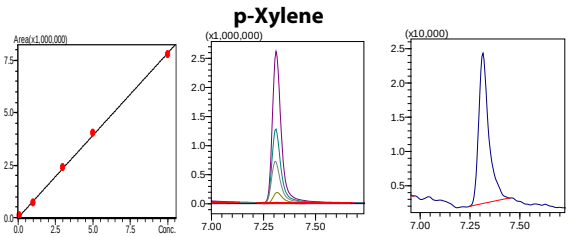

Figure 21: Data for p-Xylene

■ Results and Discussion :

Samples from all the extraction experiments were analyzed by the GC-MS/MS and TD instruments as mentioned in Method-I, Method-II and Method-III. Targeted extractables were quantified from MRM acquisition by Method-I and Method-II. Targeted extractables were monitored in samples from each experiments for Method-I and II. Highest observed content in respective experiment of each method is reported in Table 5 & 6 separately. For e.g Isobutyl benzene in Method-I was observed as 10 ppb, 34.47 ppb and 4.5 ppb in Experiment-D, E & G, respectively. Hence 34.47 ppb was reported from Experiment-E. Additionally targeted extractables data under Method-III is shown in Table 7.

Unknown extractables were semi-quantified from the scan acquisition performed in Method-I.

Table 5 : Results of Targeted compounds from Method-I

Name of the Targeted extractables	LOQ (ppb)	Content (ppb)	Observed in Expt.
Isobutyl Benzene	5	34.47	E
Decamethyl Cyclopentasiloxane	5	BLQ	A2
1,1'-Biphenyl, 2-fluoro-	25	BLQ	E
Dimethyl phthalate	50	BLQ	E
Tetradecamethyl Cycloheptasiloxane	5	BLQ	C
3-tert-Butyl-4-hydroxyanisole (BHA)	25	BLQ	B
Butylated Hydroxytoluene (BHT)	5	28.13	B
2-6-Di-tert-butyl-4-ethyl phenol (BHEB)	5	ND	NA
Diethyl Phthalate	25	236.43	E
3,5-di-tert-Butyl-4hydroxybenzaldehyde	10	208.89	C
Di-isobutyl phthalate	10	1938.92	E
Diamyl phthalate	5	8.92	D
Benzyl butyl phthalate	25	BLQ	B
Dicyclohexyl phthalate	5	349.77	B
Diphenyl phthalate	10	BLQ	D
Dinonyl Phthalate	5	14.42	A2
Di-n-octyl phthalate	10	BLQ	B

Continued…..

Where, BLQ is below limit of quantification

Table 6 : Results of Targeted extractables from Method-II

Name of the Targeted extractables (LOQ for all the PAH is 1ppb)	Content (ppb)	Observed in Experiment
Naphthalene	61.9	B
Acenaphthylene	1.5	B
Acenaphthene	10.5	E
Fluorene	12.9	C
Phenanthrene	36.6	E
Anthracene	9.0	B
Fluoranthene	12.2	E
Pyrene	7.6	E
Benz[a]anthracene	BLQ	B
Chrysene	BLQ	D
Benzo[b]fluoranthene	ND	NA
Benzo[k]fluoranthene	ND	NA
Benzo[a]pyrene	ND	NA
Indeno[1,2,3-cd]pyrene	ND	NA
Dibenz(a,h)anthracene	ND	NA
Benzo[g,h,i]Perylene	ND	NA

Where, BLQ= below limit of quantification ND=Not Detected & NA = Not Available.

Table 7 : Results of Targeted extractables from TD analysis

Name of the Targeted extractables	LOQ (ppb)	Content (ppb)
Benzene	0.4	1.5
Toluene		178
Ethyl Benzene		23
o-Xylene		32
p-Xylene		22

Results which are reported in Table 5 & 6 are quantified based on the calibration curve of targeted compound. As explained under experimental design section, all the samples were injected in GC-MS/MS as per Method-I and Method-II. For TD (Method-III) results are summarized in Table 7.

Only the highest extractables were reported for all targeted extractables.

Under this study, the screening for unknown extractables is done using only Method-I & III, as Method-II is specific for PAH analysis. The results are shown in Table 8 & 9 respectively.

Table 8 : Results of Unknown compounds from Method-I

Sr.No.	Name of Unknown extractable	RT (min)	SI	Content (ppm)
1	Tetrachloroethylene	4.1	100	34.0
2	Heptane, 2,4-dimethyl-	4.3	99	1.2
3	Ethylbenzene	4.9	97	1.4
4	2-Butanone, 3-methyl-1-phenyl-	5.0	97	1.2
5	1-Butanol, 3-methyl-, acetate	5.1	99	1.1
6	1,3,5,7-Cyclooctatetraene	5.4	99	3.7
7	D-Limonene	7.6	99	4.4
8	Octane, 5-ethyl-2-methyl-	8.0	100	1.9
9	3-Tetradecene, (Z)- #	10.1	100	4.7
10	1-Tetradecene #	13.0	100	14.1
11	Tetradecane	13.1	100	14.1
12	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	13.2	98	2.1
13	Octadecane	13.8	87	1.0
14	Heptadecane	14.0	91	1.1
15	Heptadecane	14.4	98	1.5
16	Pentadecane	14.6	100	3.7
17	Phenol, 3,5-bis(1,1-dimethylethyl)- #	14.8	100	40.2
18	Heptadecane	15.2	98	1.2
19	Heptadecane	15.9	97	1.1
20	Hexadecane, 2,6,10,14-tetramethyl-	16.0	100	5.3
21	Diethyl Phthalate	16.4	89	1.3
22	1-Heptadecene #	16.5	100	21.7
23	Hexadecane	16.7	100	53.4
24	Cyclopentane, undecyl-	18.4	99	2.9
25	Octadecane, 1-chloro-	18.6	88	1.1
26	Heptadecane	19.9	98	4.1
27	2,6,10-Trimethyltridecane	20.1	96	1.0
28	Tetradecane, 2,2-dimethyl-	20.4	98	1.9
29	Eicosane	21.0	99	2.6
30	3-Ethyl-3-methylheptadecane	21.2	96	1.7
31	Octacosane	21.4	97	1.6
32	Heptadecane, 3-methyl-	21.5	100	16.4
33	2-Ethylhexyl acrylate	21.7	89	1.9
34	1-Heptadecene #	21.8	100	18
35	Heneicosane	21.9	100	56.1
36	Eicosane	22.4	94	1.1
37	Phthalic acid, bis(7-methyloctyl) ester	22.6	95	2.4
38	Cyclopentane, undecyl-	22.6	99	5.6
39	Undecane, 3-methylene-	22.8	94	1.6
40	Octyl tetradecyl ether	22.9	82	1.4
41	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.0	97	10.3
42	2,2-Dimethyloctadecane	23.1	99	4.5
43	Triacontanoic acid, methyl ester	23.2	77	2.6
44	2-methyloctacosane	23.3	97	2.2
45	Dibutyl phthalate	23.4	98	8.8
46	3-methyl heptadecane	23.5	93	26.9
47	Undecane, 3-methylene-	23.6	70	2.1
48	Pentafluoropropionic acid, heptadecyl ester #	23.7	63	19.6
49	Heneicosane	23.7	97	57.0
50	Nonacos-1-ene	23.8	90	1.0
51	Heneicosane	23.9	94	2.1
52	1-Cyclopentyleicosane	24.1	99	12.3
53	n-Nonadecanol-1 #	24.2	100	24.1
54	Heneicosane	24.3	98	7.0
55	Tetradecane, 2,2-dimethyl-	24.4	99	8.7
56	Octyl tetradecyl ether	24.5	92	4.9
57	1-iodo hexacosane	24.7	94	37.1
58	Heneicosane	24.8	88	72.5
59	Hexacosane, 1-iodo-	24.9	98	5.6
60	3,3,13,13-Tetraethylpentadecane	25.1	96	4.5
61	1-Cyclopentyleicosane	25.2	96	9.1
62	Propionic acid, 3-iodo-, octadecyl ester	25.2	99	17.1
63	Tetradecane, 2,2-dimethyl-	25.3	99	9.4
64	Cyclononasiloxane, octadecamethyl-	25.4	98	5.0
65	Tetracosyl heptafluorobutyrate	25.4	79	3.3
66	1-iodo tricontane	25.6	94	43.7
67	Tetratetracontane	25.7	96	88.4
68	Tetrapentacontane, 1,54-dibromo-	25.8	96	7.8
69	Cyclononasiloxane, octadecamethyl-	26.0	95	7.7
70	3,3-Diethylpentadecane	26.1	97	3.3
71	1-Hexadecanol	26.2	99	12.1
72	2-Methylhexacosane	26.2	95	13.8
73	Tetradecane, 2,2-dimethyl-	26.3	98	9.9
74	Tetracontane	26.4	94	2.9
75	Tetrapentacontane, 1,54-dibromo-	26.5	93	5.9
76	Triacontane, 1-iodo-	26.6	93	50.2
77	Cyclononasiloxane, octadecamethyl-	26.7	93	7.7
78	Tetratetracontane	26.8	95	91.0
79	Hexacosane, 1-iodo-	26.9	99	6.0
80	Tetrapentacontane	26.9	98	5.0
81	2-Cyclohexylnonadecane	27.0	97	6.0
82	Triacontane, 1-iodo-	27.1	88	3.1
83	Hexacosyl nonyl ether	27.2	96	2.1
84	Pentacosane	27.3	97	2.3
85	Docosyl heptafluorobutyrate	27.5	80	21.3
86	Dodecane, 3-methyl-	27.6	84	16.4
87	Tetracontane	27.7	98	4.0
88	2-Methylhexacosane	27.9	98	6.4
89	Triacontane, 1-iodo-	28.0	95	2.0
90	Triacontane, 1-iodo-	28.1	100	47.8
91	13-Docosenamide	28.2	91	240.8
92	Tetracontane	28.3	96	99.6
93	Behenic amide	28.4	71	18.5
94	Squalene	28.5	95	20.4
95	2-Cyclohexylnonadecane	28.7	93	10.4
96	Cyclononasiloxane, octadecamethyl-	28.7	90	9.8
97	2-Methylhexacosane	29.0	94	1.2
98	Triacontane, 1-iodo-	29.1	95	2.2
99	Tetrapentacontane, 1,54-dibromo-	29.3	100	19.9
100	2,2-Dimethyleicosane	29.5	99	10.0
101	Tetracontane	29.6	96	3.6
102	2-Methylhexacosane	29.9	98	6.0

Continued…..

 # extractables are reported from experiment-B sample, however rest of all are reported from experiment-C sample.

Here, only those extractables are reported which were detected above the LOQ level i.e 1 ppm. First hit is reported from the library with its similarity index and total ion responses observed in the mass spectra of those unknown impurities were taken for semi-quantification considering worst case scenario.

Calibration curve for standards was used for the semi-quantification of the unknown extractables.

Although, this semi-quantification doesn't provide the exact content of extractable, it gives idea about its probable content, which can be compared with AET for CCP evaluation. If any extractable is detected above the qualification threshold, user can procure that standard for quantification and further toxicological assessment process can be performed.

Compounds which are reported in the Table 9 are detected in the TD analysis. Reported content is with respect to one CCP which was used in the analysis. Only those unknown impurities are reported, which were observed above 20 ppb.

Table 9 : Results of Unknown extractables from TD analysis

Sr. No.	Name of Unknown extractables	RT (min)	SI	Content (ppb)
1	Sabinyl stearate	10.1	100	73
2	Octacosane, 2-methyl-	12.3	100	28
3	Hexadecane, 1,1-bis(dodecyloxy)-	12.4	100	26
4	Octacosane, 2-methyl-	15.3	100	95
5	Hexadecane, 1,1-bis(dodecyloxy)-	15.6	100	21
6	Octacosane, 2-methyl-	18.7	100	78
7	Heptafluorobutyric acid, n-tetradecyl ester	20.1	100	30
8	Octacosane, 2-methyl-	20.3	100	515
9	Pentadecafluorooctanoic acid, tetradecyl ester	20.8	92	31
10	Docosane, 1,22-dibromo-	20.9	96	25
11	Tetratetracontane	20.9	95	21
12	Eicosane, 1-iodo-	21.1	92	22
13	Octacosane, 2-methyl-	21.2	100	175
14	Docosane, 1-iodo-	21.6	98	24
15	5-Methylnonacosane	21.7	98	29
16	Octacosane, 2-methyl-	21.8	100	146
17	Octacosane, 2-methyl-	21.8	99	29
18	Phthalic acid, bis-(10-hydroxy-decyl ester	22	81	84
19	Eicosane, 1-iodo-	22.1	99	569
20	Pentadecafluorooctanoic acid, dodec-2 -en-1-yl ester	22.1	100	26
21	Benzhydrazide, N2-benzoyl-2 -bromo-3-nitro-N1-phenyl-	22.3	100	173
22	Heptafluorobutyric acid, hexadecyl ester	22.5	97	88
23	Docosyl octyl ether	22.6	97	67
24	Eicosane, 1-iodo-	22.7	100	190
25	Butyl hexacosyl ether	22.8	96	41
26	Tetratriacontane	23	99	47
27	Dotriacontane, 1-iodo-	23.1	96	29
28	Tetracosane, 1-iodo-	23.2	99	236
29	Sulfurous acid, octadecyl pentyl ester	23.2	98	35
30	Hexatriacontane	23.3	100	290
31	Tetracosanoic acid, isopropyl ester	23.4	97	34
32	Hexatriacontane	23.6	97	40
33	Pentadecafluorooctanoic acid, octadecyl ester	23.7	86	132
34	Octacosane, 2-methyl-	23.9	96	58
35	N-(2-Hydroxy-4-octanamidophenyl)-3,5-bis(1,1-dimethylpropyl)phenoxyacetamide	24	97	170
36	Phthalic acid, butyl tridecyl ester	24.2	99	75
37	Hexacosane, 1-iodo-	24.3	99	185
38	Tetratriacontane	24.4	99	220
39	Eicosyl heptafluorobutyrate	24.8	100	87
40	Tritetracontane	24.9	97	41
41	Triacontane, 1-iodo-	25.2	100	126
42	Tetratriacontane	25.4	99	175
43	Docosyl heptafluorobutyrate	25.7	99	30
44	Hexatriacontane	26.1	100	83
45	Tetratriacontane	26.2	99	132
46	Dotriacontane, 1-iodo-	27.1	100	43
47	Hexatriacontane	27.2	100	87
48	Hexacosane, 1-iodo-	28.4	100	21
49	Tetratriacontane	28.6	100	45
50	Squalene	28.7	99	31

Continued…..

Few representative chromatograms are reported here. There are some highlighted area in the chromatogram where closely eluting peaks were detected. Figure 22 to 26 depicts, overlayed chromatograms for experiment-A samples i.e for aqueous incubation at different pH medium, Chromatogram of ethanol reflux sample, Hexane reflux sample, Isopropanol reflux sample, DCM reflux sample and overlayed chromatogram of reflux sample with highlighted critical areas, respectively.

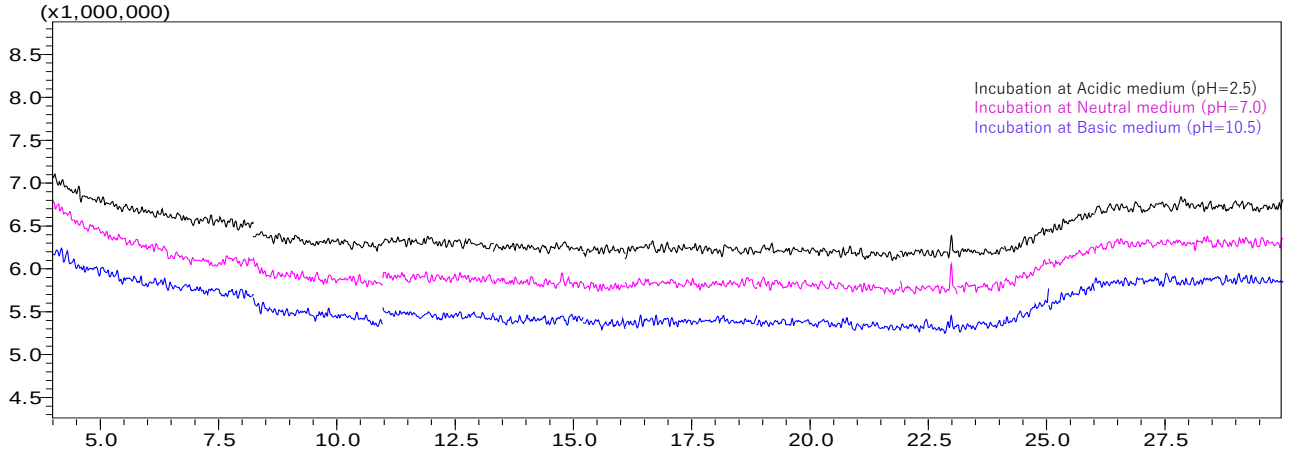

Figure 22: Overlayed chromatograms for aqueous incubation experiment sample at different pH medium

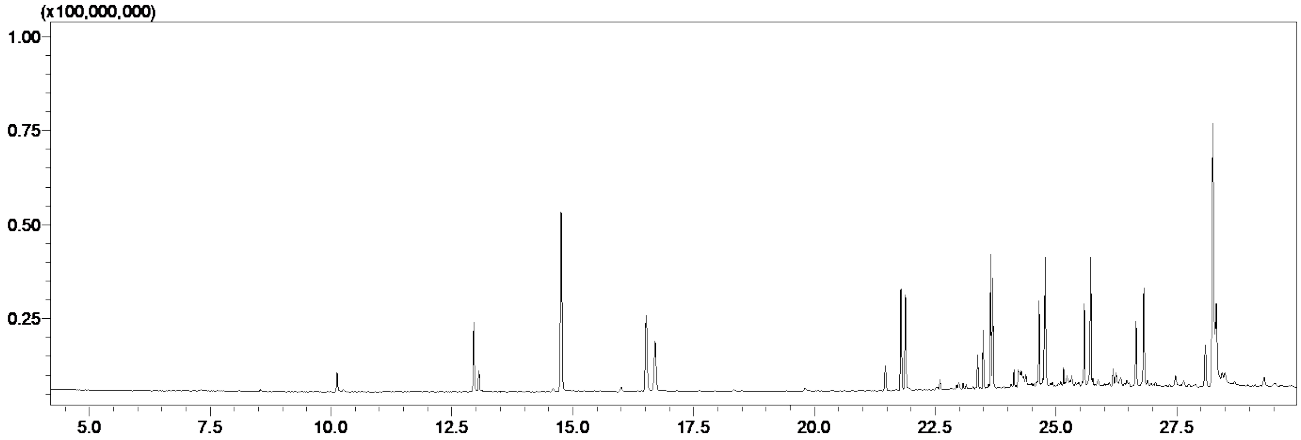

Figure 23: Chromatogram for Ethanol reflux sample at 75°C for 2 hours

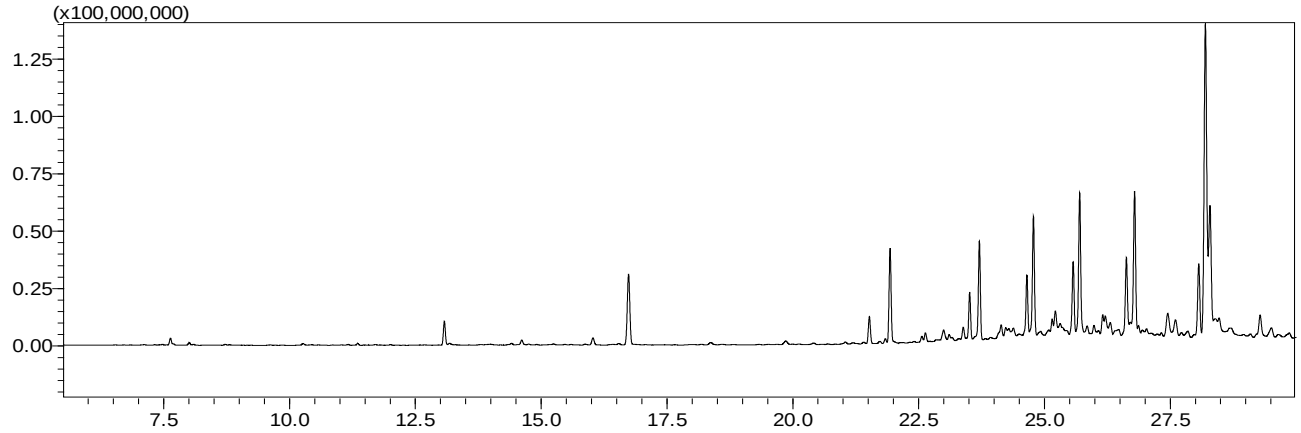

Figure 24: Chromatogram for Hexane reflux sample at 65°C for 2 hours

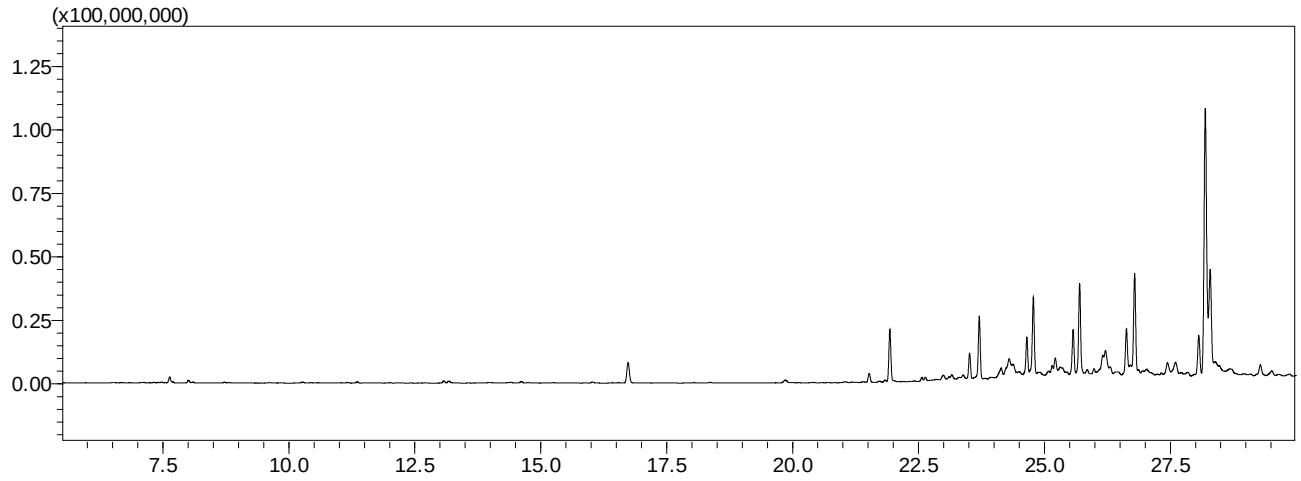

Figure 25: Chromatogram for Isopropanol reflux sample at 80°C for 2 hours

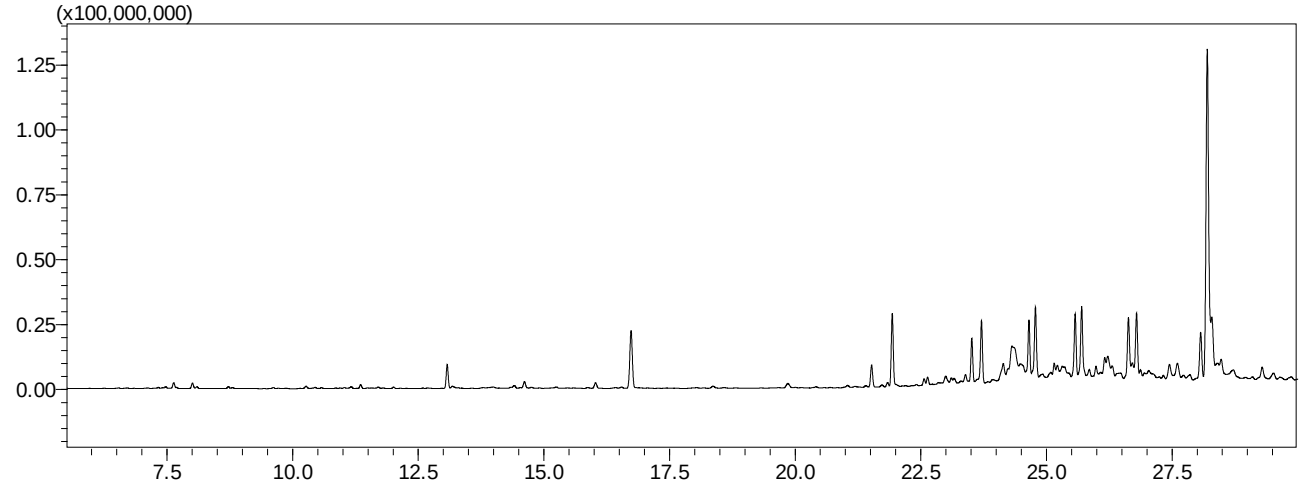

Figure 26: Chromatogram for Dichloromethane reflux sample at 38°C for 2 hours

Extraction profile is similar for the Experiment-A This experiment depicts that the CCP under study doesn't leach any unknown compound. Refer Figure 22.
Experiment-B, C, D and E were performed with refluxing the CCP sample with the solvents at their boiling points. Extraction profile was comparable with each other except few of the compounds. Response for each unknown compound identified in these experiments were different due to extraction affinity of the solvents. Maximum unknown compounds were detected with highest intensity in Hexane reflux sample (Experiment-C). Hence, this sample was considered for reporting unknown compounds in Table 8.

From 23.5 min to 28 min multiple unknown compounds were eluted. Refer focused TIC window for this time frame. Extraction profile observed in this time frame was found to be similar in all the reflux sample. CCP under study is high density polymeric bottle. So, it tends to leach the compounds which are non-polar in nature. It is reflected in the Experiment-A as well as late eluting profile nature in the reflux sample too. Semi-quantified results which are summarized in Table 8, provides tentative amount of the extractable compounds which can be leached in the sample if it is stored in respective CCP. Also, their concentration levels designs way-forward for material characterization.

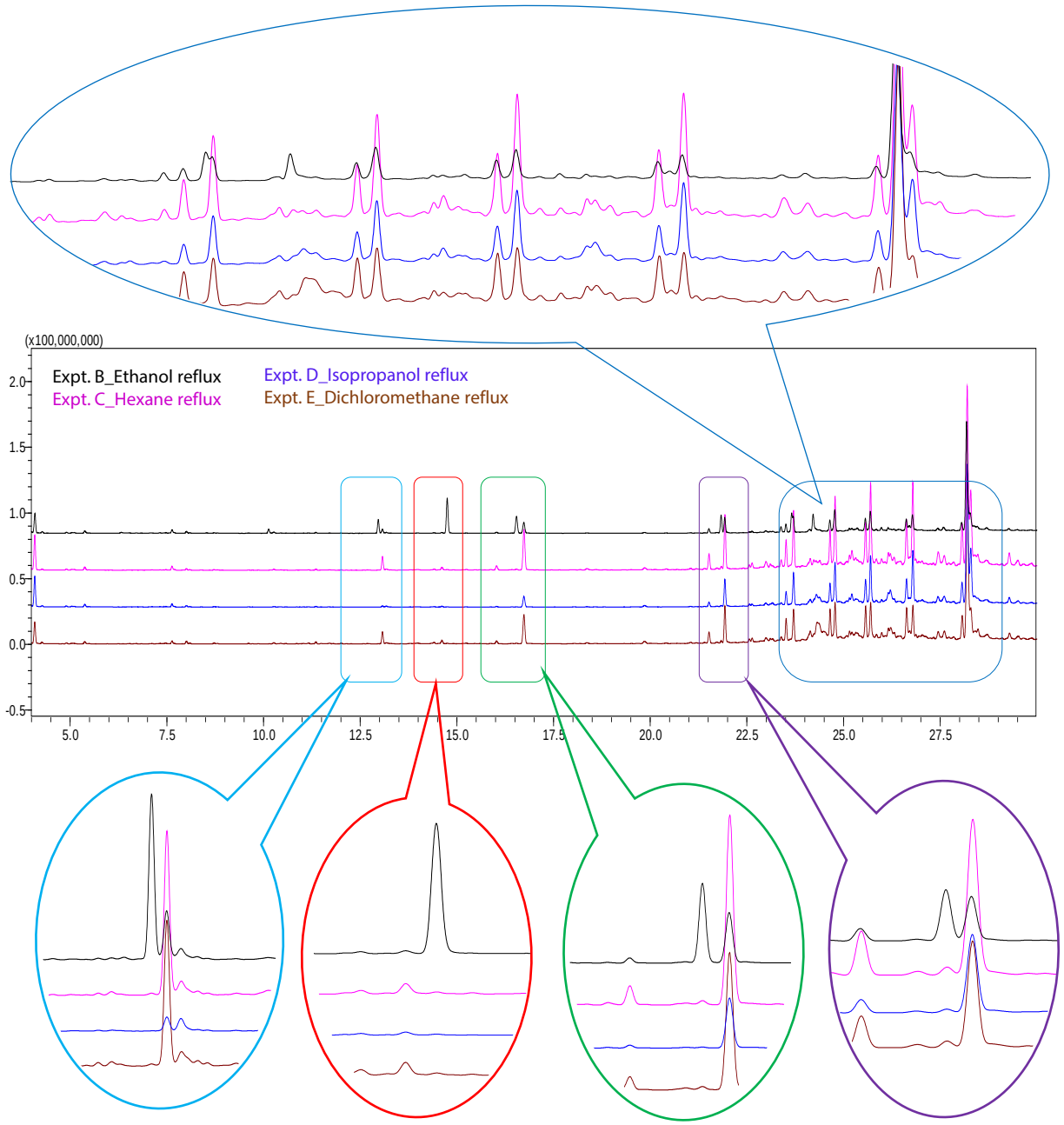

Figure 27: TIC overlay for organic-reflux experimental samples, highlighting specific unknown extractables only detected in Experiment-B (Ethanol reflux)

Figure 27 represents comparison between reflux samples. There are some critical areas where specific compounds were detected. Those regions from the overlayed chromatogram are focused and zoom views are presented for clear elucidation. One of the unknown compound which is detected at around 13 min, has potential to leach in the Ethanol medium at specific condition. This unknown compound was not detected in any of the other refluxed samples. Refer to TIC at 13 min in Figure 27. Similarly, few more compounds which were specifically detected in the Ethanol reflux sample and not in others, refer focused TIC windows at 15 min, 17 min and 22 min.

Thermal desorption system is becoming very useful technique for the extractable analysis. In this technique, those compounds can be easily identified which have tendency to leach in the sample under thermal condition. Here, sample is desorbed at high temperature. Evolved gas is trapped on the secondary trap with suitable adsorbent material and then injected in the GCMS instrument. The CCP was analyzed using Shimadzu's TD-30R instrument as explained under Experiment-F under Figure 4. All the peaks were identified and reported in Table 9.

Figure 28 depicts chromatogram of sample analyzed by heating the CCP sample at 200°C for 15 min. Some region in the chromatogram from 19 min to 29 min is presented in zoom view to showcase the elaborated elution profile.

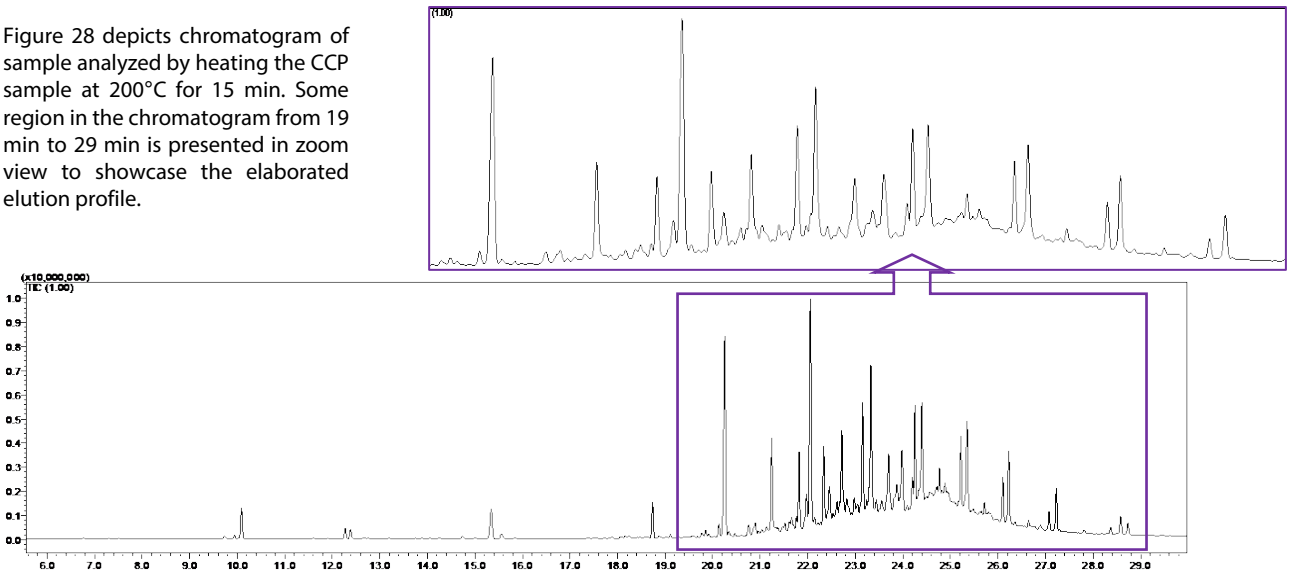

Figure 28: Chromatogram of sample analyzed by Thermal Desorption System

■ Conclusion:

- The chromatographic profile of solvent reflux experiment shows higher number of extractables than aqueous incubation. Furthermore, in aqueous incubation, the content of extractables is well below the AET level. Since, placebo of the ophthalmic drug product is completely aqueous, CCP may be used for the storage.

- Chromatographic profile from different experiments conclude that, the compounds detected in solvent extraction are different than the compounds obtained in TD analysis. Thus, additional information obtained from TD profile will further facilitate material characterization.

- Shimadzu's GCMS-TQ8040 NX triple quadrupole mass spectrometer provides high scan speed of 20000 amu/sec with ASSP technology which enables simultaneous SCAN/MRM analysis which is critical in E&L study. The UFsweeper technology enables 800 MRMs/sec which minimizes cross-talk, enhances selectivity & sensitivity.

■ References:

[1] USP <1663> Assessment of extractables associated with pharmaceutical packaging/delivery systems
[2] Extractables and leachable recommendation by Product Quality Research Institute (PQRI)

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