MP 206

# Simultaneous determination of known and unknown Extractables in pharmaceuticals packaging material by tandem mass spectrometry using Scan-MRM mode

Hemant Kesarkar[1]; Sanket Anand Chiplunkar[1]; Prashant Hase[1]; Durvesh Sawant[1]; Aseem Wagle[1]; Rahul Dwivedi[1]; Satyendra Singh[2]; Mohit Sharma[2], Jitendra Kelkar[1]; Pratap Rasam[1], Dheeraj Handique[1]

[1]Shimadzu Analytical (India) Pvt. Ltd., 1 A/B Rushabh Chambers, Makwana Road, Marol, Andheri (E), Mumbai-400059, Maharashtra, India. [2]Shimadzu Analytical (India) Pvt. Ltd., Plot No-2, Sethi Chamber, M O R Land, New Rajender Nagar, New Delhi, Delhi 110060.

## 1. Introduction

The purpose of Extractable & Leachable (E&L) studies in the regulatory references, is to identify the traces of potential chemical substances. Because these substances may be harmful to patient due to their toxicity, or the activity of drug product may impact because of their presence. Hence, to ensure the safety and efficacy of the drug till its shelf-life period, E&L study plays an important role.

Extractables are organic or inorganic entities which are released from container closure system (CCS) into an extraction medium under laboratory condition. Whereas, leachable are the foreign organic or inorganic chemical substances which are present in the packaged drug product during normal as well as accelerated stability conditions.

Samples of CCS will be used for identification of extractables. However, packaged drug product sample will be used for the evaluation of leachable. Ophthalmic drug product packaging material was used for this study (Figure 1) and the analysis was carried out on Shimadzu triple quadrupole system GCMS-TQ8040 NX equipment (Figure 2).

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Figure 1: Ophthalmic packaging system

Figure 2: GCMS-TQ8040 NX

## 2. Method development

Total 33 commonly identified potential extractable compounds were selected as targeted extractable which includes 9 phthalates, 2 siloxanes, 16 polyaromatic hydrocarbons (PAH) and 6 other additives. Two methods were developed to quantitate the above targeted extractables. Method-1 is used for quantification of targeted components (except PAH's) and unknowns. Method-2 is used for quantification of PAHs only.

### 2.1 Analytical conditions for method-1:

Instrument used : GCMS-TQ8040 NX with AOC-20i
Column : Rxi-5Sil MS 0.25mm I.D. X 30m d.f=0.25µm (P/N: 221-75954-30)
Injection Mode : Split (1:10)
Injection Temp. : 250 ℃
Flow control mode : Linear velocity @ 36.5 cm/sec
Carrier gas : Helium
Temp. Program : 40 ℃ (1 min), 10 ℃/min to 160 ℃ (7 min), 25 ℃/min to 280 ℃ (5.20 min)
Ionization Mode : Electron Impact
Ion source Temp. : 250 ℃
Acquisition mode : Scan (from 40 m/z to 500 m/z) & MRM

### 2.2 Analytical conditions for method-2:

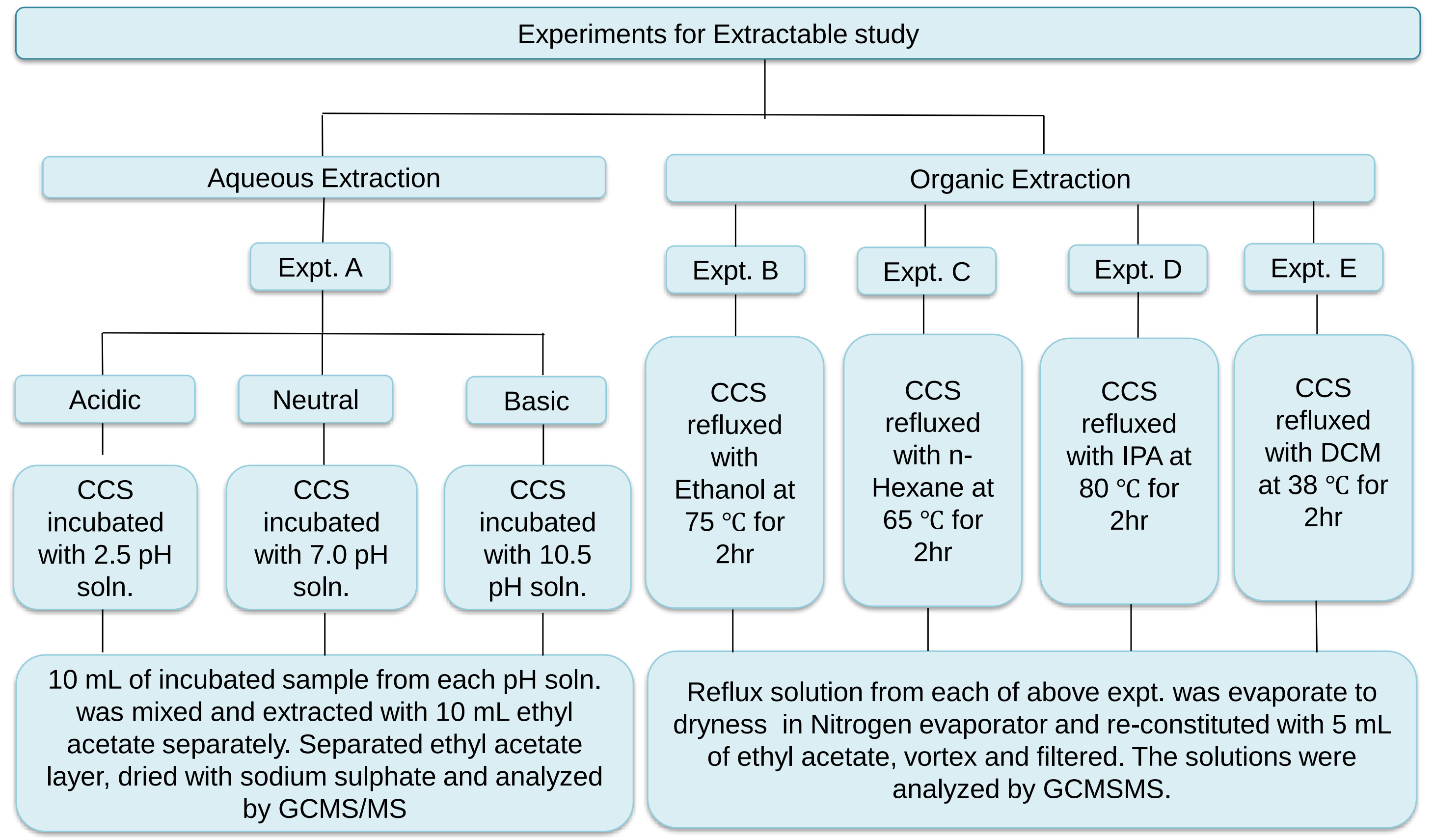
Instrument used : GCMS-TQ8040 NX with AOC-20i
Column : SH-I-PAH 0.25 mm I.D. X 30m d.f=0.1 µm (P/N: 227-36074-01)
Injection Mode : Split (1:10)
Injection Temp. : 320 ℃
Flow control mode : Linear velocity @ 52 cm/sec
Carrier gas : Helium
Temp. Program : 50 ℃ (2 min), 12 ℃/min to 270 ℃ (2 min), 30 ℃/min to 300 ℃ (6.67 min)
Ionization Mode : Electron Impact
Ion source Temp. : 250 ℃
Interface Temp. : 300 ℃

### 2.3 Standard preparation:

Standard solutions of linear concentration as mentioned in Table-1 and Table-2, were prepared using Ethyl Acetate as diluent.

### 2.4 Sample preparation:

The sample extraction procedure was carried out as per following flow diagram (Figure 3).



Experiments for Extractable study

Aqueous Extraction

Expt. A

Acidic — CCS incubated with 2.5 pH soln.

Neutral — CCS incubated with 7.0 pH soln.

Basic — CCS incubated with 10.5 pH soln.

10 mL of incubated sample from each pH soln. was mixed and extracted with 10 mL ethyl acetate separately. Separated ethyl acetate layer, dried with sodium sulphate and analyzed by GCMS/MS

Organic Extraction

Expt. B — CCS refluxed with Ethanol at 75 ℃ for 2hr

Expt. C — CCS refluxed with n-Hexane at 65 ℃ for 2hr

Expt. D — CCS refluxed with IPA at 80 ℃ for 2hr

Expt. E — CCS refluxed with DCM at 38 ℃ for 2hr

Reflux solution from each of above expt. was evaporate to dryness in Nitrogen evaporator and re-constituted with 5 mL of ethyl acetate, vortex and filtered. The solutions were analyzed by GCMSMS.

Figure 3: Flow diagram for sample preparation

## 3. Results and Discussion:

Results of Linearity and precision at LOQ for targeted compounds analyzed in both the methods are reported in below Table-1 and Table-2.

Table-1: Results for targeted compounds analysed by method-1

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

Table-2: Results for targeted compounds analysed by method-2

| Sr. No. | Compound Name | Linearity range (ppb) | Correlation Coefficient ($r^2$) | LOQ precision (%RSD, n=6) | S/N ratio at LOQ |
|---|---|---|---|---|---|
| 1 | Naphthalene | 1 ppb to 15 ppb<br>(LOQ =1 ppb) | 0.998 | 4.4 | 66 |
| 2 | Acenaphthylene | | 0.999 | 7.1 | 23 |
| 3 | Acenaphthene | | 0.999 | 13.0 | 17 |
| 4 | Fluorene | | 0.998 | 7.4 | 10 |
| 5 | Phenanthrene | | 0.997 | 12.4 | 13 |
| 6 | Anthracene | | 0.999 | 7.0 | 12 |
| 7 | Fluoranthene | | 0.998 | 3.5 | 50 |
| 8 | Pyrene | | 0.998 | 2.8 | 42 |
| 9 | Benz[a]anthracene | | 0.999 | 3.7 | 12 |
| 10 | Chrysene | | 0.999 | 7.9 | 17 |
| 11 | Benzo[b]fluoranthene | | 0.999 | 4.9 | 11 |
| 12 | Benzo[k]fluoranthene | | 0.999 | 7.3 | 10 |
| 13 | Benzo[a]pyrene | | 0.999 | 4.7 | 11 |
| 14 | Indeno[1,2,3-cd]pyrene | | 0.997 | 4.1 | 13 |
| 15 | Dibenz(a,h)anthracene | | 0.997 | 7.5 | 16 |
| 16 | Benzo[g,h,i]Perylene | | 0.998 | 6.6 | 13 |

Representative calibration curve and chromatogram at LOQ level is depicted in Figure 4.

Isobutyl Benzene, Decamethyl Cyclopentasiloxane, Dicyclohexyl Phthalate and 2-6-Di-tert-butyl-4-ethyl phenol were analysed in method-1. Naphthalene, Acenaphthalene, Fluorene and Fluoranthene were analysed in method-2.

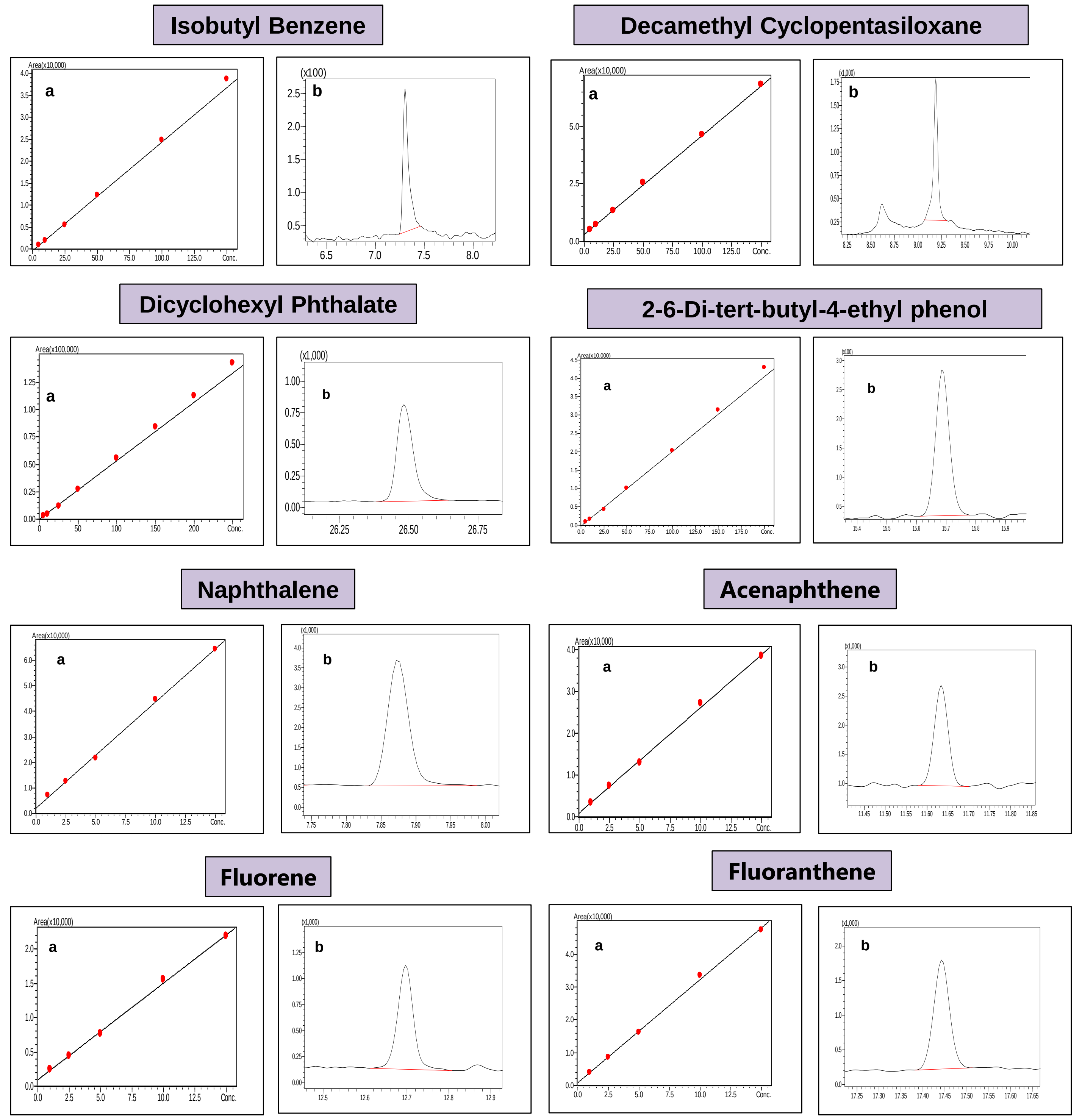


Figure 4: a) Calibration curve, b) Chromatograms of LOQ level

**Extraction profile from aqueous experiments**

Aqueous sample extraction experiments covers wide pH range. This provides suitable medium for potential extractables to get extracted from packaging material when exposed to acidic or basic medium. In this CCS, extractables were not detected in scan mode. However, two targeted extractable i.e. Decamethyl Cyclopentasiloxane and Dinonyl Phthalate were quantified in MRM mode (method-1).

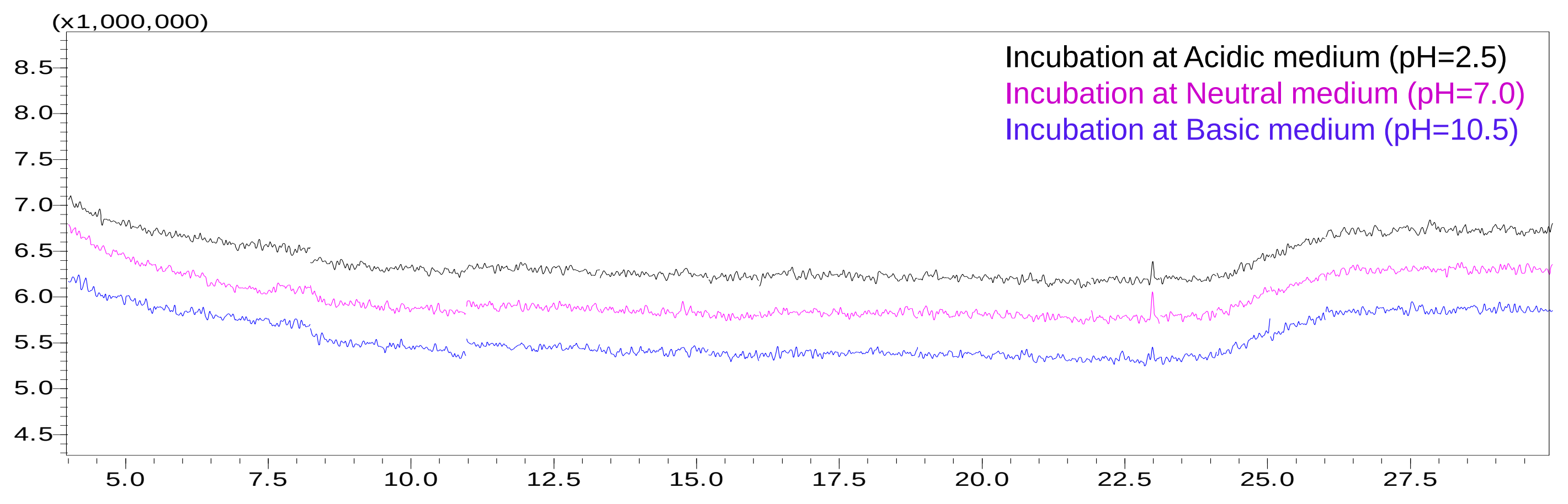


Figure 5: Overlayed total ion chromatograms for aqueous extracted experiment samples

**Extraction profile from organic-reflux experiments:**

Sample was refluxed with organic solvents such as Ethanol, Hexane, Isopropanol and Dichloromethane, with wide range of polarities. Extractables observed in this experiment are depicted in Figure 6.

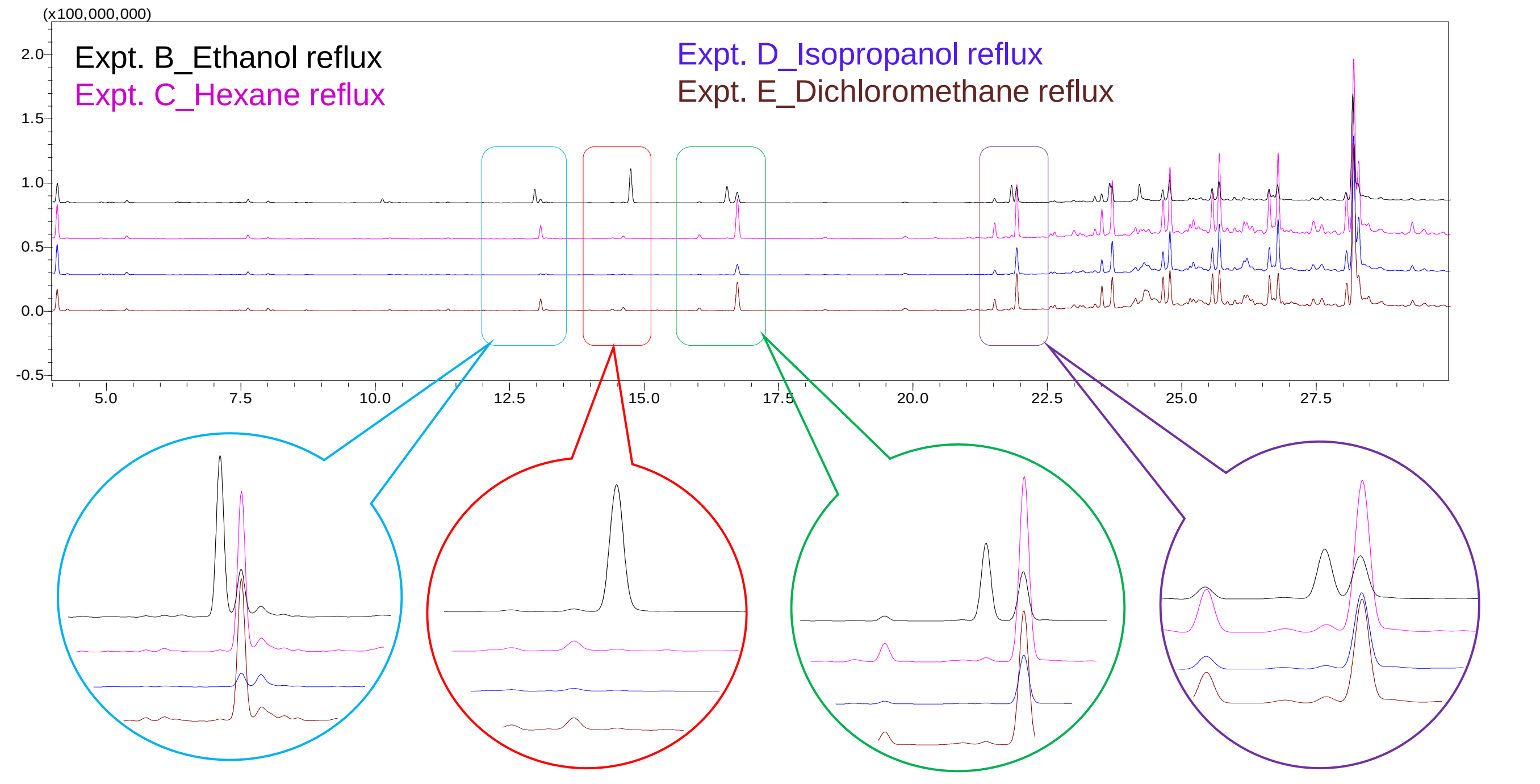


Figure 6: TIC overlay for organic-reflux experimental samples, highlighting specific unknown extractables only detected in Experiment-B (Ethanol reflux)

Targeted compounds which were detected in aqueous and organic experiments were quantified using standard linearity plot and reported in Table-3. About 88 unknown impurities were identified using scan data and semi-quantified based on average slope method. However, these are not reported here. .

Table-3: Content of targeted extractable compounds

| Name of the Targeted comp. (Method-1) | Content (ppb) | Expt. |
|---|---|---|
| Isobutyl Benzene | 34 | E |
| Decamethyl Cyclopentasiloxane | 3 | A (pH 7) |
| 1,1'-Biphenyl, 2-fluoro- | 12 | E |
| Dimethyl phthalate | 30 | E |
| Tetradecamethyl Cycloheptasiloxane | 3 | C |
| 3-tert-Butyl-4-hydroxyanisole (BHA) | 24 | B |
| Butylated Hydroxytoluene (BHT) | 28 | B |
| 2-6-Di-tert-butyl-4-ethyl phenol (BHEB) | ND | NA |
| Diethyl Phthalate | 236 | E |
| 3,5-di-tert-Butyl-4-hydroxybenzaldehyde | 209 | C |
| Di-isobutyl phthalate | 1939 | E |
| Diamyl phthalate | 9 | D |
| Benzyl butyl phthalate | 12 | B |
| Dicyclohexyl phthalate | 350 | B |
| Diphenyl phthalate | 6 | D |
| Dinonyl Phthalate | 14 | A (pH 7) |
| Di-n-octyl phthalate | 9 | B |

| Name of the Targeted comp. (Method-2) | Content (ppb) | Expt. |
|---|---|---|
| Naphthalene | 62 | B |
| Acenaphthylene | 1 | B |
| Acenaphthene | 10 | E |
| Fluorene | 13 | C |
| Phenanthrene | 37 | E |
| Anthracene | 9 | B |
| Fluoranthene | 12 | E |
| Pyrene | 8 | E |
| Benz[a]anthracene | 0 | B |
| Chrysene | 1 | 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 |

ND= Not Detected, NA= Not Applicable

## 4. Conclusion

- The migration of extractables from the CCS includes a wide range of compounds. Highly sensitive and robust techniques is required for detection at trace level. Also, Data need to be acquired in both SCAN and SIM/MRM modes to detect known and unknown extractables simultaneously without affecting sensitivity.
- Shimadzu's GCMS TQ-8040 NX triple quadrupole ultra fast mass spectrometer offers a SCAN speed of 20,000 amu/sec, 800 MRM transitions per second along with Advanced Scan Speed Protocol (ASSP) function, facilitates detection and identification extractables even at trace levels.