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BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा

वस्त्रादि – परीक्षण की विधि – क्लोरोबेंजीन और क्लोरोटोलुईन आधारित क्लोरीनयुक्त कार्बनिक कैरियर्स की मात्रा का निर्धारण

Draft *Indian Standard*

Textiles — Method of Test — Determination of The Content of Chlorinated Organic Carriers as Chlorobenzenes and Chlorotoluenes

ICS 59.080.01

Chemical Methods of Test Sectional Committee TXD 05	Last date for receipt of comments 25 October 2025

FOREWORD

(*Formal clauses will be added later***)**

Chlorinated Organic Carriers (COCs), including various chlorobenzenes and chlorotoluenes, are chlorinated aromatic compounds characterized by the presence of one or more chlorine atoms attached to a benzene or toluene ring.

Chlorinated benzenes and toluenes are chlorinated aromatic hydrocarbons commonly used as intermediates in the synthesis of various chemicals, as well as dye carriers and leveling agents in textile processing. In the apparel and footwear supply chains, these compounds are primarily associated with the dyeing of synthetic fibers, especially polyester and its blends, where they help facilitate dye uptake by acting as carriers. Their solvent properties also make them useful in chemical formulations involving dyestuffs with high melting points.

Apart from intentional use, chlorinated benzenes and toluenes may also be present as impurities in dye formulations, solvents, or other chemical mixtures used in textile manufacturing. Despite their functional roles, these substances are of concern due to their toxicity, persistence in the environment, and potential health hazards.

COCs and their isomers are associated with potential health risks. They are known to cause liver dysfunction, mucous membrane irritation, and skin disorders. Due to these hazardous effects, many leading apparel and footwear brands have prohibited the use of chlorobenzenes and chlorotoluenes in their products. Some compounds, such as Pentachlorobenzene (PCB) and Hexachlorobenzene (HCB), are categorized as Persistent Organic Pollutants. In addition, certain chlorobenzenes and chlorotoluenes are toxic to aquatic organisms even at low concentrations.

In the preparation of this standard, considerable assistance has been derived from 'BS EN 17137 : 2018 Textiles — Determination of the content of compounds based on chlorobenzenes and chlorotoluenes.'

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2: 2022 'Rules for rounding off numerical values (*second revision*)'.

Draft *Indian Standard*

TEXTILES — METHOD OF TEST — DETERMINATION OF THE CONTENT OF CHLORINATED ORGANIC CARRIERS AS CHLOROBENZENES AND CHLOROTOLUENES

1 SCOPE

1.1 This standard specifies an analytical method for determining the content of chlorobenzenes and chlorotoluenes in textiles such as dyed fibres, yarns, fabrics, and textile products, including outer fabrics, interlinings, linings, plastic slide fasteners, plastic buttons, labels, threads, and appliqués.

1.2 This method is applicable for the determination of individual isomers within the concentration range of 0.1 mg/kg to 10 mg/kg. Concentrations outside this range, both higher and lower, may also be determined by adjusting the test sample mass or by applying suitable dilution procedures.

1.3 This method is capable of detecting concentrations as low as 0.01 mg/kg when using high-sensitivity Gas Chromatography–Mass Spectrometry (GC-MS) operated in Selected Ion Monitoring (SIM) mode.

2 TERMINOLOGY

2.1 Terms and Definitions

For the purposes of this document, the following terms and definitions shall apply.

2.1.1 *Component*

An individual part of a textile product sample, consisting of a single material.

2.1.2 *Composite Test Specimen*

A test specimen prepared by combining sub-specimens from different components of the textile product.

2.1.3 *Chlorinated Organic Carriers (COCs)*

A group of aromatic compounds having one or more chlorine atoms attached to either a benzene or toluene ring.

2.1.4 *GC-MS (Gas Chromatography– Mass Spectrometry)*
An analytical technique that combines gas chromatography and mass spectrometry to separate, detect, and quantify compounds in complex mixtures with high sensitivity and specificity.

2.2 Abbreviations

The list of abbreviations is given in **Annex A**.

3 PRINCIPLE

A known weight of the sample, cut into small pieces, is extracted in an ultrasonic bath at ambient temperature for 30 minutes using a definite volume of a non-polar solvent (dichloromethane) in a closed container. The extract is then filtered through membrane filters to remove fibres and particulates. Without further purification, the filtered solution is analysed to determine the content of chlorobenzenes and chlorotoluenes by GC-MS with SIM mode, using an internal standard for quantification.

4 APPARATUS

4.1 Glass Vials with Tight Closure — of capacity 50 mL.

4.2 Graduated Pipette — of capacities 1 mL and 10 mL.

4.3 Micropipettes — of capacities 10 - 100 µL.

4.4 Volumetric Flask — of capacity 10 mL.

4.5 Ultrasonic Bath for Extraction.

4.6 Analytical Balance, with an accuracy of 0.1 mg.

4.7 Syringes with Luer Lock, 2mL, with disposable syringe filters, made of polytetrafluoroethylene (PTFE) /nylon membrane of pore size 0.20 µm has been found suitable.

4.8 Gas Chromatography with Mass Selective Detector (MSD) equipped with an Autosampler.

4.9 GC Glass Vials having Screw Cap with Silicon Septa — of capacity 2 mL.

5 REAGENTS

5.1 Dichloromethane for Residue Analysis (Analytical Grade).

5.2 Certified Reference Materials

Certified Reference Materials with traceability are listed in **Table 1**.

Table 1 Certified Reference Materials

(*Clause* 5.2)

Sl. No. (1)	Name of component (2)	CAS number (3)
i)	Chlorobenzene	108-90-7
	Dichlorobenzene isomers	
ii)	1,2 Dichlorobenzene	95-50-1
iii)	1,3 Dichlorobenzene	541-73-1
iv)	1,4 Dichlorobenzene	106-46-7
	Trichlorobenzene isomers	
v)	1,2,3 Tri chlorobenzene	87-61-6
vi)	1,2,4 Tri chlorobenzene	120-82-1
	Tetrachlorobenzene isomers	
vii)	1,2,3,4 Tetra chlorobenzene	634-66-2
viii)	1,2,3,5 Tetra chlorobenzene	634-90-2
ix)	1,2,4,5 Tetra chlorobenzene	95-94-3
x)	Pentachlorobenzene	608-93-5
xi)	Hexachlorobenzene	118-74-1
	Chlorotoluene isomers	
xii)	2- Chlorotoluene	95-49-8
xiii)	3- Chlorotoluene	108-41-8
xiv)	4- Chlorotoluene	106-43-4
	Dichlorotoluene isomers	
xv)	2,3- Dichlorotoluene	32768-54-0
xvi)	2,4- Dichlorotoluene	95-73-8
xvii)	2,5- Dichlorotoluene	19398-61-9
xviii)	2,6- Dichlorotoluene	118-69-4
xix)	3,4- Dichlorotoluene	95-75-0
xx)	3,5- Dichlorotoluene	25186-47-4
	Trichlorotoluene isomers	
xxi)	2,3,4- Trichlorotoluene	7359-72-0
xxii)	2,3,6- Trichlorotoluene	2077-46-5
xxiii)	2,4,5- Trichlorotoluene	6639-30-1
xxiv)	Benzyl chloride	100-44-7
xxv)	Benzo trichloride	98-07-7
xxvi)	4 Chlorobenzotrichloride	5216-25-1
	Tetrachlorotoluene isomers	
xxvii)	α,α,2,3- Tetrachlorotoluene	57058-14-7
	Pentachlorotoluene	
xxviii)	2,3,4,5,6- Pentachlorotoluene	877-11-2

6 SAMPLING AND TEST SPECIMEN PREPARATION

6.1 The test specimen is generally a polyester fibres/yarn/fabric dyed with disperse dyes and composite test specimen of sub-test specimens of different components. Attention has to be paid to take equivalent masses of the different selected components. For multicoloured and differently coloured products all available colours shall be selected and be tested. Up to four colours may be tested together.

6.2 In order to gather four colours, the following rules shall be applied. The rules have been listed in order of preference:

a) Select the four colours from the same part of the textile;

b) If the four colours do not come from the same part of the textile article, select these four colours from textile parts made of the same type of textile fibre.

6.3 Each colour shall have approximately the same mass in order to obtain the total mass of 2 g.

6.4 If the rules cannot be applied (e.g. Due to a complex printed pattern, plastic buttons), the sampling description of the selected test specimens shall be reported.

6.5 The test specimens are cut into pieces (approx. 5 mm wide) and stored in sealed glass vials until further processing. No conditioning of the samples is required for the analysis of COCs.

7 PROCEDURE

7.1 Extraction

(2.0 ± 0.1) g of the cut test specimen is weighed in a glass vial of 50 mL capacity and overlaid with 20 mL of dichloromethane. After adding 100 µL (1000 µg/mL) internal standard for scan mode analysis or 400 µL (10 µg/mL) internal standard for SIM mode analysis, close the vial and the test specimen is extracted for (30 ± 1) min in an ultrasonic bath (starting at laboratory ambient temperature). The weight of the test specimen and volume of the extract may be varied as per the concentration of the analyte present in the sample.

7.2 Filtration

1-2 mL of the extract is taken with a disposable syringe and cleaned of interfering particles and fibres with the help of membrane filters. The filtrate is directly transferred into a 2 mL glass vial with screw cap and silicone septa. The vial is loaded into the autosampler of the GC-MS for analysis.

7.3 Gas Chromatographic Determination

The substances of the extract are separated on a capillary column, and analysed using a mass-selective detector either in scan mode (in the range of 25 mg/kg to 500 mg/kg COCs) or SIM for trace analysis (in the range of 1.0 mg/kg to 10 mg/kg COCs).

Examples of instrumental parameters are given in **Annex B**.

7.4 Calibration

Calibration of COCs shall be done either in scan mode or in SIM mode.

7.4.1 *Calibration in Scan Mode*

Individual stock solutions of standards are prepared for calibration of the reference substances (e.g. 10 mg to 10 mL of dichloromethane (DCM), with a mass concentration S_1 = 1 mg/mL). A suitable internal standard (e.g., aniline, xylene, 2,4,5-trichloroaniline, or tetrachloro-m-xylene) is also prepared at the same concentration, IS_1 = 1 mg/mL. Further, a series of calibration solution mixtures are prepared as specified in **Table 2**.

Table 2 Preparation of Calibration Solutions for Scan Mode

(*Clause* 7.4.1)

Sl No. **(1)**	**Calibration Standard** **(2)**	**Volume of Stock Solution (S_1/IS_1) (µL)** **(3)**	**Dilution with DCM (mL)** **(4)**	**Concentration of Calibration Standard (µg/mL)** **(5)**	**Concentration of COC in Sample (mg/kg)** **(5)**
i)	1	25	10	2.5	25
ii)	2	50	10	5.0	50
iii)	3	100	10	10	100
iv)	4	250	10	25	250
v)	5	500	10	50	500
	Internal Standard	100	10	10	---

NOTE — The COC concentration is based on a 2.0 g sample weight and a 20 mL extract volume.

7.4.2 *Calibration in SIM Mode*

Individual stock solutions of standards are prepared for calibration of the reference substances (e.g. 10 mg to 10 mL of dichloromethane, with a mass concentration S_1 = 1 mg/mL). An intermediate solution, S_2, is prepared in dichloromethane by diluting 100 µL of S_1 to 10 mL of dichloromethane, resulting in a mass concentration S_2 = 10 µg/mL. A suitable internal standard (aniline, xylene, 2,4,5-trichloroaniline, or tetrachloro-m-xylene) is also prepared at the same concentration, IS_2=10 µg/mL. Further, a series of mixtures of calibration solutions are prepared as specified in **Table 3**.

Table 3 Preparation of Calibration Solutions for SIM Mode

(*Clause* 7.4.2)

Sl. No.	**Calibration Standard**	**Volume of stock solution**	**Dilution with DCM**	**Concentration of calibration**	**Concentration of COC in the**

(1)	(2)	(S_2/IS_2) (µL) (3)	(mL) (4)	standard (µg/mL) (5)	sample (mg/kg) (6)
i)	1	100	10	0.1	1.0
ii)	2	200	10	0.2	2.0
iii)	3	400	10	0.4	4.0
iv)	4	800	10	0.8	8.0
v)	5	1000	10	1.0	10.0
	Internal Standard	400	10	0.4	----

NOTE — The COC concentration is based on a 2.0 g sample weight and a 20 mL extract volume.

8 CALCULATION AND EXPRESSION OF THE RESULTS

The concentrations of chlorobenzenes or chlorotoluenes are expressed as a mass fraction in µg/mL. These concentrations are calculated using the following formula, which is based on a calibration graph and the use of an internal standard:

$$C = \frac{A_{\text{sample}} \times \beta_{\text{ISTD}} \times V}{F_{\text{ISTD}} \times m \times E}$$

where

C = concentration of COC (mg/kg);

A_{sample} = measured valued of the COC (area value);

F_{ISTD} = measured value of the internal standard (area value);

β_{ISTD} = mass concentration of the internal standard in the extract (µg/mL);

m = slope of the calibration;

V = extraction volume (mL);

E = initial mass of sample (g).

Alternatively, the calculation can be performed based on a single standard, using the formula:

$$C = \frac{C_{\text{standard}} \times A_{\text{isd.standard}} \times A_{\text{sample}} \times V}{A_{\text{standard}} \times A_{\text{isd.sample}} \times W}$$

where

C = concentration of COC (mg/kg);

C_{standard} = concentration of standard (µg/mL);

$A_{standard}$ = peak area of standard;

$A_{isd.sample}$ = peak area of internal standard in sample;

$A_{isd.standard}$ = peak area of internal standard in standard;

A_{sample} = peak area of sample;

W = initial mass of the sample (g);

V = extraction volume (mL).

9 RELIABILITY OF THE METHOD

For the reliability of the method, *see* **Annex C**.

10 TEST REPORT

The test report shall include at least the following particulars:

a] Reference of this document;

b] Identification of the submitted sample;

c] Description of the sampling of individual components;

d] Date of analysis;

e] Content of the particular compound, the nature and content as a mass fraction in mg/kg of product;

f] Detection limit of each COC in mg/kg; and

g] Any deviation from the given procedure.

ANNEX A

(C*lause* 2.2)

LIST OF ABBREVIATIONS

C	
COC	Chlorinated organic carrier
D	
DCM	dichloromethane
G	
GC-MS	gas chromatography-mass spectrometry
I	
IQR	inter quartile range
IS	internal standard
IDL	instrument detection limit
ILC	Inter-laboratory comparison
L	
LOD	limit of detection
LOQ	limit of quantification
M	
MDL	method detection limit

R

RT	retention time
RSD	relative standard deviation

S

SD	standard deviation
SIM	selected ion monitoring

ANNEX B

(*Clause* 7.3)

(Informative only)

EXAMPLE OF CHROMATOGRAPHIC METHOD

B-1 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSES

B-1.1 Instrument Conditions — Set up a GC-MS system using the conditions specified in **Table 4**, after proper calibration of the equipment employed.

Table 4 — Example of Gas Chromatographic Conditions

(*Clause* B-1.1)

Sl. No.	Carrier gas	Helium
(1)	(2)	(3)
i)	Injector temperature	250 °C
ii)	Flow rate	2 mL/min
iii)	Oven program	60 °C (10 min) 60 °C to 150 °C (2 °C/min) 150 °C to 200°C (20 °C/min) (Total Run Time — 57.5 min)
iv)	Injection mode	Spit, Split ratio (10:1)
v)	Column	5% Diphenyldimethylpolysiloxane & 95% Dimethylpolysiloxane (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm)
vi)	Injection volume	2 µL
vii)	MSD transfer line temperature	260 °C
viii)	Acquisition type	SIM/Scan
ix)	Scan rate (m/z)	Start mass = 40, End mass = 400
x)	Scan speed /sec	1666 M/Z

B-1.2 Retention Time and Mass Fragmentation Characteristics of Reference Chlorinated Organic Carriers (COCs)

B-1.2.1 The retention times (RT) and characteristic mass fragmentation patterns of selected reference COCs are determined using GC-MS under the conditions specified in **Table 4**. A variety of chlorinated benzenes and toluenes, including their positional isomers (compounds with the same molecular formula but differing in the position of chlorine substituents), were analyzed. These compounds often exhibit similar boiling points and molecular weights, which can result in co-elution (i.e., same retention time) during chromatographic separation. To minimize overlap and improve identification, the COCs were grouped into two distinct mixtures based on their retention times, as detailed in **Table 5**.

Table 5 — List of COCs in Group 1 & Group 2

(*Clause* B-1.2.1)

Sl No (1)	**Group-1 (15 COCs) (2)**		**Group-2 (13 COCs) (3)**	
	COCs	**RT (min)**	**COCs**	**RT (min)**
i)	Chlorobenzene	3.30	3-Chlorotoluene	8.58
ii)	2-Chlorotoluene	6.26	1,4 Dichlorobenzene	12.63
iii)	4-Chlorotoluene	6.58	2,4 Dichlorotoluene	20.03
iv)	1,3 Dichlorobenzene	8.99	3,4 Dichlorotoluene	20.32
v)	Benzylchloride	9.52	3,5 Dichlorotoluene	22.42
vi)	1,2 Dichlorobenzene	10.70	1,2,3 Trichlorobenzene	26.94
vii)	2,3/2,5 Dichlorotoluene	17.29	Benzotrichloride	27.81
viii)	2,6 Dichlorotoluene	18.92	2,3,4/2,3,6 Trichlorotoluene	33.58
ix)	1,2,4 Trichlorobenzene	21.17	1,2,4,5 Tetrachlorobenzene	35.12
x)	1,2,3,5 Tetrachlorobenzene	31.34	2,4,5 Trichlorotoluene	35.35
xi)	1,2,3,4 Tetrachlorobenzene	34.54	Pentachlorobenzene	46.72
xii)	4 Chlorobenzotrichloride	35.69	Hexachlorobenzene	56.41
xiii)	2,3,3,4,5 Pentachlorotoluene	55.49		
xiv)	α,α, 2,3 Tetrachlorotoluene	46.0		

B-1.2.2 Mass spectral identification is aided by the characteristic mass-to-charge ratios (m/z) of the target and qualifier ions, along with their relative response percentages, as provided in **Table 6**. These parameters assist in confirming compound identity during analysis in both scan and SIM modes.

Table 6 — Parameters for Mass Selective Detector

(*Clause* B-1.2.2)

SI. No. (1)	Name of component (2)	m/z Target ion (3)	m/z Qualifier ion-1 (4)	% Response Qualifier ion-1 (5)	m/z Qualifier ion -2 (6)	% Response Qualifier ion-2 (7)
i)	Chlorobenzene	114	112	100	72	35
ii)	3- Chlorotoluene	91	126	40	128	15
iii)	2- Chlorotoluene	91	126	40	128	20
iv)	1,3 Dichlorobenzene	146	148	65	111	52
v)	1,4 Dichlorobenzene	146	148	68	111	50
vi)	Benzyl chloride	159	161	65	194	45
vii)	1,2 Dichlorobenzene	146	148	70	111	55
viii)	4- Chlorotoluene	91	126	40	128	15
ix)	3,5- Dichlorotoluene	125	160	50	162	35
x)	2,4- Dichlorotoluene	125	160	40	162	25
xi)	2,6- Dichlorotoluene	125	160	45	162	27
xii)	2,5- Dichlorotoluene	125	160	50	162	30
xiii)	2,3- Dichlorotoluene	125	160	40	162	26
xiv)	3,4- Dichlorotoluene	125	160	40	162	28
xv)	1,2,4 Tri chlorobenzene	180	182	100	145	40
xvi)	1,2,3 Tri chlorobenzene	180	182	100	145	40
xvii)	Benzotrichloride	159	161	65	88	25
xviii)	2,4,5- Trichlorotoluene	159	194	50	196	51
xix)	2,3,6- Trichlorotoluene	159	194	60	196	57
xx)	1,2,3,5 Tetra chlorobenzene	216	214	79	218	48
xxi)	1,2,4,5 Tetra chlorobenzene	216	214	80	218	49
xxii)	2,3,4- Trichlorotoluene	159	194	49	196	47
xxiii)	1,2,3,4 Tetra chlorobenzene	216	214	80	218	49
xxiv)	4- Chlorobenzotrichloride	193	194	95	197	38
xxv)	Pentachlorobenzene	250	252	63	248	62
xvi)	α,α,2,3 Tetrachlorotoluene	193	230	75	228	60

xvii)	Hexachlorobenzene	284	286	80	282	52
xviii)	2,3,4,5,6 Pentachlorotoluene	229	264	65	262	49

B-1.2.3 The GC MS program is found to be suitable for analyzing all the COC isomers in SIM mode. Parameters for mass selective detector for Group 1 and Group 2 are given in **Table 7** and **Table 8.**

Table 7 — Parameters for Selective Ion Monitor for COCs Group 1

(*Clause* B-1.2.3)

Sl No. **(1)**	**COCs** **(2)**	**Time window (min)** **(3)**	**m/z** **(4)**
i)	Chlorobenzene 3-Chlorotoluene 4-Chlorotoluene	3.5 to 10.0	128,126,114,112,91,77
ii)	1,3 Dichlorobenzene Benzyldichloride	10.0 to 15.0	148,146,126,111,91,65
iii)	2,4 Dichlorotoluene 2,5 Dichlorotoluene 3,4 Dichlorotoluene 2,4,5 Trichlorotoluene	15.0 to 25.0	162,160,125
iv)	1,2,3 Trichlorobenzene 2,3,4 trichlorotoluene 1,2,3,5 Tetrachlorobenzene	25.0 to 37.0	218,216,214, 194,182,180,160,159,145
v)	4 Chlorobenzotrichloride α,α 2,3,Tetrachlorotoluene	37.0 to 47.0	230,228,197,194,193
vi)	2,3,4,5,6 Pentachlorotoluene	47.0 to 57.0	229,264,228

Table 8 — Parameters for Selective Ion Monitor for COCs Group 2

(*Clause* B-1.2.3)

Sl No. **(1)**	**COCs** **(2)**	**Time window (min)** **(3)**	**m/z** **(4)**
i)	2 Chlorotoluene 1,4 Dichlorobenzene	7.0 to 13.0	148,146,128,126,111,91

ii)	1,2 Dichlorobenzene 3,5 Dichlorotoluene 2,6 Dichlorotoluene 2,3 Dichlortoluene	13.0 to 23.0	162,160,148,146,125,111
iii)	1,2,4 trichlorobenzene Benzotrichloride	23.0 to 30.0	182,180,160,159, 145, 89
iv)	2,3,6 Trichlorotoluene 1,2,4,5 Tetrachlorobenzene 1,2,3,4 Tetrachlorobenzene	30.0 to 40.0	218,216,214,196, 194,159
v)	Pentachlorobenzene Hexachlorobenzene	40.0 to 57.5	284,286,282,252,250,248

ANNEX C

(*Clause* 9)

(Informative only)

METHOD VALIDATION

C-1 RELIABILITY OF THE METHOD

C-1.1 General

The in-house developed test method has been validated as per the validation protocol covering the parameters such as extraction efficiency of solvents, accuracy, precision, linearity, range of detection, spike recovery, repeatability, reproducibility, limit of detection (LOD) and limit of quantification (LOQ). Polyester fibres which are free from chlorobenzenes and chlorotoluenes were used as control specimens. All validations were done by spiking different concentration levels on a polyester fibre.

C-1.2 Extraction Efficiency of Solvents

The extraction efficiency is determined by comparing the spiked concentration to the recovered concentration of several common COCs. Among the six solvents tested, Dichloromethane (DCM) proved to be the most suitable, demonstrating an extraction efficiency of 95-99%. The extraction efficiency for each solvent is detailed in **Table 9**.

Table 9 Extraction Efficiency of Solvents

(*Clause* C-1.2)

Sl No. (1)	COCs (2)	Acetone (3)	N Hexane (4)	Cyclohexane (5)	Toluene (6)	Ethyl acetate (7)	Dichloromethane (8)
i)	1,2 Dichlorobenzene	5.5 %	18.5 %	18.8%	19.5 %	25.5 %	98.5%
ii)	1,2,3 Trichlorobenzene	6.2 %	12.1 %	10.5%	15.5%	20.1%	95.2%
iii)	1,2,3,4 Tetrachlorobenzene	5.8 %	10.7%	13.4%	18.3%	15.9%	99.0%
iv)	2 Chlorotoluene	4.5 %	11.9%	11.9%	14.6%	16.4%	98.9%
v)	2,3 Dichlorotoluene	6.9 %	15.0%	10.8%	11.9%	19.5%	95.0%

C-1.3 Accuracy (Spike Recovery Rate)

The accuracy of an analytical procedure expresses the closeness of agreement between a value accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. For quantitative approaches, accuracy can be determined by spiking at two levels, with at least five replicates obtained for each. The

percentage recovery or the difference between the mean and the accepted true value, together with the confidence level, are recommended. For accuracy, a mean recovery rate within 90% to 110% of the theoretical value and a relative standard deviation (RSD) of replicates below 10% may be taken as acceptance criteria. To establish accuracy, a textile material (polyester yarn) that is free from COCs was spiked at two levels, and five replicate analyses were conducted. Spike recovery at 0.1 mg/kg for Group 1 and Group 2 COCs is provided in **Table 10** and **Table 11**, respectively. Spike recovery at 1.0 mg/kg for Group 1 and Group 2 COCs is provided in **Table 12** and **Table 13**, respectively.

Table 10 Spike Recovery @ 0.1 mg/kg COCs (Group 1)

(*Clause* C-1.3)

Sl No (1)	**COCs (2)**	**Recovered concentration (%) (3)**							
		R1	**R2**	**R3**	**R4**	**R5**	**Mean**	**SD**	**RSD %**
	Chlorobenzene	101.21	107.71	109.4	105.17	104.55	105.61	3.13	2.97
	3-Chlorotoluene	101.02	109.16	109.49	105.58	105.06	106.06	3.46	3.26
	4-Chlorotoluene	101.17	109.55	109.55	105.70	105.15	106.22	3.0	3.29
	1,3 Dichlorobenzene	96.48	106.76	108.20	105.39	105.61	104.49	4.61	4.41
	Benzylchloride	100.74	109.99	109.25	105.38	104.88	106.05	3.73	3.52
	2,4 Dichlorotoluen	96.80	108.87	107.98	105.44	104.82	104.78	4.77	4.55
	2,5 Dichlorotoluene	96.80	108.87	107.98	105.44	104.82	104.78	4.77	4.55
	3,4 Dichlorotoluene	96.47	108.46	107.59	105.33	104.86	104.54	4.75	4.54
	2,4,5 Trichlotoluene	96.04	108.71	107.31	104.96	104.72	104.35	4.93	4.72
	1,2,3 Trichlorobenzene	94.73	108.50	106.37	103.96	104.24	103.56	5.26	5.08
	2,3,4 Trichlorotoluene	94.10	107.35	103.84	103.42	103.65	102.47	4.95	4.83
	1,2,3,5 Tetrachlorobenzene	92.91	106.55	103.42	103.37	103.46	101.94	5.22	5.12

	4 Chlorobenzotrichloride	104.911	115.37	105.21	106.23	104.58	107.26	4.57	4.26
	α,α,2,3 Tetrachlorotoluene	93.39	104.18	99.10	102.65	102.807	100.42	4.35	4.34
	2,3,4,5,6 Pentachlorotoluene	95.14	108.85	99.74	106.65	106.73	103.42	5.76	5.57

Table 11 Spike Recovery @ 0.1 mg/kg COCs (Group 2)

(*Clause* C-1.3)

COCs	**Recovered concentration (%)**							
	R1	**R2**	**R3**	**R4**	**R5**	**Mean**	**SD**	**RSD (%)**
2-Chlorotoluene	91.99	79.68	87.81	82.51	87.82	85.96	4.86	5.65
1,4 Dichlorobenzene	88.69	77.42	85.18	80.66	87.09	83.81	4.66	5.57
1,2 Dichlorobenzene	88.79	76.86	84.99	81.00	87.36	83.80	4.87	5.81
3,5 Dichlorotoluene	83.52	75.00	85.71	81.91	88.42	82.91	5.05	6.09
2,6 Dichlorotoluene	83.66	76.79	86.89	82.72	89.06	83.82	4.68	5.58
2,3 Dichlorotoluene	83.72	75.79	86.09	82.34	89.54	83.49	5.09	6.1
1,2,4 Trichlorobenzene	89.09	77.28	84.77	80.71	88.43	84.06	5.05	6.01
Benzotrichloride	94.33	79.42	87.97	83.43	89.01	86.83	5.67	6.53
2,3,6 Trichlorotoluene	84.05	75.06	83.77	80.22	89.52	82.52	5.33	6.46
1,2,4,5 Tetrachlorobenzene	91.59	80.24	88.19	83.57	89.55	86.63	4.62	5.34
1,2,3,4 Tetrachlorobenzene	90.59	79.69	86.10	84.56	90.38	86.25	4.49	5.21
Pentachlorobenzene	95.56	85.50	93.65	88.17	93.08	91.19	4.18	4.59
Hexachlorobenzene	92.06	81.69	89.83	86.15	90.80	88.11	4.2	4.77

Table 12 Spike Recovery @ 1.0 mg/kg COCs (Group-1)

(*Clause* C-1.3)

COCs	**Recovered concentration (%)**							
	R1	**R2**	**R3**	**R4**	**R5**	**Mean**	**SD**	**RSD %**

Chlorobenzene	96.86	97.33	95.27	96.93	97.26	96.73	0.84	0.86
3-Chlorotoluene	97.24	97.59	95.64	97.11	97.41	97.00	0.77	0.80
4-Chlorotoluene	96.95	97.30	95.60	97.10	97.41	96.87	0.73	0.75
1,3 Dichlorobenzene	97.61	98.20	96.06	97.26	97.64	97.35	0.79	0.81
Benzylchloride	97.80	98.30	96.02	97.00	97.53	97.33	0.87	0.89
2,4 Dichlorotoluen	96.66	97.83	95.28	96.57	96.89	96.64	0.91	0.94
2,5 Dichlorotoluene	96.66	97.83	95.28	96.57	96.89	96.64	0.91	0.94
3,4 Dichlorotoluene	96.76	97.83	95.31	96.52	96.92	96.67	0.90	0.94
2,4,5 Trichlotoluene	96.73	977.62	95.18	96.53	96.93	96.60	0.89	0.92
1,2,3 Trichlorobenzene	98.49	99.50	96.85	97.96	97.77	98.11	0.97	0.99
2,3,4 Trichlorotoluene	98.55	99.53	97.38	97.84	98.01	98.26	0.82	0.83
1,2,3,5 Tetrachlorobenzene	97.19	98.38	97.77	97.45	97.79	97.52	0.60	0.62
4 Chlorobenzotrichloride	99.83	99.47	97.84	98.62	97.65	98.68	0.96	0.97
α,α,2,3 Tetrachlorotoluene	97.42	98.63	97.41	98.55	98.21	98.04	0.59	0.60
2,3,4,5,6 Pentachlorotoluene	99.88	100.81	99.52	99.99	97.65	99.57	1.17	1.17

Table 13 Spike Recovery@ 1.0 mg/kg COCs (Group -2)

(*Clause* B-1.3)

COCs	**Recovered concentration (%)**							
	R1	**R2**	**R3**	**R4**	**R5**	**Mean**	**SD**	**RSD (%)**
2-Chlorotoluene	99.00	96.98	91.97	90.70	97.47	95.22	3.65	3.84
1,4 Dichlorobenzene	97.5	95.42	88.28	89.94	97.68	93.77	4.38	4.67
1,2 Dichlorobenzene	100.84	99.02	90.58	91.49	97.90	95.97	4.63	4.82
3,5 Dichlorotoluene	100.47	98.33	87.77	88.61	97.79	94.59	5.93	6.27
2,6 Dichlorotoluene	100.96	98.71	88.01	90.42	97.50	95.12	5.59	5.88
2,3 Dichlorotoluene	102.45	100.28	88.66	90.19	98.67	96.05	6.21	6.47
1,2,4 Trichlorobenzene	105.73	104.62	84.88	92.75	97.09	97.01	6.19	6.38
Benzotrichloride	102.86	101.70	98.85	91.16	99.19	98.75	4.56	4.62
2,3,6 Trichlorotoluene	99.02	97.44	81.47	89.21	97.30	92.89	4.03	4.34
1,2,4,5 Tetrachlorobenzene	97.24	95.88	75.75	89.42	96.66	90.99	3.62	3.99

1,2,3,4 Tetrachlorobenzene	100.64	99.72	79.01	93.68	97.74	94.16	3.08	3.28
Pentachlorobenzene	105.2	105.04	72.64	95.23	93.78	94.38	6.15	6.52
Hexachlorobenzene	97.49	97.47	94.15	91.92	86.29	93.29	5.35	5.73

C-1.4 Range and Detector Linearity

C-1.4.1 Linearity is a method validation parameter that measures how well an analytical method produces test results that are directly proportional to the concentration of an analyte in the sample. Linearity should be demonstrated across the entire range of the analytical procedure. Linearity is evaluated by plotting signals as a function of analyte concentration. A minimum of five concentrations is recommended for plotting linearity curves. The resulting plot can be analyzed using statistical methods such as regression analysis, correlation coefficient, slope, y-intercept, and the residual sum of squares. The coefficient of determination (R^2) should be at least 0.98. The slope of the regression line indicates the sensitivity of the method. A steeper slope means the method can better distinguish small differences in the concentration.

C-1.4.2 Linearity values of all COCs in scan mode in the range of 2.5 µg/mL to 50 µg/mL concentration with Group 1 and Group 2 are given in **Table 14** and **Table 15**, respectively.

Table 14 Linearity of COCs in Scan Mode (Group-1)

(*Clause* C-1.4.2)

COCs	Concentration (µg/mL) versus peak area					
	2.5 ppm	**5.0 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**R^2**
Chlorobenzene	607120	1170752	2401914	6860825	16519257	0.9932
2-Chlorotoluene	4850946	8743464	14794421	19796531	99741130	0.9957
4-Chlorotoluene	3745216	6483098	12983126	17424238	93306146	0.9975
1,3 Dichlorobenzene	3528059	6019118	12491111	44924231	86956814	0.9965
Benzylchloride	891129	1594011	2142571	23495157	48565595	0.9987
1,2 Dichlorobenzene	776092	1402447	2815795	18873654	39844520	0.9909
2,3 Dichlorotoluene	5495893	10458782	17423232	47526312	90270327	0.9989
2,5 Dichlorotoluene	5495893	10458782	17423232	47526312	90270327	0.9989
2,6 Dichlotoluene	2455687	4103152	8168645	23371697	40920337	0.9940
1,2,4 Trichlorotoluene	1528383	3083507	5607084	16821264	30082306	0.9952
1,2,3,5 Tetrachlorobenzene	2664591	4736074	9358102	23534644	44092769	0.9990
1,2,3,4 Tetrachlorobenzene	2198997	4090559	7961259	20677714	38344790	0.9985
4	349537	1207642	2683754	11458436	24622431	0.9968

Chlorobenzotrichloride						

Table 15 Linearity of COCs in Scan Mode (Group 2)

(*Clause* C-1.4.2)

COCs	Concentration (µg/mL) versus peak area					
	2.5 ppm	**5.0 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**R^2**
3-Chlorotoluene	374299	725690	1544949	3647476	6407165	0.9947
1,4 Dichlorobenzene	206827	588503	1303898	3410596	1263579	0.9999
2,4 Dichlorotoluene	250591	539429	1196975	2841168	5374252	0.9986
3,4 Dichlorotoluene	209724	456744	1048098	2339852	4512973	0.9985
3,5 Dichlorotoluene	294976	651087	1438803	3627873	6174868	0.9910
1,2,3 Trichlorobenzene	150722	314276	824698	1979887	3618113	0.9959
Benzotrichloride	129375	240211	670061	1293750	4387881	0.9991
2,3,4 Trichlorotoluene	307473	707804	1529384	3495004	6989742	0.9994
2,3,6 Trichlorotoluene	307473	707804	1529384	3495004	6989742	0.9994
1,2,4,5 Tetrachlorobenzene	316270	660930	1256027	2696426	5020075	0.9980
2,4,5 Trichlorotoluene	210153	512126	1260638	2477617	5608496	0.9941
Pentachlorobenzene	136292	214805	553361	1404749	2392394	0.9906
Hexachlorobenzene	131462	301268	467264	1084052	2229614	0.9985

C-1.4.3 Linearity values of all COCs in selected ion monitor (SIM) mode in the range of 0.1 µg/mL to 1.0 µg/mL with Group 1 and Group 2 are given in **Table 16** and **Table 17**, respectively.

Table 16 Linearity of COCs in SIM Mode (Group 1)

(*Clause* C-1.4.3)

COCs	Concentration of COCs (µg/mL) versus peak area					
	0.1 ppm	**0.2 ppm**	**0.4 ppm**	**0.8 ppm**	**1.0 ppm**	**R^2**
Chlorobenzene	208006	336946	750656	1590451	1830450	0.9932

3-Chlorotoluene	377513	623501	1403833	2923425	3365651	0.9947
4-Chlorotoluene	1141020	188579	4095442	8165252	9296106	0.9935
1,3 Dichlorobenzene	372824	608928	1396506	2948202	3414807	0.9950
Benzylchloride	467939	781252	1826241	3858520	4467812	0.9950
2,4 Dichlorotoluene	307865	510739	1158510	2534169	2918656	0.9943
2,5 Dichlorotoluene	307865	510739	1158510	2534169	2918656	0.9982
3,4 Dichlorotoluene	330939	549291	1242467	2715160	3124681	0.9942
2,4,5 Trichlorotoluene	386145	640328	1442966	3157926	3633698	0.9942
1,2,3 Trichlorobenzene	439406	719649	1583722	3458859	3999080	0.9947
2,3,4 Trichlorotoluene	374945	616422	1351334	2910648	3395594	0.9958
1,2,3,5 Tetrachlorobenzene	513211	845958	1819400	3791841	4363571	0.9950
4 Chlorobenzotrichloride	47224	78146	195425	493721	587298	0.9931
α,α,2,3 Tetrachlorotoluene	474051	782735	1693633	3497579	4027719	0.995
2,3,4,5,6 Pentachlorotoluene	40269	67259	147270	308782	360908	0.9963

Table 17 Linearity of COCs in SIM Mode (Group 2)

(*Clause* C-1.4.3)

COCs	**Concentration of COCs (µg/mL) versus peak area**					
	0.1 ppm	**0.2 ppm**	**0.4 ppm**	**0.8 ppm**	**1.0 ppm**	**R^2**
2-Chlorotoluene	580589	1052040	2101251	4082866	4542526	0.9945
1,4 Dichlorobenzene	1589233	2856832	5600631	10665289	11836177	0.9901
1,2 Dichlorobenzene	519025	944095	1894561	3684344	4165617	0.9931
3,5 Dichlorotoluene	342024	635944	1279853	2493655	27792240	0.9908
2,6 Dichlorotoluene	285652	531043	1076789	2094996	2313959	0.9892
2,3 Dichlorotoluene	226963	430307	862042	1710343	1886799	0.9894
1,2,4 Trichlorobenzene	214436	392726	798283	1548834	1804108	0.9965
Benzotrichloride	258811	516145	1156099	2471337	2886517	0.9964
2,3,6 Trichlorotoluene	295730	559644	1124536	2180574	2443418	0.9915
1,2,4,5 Tetrachlorobenzene	519780	978051	1927527	3714458	4214422	0.9935
1,2,3,4 Tetrachlorobenzene	298424	570706	1145438	2242551	2484783	0.9898
Pentachlorobenzene	246656	469231	932262	1793741	2069927	0.9954
Hexachlorobenzene	200387	371114	721747	1357990	1579307	0.9961

C-1.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

C-1.5.1 LOD and LOQ are two important parameters for analytical method validation, especially in case of impurity determination. LOD can be defined as the lowest amount of the analyte in a sample which can be detected but not necessarily quantified as an exact value. This is also known as instrument detection limit (IDL). LOQ of an analytical procedure can be defined as the lowest amount of the analyte in a sample that can be quantified with suitable accuracy and precision as an exact value. This is also known as method detection limit (MDL).

C-1.5.2 LOD and LOQ can be calculated based on signal to noise ratio (S/N) or based on standard deviation of the response and the slope of a calibration curve having a series of analyte concentrations near or with LOD and LOQ using the formulas:

$$LOD - \frac{3.3\sigma}{m}$$

and

$$LOQ = \frac{10\sigma}{m}$$

Where,

m is the slope of the calibration graph.

σ can be estimated by various ways; it can be the magnitude of analytical background response estimated by calculating standard deviation of the number of blank determinations, or it can be the residual standard deviation of the regression line.

C-1.5.3 LOD and LOQ of COCs are calculated based on the calibration graph in the range of 0.01 mg/kg to 0.10 mg/kg and are given in **Table 18** and **Table 19**.

Table C 18 LOD and LOQ of COCs (Group 1)

(*Clause* C-1.5.3)

	Concentration (µg/mL) versus peak area									
COCs	**0.01**	**0.02**	**0.04**	**0.08**	**0.1**	**R^2**	**M**	**STEYX**	**LOD (mg/kg)**	**LOQ (mg/kg)**
CB	12156	264249	490880	908024	110638	0.998	1081022	20885.0	0.006	0.01

	1				7	1	7	9	3	93
3CT	256520	604096	1145719	211010 8	260416 1	0.9978	2561173 7	53375.54	0.0068	0.0208
4CT	656331	155010 1	2926633	531930 7	651716 4	0.9970	6390246 8	156028.4	0.0080	0.0244
1,3 DCB	166970	396160	754248	138323 1	171054 3	0.9970	1681966 7	36228.96	0.0071	0.0215
BC	302180	744004	142884 7	259738 3	320877 7	0.9970	3161075 8	77549.14	0.0080	0.0245
2,4 DCT	179261	438148	844238	150836 1	185779 7	0.9961	1823057 0	50814.23	0.0091	0.0278
2,5 DCT	179261	438148	844238	150836 1	185779 7	0.9961	1823057 0	50814.23	0.0091	0.0278
3,4 DCT	158614	389337	749796	134023 2	165161 6	0.9961	1621085 5	45155.27	0.0091	0.0278
2,4,5 TCT	179438	445817	859797	152643 0	188083 9	0.9956	1844747 5	54554.58	0.0097	0.0295
1,2,3 TCB	229746	568309	1082939	189063 9	232056 5	0.9945	2261315 3	75165.27	0.0109	0.0332
2,3,4 TCT	166204	415692	773527	131342 6	159227 4	0.9915	1536038 2	63535.25	0.0136	0.0413
1,2,3,5 TECB	440756	110938 2	2076595	351732 6	429007 4	0.9917	4139097 2	169709	0.0135	0.0410
4CBTC	29199	77377	166941	315799	402619	0.9985	4074373	7071.76	0.0057	0.0173
α,α,2,3 TCT	179070	441041	838105	141109 9	171997 4	0.9914	1659276 5	68993.63	0.0137	0.0415
2,3,4,5,6 PCT	4600	11365	25112	43515	49090	0.9828	497313.3	2956.36	0.0196	0.0594

Table C.19 — LOD and LOQ of COCs (Group 2)

(*Clause* C-1.5.3)

			Concentration µg/mL versus peak area							
	0.01	**0.02**	**0.04**	**0.08**	**0.10**	**R^2**	**M**	**STEYX**	**LOD**	**LOQ**
2CT	68134	192735	335408	441524	710563	0.9907	6152063	72314.4	0.0387	0.1175
1,4 DCB	180273	505761	870939	115542 4	186008 8	0.9901	1609566 3	187805.9	0.0385	0.1166

1,2 DCB	58343	16542 0	284920	376737	61079 8	0.9902	5282748	62646.6	0.039 1	0.11 85
3,5 DCT	40014	11292 8	191408	253126	41478 4	0.9908	3571750	43324.2	0.040 0	0.12 12
2,6 DCT	33692	94421	166527	212430	32756 7	1.000	2817612	32918.9	0.038 5	0.11 68
2,3 DCT	29976	77366	138379	173436	28561 6	0.9869	2450012	31654.8	0.042 6	0.12 92
1,2,4 TCB	22039	61693	101380	137555	22996 9	0.9932	1979825	24339.3	0.040 5	0.12 29
BTC	33941	10270 2	178049	243354	43076 1	0.9962	3769913	38706.4	0.033 8	0.10 26
2,3,6 TCT	33654	96224	158592	208520	35112 9	0.9915	2998875	38706.4	0.042 5	0.12 90
1,2,4 ,5 TEC B	50173	13834 2	223823	302314	51965 9	0.9941	4442827	57893.0	0.043 0	0.13 03
1,2,3 ,4 TEC B	30319	83726	138057	184045	31846 7	0.9943	2723265	36043.1	0.043 6	0.13 23
PCB	8948	16538	23515	47127	11630 5	0.9802	1023308	21180.3	0.068 3	0.20 69
HCB	1631	3727	5670	10704	21423	0.9805	193086	2947.0	0.503	0.15 26

Where,

R^2 = Coefficient of determination

M = slope of calibration graph

STEYX = Standard error Y upon X

LOD = 3.3 (STEYX/M)

LOQ =10 (STEYX/M)

C-1.6 Precision

In analytical method validation, precision is a measure of how closely a series of measurements agree with each other when the same procedure is repeated on multiple samples. Precision is measured by injecting a series of standards or analyzing multiple samples from a homogeneous lot. It is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation or relative standard deviation,

calculated from the results obtained by carrying out replicate measurements on a suitable material under specified conditions. There are three levels of precision: repeatability (intra-assay precision), intermediate precision and reproducibility.

C-1.6.1 *Repeatability (Intra-assay Precision)*

Repeatability is the precision established under the same operating conditions over a short period of time. Repeatability, expected to give the smallest variation in results, is a measure of the variability in the results when a measurement is performed by a single analyst using the same equipment over a short time scale. It is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation or relative standard deviation calculated from the results obtained by carrying out replicate measurements on a suitable material under a specified condition. To determine the repeatability, 1mg/kg COC standard is spiked on a polyester yarn and recovery is calculated. The same experiment is repeated in five days in duplicate, and the standard deviation of the repeatability is calculated. Repeatability values of 28 COCs are given in **Table** 20 and **Table C 21**.

Table 20 Repeatability of COCs (Group 1)

(*Clause* C-1.6.1)

	Concentration of COCs (mg/kg)												
COC	**Day-1**		**Day-2**		**Day-3**		**Day-4**		**Day-5**		**Mean**	**SD**	**RSD (%)**
CB	0.76	0.76	0.77	0.78	0.78	0.81	0.81	0.85	0.87	0.83	0.80	0.037	4.66
3 CT	0.71	0.74	0.76	0.77	0.77	0.80	0.80	0.84	0.86	0.83	0.79	0.046	5.85
4 CT	0.72	0.75	0.77	0.78	0.78	0.80	0.81	0.84	0.86	0.83	0.79	0.043	5.46
1,3DCB	0.77	0.77	0.78	0.78	0.78	0.80	0.81	0.84	0.85	0.82	0.80	0.028	3.54
BDC	0.70	0.74	0.76	0.77	0.76	0.79	0.79	0.83	0.84	0.81	0.78	0.041	5.34
2,4 DCT	0.76	0.78	0.80	0.80	0.80	0.82	0.82	0.86	0.87	0.84	0.82	0.032	3.98
2,5 DCT	0.76	0.78	0.80	0.80	0.80	0.82	0.82	0.86	0.87	0.84	0.82	0.032	3.98
3,4 DCT	0.76	0.78	0.79	0.80	0.80	0.82	0.82	0.85	0.87	0.83	0.81	0.033	4.07
2,4,5 TCT	0.76	0.77	0.79	0.80	0.79	0.81	0.82	0.86	0.87	0.83	0.81	0.034	4.28
1,2,3 TCB	0.74	0.74	0.75	0.76	0.76	0.78	0.78	0.82	0.83	0.80	0.78	0.032	4.14
2,3,4 TCT	0.77	0.76	0.77	0.78	0.78	0.79	0.82	0.84	0.84	0.81	0.79	0.029	3.63
1,2,3,5-TCT	0.77	0.77	0.78	0.79	0.79	0.80	0.81	0.85	0.86	0.83	0.80	0.031	3.93
4-CBT	0.76	0.74	0.71	0.74	0.71	0.73	0.72	0.76	0.76	0.72	0.74	0.019	2.61
αα2,3-	0.75	0.74	0.75	0.77	0.77	0.78	0.78	0.82	0.83	0.80	0.78	0.030	3.86

TCT													
2,3,4,5,6-PCT	0.70	0.72	0.71	0.75	0.74	0.76	0.74	0.78	0.78	0.75	0.74	0.026	3.56

Table 21 Repeatability of COCs (Group 2)

(*Clause* C-1.6.1)

	Concentration of COCs (mg/kg)												
COC	**Day -1**		**Day -2**		**Day -3**		**Day -4**		**Day -5**		**Mean**	**SD**	**RSD (%)**
2-CT	1.09	1.05	1.04	1.07	1.04	1.05	1.07	1.07	1.13	1.10	1.07	0.029	2.74
1,4-DCB	1.08	1.04	1.03	1.05	1.04	1.04	1.05	1.05	1.11	1.08	1.06	0.025	2.40
1,2-DCB	1.08	1.05	1.04	1.07	1.04	1.04	1.06	1.05	1.11	1.08	1.06	0.024	2.26
3,5 DCT	1.08	1.46	1.03	1.05	1.03	1.04	1.05	1.05	1.11	1.08	1.10	0.025	2.27
2,6-DCT	1.08	1.04	1.03	1.05	1.03	1.04	1.05	1.05	1.11	1.08	1.06	0.025	2.36
2,3-DCT	1.08	1.03	1.02	1.06	1.03	1.04	1.04	1.05	1.10	1.07	1.05	0.024	2.28
1,2,4 TCB	1.04	1.07	1.05	1.06	1.03	1.03	1.04	1.04	1.08	1.05	1.05	0.017	1.65
BTC	1.07	1.03	1.02	1.03	1.03	1.05	1.05	1.05	1.11	1.07	1.05	0.027	2.58
2,3,6 TCT	1.04	1.02	1.00	1.04	1.03	1.04	1.03	1.03	1.08	1.03	1.03	0.018	1.74
1,2,4,5-TCB	1.04	1.06	1.02	1.05	1.04	1.04	1.03	1.02	1.07	1.03	1.04	0.015	1.51
1,2,3,4-TCB	1.09	1.09	1.06	1.09	1.08	1.08	1.08	1.07	1.14	1.07	1.08	0.022	2.09
PCB	1.02	1.11	1.10	1.12	1.08	1.08	1.06	1.03	1.06	1.01	1.07	0.037	3.50
HCB	0.91	1.17	1.13	1.16	1.08	1.06	1.05	1.01	0.93	0.87	1.04	0.10	10.0

C-1.6.2 *Intermediate Precision*

Intermediate precision, also known as laboratory reproducibility, is a measure of precision that takes into account variations within a laboratory over a period of time. It's used in method validation to assess the variability of results under different conditions, such as different analysts, different days, different equipment, different batches of reagents, etc. Intermediate precision measures the effects of random events on the precision of the analytical procedure. To determine the laboratory reproducibility, 1 mg/kg COC standards is spiked on a polyester yarn and recovery is calculated. The same experiment is repeated in five to seven days by different operators, and the standard deviation of the reproducibility is calculated. Intermediate precision of 28 COCs is given in **Table** 22 and **Table** 23.

Table 22 Reproducibility of COCs (Group 1)

(*Clause* C-1.6.2)

COC	Day-1 Operator-1	Day-2 Operator-2	Day-3 Operator-3	Day-4 Operator-1	Day-5 Operator-2	Day-6 Operator-3	Day-7 Operator-1	Mean	SD	RSD(%)
	Concentration of COCs (mg/kg)									
CB	0.96	0.95	0.97	0.97	0.98	0.99	0.99	0.97	0.015	1.58
3CT	0.97	0.96	0.97	0.97	0.98	0.99	0.99	0.98	0.013	1.41
4CT	0.95	0.95	0.97	0.97	0.98	0.99	0.99	0.97	0.018	1.85
1,3 DCB	0.97	0.96	0.97	0.98	0.98	0.99	1.00	0.98	0.011	1.20
BDC	0.96	0.95	0.97	0.98	0.98	0.99	0.99	0.97	0.016	1.64
2,4 DCT	0.98	0.97	0.97	0.98	0.99	0.99	0.99	0.98	0.008	0.86
2,5 DCT	0.98	0.97	0.97	0.98	0.99	0.99	0.99	0.98	.008	0.86
3,4 DCT	0.98	0.97	0.97	0.98	0.99	0.99	1.00	0.98	0.009	0.96
2,4,5 TCT	0.98	0.97	0.97	0.98	0.98	0.99	0.99	0.98	0.009	0.93
1,2,3 TCB	0.97	0.97	0.97	0.99	0.98	1.00	1.00	0.98	0.012	1.29
2,3,4 TCT	0.97	0.97	0.97	0.98	0.98	1.00	1.00	0.98	0.013	1.32
1,2,3,5 TCT	0.96	0.95	0.96	0.98	0.98	0.99	1.00	0.98	0.017	1.74

4 CBTC	0.96	0.92	0.95	0.99	0.96	1.01	1.01	0.97	0.03	3.29
αα2,3-TCT	0.96	0.95	0.96	0.99	0.97	1.00	0.99	0.97	0.018	1.92
2,3,4,5,6 PCT	0.97	0.94	0.96	0.99	0.97	0.99	0.98	0.97	0.019	1.96

Table 23 — Reproducibility of COCs (Group 2)

(*Clause* C-1.6.2)

COC	**Day-1 Operator-1**	**Day-2 Operator-2**	**Day-3 Operator-3**	**Day-4 Operator-1**	**Day-5 Operator-2**	**Mean**	**SD**	**RSD (%)**
	Concentration of COCs (mg/kg)							
2CT	1.10	1.09	1.07	1.07	1.13	1.09	0.0263	2.40
1,4 DCB	1.08	1.08	1.05	1.05	1.11	1.08	0.0232	2.15
1,2 DCB	1.08	1.08	1.05	1.06	1.11	1.08	0.0240	2.21
3,5 DCT	1.08	1.08	1.05	1.05	1.11	1.07	0.0246	2.29
2,6 DCT	1.08	1.08	1.05	1.05	1.11	1.07	0.0251	2.33
2,3 DCT	1.07	1.08	1.05	1.04	1.10	1.07	0.0236	2.20
1,2,4 TCB	1.05	1.04	1.04	1.04	1.08	1.05	0.0194	1.84
BTC	1.07	1.07	1.05	1.05	1.11	1.07	0.0262	2.44
2,3,6 TCT	1.03	1.04	1.03	1.03	1.08	1.04	0.0194	1.85
1,2,4,5 TCB	1.03	1.04	1.02	1.03	1.07	1.04	0.0197	1.89
1,2,3,4 TCB	1.07	1.09	1.07	1.08	1.14	1.09	0.0302	2.76
PCB	1.01	1.02	1.03	1.06	1.06	1.04	0.022	2.14

							3	
HCB	0.87	0.91	1.01	1.05	0.93	0.95	0.0740	7.72

C-1.6.3 *Reproducibility (Inter-Laboratory Comparison)*

C-1.6.3.1 Reproducibility measures the ability of different analysts in different laboratories to get similar results. It is a measure of how sensitive a method is to change in a laboratory, such as variation in the operator's technique, equipment performance, or the lab environment. It is also known as between-lab reproducibility. Inter-Laboratory Comparison (ILC) can be performed on a homogenized sample having a known concentration of analyte or a blank sample spiked with a known concentration of analyte. To estimate the reproducibility of the test method, two polyester materials (one fibre and one Yarn) were dyed with disperse dyes (Disperse blue and Disperse red) at 100 °C using two common carriers: 1,2 dichlorobenzene and 3 chlorotoluene. The dyed materials were washed and dried. COC content in the dyed samples was measured as per the developed test method. Stability and homogeneity of the COCs within the samples were also studied.

C-1.6.3.2 For establishing the homogeneity of samples, analyses were conducted in triplicate and variation (RSD %) were calculated. Similarly, to check the stability of COCs, samples were analyzed on different days within a span of 10 days intervals and variations (RSD %) were calculated. Homogeneity and stability values are given in **Table 24** and **Table 25**, respectively. From **Table 26** and **Table C 27** it can be seen that the RSD % of COCs are below 10 % which indicates that samples have homogeneity. Again, the variation within the average value of homogeneity and stability is below 5.0 % which indicates that samples have stability.

Table 24 Homogeneity of COCs in Sample

(*Clause* C-1.6.3.2)

	Blue fibre		**Red yarn**	
Trials	**3 Chlorotoluene**	**1,2 Dichlorobenzene**	**3 Chlorotoluene**	**1,2 Dichlorobenzene**
1	115.50	658.41	3916.84	10361.98
2	111.59	626.94	4179.42	10242.60
3	115.22	685.11	4053.61	10764.40
4	108.66	651.32	4142.80	10461.83
5	102.97	652.42	4321.90	10656.19
Average	110.78	654.84	4122.91	10497.40
SD	5.1994	20.7706	150.37	212.73
RSD	4.69	3.17	3.64	2.02

(%)				

Table 25 Stability of COCs in Sample

(*Clause* C-1.6.3.2)

	Blue fibre		Red yarn	
Trials	**3 Chlorotoluene**	**1,2 Dichlorobenzene**	**3 Chlorotoluene**	**1,2 Dichlorobenzene**
1st Day	108.20	650.20	3961.44	10450.5
2nd Day	105.03	610.21	4369.93	10465.1
5th Day	101.88	597.70	4064.93	10689.0
8th Day	111.26	639.25	4455.44	10856.1
10th Day	112.83	660.35	4021.33	10700.2
Average	107.84	631.54	4174.61	10632.18
SD	4.47	26.63	222.47	172.423
RSD (%)	4.15	4.21	5.32	1.62

C-1.6.3.3 The dyed specimens were sent to nine NABL-accredited laboratories for an inter-laboratory comparison (ILC) study. The round robin test results were collected, and a robust Z score was calculated. The Z score values of 3 chlorotoluene and 1,2 dichlorobenzene in blue fibre are given in **Table C.18**. The Z score values of 3 chlorotoluene and 1,2 dichlorobenzene in red yarn are given in **Table C.19**.

C-1.6.3.4 The Z score value is calculated using the following Equation (1):

$$Robust\ Z\ Score = \frac{Xi - Median}{NIQR} \quad (1)$$

Where,

*X*i is the result provided by the participant laboratory

$$Median = \mathrm{X}\left[\frac{n}{2} + \left(\frac{1}{2}\right)\right]$$

Normalized IQR (*NIQR*) = IQR X 0.7413 at Normal distribution

Inter Quartile Range (IQR) = Upper Quartile (Q_3) - Lower quartile (Q_1)

Where,

$$Q_3 = \mathrm{X}\left[\frac{3n}{4} + \left(\frac{1}{2}\right)\right]$$

and

$$Q_1 = \mathrm{X}\left[\frac{n}{4} + \left(\frac{1}{2}\right)\right]$$

X is the result provided by the participant laboratory

n is the number of participating laboratories

C-1.6.3.5 The Interpretation of Z score is based on the following criteria:

Acceptable range: I Z I ≤ +/- 2 .0

Straggler: +/-2.0 < I Z I ≤ 3.0

Outlier: I Z I > 3.0

Table 26 COC Content in Blue Fibre and Z Score Values

(*Clause* B-1.6.3.3)

	3 Chlorotoluene		1,2 Dichlorobenzene	
Lab code	**Reported value(mg/kg)**	**Z score**	**Reported value(mg/kg)**	**Z score**
A	119	0.00	710	0.26
B	105	-0.33	617	-0.07
C	120	0.01	785	0.53
D	159	0.95	975	1.19
E	96.27	-0.54	528.7	-0.37
F	130.12	0.26	967.8	1.16
G	91.1	-0.66	582.9	-0.18
H	191	1.71	1106	1.65
I	102.3	-0.40	654.3	0.07
J	160	0.98	529.3	-0.37
Median(Q_2)	119		635.65	
LQ (Q_1)	102.3		582.9	
UQ(Q_3)	159		967.8	
IQR	56.7		384.9	
NIQR	42.03		285.32	

Table 27 COC Content in Red Yarn and Z Score Values

(*Clause* B-1.6.3.3)

	3 Chlorotoluene	1,2 Dichlorobenzene

Lab code	Reported value (mg/kg)	Z score	Reported value (mg/kg)	Z score
A	4277	-0.09	10548	0.72
B	4026	-1.02	10121	-0.31
C	4313	0.03	10679	1.07
D	4696	1.47	10729	1.16
E	4153.1	-0.55	10258.4	0.02
F	4236.7	-0.24	10238.5	-0.02
G	4719.9	1.56	10982.6	1.77
H	4300	0.00	10117	0.32
I	4357	0.21	10171	-0.19
J	4600	1.11	10900	1.58
Median(Q_2)	4300		10248.45	
LQ (Q_1)	4236.7		10171	
UQ(Q_3)	4600		10729	
IQR	363.3		558	
NIQR	269.31		413.64	