Development and validation of test method for determination of chlorinated organic carriers in textiles

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Abstract

A test method for analyzing chlorobenzene and chlorotoluenes (commonly known as COC,s) in textiles and textile products has been developed and the same has been validated as per international validation protocol. 28 restricted COC,s in textiles as per Oekotex were standardized by using GC MS. As a part of method validation, Detector linearity, Accuracy, Repeatability, Spike recovery, LOD, LOQ, etc. is established. A sample preparation method using a solvent extraction method is also developed based on extraction efficiency of few solvents.

Keywords

Chlorobenzene, Chlorotoluene, Chlorinated organic carrier, GC MS, Method validation,

Citation

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1.0 Introduction:

Chlorinated Benzenes and Toluenes generally called chlorinated carriers are a group of organic compounds having one or more chlorine atoms attached to either a benzene or toluene ring. Chlorobenzenes and Chlorotoluenes have diversified application in several industries including textile and footwear industries [1]. Chlorinated carriers are typically used as intermediates or precursors in the manufacture of textile dyes and auxiliaries. It is also used in polyester dyeing as a dispersing and leveling agent [1]. After dyeing, polyester materials are washed with water to remove unfixed dyes and carriers. However, trace amounts of carriers may remain as impurities in the final dyed materials. COC,s and their isomers have potential health hazards [2]. These compounds are harmful and may induce lever malfunction, irritation to mucous membrane and skin. Due to the hazardous nature of these chemicals, many leading apparel and footwear brands have banned the use of chlorobenzenes and chlorotoluenes in the production and manufacturing of their products [2]. Pentachlorobenzene (PCB) and Hexachlorobenzene (HCB) are categorized as persistent organic pollutants. According to the Stockholm Convention, both these carriers have been banned globally. Some chlorobenzene and chlorotoluene can be very toxic to aquatic organisms at certain concentrations. Hence, chemical suppliers that require chemical formulations are also required to comply with current ZDHC MRL limits of COC,s [3]. Many of the EU regulations like Oekotex 100, GOTS, ECHA's candidate list of substances of very high concern (SVHC) have restricted these substances [4-6]. List of restricted chlorobenzene and chlorotoluene as per the Oekotex 100, ver-2023 is given in Table-1.The limit value for the sum of the chlorobenzene and chlorotoluene is 1.0 mg/kg [6]. Though the list is exhaustive, only few common COC,s are reported in textiles.

Many commercial testing laboratories both in India and abroad are testing the content of COC, either adopting international test method DIN EN/ EPA/ in-house developed test methods to cater their customers [7] . At present there are no Indian/ISO standards to test COC,s for textile materials. Hence, in this R&D project, a validated test method has been developed to formulate the same as the national standard.

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2.0 Materials, Equipments and analytical methods

2.1 Materials

28 Certified Reference Materials (CRM) of each COC,s were purchased from DR EHRENSTORFER as per the list given in Table-1. Acetone, N-Hexane, Toluene, Ethyl acetate, Cyclohexane, Dichloromethane were purchased from Loba Chemie Pvt. Ltd.

Table-1 Certified Reference Materials of COC,s

Sr. No	Chlorinated carriers	Case number
1.	Chlorobenzene	108-90-7
	Dichlorobenzene isomers	
2.	1,2 Dichlorobenzene	95-50-1
3.	1,3 Dichlorobenzene	541-73-1
4.	1,4 Dichlorobenzene	106-46-7
	Trichlorobenzene isomers	
5.	1,2,3 Tri chlorobenzene	87-61-6
6.	1,2,4 Tri chlorobenzene	120-82-1
	Tetrachlorobenzene isomers	
7.	1,2,3,4 Tetra chlorobenzene	634-66-2
8.	1,2,3,5 Tetra chlorobenzene	634-90-2
9.	1,2,4,5 Tetra chlorobenzene	95-94-3
10.	**Pentachlorobenzene**	608-93-5
11.	**Hexachlorobenzene**	118-74-1
	Chlorotoluene isomers	
12.	2- Chlorotoluene	95-49-8
13.	3- Chlorotoluene	108-41-8
14.	4- Chlorotoluene	106-43-4
	Dichlorotoluene isomers	
15.	2,3- Dichlorotoluene	32768-54-0
16.	2,4- Dichlorotoluene	95-73-8
17.	2,5- Dichlorotoluene	19398-61-9
18.	2,6- Dichlorotoluene	118-69-4
19.	3,4- Dichlorotoluene	95-75-0
20.	3,5- Dichlorotoluene	25186-47-4
	Trichlorotoluene isomers	
21.	2,3,4- Trichlorotoluene	7359-72-0
22.	2,3,6- Trichlorotoluene	2077-46-5
23.	2,4,5- Trichlorotoluene	6639-30-1
24.	Benzyl chloride	100-44-7
25.	Benzotrichloride	98-07-7
26.	4- Chlorobenzotrichloride	5216-25-1
	Tetrachlorotoluene	
27.	**α α 2,3 tetrachlorotoluene**	57058-14-7
	Pentachlorotoluene	
28.	**2,3,4,5,6-Pentachlorottoluene**	877-11-2

2.2 Equipments

The following equipments were used for the analysis of coc,s.

Ultrasonic bath from PCI analytical, Semi micro balance from Shimadzu (AUW 220D), GC MS from Shimadzu (QP2020 NX).

2.3 Analytical Methods

2.3.1 Selection of solvents for extraction of COC,s

To determine the suitability of solvents for extraction of COC,s from textile material, five COC standards were spiked in a blank matrix (polyester fibre) in triplicate with the target substance of 10 mg/kg. Six different solvents were used for extraction of COC,s from the spiked matrix. Each solvent extracts were made up to a certain volume and analyzed by GC MS under identical conditions.

2.3.2 Standardization of COC,s using GC MS

1000 µg/ml of individual COC,s stock solutions (10mg/10 ml) is prepared in dichloromethane. A further dilution of 1ml to 10 ml in dichloromethane is prepared to get a concentration of 100µg/ml and analyzed by GC MS scan mode to identify the retention times of each standard as per the following instrument parameters.

- Instrument make and model: Shimadzu QP 2020NX
- Column: DB5 MS (30m X 0.25mm X 0.25µm)
- Carrier gas Helium
- Flow: 2.0 ml/min
- Injector temperature: 2500C
- Ion source temperature: 2600C
- Interface temperature: 2700C
- Oven temperature: 600C (10 min), @ 20C/min 150, @200C/min 2000C
- Mass range (M/Z) : 40-400 amu
- Injection volume: 2µl
- Split ratio 1:10
- Ionization voltage: 70 ev
- Library: NIST23
- Retention time of each COC,s is given in Table-2

Table-2 Retention time of COC,s

Sr. No.	Name of component	CAS number	Retention time min
1.	Chlorobenzene	108-90-7	3.1
	Dichlorobenzene isomers		
2.	1,2 Dichlorobenzene	95-50-1	10.7
3.	1,3 Dichlorobenzene	541-73-1	9.2
4.	1,4 Dichlorobenzene	106-46-7	9.57
	Trichlorobenzene isomers		
5.	1,2,3 Tri chlorobenzene	87-61-6	23.6
6.	1,2,4 Tri chlorobenzene	120-82-1	21.46
	Tetrachlorobenzene isomers		
7.	1,2,3,4 Tetra chlorobenzene	634-66-2	34.91
8.	1,2,3,5 Tetra chlorobenzene	634-90-2	31.69
9.	1,2,4,5 Tetra chlorobenzene	95-94-3	31.96
10.	**Pentachlorobenzene**	608-93-5	43.5
11.	**Hexachlorobenzene**	118-74-1	53.63
	Chlorotoluene isomers		
12.	2- Chlorotoluene	95-49-8	6.39
13.	3- Chlorotoluene	108-41-8	6.27
14.	4- Chlorotoluene	106-43-4	14.37
	Dichlorotoluene isomers		
15.	2,3- Dichlorotoluene	32768-54-0	19.2
16.	2,4- Dichlorotoluene	95-73-8	17.27
17.	2,5- Dichlorotoluene	19398-61-9	17.54
18.	2,6- Dichlorotoluene	118-69-4	17.37
19.	3,4- Dichlorotoluene	95-75-0	19.33
20.	3,5- Dichlorotoluene	25186-47-4	17.02
	Trichlorotoluene isomers		
21.	2,3,4- Trichlorotoluene	7359-72-0	32.1
22.	2,3,6- Trichlorotoluene	2077-46-5	30.4
23.	2,4,5- Trichlorotoluene	6639-30-1	29.08
24.	Benzyl chloride	100-44-7	9.71
25.	Benzotrichloride	98-07-7	24.62
26.	4- Chlorobenzotrichloride	5216-25-1	36.17
	Tetrachlorotoluene		
27.	**α α 2,3 tetrachlorotoluene**	57058-14-7	46.07
	Pentachlorotoluene		
28.	**2,3,4,5,6-Pentachlorotoluene**	877-11-2	55.49

Since few COC isomers are co-eluted and have the same retention time, it will be difficult to identify all 28 COC,s unambiguously in a single analysis. Hence, based on the retention time, they have been made into a mixture of two groups and both the groups are further analyzed by GC MS scan mode to confirm their separation and retention time. List of COC,s in group 1 and group 2 is given in Table 3.

Table-3 : COC,s (Group-1& Group-2)

Group-1		Group-2	
COC,s	**Rt (min)**	**COC,s**	**Rt(min)**
Chlorobenzene	3.30	3-Chlorotoluene	8.58
2-Chlorotoluene	6.26	1,4 Dichlorobenzene	12.63
4-Chlorotoluene	6.58	2,4 Dichlorotoluene	20.03
1,3 Dichlorobenzene	8.99	3,4 Dichlorotoluene	20.32
Benzylchloride	9.52	3,5 Dichlorotoluene	22.42
1,2 Dichlorobenzene	10.70	1,2,3 Trichlorobenzene	26.94
2,3/2,5 Dichlorotoluene	17.29	Benzotrichloride	27.81
2,6 Dichlorotoluene	18.92	2,3,4/2,3,6 Trichlorotoluene	33.58
1,2,4 Trichlorobenzene	21.17	1,2,4,5 Tetrachlorobenzene	35.12
1,2,3,5 Tetrachlorobenzene	31.34	2,4,5 Trichlorotoluene	35.35
1,2,3,4 Tetrachlorobenzene	34.54	Pentachlorobenzene	46.72
4 Chlorobenzotrichloride	35.69	Hexachlorobenzene	56.41
2,3,3,4,5 Pentachlorotoluene	55.49		
α,α 2,3 Tetrachlorotoluene	46.0		

3.0 Results and discussion

The extraction efficiency is calculated from the spiked concentration and recovered concentration. Out of six solvents, Dichloromethane is found to be the suitable solvent with an extraction efficiency of 95-99%. Extraction efficiency of each solvent is given in Table-4.

Method of sample analysis

A known weight of textile material (1-2g) depending the concentration of coc,s is weighed in a 25 ml stopper glass vial, and overlaid 10 ml dichloromethane (as per the extraction efficiency of solvents) .The test specimen is extracted for 20 min in an ultrasonic bath at ambient temperature. About 2.0 ml of the extract is taken with a disposable syringe and cleaned of interfering particles and fibres with the help of a membrane filter. After filtration, the extract is transferred into a GC vial. 2.0 µl of the extract is

Table-4 Extraction efficiency of solvents

COC	Acetone	N Hexane	Cyclohexane	Toluene	Ethyl acetate	Dichloro methane
1,2 Dichlorobenzene	5.5 %	18.5 %	18.8%	19.5 %	25.5 %	98.5%
1,2,3 Trichlorobenzene	6.2 %	12.1 %	10.5%	15.5%	20.1%	95.2%
1,2,3,4 Tetrachlorobenzene	5.8 %	10.7%	13.4%	18.3%	15.9%	99.0%
2 Chlorotoluene	4.5 %	11.9%	11.9%	14.6%	16.4%	98.9%
2,3 Dichlorotoluene	6.9 %	15.0%	10.8%	11.9%	19.5%	95.0%

injected into GC MS using an auto injector. The substances of the extract are separated on a capillary column and analyzed using a mass selective detector in selected ion mode (SIM) as per the conditions mentioned in section 2.3.2. Detected chlorinated carriers are quantified against the corresponding standards under the same test conditions. A five point calibration graph may be plotted to quantify the coc,s. (please refer to the method validation section).

The concentrations of chlorobenzenes or chlorotoluenes in the sample are expressed in the mass fraction as mg/kg and are calculated as per the following formula.

$$C = \frac{C.std \times A.sample \times V}{A.std \times W}$$

Where

C is the mass fraction of coc in sample (mg/kg)

C.std is concentration of coc standard (ug/ml)

A.sample is the peak area of the sample (abundance value)

A.std is the peak area of the standard (abundance value)

V is the volume of extract (ml)

W is the weight of sample (g)

COC method validation

Method validation is carried out as per international validation protocol (Eurachem and ICH guidelines) by conducting linearity, Precision, Accuracy, recovery rate, Limit of detection (LOD) and Limit of quantification (LOQ)

Linearity

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 demonstrates 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 (R2) should be at least 0.95. 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.

Linearity values of all COC,s in scan mode in the range of 2.5 µg/ml to 50 µg/ml concentration with group -1 and group-2 is given in Table- and Table-5 & 6 respectively.

Table- 5 Linearity of COC,s in scan mode (Group-1)

COC,s	Concentration (µg/ml) verses 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 Chlorobenzotrichloride	349537	1207642	2683754	11458436	24622431	0.9968

Table-6 : Linearity of COC,s in scan mode (Group-2)

COC,s	Concentration (µg/ml) verses 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

From Table 5 and Table 6, it can be seen that the coefficient of determination (R2) of all the 28 COC,s within a working range of 2.5 µg/ml to 50 µg/ml is linear and the value is above 0.95 which is well accepted. Once the detector linearity in scan mode (>0.95) is confirmed, the same experiments repeated in SIM mode in the range of 0.1 µg/ml to 1.0 µg/ml concentrations of COC,s. Based on the retention time and M/Z of target ion and two qualifier ions, the following GC MS program found to be suitable for analyzing all the COC isomers in SIM mode. Parameters for mass selective detectors for group-1 and group 2 are given in Table 7 and Table 8 respectively.

Table 7 : Parameters for mass selective detector for group-1

COC,s	Time window (min)	M/Z
Chlorobenzene 3-Chlorotoluene 4-Chlorotoluene	3.5 to 10.0	128,126,114,112,91,77
1,3 Dichlorobenzene Benzyldichloride	10.0 to 15.0	148,146,126,111,91,65
2,4 Dichlorotoluene 2,5 Dichlorotoluene 3,4 Dichlorotoluene 2,4,5 Trichlorotoluene	15.0 to 25.0	162,160,125
1,2,3 Trichlorotoluene 2,3,5 trichlorotoluene 1,2,3,4 Tetrachlorotoluene	25.0 to 37.0	218,216,214, 194,182,180,160,159,145
4 Chlorobenzotrichloride α,α 2,3,Tetrachlorotoluene	37.0 to 47.0	230,228,197,194,193
2,3,4,5,6 Pentachlorotoluene	47.0 to 57.0	229,264,228

Table 8 : Parameters for mass selective detector for group-2

COC,s	Time window (min)	M/Z
2 Chlorotoluene 1,4 Dichlorobenzene	7.0 to 13.0	148,146,128,126,111,91
1,2 Dichlorobenzene 3,5 Dichlorotoluene 2,6 Dichlorotoluene 2,3 Dichlortoluene	13.0 to 23.0	162,160,148,146,125,111
1,2,4 trichlorotoluene Benzotrichloride	23.0 to 30.0	182,180,160,159, 145, 89
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
Pentachlorobenzene Hexachlorobenzene	40.0 to 57.5	284,286,282,252,250,248

Linearity values of all COC,s 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 is given in Table-9 and Table-10 respectively.

Table-9 Linearity of COC,s in SIM mode (Group-1)

COC,s	Concentration (µg/ml) verses 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 Dichlorotoluen	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 Trichlotoluene	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-9 Linearity of COC,s in SIM mode (Group-1)

COC,s	Concentration (µg/ml) verses 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

From Table 9 and table 10 it has been confirmed that the detector linearity (R2) of all the 28 COC,s in the working range of 0.1µg/ml to 1.0µg/ml is within the acceptable value of more than 0.980.

Accuracy (Spike recovery rate)

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed as trueness. For the quantitative approaches, accuracy can be determined by spiking a known concentration in the working range and at least 5 replicates should be obtained. The percentage recovery or the difference between the mean and the accepted true value together with the confidence level are recommended. For accuracy, the mean recovery within a rate of 90 to 110 percent of the theoretical value and relative standard deviation of replicates below 10 % may be taken as acceptance criteria. To establish the accuracy, a textile material (polyester yarn) which is free from COC,s is spiked at 1.0 mg/kg and five replicates of analysis were conducted. Spike recovery at 1.0 mg/kg of group 1 and group 2 coc,s is given in Table 11 and Table 12 respectively.

Table 11 Spike recovery@ 1.0 mg/kg COC,s (Group-1)

COC,s	Recovery (%)							
	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	977.64	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-12 Spike recovery @ 1.0 mg/kg COC,s (Group-2)

COC,s	Recovery (%)							
	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		91.92	86.29	93.29	5.35	5.73

From Table 11 and Table 12 it can be seen that the spike recovery @ 1.0 mg/kg of all the 28 coc,s are above 90% with a relative standard deviation of below 10% which is well accepted within the criteria.

Limit of detection (LOD) and Limit of quantification (LOQ)

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).

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 formula,

$LOD = 3.3\sigma/M$ and $LOQ = 10\,\sigma/M$

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 determination or it can be the residual standard deviation of the regression line. M is the slope of the calibration graph. LOD and LOQ of COC,s are calculated based on the calibration graph in the range of 0.1 mg/kg to 1.0 mg/kg and is given in Table 13 and Table 14.

Table 13 LOD and LOQ of coc,s (Group 1)

	Concentration (µg/ml) verses peak area					Statistical parameters			
COC,s	0.1	0.2	0.4	0.8	1.0	M	STEYX	LOD (mg/kg)	LOQ (mg/kg)
CB	208006	336946	750656	1590451	1830450	1888348	62609.69	0.1094	0.3315
3-CT	377513	623501	1403833	2923425	3365651	3469024	113581.8	0.1080	0.3274
4-CT	1141020	1868579	4095442	8165252	9296106	9451838	342126.3	0.1194	0.3619
1,3- DCB	372824	608928	1396506	2948202	3414807	3534009	111564.2	0.1041	0.3156
BC	467939	781252	1826241	3858520	4467812	4645478	147958.8	0.1051	0.3185
2,4- DCT	307865	510739	1158510	2534169	2918656	3045600	103504.7	0.1121	0.3398
2,5- DCT	307865	510739	1158510	2534169	2918656	3045600	103504.7	0.1121	0.3398
3,4- DCT	330939	549291	1242467	2715160	3124681	3259132	111243.7	0.1126	0.3413
2,4,5 -TCT	386145	640328	1442966	3157926	3633698	3788956	129569.7	0.1128	0.3419
1,2,3- TCB	439406	719649	1583722	3458859	3999080	4145281	134860	0.1073	0.3253
2,3,4 -TCT	374945	616422	1351334	2910648	3395594	3501589	101776.7	0.0959	0.2906
1,2,3,5 -TECB	513211	845958	1819400	3791841	4363571	4463877	142026.8	0.1049	0.3181
4- CBTC	47224	78146	195425	493721	587298	633149	23552.1	0.1227	0.3719
α,α,2,3 TECT	474051	782735	1693633	3497579	4027719	4115548	129869.4	0.1041	0.3155
2,3,4,5,6 PCT	40269	67259	147270	308782	360908	370127.2	10043.9	0.0895	0.2713

Table-14 LOD and LOQ of coc,s (Group 2)

	Concentration (µg/ml) verses peak area					Statistical parameters			
COC,s	0.1	0.2	0.4	0.8	1.0	M	STER	LOD (mg/kg)	LOQ (mg/kg)
2-CT	580589	1052040	2101251	4082866	4542526	4563584	197793	0.1430	0.4334
1,4-DCB	1589233	2586832	5600631	10665289	11836177	11909782	561607.6	0.1556	0.4715
1,2-DCB	19025	944095	1894561	3684344	4165617	4513029	292471.8	0.2138	0.6480
3,5-DCT	342024	635944	1279853	2793655	27792240	2953564	251638.2	0.2811	0.8519
2,6-DCT	285652	531043	1076789	2094996	2313959	2340376	109436.4	0.1543	0.4676
2,3-DCT	226963	430307	862042	1710343	1886799	1917368	88836.2	0.1528	0.4633
1,2,4-TCB	214436	392726	798283	1548834	1804108	1805473	47874.9	0.0875	0.2651
BTC	258811	516145	1156099	2471337	2886517	3017803	80665.5	0.0882	0.2672
2,3,6-TCT	295730	559644	1124536	2180574	2443418	2462071	101741.2	0.1363	0.4132
1,2,4,5-TCB	519780	978051	1927527	3714458	4214422	4212447	152855.9	0.1197	0.3628
1,2,3,4-TCB	298424	570706	1145438	2242551	2484783	2516719	114014.2	0.1494	0.4530
PCB	246656	469231	932262	1793741	2069927	2067380	62644.3	0.0999	0.3030
HCB	200387	371114	721747	1357990	1579307	1555645	43306.3	0.0918	0.2783

LOD and LOQ have been calculated based on the slope and standard error of the calibration graph in the liner range of 0.1 µg/ml to 1.0 µg/ml of coc,s.

M is the slope of the calibration graph

STEYX is the standard error of the predicted y- value in a linear regression

LOD =3.3 X (STEYX/M)

LOQ= 10 X (STEYX/M)

From Table 13 and Table 14 it can be seen that theoretical values of LOD and LOQ are below 0.2 mg/kg and 0.6 mg/kg respectively. This clearly indicates that with this test method all coc,s can be estimated below 1.0 mg/kg which is the requirement of most of the regulations.

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.

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. Ideally, at 1mg/kg level, RSD percentage of repeatability should be below 10%.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 COC,s are given in Table 15 and Table 16.

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 determination or it can be the residual standard deviation of the regression line. M is the slope of the calibration graph. LOD and LOQ of COC,s are calculated based on the calibration graph in the range of 0.1 mg/kg to 1.0 mg/kg and is given in Table 13 and Table 14.

Table 15 Repeatability of coc,s (Group 1)

	Concentration of coc,s (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-TCT	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
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-16 Repeatability of coc,s(Group 2)

Concentration of coc,s (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													

From Table -15 and Table-16, it can be seen that the RSD percentage of repeatability at 1.0 mg/kg of all coc,s are below 10 which is within the acceptable norms.

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, 1mg/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 COC,s is given in Table 17 and Table 18

Table 17 Reproducibility of coc,s (Group 1)

COC	Day-1 Op-1	Day-2 Op-2	Day-3 Op-3	Day-4 Op-1	Day-5 Op-2	Day-6 Op-3	Day-7 Op-1	Mean	SD	RSD(%)
	Concentration of coc,s (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 18 Reproducibility of coc,s (Group 2)

COC	Day-1 Op-1	Day-2 Op-2	Day-3 Op-3	Day-4 Op-1	Day-5 Op-2	Mean	SD	RSD(%)
	Concentration of coc,s (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	0.0223	2.14
HCB	0.87	0.91	1.01	1.05	0.93	0.95	0.0740	7.72

From Table 17 and Table 18 it can be seen that the RSD percentage of reproducibility at 1.0 mg/kg level is below 10% which is within the acceptable limit.

4.0 Conclusions

From the extraction efficiency of various solvents, Dichloromethane is found to be the most ideal solvent for sample analysis. A test method for the analysis of coc,s in textile material has been developed . The in-house developed test method has been validated for detector linearity, accuracy, precision, repeatability, and reproducibility, limit of detection and limit of quantification. With this developed test method, chlorinated benzene/toluene down to 1.0 mg/kg can be quantified. Based on the validation parameters, the developed test method is found to fit for use.

Declaration

Authors hereby declare that there is no conflict of interest in publishing this paper.

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