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COMPREHENSIVE GC-MS METHOD FOR THE ESTIMATION OF EXTRACTABLE ORTHO-PHENYLPHENOLS (OPPS) CONTENTS IN TEXTILE AND LEATHER TEST SPECIMENS

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ABSTRACT:
A new method was established for determination of extractable ortho-phenylphenol (OPP) contents, its salts and esters in textile and leather test specimens. 0.1 M potassium hydroxide assisted with ultrasonic bath was used for alkaline digestion of test specimens. Test specimens were acetylated using acetic anhydride assisted with mechanical shaker at 200 rpm for 60 mins time. OPP contents were extracted in n-Hexane and were analyzed using fused silica capillary HP-5MS column. Helium gas 99.9 % purity was used as mobile phase. One target and two qualifier ions were selected for target analyte in GC-MS under selective ion monitoring (SIM) mode. Pre-treatment parameters as extraction method, solvent and time were optimized. Under optimized conditions recovery of standard was in the range of 93 to 98 %. Linearity was with regression value 0.9993-0.9995. Calibration curve working range was 0.1-5.0 mg L^{-1}. Relative standard deviation (RSD) was up to 10% for test specimens and 5% for pure standards. Limit of detection was up to 0.006 mg L^{-1} level. From actual test specimens OPP contents were successfully analyzed using developed analytical method. OPP contents found were 1.15 to 35.52 mg L^{-1}.

KEYWORDS*:* Ortho-phenylphenol (OPP), Alkaline digestion, Leather & Textile samples, Solvent extraction, GC-MS Analysis.

INTRODUCTION:

Ortho-phenylphenol (OPP), a white crystalline compound with notable biocidal properties, has been extensively utilized for decades in diverse preservation applications.[1, 2] Its antimicrobial and antifungal efficacy has rendered it a staple in industries ranging from agriculture to manufacturing. Historically, OPP has been employed to protect stored fruits and vegetables through antifungal wax coatings, as well as in water-extendible paints and oil-water emulsions.[4, 5] Within the textile and leather sectors, OPP serves critical roles: as a preservative in wet blue leather processing, a dye carrier for synthetic fibers, and a germicide in finished consumer products.[3] Despite its utility, concerns over its toxicity have led to regulatory scrutiny. Once used in household disinfectants and cleaning agents, OPP has been phased out from such applications due to evidence of adverse health effects.[6–11] Animal studies have linked OPP exposure to mucous membrane irritation, skin discoloration, and potential carcinogenicity, prompting its classification as a hazardous substance.[12–16]

The growing recognition of OPP's risks has spurred regulatory action. Notably, OPP was recently added to the Manufacturing Restricted Substances List (MRSL) in 2016, compelling industries to monitor and limit its presence in consumer goods (MRSL, 2016). However, while regulatory frameworks emphasize compliance, analytical methodologies for detecting OPP in complex matrices such as textiles and leather remain underdeveloped. Existing techniques, including high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS), have been successfully applied to quantify OPP in food products (e.g., fruits, vegetables), biological samples (e.g., human urine), and beverages.[17–20] Yet, these methods are not optimized for the heterogeneous and chemically treated matrices typical of textiles and leather, where interfering substances (e.g., dyes, tanning agents) complicate detection. This gap in analytical capability poses a significant challenge for industries striving to meet MRSL standards while ensuring product safety.

The absence of tailored methods for textiles and leather is particularly concerning given OPP's persistent use in these sectors. For instance, OPP residues may linger in dyed fabrics or treated hides, posing risks of dermal exposure to consumers. Current regulatory limits for OPP in finished goods demand detection methods with exceptional sensitivity and selectivity, as permissible concentrations often fall in the parts-per-million (ppm) or sub-ppm range. Conventional approaches may lack the robustness to distinguish OPP from structurally similar compounds or to overcome matrix-induced signal suppression. Furthermore, the diversity of textile and leather materials—ranging from synthetic fibers to vegetable-tanned hides—necessitates a versatile analytical protocol adaptable to varied sample types.

In response to this critical need, we present a novel analytical method designed to quantify OPP and its derivatives in textile and leather matrices with high precision, sensitivity, and efficiency. Our approach addresses the limitations of existing techniques by incorporating advanced extraction and detection strategies tailored to these challenging matrices. The method achieves a remarkably low limit of detection (LOD), ensuring compliance with stringent regulatory thresholds. Additionally, it offers adaptability across diverse material types, from printed polyester fabrics to chrome-tanned leathers, without compromising analytical performance. By bridging this methodological gap, our work empowers industries to meet evolving safety standards, safeguards consumer health, and supports sustainable manufacturing practices.
This study not only advances analytical chemistry but also aligns with global regulatory trends emphasizing the reduction of hazardous substances in consumer products. The following sections detail the method's development, validation, and application, underscoring its significance for quality control in the textile and leather industries.

EXPERIMENTAL

SOLVENTS AND CHEMICALS:

Pesticide grade chemicals and organic solvents were procured from available suppliers. Acetone, Potassium hydroxide, Acetic anhydride, n-Hexane were purchased from Merck (Germany), 4-dimethylaminopyridine (DMAP), Sodium sulfate (anhydrous) from Dae-Jung (Korea) and working standard Ortho-phenylphenol (OPP) and internal standard anthracene d-10 from chem service (UK). Grade three water was used produced by Milli Q instrument.

Reagent preparation:

Potassium hydroxide 1 mol L^{-1} was prepared by dissolving 56.11 g in 1000 mL of grade three water. 0.1 g 4-dimethylaminopyridine (DMAP) was weighed and dissolved in 5mL of grade three water. Ortho-phenylphenol (OPP) (purity ≥ 98%) was weighed 0.01 g in 10 mL measuring flask. Solution was diluted using acetone upto specified mark to obtain primary

stock solution (1000 mg L^{-1}). Primary stock solution 1.0 mL was diluted to 10 mL in a volumetric flask with acetone for acquiring working stock solution (100 mg L^{-1}). Appropriate dilutions were made for the calibration curve points (*i.e.* 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg L^{-1}). The acetylation step for each curve point was performed in 40 mL screw capped vial containing 30 mL of potassium hydroxide 1mol L^{-1} solution. Anthracene d-10 was weighed 0.01 g in 50 mL measuring flask. Solution was diluted using acetone for acquiring 200 mg L^{-1} internal standard solution.

TEST SPECIMEN COLLECTION:

Textile commercial products, finished garments, printed and non-printed fabrics were collected during the period January to May 2019 from retail stores and textile process industries in the Lahore city, Pakistan.

INSTRUMENTATION:

Chromatographic system GC-2010plus (Shimadzu) was used with auto sampler AOC-20s, auto injector AOC-20i, and mass selective detector QP2010ultra-plus. Software used was GC-MS solution 2.70 version, Shimadzu corporation with mass spectral libraries NIST 127 & 147. HP-5MS (0.25mm × 30m × 0.25µm) capillary column fused-silica with 340 °C maximum temperature capacity was used for screening of OPP contents. Electron impact (EI) mass spectra was utilized to confirm the presence and structure of OPP contents. For quantification purpose and peak identification 1.2kV electron multiplier setup with 70eV EI ionization (m/z, 50 to 350) full scan operation.

CHROMATOGRAPHIC CONDITIONS:

Auto tuning was performed for mass-selective detector using perfluorotributylamine (PFTBA, m/z 69, 219, 502). Split-less injection system mode was functioned at chromatographic system and 3.0 mL min^{-1} was the purge flow after the test specimen injection. Highly pure 99.99 % helium was used as carrier gas with flow rate 1.0 mL min^{-1}. 1.0 µL was the test specimen injection volume. 250 °C was set injector temperature and 320 °C interface temperature.

EQUIPMENT TEMPERATURE PROGRAM:

100 °C was initial column temperature, after injection it was retained for 1.0 min, then rise @ 20 °C min^{-1} from 100 to 200 °C retained for 2.0 mins and finally from 200 °C to 300 °C @ 40 °C min^{-1} retained for 6 mins. 16.5 mins was total run time for analysis.

TEST SPECIMEN PROCESSING:

From different parts the test specimen was selected randomly for analysis. Textile test specimen was cut into pieces approximately 5 mm × 5 mm, if it was homogeneous single colored. If the test specimen was color with pattern or multi colored, it was selected according to the percentage of color and small pieces approximately 5 mm × 5 mm were made. If removable coating was present on specimen, the coting was removed with blade or the test specimen was selected from portion which don't have coating.

A, B and C these three samples were selected as matrix blank. These samples were used to spiked with pure standard and were analyzed using different digestion solvents, time and methods. Using ultrasonic method, 0.1 mg L^{-1} KOH solvent at 60 mins time gives best recovery. So, 0.1 mg L^{-1} KOH was chosen digestion solvent, 60 mins extraction time and ultrasonic as extraction method (results were summarized in figure 3 and table a, b and c in supplementary data).

TEST SPECIMEN EXTRACTION:

Test specimen was weighed 5.00 g accurately using Mettler Toledo Model: ML 204/01 analytical balance in a 100 mL reagent bottle. 1.0 mg L^{-1} potassium hydroxide solution 60 mL was added to the reagent bottle, capped and sonicated for 60 mins at 70 °C.

ACETYLATION:

The extract was cooled to room temperature and 30 mL of extract was taken in 40 mL capacity capped vial. DMAP solution 0.5 mL and 2.5 mL acetic anhydride was added to the vial, capped and shake for 5 mins on vortexed shaker followed by orbital shaking for 60 mins at 200 rpm. Then 5 mL n-Hexane was added and vortexed again for 5 mins followed by 30 mins orbital shaking at 200 rpm. 30 mins extraction process was enough for whole extraction of OPP contents and there was no need for further extraction. Extract (Organic n-Hexane layer that contain extracted target analyte) was taken 1mL into a 1.5 mL GC vial, internal standard 10 mg L^{-1} was added and analyzed on GC-MS.

INSTRUMENTAL ANALYSIS BY GC-MS:

Following GC-MS instrument parameters were ensured before performing any batch of test specimens. Instrument parameters were established as (section 2.4.1.) and (section 2.4.2.) temperature program. Concentration of analyte vs peak area, calibration curve was established for OPP contents. Linear regression coefficient (r^2) was > 0.9995. Method blank and specimen blank were run on instrument to check any contamination. 0.1 mg L^{-1} standard solution was run on instrument for the estimation of instrument sensitivity. For estimation of experimental recovery 1.0 mg L^{-1} each, laboratory quality control, specimen spike and calibration standard check solutions were injected to the instrument. In the range of 85 – 115 % was recovery of these solutions (results were summarized in table d in supplementary data). Testing samples were injected for analysis to the instrument. By comparing the mass spectrum and retention time, presence or absence of target analyte was confirmed. Test specimen component retention time (RT) was within the range of ± 0.01 RT units of the standard component relative RTs. Test specimen extract was diluted for required range, if quantitated result exceeds from the range of prepared calibration curve. For out-of-range test specimens, retest was performed with increasing final volume of n-Hexane or by decreasing test specimen weight.

RESULTS AND DISCUSSION
ANALYTICAL METHOD VALIDATION

Working range and linearity: A calibration curve of 6 points was established, curve points were 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg L^{-1}. 0.9993 to 0.9995 was regression value for this curve. It was linear with 0.1 mg L^{-1} to 5.0 mg L^{-1} working range (as shown in figure “I” in supplementary data). Respective curve levels were prepared and analyzed three times.

Specificity: For chromatographic technique to perceive the consequence of any conceivable interference matrix or reagent, matrix blank, reagent blank and pure standard was analyzed. Method blank and matrix blank did not show any detection it means there is no any contamination from reagents or matrix. Results of sensitivity check, lab quality check,

specimen spike and calibration standard check were 100 ± 15%. (results were summarized in figure II and table d in supplementary data).

Precision: A specimen solution was prepared that contain target level of analyte (3.0 mg L^{-1}). Using final method practice, 10 replicates were made from this solution. In the consequent six days these replicates were analyzed. Relative standard deviation for these replicates was 1.4 % for the intra-day and 2.2 % for inter-days (results were summarized in table 3).

Accuracy: A specimen and a blank specimen were spiked with pure standard of concentrations 1.0 mg L^{-1}, 3.0 mg L^{-1} and 5.0 mg L^{-1}. Each concentration level for these individually prepared replicates was analyzed three time. 100 ± 15% was the recovery for these individually prepared replicates (results were summarized in table e in supplementary data).

LOD and LOQ: Spike solutions and blank solution with gradually falling concentrations of analyte were organized for the proposed analytical method to calculate limit of detection and limit of quantitation. Using the developed method these prepared solutions were screened. By assessing the lowest concentration of analyte quantified and detected with accuracy the LOQ & LOD were determined (Limit of detection was calculated from signal-to-noise ratio 3:1 and limit of quantitation from 10:1). Limit of detection was 0.006 mg L^{-1} and limit of quantification was 0.018 mg L^{-1}.

Robustness: Chromatographic parameters of presented method were changed purposely to evaluate the robustness of the method. From the results obtained it was determined that varying the conditions, column oven temperature from 100-300 ˚C to 95-295 ˚C and 105-305 ˚C, flow rate of carrier gas from 3.0 mL min^{-1} to 2.95 mL min^{-1} and 3.05 mL min^{-1} had no appreciable effect on the analyte. Deviation of the analyte was within 90 to 110% (results were summarized in table 2).

Stability: Percentage deviation of result was observed that was attained after three days period. Stability of analyte content was estimated, in occurrence of other analyte in solution. Deviation of analyte was < 3 % in the three days period.

Selectivity: By formulating mixtures of analyte with textile test specimens, selectivity for presented GC-MS method was evaluated. Recovery was 100 ± 15% for OPP contents in the presence of interferences (results were summarized table e in supplementary data).

TEXTILE SAMPLES SCREENED FOR OPP CONTENTS:

For test specimens screened for OPP contents, 1.15 mg L^{-1} was the lowest value and 35.52 mg L^{-1} was the uppermost value. OPP contents 4.28 mg L^{-1} were detected in sample D, 1.15 mg L^{-1} in sample E, 2.08 mg L^{-1} in sample F, 3.93 mg L^{-1} in sample G, 10.15 mg L^{-1}, in sample H, 3.77 mg L^{-1} in sample I and 35.52 mg L^{-1} in sample J. In the five months period total 122 test specimens were analyzed; 7 test specimens show high detection (results were summarized in table 1).

Table 1: Results of detectable test specimens analyzed on GC-MS

Test specimens	Test specimen description	OPP mg L^{-1}	Test specimens	Test specimen description	OPP mg L^{-1}
A	Black fabric	BDL	F	Blue denim pant	2.08
B	Blue denim fabric	BDL	G	Black denim pant	3.93
C	Black denim fabric	BDL	H	Brown leather	10.15
D	Black foam + fabric	4.28	I	Yellow fabric	3.77
E	Black leather	1.15	J	Black denim pant	35.52

Peak resolution was good in detected test specimens and the target analyte elute within 17 mins. OPP elute at 13.42 mins and internal standard Anthracene d-10 at 14.92 mins, (as shown in figure 1). For OPP target ion was *m/z* 170 mass fragment and for Anthracene d-10 *m/z* 188 mass fragment (Mass spectrum showed as figure 2). Internal standard method was utilized for quantification of test specimens. OPP contents in the range 1.15 mg L^{-1} to 35.52 mg L^{-1} leach out from test specimens (results were summarized in table 1, only detected test specimens were summarized). Textile samples A, B and C were used as blank matrix for examining the parameters, extraction time, method and digestion solvent for OPP contents (results were summarized in figure 3 and table a, b, c in supplementary data).

Table 2: Results of robustness study of OPP for GC-MS instrument.

Test specimen	Spike level (mg L^{-1})[b]	Flow rate, 3.0 mL min^{-1}	Flow rate, 3.05 mL min^{-1}	Flow rate, 2.95 mL min^{-1}	Column oven temperature 100-300 ˚C	Column oven temperature 95-295 ˚C	Column oven temperature 105-305 ˚C
A	0.5	0.48	0.47	0.47	0.47	0.46	0.45
B	2	2.03	2.08	2.06	2.04	2.05	2.09
C	5	5.05	5.09	5.06	4.97	4.92	4.94

[b]=Three replicate measurements

Table 3: Regression equation, the limit of detection (LOD), the limit of quantification (LOQ), and relative standard deviation (RSD) for OPP.

Target	Regression equation	Correlation coefficient	LOD (mg L^{-1})	LOQ (mg L^{-1})	RSD in the same day (%)[a]	RSD in 6 days (%)[a]
OPP	Y=0.7475X+2.1476	0.9995	0.006	0.018	1.4	2.2

Y = Peak area, X = Mean concentration (mg L^{-1}) Linear range = 0.1 - 5.0 mg/L,
[a] =Ten replicate measurements

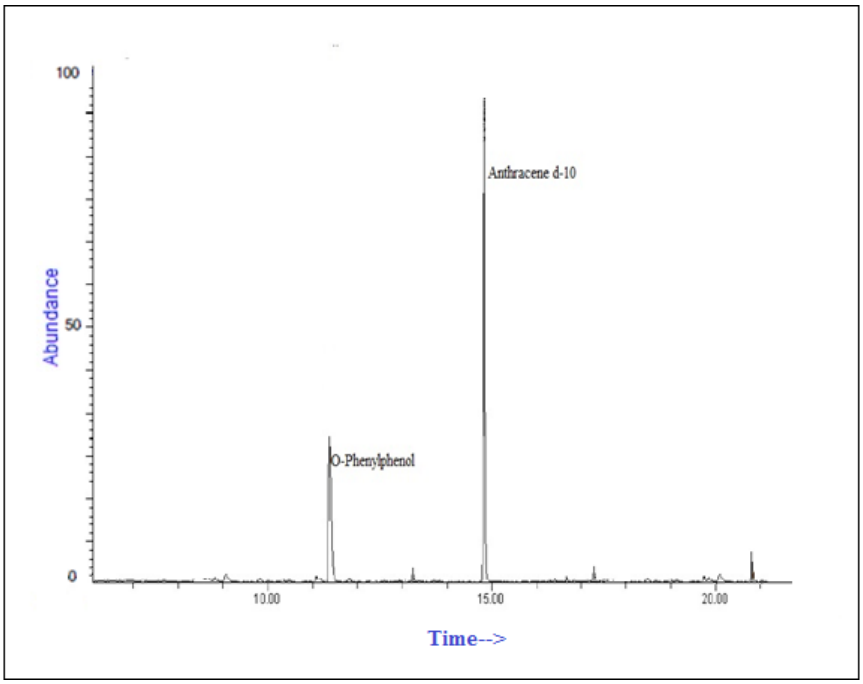
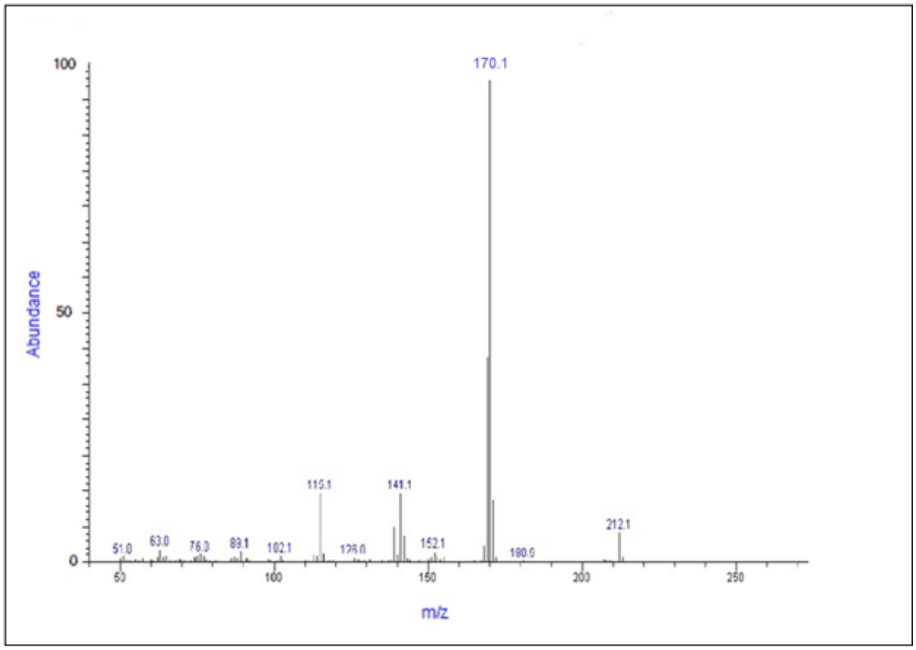


Figure 1: GC-MS chromatogram of O-Phenylphenol and internal standard d-10, chromatographic method used was mentioned in section "equipment temperature program".



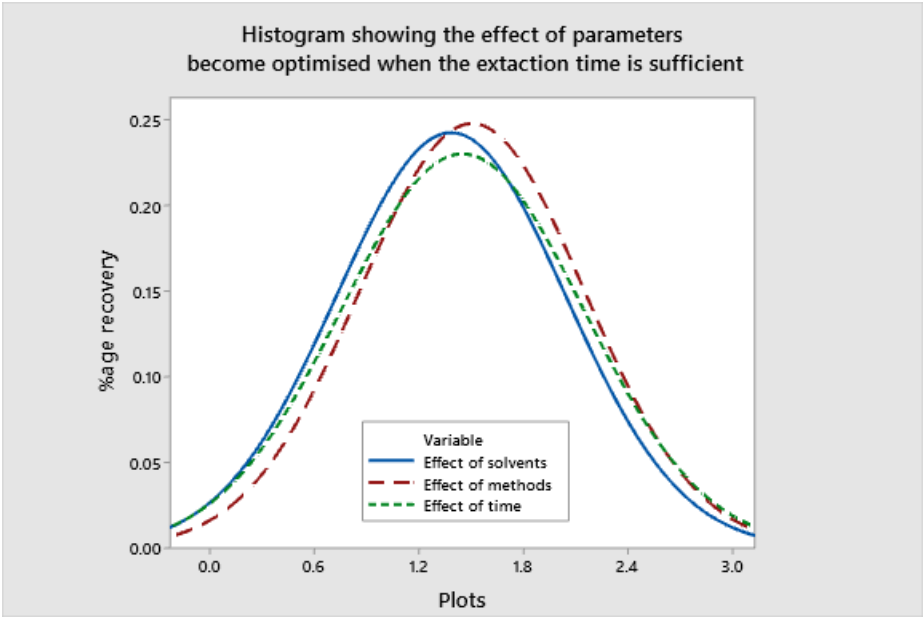


Figure 3. Shows extraction efficiencies of OPP contents (average results in mg L^{-1}) for textile samples spiked with known concentrations of analyte for extraction, method, time and solvent.

CONCLUSIONS:

For the quantitative analysis and separation of Ortho-phenylphenol contents from the textile and leather test specimens sensitive, rapid and a novel method was developed. For determination of OPP contents from real test specimens the suitability of solvent extraction was described. Target analyte from the test specimen was extracted in n-Hexane by alkaline digestion using 0.1 M potassium hydroxide solution and acetylated using acetic anhydride. OPP contents were analyzed on fused silica capillary HP-5MS column using helium as carrier gas. In the range of 0.1 to 5.0 mg L^{-1} the target analyte had good linearity ($r^2 \geq 0.9995$) under the optimized conditions. For target analyte recovery was 93 to 98 % with the relative standard deviation ≤ 3 %. Limit of detection was 0.006 mg L^{-1} and limit of quantification was 0.018 mg L^{-1}. To control the safety of leather and textile test specimens the proposed method could be a useful tool. Target analyte can be successful determination by the presented analytical method. So, for tracking the fate of this toxic chemical agent connected with human exposure by direct contact, this method will be a convenient tool.

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AUTHOR STATEMENT: All authors contributed equally to this work.

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