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Extraction of Residual Chlorinated Pesticides from Cotton Matrix

As a part from certification method of cotton reference material

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ABSTRACT

As a part of developing a certified testing method for residual pesticides analysis in cotton materials using gas chromatography (GC), the target pesticides listed in Oeko-tex standards should be isolated from a homogenized cotton matrix. The sample homogeneity and extraction procedures are the most effective steps in this analysis. Any error in this procedure must leads to incorrect results. Two extraction methods were used throughout this work with different solvents e.g. methanol, hexane, hexane/acetone (1:1 v/v) and dichloromethane. Extraction methods were soxhlet (SOX) and accelerated solvent extraction (ASE). The resulted extracts were concentrated then injected in a GC equipped with Electron Capture Detector (ECD). The qualitative results when compared with those of the NIST standard reference materials (SRM-2261) that certified by the National Institute of Standards and Technology USA, proved that the ASE and SOX techniques with dichloromethane as extracting solvent is more selective for all concerned pesticides for homogenized cotton samples. The ASE extraction with dichlormethane is better for hexachlorobenzene, gamma-HCH, heptachlor, Cis-chlordane, trans-nonchlor and SOX extraction technique with dichloromethane is better for Heptachlor epoxide, 4,4'-DDE, Dieldrin, 2,4'-DDD, 4,4'DDT and Mirex.

Keywords: Cotton, Oeko-Tex, gas chromatography; organochlorine pesticides, cryogenic homogenization, liquid extraction

Introduction

Pesticides are biologically active compounds, which control the growth of organisms e.g. bacteria, fungi, algae, insects or plants. The main objective of using pesticides is to improve a crop yield and quality by controlling the growth of the organisms. It has been observed that 18 percent of the world's production of pesticides is used in cotton plantations [1-3] Excessive use of pesticides causes severe environmental degradation and research efforts are being made allover the world to find alternatives for these harmful chemicals [4,5].

Oeko-tex standard 200 includes list containing 2,4,5-T, 2,4-D, aldrin carbaryl, DDD, DDE, DDT, dieldrin, α and β-endosulfan, endrine, heptachlor, heptachloroepoxide, hexachlorobenzene, α and β and δ-hexachlorcyclohexan, lindane, methoxychlor, mirex, toxaphene and trifluralin. These pesticides might be used for natural fibers and these are critical because of their toxicity and persistence. This Oeko-tex standard establishes testing and cleaning-up procedures by gas

chromatography (GC) with selective detection, e.g., Electron Capture Detector (ECD) or Mass Selective Detector (MSD)[6,7]. The limits of these pesticides referring to the sample weights are prescribed in the Oeko-tex 103[8]

Organochlorine pesticides include aldrin, chlordane, dicophane (DDT), dieldrin (also a metabolite of aldrin), endrin, heptachlor, hexachlorobenzene (HCB), 1,2,3,4,5,6-hexachorocyclohexane (HCH= benzene hexachloride, BHC), and lindan (gamma-HCH). These compounds were widely used but persist in the environment, and lindan, which has a relatively short half-life in vivo, is now the only member of this group to remain in common use in Europe and North America [9].

The analyte is generally contained in a liquid or a solid matrix. Interfering species that may lead to unwanted interactions, particularly during trace analysis in the presence of abundant matrix components, have to be eliminated. As a result, analysts have long acknowledged the need for efficient and reproducible sample preparation methods. The pre-treatment process has to take into account the analyte, matrix and measurement technique chosen [10]. Solvent extraction followed by GC-ECD remains the method of choice for the analysis of these compounds. There are many extraction procedure e.g., ultrasonic, soxhlet, and accelerated solvent extraction (ASE)[1,11].

The cotton fiber is collapsed tube composed of micro fibrils in helical windings with frequent reversal in direction and with numerous convolutions along the fiber length. The cellulose chain has in its chemical structure on each building unit (glucopyranose) three active hydroxyl groups one primary in 6 position and two secondary in 3,4 positions. Most of the chemistry of the cottons depends upon the reactivity of these hydroxyl groups [12].

This work was carried out to assist the researchers, who are involved in eco-tex analysis and residual pesticides analysis in cotton samples, in selecting the best extraction technique in their measurements on cotton matrices.

Experimental

Materials

Egyptian cotton variety namely Giza 86 (G86) was collected from the cultivation season 2000/2001. It was obtained from Cotton Research Institute (CRI)- Ministry of Agriculture-Egypt. This variety is one of the most long staple Egyptian cotton exports. The sample was mechanically cleaned up from its seeds, the seed coats and other foreign contaminants.

Reagents and Apparatus

HPLC grades Hexane, Acetone, Methanol and dichloromethane solvents were used for the extraction procedure. Anhydrous and granular sodium sulfate was used as dehydrating agent throughout the extraction process. Glass wool was used as supporting material in soxhlet extortion after cleaned with acetone and alcohol several times. Gas Chromatograph -HP H5890a in an analytical system was equipped with a temperature programming capability, splitless injector (0.5 min splitless mode), capillary columnDB-5, and lineralized ECD. It is equipped with an automatic sample injector. The columns of 0.25 inside diameter by 60 m long fused silica capillary, with a chemically bonded phenylmethyl polysiloxane phases was supplied from J. and W. Scientific, CA. The operating condition of the gas chromatograph analytical system were adjusted to the following conditions: Helium carrier gas with a flow rate of 1.2 ml/min, Injector temperature 250^{o}C, column temperature (oven) 250^{o}C started at 60^{o}C and increased with the rate 3^{o}C/min, holding time 30min, detector temperature 275^{o}C. Accelerated solvent extractor (Dionex ASE 200): stainless steel extraction cell of 22ml capacity was used at 100^{o}C under 2000 pci pressure for five minutes.

Soxhlet apparatus: 250ml round flask with soxhlet and condenser glassware was used to conduct the soxhlet extraction.

TurboVap(TM) water bath using nitrogen gas to evaporate the excess solvent and reduce the volume to 0.5 ml was also used.

All the above experiments were done at the Analytical Chemistry Division at the National Institute of Standards and Technology (NIS-USA).

Cryogenic Homogenization

It is necessary for the determination of the residual pesticides in cotton samples to turn the sample into finely chopped or ground powder. This chopping grinding procedure should be done carefully to avoid heat generation [13]. The standard protocol for cryogenic homogenization, detailed elsewhere [14,15], was followed for the initial homogenization. The frozen sample is smashed in a pre-chilled Teflon chamber resulting in small frozen pieces ~ 3 cm in diameter. These frozen pieces of sample are arranged around the inside of a pre-chilled Teflon mill containing a concentric Teflon ring and Teflon puck. The mill is placed on a shaker for approximately 5 minutes. After the shaking, the mill is returned to the liquid nitrogen freezer and the resulting powder is sampled. In addition to the Teflon puck mill, a Teflon ball mill was also used to evaluate any difference in the resulting powder. There was no significant difference in the final powder from the two methods; therefore, the standard Teflon puck mill procedure was used for the remainder of the raw cotton sample. The raw cotton, which had been transferred to a clean Teflon bag and sealed, was held overnight in liquid nitrogen vapor; however, this did not produce any rigidity in the sample. Since the raw cotton had to be extremely cold, the raw cotton contained in the sealed Teflon bag was immersed in liquid nitrogen overnight before homogenization could be accomplished. Only ~ 2 - 3 g of the raw cotton would fit into the mill at a time and grind successfully, normally a 150 g sample can be homogenized during one grinding. After each successful homogenization, the resulting powder was pooled in another clean Teflon bag. Once the entire sample, ~ 30 g was homogenized and blended by manual inversions, the powder was sampled, cleaned, chilled, pre-weighed, and labeled Teflon jars for storage and for analysis.

Extraction Procedures

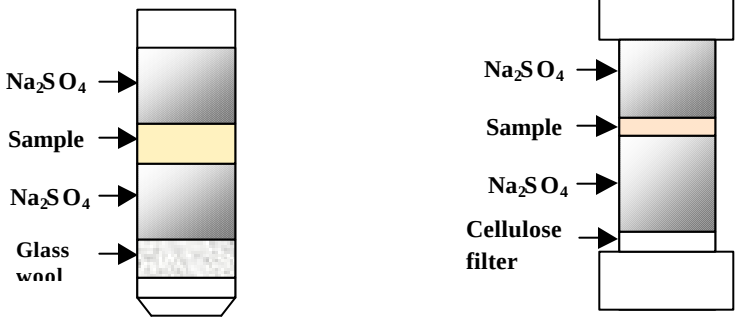
Two different extraction methods were used to extract chlorinated pesticides from an Egyptian cotton matrix. Four different solvents with different polarities, methanol, hexane, hexane/acetone (1/1 v/v) and dichloromethane were also used. All of these solvents were of high purity HPLC grades.

Soxhlet Extraction (SOX)

A total of 0.5 gm ±0.1 mg was transferred to the soxhlet thimble in between two layers of dehydrated sodium sulfate over a glass wool layer as shown in Figure 1. The thimble was placed in the extraction apparatus charged with 200 ± 10 ml of the studied solvent. Sample was extracted for overnight. The extract then concentrated by turbo evaporator to 0.5 ml then excess amounts of hexane (about 2 ml) was used and extract was re-concentrated. At the end of the concentration procedure we have 0.5 ml of the extracted chlorinated pesticides is dissolved in 0.5 ml of hexane.

Accelerated Solvent Extraction (ASE)

The extraction procedure was carried out using Dionex ASE system, 0.5g (standard deviation 0.002g) was transferred to the extraction cell of 22 ml capacity as shown in Figure 1. The sample was placed in between two layers of dehydrated pure sodium sulfate. The extraction operating conditions were 100°C and pressure 2000 pci for 5 minutes.



Homogenized Sample in the glass thimble for Soxhlet extraction

Homogenized Sample in the ASE cell

Figure 1: Schematic diagram illustrates the positioning of the homogenized sample in the glass thimble and in the ASC extraction cell

Identification of Chlorinated Pesticides

The qualitative analysis simply is a comparison of retention data from the known and unknown samples. The known samples used, through out this work, have the standard reference material number SRM22611 for the chlorinated pesticides in hexane. This SRM 2261 was certified by the National Institute for Standards and Technology (NIST) in the United States [10].

After extraction and evaporation the concentrate was .5 ml and 2 ml of hexane was added and re-evaporated the extract to 0.5 ml to replace the extract solvent with hexane to match the solvent of the standard used. The extract was then transferred to GC vials to do the injection into GC.

Results and Discussion

In this work we have succeeded to produce a homogenized cotton powder form the Egyptian cotton fibers of variety Giza 89 without any degradation in residual pesticides that might exist in it. The homogenization was carried out cryogenically to avoid the degradation of the pesticides analytes. Figure 2 shows the started and final forms of the cotton samples.

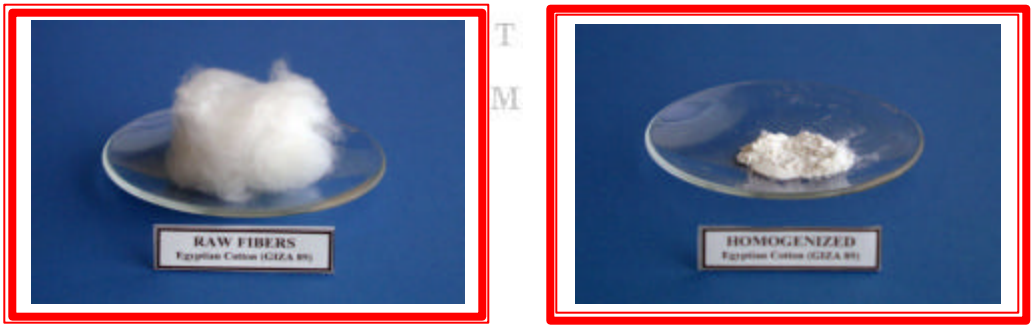


Figure 2: Homogenized Egyptian Cotton by Cryogenic Homogenization Method.

The residual chlorinated pesticides mentioned in the Oeko-tex standards for the textile materials were extracted by using SOX and ASE extraction methods. The cryogenic homogenized samples were subjected to the following steps as shown in Figure 3.

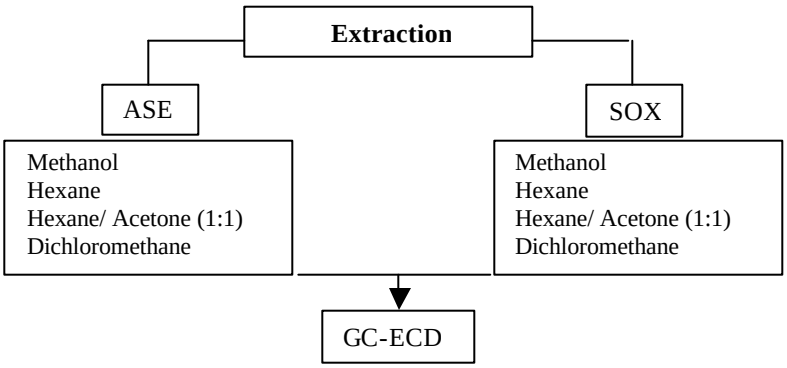


Figure 3: GC Analysis of the Homogenized Cotton by Accelerated Solvent Extraction (ASE) and Soxhlet Extraction (SOX) Methods

The resulting chromatogram of each injection comparison was compared in terms of the retention time of each pesticide. The integration of each peak in comparing to the NIST SRM2261 for the organochlorinated pesticides for Hexachlorobenzen, Gamam-HCH, Heptachlo, Aldrin, Heptachlor epoxide, 2,4'-DDE, Cis-chlordane, Trans-nonachlor, 2,4'-DDE, Dieldrin, 2,4'-DDD, 4,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT and Mirex.

Tables (1 and 2) show the chromatogram of the NIST SRM 2261 and the different extracts. The integration of the area under each peak at specified retention time (as those of SRM 2261) was performed to compare the efficiency of the extraction procedure.

Table 1: Certified Concentration of Chlorinated Pesticides in Hexane of SRM2261

Pesticide Name [2,15]	**Concentration (Mg/kg)**	**Retention time (Min)**
Hexachlorobenzene	3.005± 0.014	9
Gamam-HCH	3.012± 0.015	10.13
Heptachlo	3.020± 0.023	14.44
Aldrin	3.029± 0.039	---
Heptachlor epoxide	3.020± 0.026	21.66
2,4'-DDE	3.019± 0.026	--
Cis-chlordane	3.012± 0.019	26.79
Trans-nonachlor	3.034± 0.022	27.25
4,4'-DDE	3.019± 0.015	30.88
Dieldrin	3.012± 0.020	31.5
2,4'-DDD	3.013± 0.026	32.05
4,4'-DDD	3.043± 0.042	--
2,4'-DDT	3.993± 0.014	--
4,4'-DDT	3.004± 0.018	43.44
Mirex	3.041± 0.042	63.00

Table 1 shows that four pesticides were not detected. Aldrin was not detected because its instability and it tends to oxidize rapidly. 4,4' DDD, 2,4'-DDE and 2,4' DDT were not separated by the operating condition that previously mentioned in the experimental part.

Table 2: Comparison: Soxhlet (SOX) and Accelerated Solvent Extraction (ASE) Methods (with methanol)

Pesticide Name [2,15]	Retention time (Min)	Peak area	
		(SOX)	(ASE)
Hexachlorobenzene	9	7826.50	14122.50
Gamam-HCH	10.13	No peak	2110.50
Heptachlo	14.44	468.00	642.00
Aldrin	---	--	--
Heptachlor epoxide	21.66	No peak	2584.00
2,4'-DDE	--	--	--
Cis-chlordane	26.79	1692.00	22618.00
Trans-nonachlor	27.25	775.00	1053.00
4,4'-DDE	30.88	96062.00	18322.00
Dieldrin	31.5	329.00	5672.00
2,4'-DDD	32.05	7985.00	17391.00
4,4'-DDD	--	--	--
2,4'-DDT	--	--	--
4,4'-DDT	43.44	27632.00	171864.00
Mirex	63.00	155146.50	115953.00

The area data of the chromatograms resulted from the SOX and ASE extraction reveals that there is broadens in both of them and this may be attributed to the hydrogen bonding that was resulted polarity of the hydroxyl groups in the molecular structure of the methanol. The peak-separations by both extraction techniques were not good. It is clear also that ASE extraction technique has a better efficiency to extract most of the studied pesticides except for 4,4'-DDE and Mirex these may be attributed to the rinsing time is more efficient than the pressurized ASE extraction technique. The sharpness of the peak for these two pesticides is more broad in SOX this give the advantage to the ASE technique.

Table 3: Comparison: Soxhlet (SOX) and Accelerated Solvent Extraction (ASE) Methods (with Hexane)

Pesticide Name [2,15]	Retention time (Min)	Peak area	
		(SOX)	(ASE)
Hexachlorobenzene	9	29650.50	9848.00
Gamam-HCH	10.13	No peak	No peak
Heptachlor	14.44	19108.00	No peak
Aldrin	---	--	--
Heptachlor epoxide	21.66	933.00	543.00
2,4'-DDE	--	--	--
Cis-chlordane	26.79	1708.00	No peak
Trans-nonachlor	27.25	1293.00	1793.50
4,4'-DDE	30.88	32127.00	5498.00
Dieldrin	31.5	309.00	300.00
2,4'-DDD	32.05	178.00	107.00
4,4'-DDD	--	--	--
2,4'-DDT	--	--	--
4,4'-DDT	43.44	39300.00	14020.50
Mirex	63.00	392.50	27896.00

Extraction with hexane as a solvent by SOX and ASE extraction techniques shows a good separation of some peaks either without a high detection or an absent from the chromatogram. It is clear from the data included in Table 3 that, ASE extraction does not give peaks either for gamma-HCH, heptachlor or cis-nanochlor while all peaks appeared with SOX extraction but still with low values when compared to dichloromethane extraction as in Table 5.

Table 4: Comparison: Soxhlet (SOX) and Accelerated Solvent Extraction (ASE) Methods (with Hexane/acetone)

Pesticide Name [2,15]	**Retention time (Min)**	**Peak area**	
		(SOX)	**(ASE)**
Hexachlorobenzene	9	10826.00	1551.50
Gamam-HCH	10.13	948340.50	209749.50
Heptachlo	14.44	10595.00	No peak
Aldrin	---	--	--
Heptachlor epoxide	21.66	7265.00	22093.50
2,4'-DDE	--	--	--
Cis-chlordane	26.79	No peak	6502.50
Trans-nonachlor	27.25	12356.50	1457.50
4,4'-DDE	30.88	29436.00	10481.00
Dieldrin	31.5	264.00	93.50
2,4'-DDD	32.05	124.00	56093.00
4,4'-DDD	--	--	--
2,4'-DDT	--	--	--
4,4'-DDT	43.44	37640.50	34975.50
Mirex	63.00	1468.00	79116.00

Table 5: Comparison: Soxhlet (SOX) and Accelerated Solvent Extraction (ASE) Methods (with Dichloromethane)

Pesticide Name [2,15]	**Retention time (Min)**	**Peak area**	
		(SOX)	**(ASE)**
Hexachlorobenzene	9	20385.00	386558.50
Gamam-HCH	10.13	63.00	30417.00
Heptachlo	14.44	12304.50	13796.00
Aldrin	---	--	--
Heptachlor epoxide	21.66	6617.00	4293.00
2,4'-DDE	--	--	--
Cis-chlordane	26.79	22629.00	2180.00
Trans-nonachlor	27.25	663.00	17037.46
4,4'-DDE	30.88	26412.00	3260.00
Dieldrin	31.5	144811.00	16907.00
2,4'-DDD	32.05	104118.50	30904.50
4,4'-DDD	--	--	--
2,4'-DDT	--	--	--
4,4'-DDT	43.44	3435370.00	69270.00
Mirex	63.00	170002.50	69759.00

The areas in Tables 2-4, show that the soxhlet extraction is more selective for Heptachlor epoxide, 4,4'-DDE, Dieldrin, 2,4'-DDD, 4,4'DDT and Mirex. Dichloromethane detects the entire component with good sensitivity; this can be attributed to the polarity of the solvent [12].

Conclusions

This work is the first step in producing standard reference materials from the Egyptian cotton of cotton variety Giza 89 (2000/2001). The results of this research can be concluded as follows:

1. Using methanol as an extracting liquid of pesticides from cotton matrix, the ASE technique proved to be more efficient for pesticides except for 4,4'-DDE and Mirex.
2. Using Hexane as an extracting liquid of pesticides from cotton matrix, SOX extraction technique is better than ASE except for Trans-nonchlor and Mirex.
3. Using hexane/acetone (1:1 v/v) mixture as an extracting solvent of pesticides from the cotton matrix the SOX technique proved to be better than the ASE technique except for Heptachlor epoxide, Cis-chlordane, 2,4'DDE and Mirex. Moreover the sharpness of the peak in ASE extraction shows a better sharpness and less broadening.
4. Using dichloromethane as extracting solvent of the chlorinated pesticides from the cotton matrix. ASE shows a better sharp peak but less selective for Heptachlor epoxide, 4,4'-DDE, Dieldrin, 2,4'-DDD, 4,4'DDT and Mirex.

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