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DETERMINATION OF SOME ORGANOCHLORINE COMPOUNDS IN HERBAL COLOURING AGENT HENNA (*LAWSONIA INERMIS*) AND IN TEA (*THEA SINENSIS*)*

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Henna (*Lawsonia inermis*) has been used for centuries as a herbal hair and skin dye, but very little is known about its additives and contaminants that could adversely affect human health. An analytical method was developed to determine organochlorine compounds in henna, as they are still widely used in the areas where henna is grown. Samples were sonicated with *n*-hexane, extracts cleansed on Florisil sorbent and analysed using gas chromatography with electron capture detection. The overall recoveries were 17-33 % with the extraction RSD 5-21 %, while the levels of *lindane (γ-HCH), p,p'-DDT, and p,p'-DDE* in henna samples were 7-157 μg kg^{-1}. The same procedure was successfully applied to analyse black tea samples for the same compounds, and which showed lower contamination.

KEY TERMS: *black tea, DDE, DDT, gas chromatography, lindane*

Henna plant (*Lawsonia inermis*) is a tropical and subtropical shrub, growing in North Africa, Middle East and Indian subcontinent. The powder made of dried crushed leaves is called henna (1). When applied in a form of paste onto hair or skin, it imparts a reddish-brown colouration lasting for up to twelve weeks. It was used as a hair dye as early as ancient times; for instance, the hair of Egyptian mummies was dyed with henna (2). Besides its use in cosmetics, henna was also used in Medieval Persian, Arab, Turkish and Jewish medicine to treat headaches (3), skin and teeth diseases, as well as animal bites (4). In Arab countries, it is still used in folk medicine to treat different skin conditions (5). Modern pharmacological research on henna and its constituents has confirmed its anti-inflammatory, antipyretic and analgesic effects (6), and discovered its anticarcinogenic potential (7). It can also be used to treat pediculosis (8). The active component of henna is lawsone (2-hydroxy-1,4-naphthoquinone, CAS 83-72-7), which is also the principal dye ingredient. Current research suggests that lawsone is non-problematic for external use because of its low toxicity and genotoxicity (9).

In the Western world, henna is known and used as a herbal hair dye, but has recently been gaining popularity as the so-called "black henna tattoo". Tattoos are usually made by street vendors and are temporary, lasting for up to a few weeks. However, there is an increasing number of reports of allergic reactions associated with this practice (10). Pure henna is only rarely connected with allergic reactions (11); the main causative agent for allergic dermatitis following tattoo is usually *p*-phenylenediamine (PPD) (10, 12), a highly allergenic black hair dye added to henna to produce darker colour.

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However, very limited data are available about the presence of other additives and contaminants in henna powder, which may adversely affect human health. In certain regions, henna is mixed with minerals containing lead, mercury, copper and zinc in order to strengthen the colour (5). It is a popular belief among hairdressers that lead in henna is responsible for the green discolouration of henna-dyed hair upon bleaching with H_2O_2, although this notion has not been verified.

Organochlorine insecticides (OCl) are also possible contaminants, as they are still used to control insects, especially mosquitoes (13), in several countries where henna plants are grown, because of their relative low price and high efficacy. These compounds are problematic for several reasons and were therefore banned or their use was severely restricted in industrialised countries decades ago. They are toxic [LD_{50} (oral, rats) 5-4000 mg kg^{-1}], carcinogenic and teratogenic; some of them exhibit estrogenic activity (13). Prenatal exposure to OCls has been linked with delayed infant development (14). Moreover, as they are quite persistent in the environment and highly lipophilic, they bioaccumulate along the food chain (15). Detectable concentrations of these compounds and their degradation products are still found in human milk even in industrialised countries, where they have not been in use for at least twenty years (13, 16, 17).

Organochlorine pesticides and their degradation products in various matrices are usually determined by combining an extraction method with gas chromatography (GC), usually with electron-capture detector (ECD) (16, 18-21) or mass-spectrometric detector (MS) (22-24). The crucial step in the analysis is the extraction with simultaneous preconcentration of analysed compounds, since they are usually present at quite low concentrations. For solid matrices such as soil and various foodstuffs, as well as for oleaginous liquid matrices, extraction with organic solvents and subsequent clean-up of extracts is still the preferred method (21-23), although other approaches are also successful, e.g. supercritical fluid extraction (19, 21), sonication with water-organic solvent mixture (18), and steam distillation (16). For aqueous matrices, solid-phase extraction (24) and solid-phase microextraction (18, 20) are the most common techniques, beside extraction into organic solvents.

The aim of this study was to develop a simple gas chromatographic method for the analysis of most common organochlorine insecticides and their degradation products in henna samples. To our knowledge, this is the first article on these compounds in henna. In addition to various henna samples, specimens of black tea (*Thea sinensis*) were also analysed using the same procedure.

MATERIALS AND METHODS

Materials

Our analysis included organochlorine compounds lindane, *p,p'*-DDT and *p,p'*-DDE, a metabolite of *p,p'*-DDT. They are shown in Figure 1, while Table 1 lists their chemical names and some physicochemical properties. Their standards of 96-99 % purity were purchased from PolyScience (Niles, IL, USA) or Serva (Heidelberg, Germany). Solvents *n*-hexane and acetone were of the HPLC grade, obtained from Rathburn (Walkerburn, Scotland, UK). They were used as obtained, after their purity was checked by evaporation of 10 mL of solvent to 0.1 mL and subsequent analysis by gas chromatography.

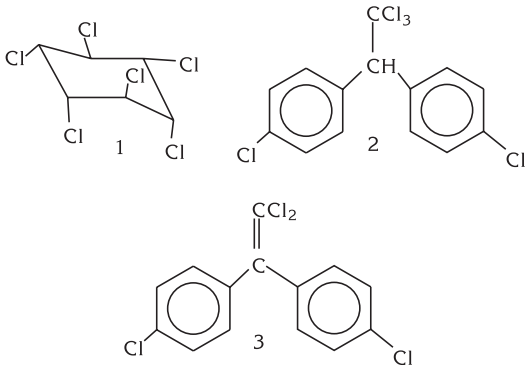

Figure 1 *Chemical structure of analysed organochlorine compounds: **1** - lindane (γ-HCH), **2** - p,p'-DDT, **3** - p,p'-DDE.*

Henna samples were purchased in Slovenian drugstores (packed in Slovenia, of unknown origin) or bought abroad in traditional markets. Black tea samples were purchased in specialised stores in Slovenia. They were imported from different countries. Table 2 gives a complete list of henna and tea samples.

Extraction cartridges for the clean-up were Supelclean LC-18 SPE (1 g of sorbent) from Supelco (Bellefonte, PA, USA) and Bakerbond SPE Florisil (1 g of sorbent) from J. T. Baker (Deventer, The Netherlands).

For gas chromatography, helium (>99.999 %) and nitrogen (>99.996 %) from Messer (Gumpoldskirchen,

Table 1 *Chemical names, abbreviations, and relevant physicochemical properties of lindane, p,p'-DDT, and p,p'-DDE (13, 25, 26).*

Trivial name	Chemical name	Abbrev.	*M* / g mol^{-1}	Solubility in H_2O/mg L^{-1}	log K_{ow}	*p*/Pa
lindane (γ-HCH)	1,2,3,4,5,6-hexachloro-cyclohexane (HCH), γ-isomere	LN	290.8	7-17 (20 °C)	3.2-3.7	$4.34 \cdot 10^{-3}$ (20 °C)
p,p'-DDT	1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane	DDT	354.5	0.001	4.0-6.1	$2.53 \cdot 10^{-5}$ (20 °C)
p,p'-DDE	1,1-dichloro-2,2-bis (4-chlorophenyl)ethene	DDE	319.0	0.008	5.7	$8.6 \cdot 10^{-4}$ (30 °C)

Austria) were used. Analytical capillary column was HP1, dimensions 25 m x 0.2 mm (i.d.), film thickness 0.11 μm, from Hewlett-Packard (Palo Alto, CA, USA).

Table 2 *Analysed samples of henna and black tea.*

Sample	Abbreviation used	Country of purchase	Country of origin
Drugstore henna 1	HnD	Slovenia	unknown
Drugstore henna 2	HnA	Slovenia	unknown
Indian henna	HnI	India	India
Turkish henna	HnT	Turkey	Turkey
Ceylon black tea	BtS	Sri Lanka	Sri Lanka
Indian black tea	BtI	Slovenia	India (Assam)
Chinese black tea	BtC	Slovenia	China
African black tea	BtK	Slovenia	Kenya

Instrumentation

The gas chromatograph was a Hewlett-Packard with electron capture detector (ECD) of HP 6890 Series (Palo Alto, CA, USA).

Laboratory centrifuge was Megafuge 1.0 (Heraeus Sepatech, Hanau, Germany), ultrasonic bath was from Iskra (Kranj, Slovenia), and the analytical balance was Mettler Toledo MX5 (Mettler Toledo, Kuesnacht, Switzerland).

Standard solutions and sample preparation

Stock standard solutions of organochlorine compounds were prepared by dissolving the weighed solid standard in *n*-hexane to obtain concentration 0.5-1.0 g L^{-1}. These solutions were kept in the refrigerator and were stable for several months. They were further diluted with *n*-hexane to obtain working solutions containing all analysed compounds in the concentration range 25-100 μg L^{-1}.

Henna and tea samples with standard addition of the organochlorine compounds were prepared as follows: 10 g of a sample was weighed in an open vessel, 5 mL of standard solution (100 μg L^{-1} of each compound in *n*-hexane) was added, mixed and left open until the solvent evaporated.

Extraction procedure

A two-gram sample was weighed in the extraction vessel and 20 mL of *n*-hexane was added. The suspension was extracted for 10 min in the ultrasonic bath, transferred to centrifuge cuvettes and centrifuged for 3 min at 3000 rpm (1400 g). The supernatant (5 mL) was passed through a Florisil® extraction cartridge, evaporated to dryness under a stream of nitrogen on water bath (ca. 50 °C) and reconstituted in 0.1 mL of *n*-hexane. The final extract was injected in the gas chromatograph (1 μL).

Gas chromatography

The optimal temperature program for the separation of analytes was 50 °C (1 min) - 20 °C min^{-1} - 200 °C - 10 °C min^{-1} - 250 °C - 30 °C min^{-1} - 280 °C (3 min). The temperature of the injector was 250 °C and the temperature of the detector 320 °C. Carrier gas (He) flow was 1 mL min^{-1}, make-up gas (N_2) flow was 50 mL min^{-1}. Manual splitless injection: 1 μL.

RESULTS AND DISCUSSION

Optimisation of gas chromatography

Gas chromatography with electron capture detection is probably the most common method

Table 3 *Relevant GC method parameters for lindane, p,p'-DDT, and p,p'-DDE (analysis of standard solutions in n-hexane). RSD - relative standard deviation for at least 5 injections.*

compound	lin. range/ μg L^{-1}	r^2	t_R / min	RSD (t_R)/%	RSD (peak area)/%
LN	25-100	0.9383	7.69	0.1	9.8
DDT	50-200	0.9733	10.81	0.4	12.8
DDE	25-100	0.9913	10.25	0.2	9.4

for the analysis of organochlorine compounds in various matrices (16,18-21). The specific nature of such detector allows for determination of very low concentrations of compounds. Our compounds of interest are nonpolar as seen from their log K_{ow} values and solubility in water (see Table 1). Therefore, the main factor affecting their separation and order of elution from the chromatographic column is expected to be their volatility, corresponding to their molecular mass (see Table 1). As shown in Figure 2, all organochlorine compounds were eluted in the expected order and well separated in less than 12 min. The total time for a chromatographic run could have been even shorter; however, we took into account possible interfering peaks appearing in the chromatograms of sample extracts. Table 3 lists some parameters for the GC method, obtained by injection of standard solutions in *n*-hexane. The linearity is acceptable, while the repeatability (shown as RSD) is excellent for the retention times and within the usual limits for the manual injection for the area of chromatographic peaks.

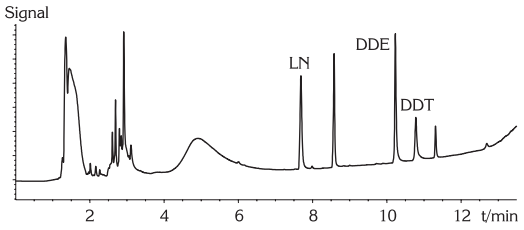

Figure 2 *GC-ECD chromatogram of the standard solution of analytes in n-hexane: 75 µg L^{-1} of LN, DDE and 150 µg L^{-1} of DDT.*

Optimisation of the extraction procedure

As a complex matrix, henna contains many compounds possibly interfering with the chromatographic analysis of organochlorine compounds. We prepared henna spiked with the compounds of interest and performed an extraction with *n*-hexane in an ultrasonic bath. Ultrasonication is one of possible aids to release compounds from the solid matrix (18, 21). The obtained extract was of reddish-brown colour. As expected, many other compounds from henna were co-extracted into *n*-hexane, completely covering the peaks for the analysed compounds in the chromatogram (shown in Figure 3a). Therefore, there was an obvious need to further cleanse the extract.

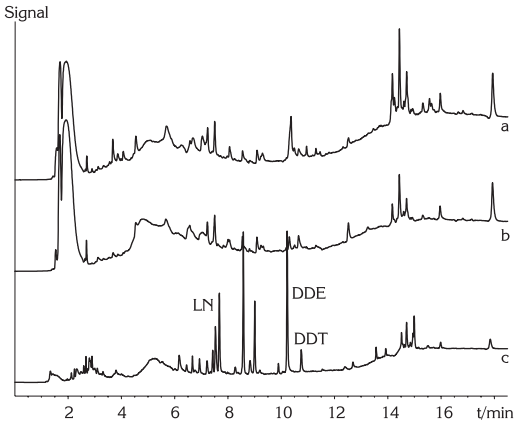

Figure 3 *GC-ECD chromatograms of differently prepared extracts of henna spiked with ca. 50 µg kg^{-1} of analysed compounds (100 µg kg^{-1} of DDT).* ***a*** *- extract after sonication with n-hexane;* ***b*** *- n-hexane extract (a) cleansed on C_{18} SPE cartridge;* ***c*** *- n-hexane extract (a) cleansed on Florisil® SPE cartridge and preconcentrated (preconcentration factor 50).*

Organochlorine compounds are hydrophobic and thus retained on hydrophobic solid sorbent if previously dissolved in aqueous matrix. After evaporation of *n*-hexane, henna extract was reconstituted in distilled water and passed through non-polar extraction cartridge (modified silica - C_{18}). The retained compounds were eluted with *n*-hexane. Figure 3b shows the chromatogram of thus obtained cleansed extract; hardly any improvement is noticed in comparison to Figure 3a.

The second cleansing procedure involved passing the *n*-hexane extract through a polar sorbent Florisil® (magnesium silicate) which adsorbs polar compounds. Organochlorine compounds were not expected to be retained on the sorbent. The effluent from the cartridge was colourless and after further

preconcentration (preconcentration factor 50), a rather clean chromatogram of organochlorine compounds was obtained (Figure 3c). However, the calculated recoveries from the overall extraction process were quite low: LN 17 % (RSD 21 %), DDT 32 % (RSD 16 %), DDE 33 % (RSD 5 %). The Florisil® sorbent was separately tested with a standard solution of organochlorine compounds and no retention was observed. Therefore, the majority of the losses was probably due to incomplete extraction of analytes into *n*-hexane. Acetone was tested as an alternative solvent, but the recoveries obtained were comparable to those with *n*-hexane. Usually the extraction times recommended for organochlorine compounds in solid matrices are longer than used in our case. However, the final procedure was fast and reliable enough to allow for the estimation of levels of organochlorine compounds in henna samples.

Estimation of levels of organochlorine compounds in the samples

In spite of the successful clean-up procedure for henna extracts, there were still many interferences present in the chromatograms of these extracts (see Figure 3c). Because of this fact and low recoveries for organochlorine compounds, all samples of henna and black tea were analysed using the method of standard addition of analytes. Both original sample and sample with a standard addition were extracted in the same manner, and the amount of analyte present in the original sample was estimated from the difference in peak areas for the same compound in chromatograms of both extracts. The results are presented in Table 4.

Table 4 *Estimated mass fractions of analysed organochlorine compounds in the samples of henna and black tea. (for abbreviations, see Tables 1 and 2)*

Sample	mass fraction / μg kg^{-1}		
	lindane	*p,p'*-DDT	*p,p'*-DDE
HnD	ND	ND	ND
HnA	13	ID	13
HnI	7	22	10
HnT	11	157	15
BtS	ND	ND	ND
BtI	ND	ND	ND
BtC	2	28	1
BtK	ND	ND	ND

ND - not detected
ID - determination impossible because of interferences

Low levels of organochlorine compounds were found in three of the four henna samples, while only one of the four black tea samples contained a very low mass fraction of the analysed compounds, which is far below the safety limit of 0.2 mg kg^{-1}, established for these compounds in tea (23). These results are not surprising, as black tea is intended for human consumption and therefore some regulations are imposed in its production. Henna is a traditional product sold mainly in local markets and is usually exported only in small quantities. As it is mostly applied externally, probably no regulations are imposed on its production, including the use of pesticides in the areas where it is grown.

CONCLUSIONS

Our simple method of extraction, clean-up and chromatographic analysis has proven to be successful in the estimation of the levels of some organochlorine compounds in henna and black tea samples. The presented method is suitable for fast sample screening, but a more reliable analysis in complex matrices requires identification of compounds, either by mass spectrometry or at least by using a second chromatographic column with different properties.

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Sažetak

ODREĐIVANJE NEKIH ORGANOKLOROVIH SPOJEVA U CRVENOJ KANI (*Lawsonia inermis*) I ČAJU (*Thea sinensis*)

Kana (*Lawsonia inermis*) jest biljka koja se već stoljećima primjenjuje za bojenje kose i kože, pa ipak se veoma malo zna o mogućim dodacima i onečišćenjima u njoj, koji bi mogli štetno utjecati na ljudsko zdravlje. Razradili smo analitičku metodu za određivanje organoklorovih spojeva u prašku kane, jer se oni kao insekticidi još često primjenjuju u područjima gdje kana raste. Uzorke kane smo ekstrahirali u ultrazvučnoj kupelji *n*-heksanom, a ekstrakte smo pročistili na sorbensu Florisilu® i analizirali plinskom kromatografijom. Analitički povrati bili su od 17 % do 33 % s ponovljivošću (RSD) 5-21 %. Razine lindana (γ-HCH), p,p'-DDT-a i *p,p'*-DDE-a u uzorcima kane bile su 7-157 μg kg^{-1}. Iste analitičke postupke uspješno smo primijenili i na određivanje tih spojeva u uzorcima čaja (*Thea sinensis*), koji su se pokazali manje onečišćenima. Primijenjena analitička metoda može se smatrati prikladnom za brzo preliminarno detektiranje i utvrđivanje masenih udjela organoklorovih spojeva u uzorcima kane i čaja.

KLJUČNE RIJEČI: *crni čaj, DDE, DDT, lindan, plinska kromatografija*

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