Heavy Metals and Para-Phenylenediamine in Hair Dye

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Research Article

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Abstract

Background: The use of cosmetics is widespread around the world, particularly in Arabian countries. Some cosmetics as hair dyes are used since very young ages. Hair dye has been one of the pollution resources of heavy metals.

Aim: The present study aimed to determine the percentage of para-phenylenediamine (PPD) and heavy metals in various hair dyes sold in Aswan, Egypt.

Methods: The study was done on four (n = 4) types of hair dyes commonly used in Aswan. Two analytical methods were used as (GC/MS and EDX). PPD was detected using gas chromatography/mass spectrometry (GC/MS), and the metal components of these dyes were determined using energy dispersive x-ray (E.D.X.).

Results: The concentration of PPD was very high in SHD (99.706%) and tancho HD (99.80%) followed by bigen cream HD (5.563%) and finally bigen powder HD (0.492%). The heavy metals content was arranged in decreasing order as follow: Al > Ca > Zn > Cu > Cl > Pb> Fe > Sc> Cr > Mn in SHD, Si > Mg> Cu > Zn > Cl > Ca in tancho HD, Pb> Cu > Zn >Si >K > Al > Ca > Cr in bigen powder HD and Al > Cu > Cl > Si > Zn > Ca > Pb> Cr in bigen cream HD.

Conclusion: Poisoning with (PPD)-containing hair dye is emerging as a method of deliberate self-harm in various developing countries including the Middle-East, and it is accompanied with high human death rate specially among females. They were also polluted with heavy metals, therefore doctors and consumers should be aware of their potential toxicity as well as the symptoms of systemic poisoning. Even if it isn't stated on the label, many hair dyes contain PPD and heavy metals.

1- Introduction:

Hair dye (HD) comes in a variety of forms, the most prevalent of which are stone hair dye and tancho hair dye, both of which are widely accessible in North Africa and the Middle East and contain the highest concentration of PPD (from 70 to 90%) **(**Khan et al., 2018). Other branded hair dyes like L'Oreal, Garnier, Bigen, Color mate, are available in powder or liquid forms, contain lesser concentrations of PPD, typically from 2 to 10% **(Patra et al., 2015).**

Accidental and suicidal ingestion of the dye are more common in developing countries across the world **(**Anugrah et al., 2010). Many incidents of poisoning and death have been documented in Sudan, Egypt, Saudi Arabia, and India as a result of oral ingesting or subcutaneous mistaken used of hair dyes **(Ahmed et al., 2013).**

Hair dyes have a variety of compounds, numerous of them are sensitizers. P-phenylenediamine (PPD) is the most used synthetic component, and it creates the natural appearance, gives the longest lasting color **(AlGhamdi and Moussa., 2011) and (Ahmed et al., 2013).**

Toxicity with paraphenylenediamine (PPD)-containing hair dye is becoming a new form of suicide across Asia, Africa, and the Middle East, and it's linked to a significant risk of human death since it damages several organs, including the heart, liver, kidneys, and muscles. (Balasubramanian et al., 2014 **and** Zaghla et al., 2019).

PPD in commercial hair color products has been detected by different techniques as inductively coupled plasma mass spectrometry (ICP/MS), high performance liquid chromatography mass spectrometry (HPLC/MS), and gas chromatography mass spectrometry (GC/MS) **(Ahmed et al., 2013).**

Many cosmetics, including hair dyes, contain heavy metals as components or contaminants (Alam et al., 2019).

Heavy metals (HMs) are a collection of elements with similar chemical properties. Some of them, such as copper, iron, zinc, play a crucial role in human organism and are referred to as essential metals, while others are not recognized to be beneficial to our health, or, to put it another way, they are toxic (Sommar et al., 2013).

Energy Dispersive X-ray (EDX) microanalysis is a type of elemental analysis that use specialized X-rays to detect the presence of elements in specimens. It is used in collaboration with electron microscopy, it is a useful technique for detecting nanoparticles of heavy metal components in these dyes (Sandonà et al., 2020)

Gas chromatography/mass spectrometry technique (GC/MS) in spitless mode, as well as energy dispersive x-ray (E.D.X.) microanalysis, were key components of this study.

2- Aim Of The Study:

The purpose of this research is to determine the chemical and microelemental features of PPD-containing commercial hair dyes currently used in Egypt, find adequate detection methods, and measure the components that ay pose a health risk if consumed.

3- Materials And Methods:

3.1. Chemicals:

- Commercially accessible and widely used hair colors in Aswan as Black hair dye, also known as “Stone Hair Dye” (SHD), Tancho dye powder and Bigen dye powder were obtained from hair supplies markets in Aswan while Bigen hair dye cream was obtained from pharmacy.

3.2. Instruments:

a- Gas chromatography/ mass spectrometry (GC/MS) analysis was carried out in Analytical Chemistry Unit (ACAL) - Faculty of Science- Assuit University using a GC/MS system (7890A-5975B) made in USA,

equipped with capillary Column DB 5ms (30m*0.250mm*0.250 µl).

b- Energy dispersive x-ray (E.D.X.) microanalysis was done in Electron Microscope Unit- Assuit University for analysis of elemental components of different hair dyes using Scanning Electron Microscope (JSM-5400LV), made in England.

3.3. Extraction procedure for dye in GC/MS:

Half gm of sample was added to 2 ml of ethyl acetate then sonicated for 15 minutes and centrifuged at 8000 rpm / 40°C for 15 minutes.

The GC/MS analysis was performed in a spitless mode using helium as the carrier gas at a flow rate of 0.5 ml/min for 10.9 minutes, then 1 ml/min per minute for 30 minutes. The injection port was set to 250°C, and the oven was preheated to 60°C for 2 minutes before being programmed at 14°C/min (11°C/min for trimethylsilyl derivatives) to a final temperature of 280°C for 20 minutes.

4- Results:

4.1. Qualitative and quantitative analysis of different hair dyes by gas chromatography / mass spectrometry (GC/MS):

a- Qualitative analysis:

PPD in SHD had a retention time of 9.286 min while the retention time of PPD in Tancho HD was 9.470 min, in Bigen HD powder was 10.37 min and finally in Bigen HD cream was 10.52 min, as illustrated in figures (1,2,3,4).

b- Quantitative analysis:

At concentration of 500 mg/ml, the concentration of PPD in SHD was 99.7% while the concentration of PPD in Tancho hair dye was 99.8%, the concentration of PPD in Bigen HD powder was 0.492% and the concentration of PPD in Bigen HD cream was 5.563%, as shown in Table 1.

Table 1
Components identified in SHD, Tancho HD, Bigen HD powder, and Bigen HD cream by (GC/MS):

Test method	Hair Dye	Analyte/ parameter	Description
ACAL-APR-133-00	**Stone hair dye**	18-Oxononahexacontanoic acid	Value: 0.022% Retention time: 34.127 min
		2-Amino-beta-picoline	Value: 0.272% Retention time: 31.454 min
		P-phenylenediamine	Value: 99.706% Retention time:9.286 min
	Tancho hair dye	P-phenylenediamine	Value: 99.80% Retention time:9.470 min
	Bigen hair dye powder	1-Methyl-2-ethylcyclopentane isomer 1	Value: 22.282% Retention time: 19.506 min
		2-Chloropropionic acid octadecyl ester	Value: 3.580% Retention time: 43.296 min
		2,4-Di-tert-butyl-phenol	Value: 4.827% Retention time: 14.761 min
		Alpha-Octadecene	Value: 13.695% Retention time: 30.907 min
		Cyclododecane	Value: 50.700% Retention time: 13.333 min
		P-phenylenediamine	Value: 0.492% Retention time: 10.37 min
		Triethyl 2-hydroxy-1,2,3-propanetricarboxylate	Value: 4.424% Retention time: 19.195 min

Test method	Hair Dye	Analyte/ parameter	Description
	Bigen hair dye cream	1-Hexadecene	Value:16.640% Retention time:25.936 min
		1-Octadecene	Value:76.569% Retention time:30.907 min
		6-Methyl-2-pyridone	Value: 0.931% Retention time:13.494 min
		Peracetyl-Maltotetraose-1-phenylflavazole	Value:0.297% Retention time:28.953 min
		P-phenylenediamine	Value:5.563% Retention time:10.52 min

4.2. Energy dispersive x-ray (E.D.X.) microanalysis of different hair dyes:

Concentrations of heavy metals contents in many hair dye products (n = 4) widely used in Aswan using EDX were shown in Table 2. SHD analysis revealed the following: Al 29.215%, Ca 28.99%, Fe 5.235%, Cu 8.96%, Zn 8.99%, Pb 6.265%, and Sc 2.15% while the highest components of Tancho HD was Si (55.8%) followed by Mg (28.44%), Cu (5.55%), Zn (5.41%), Cl (4.4%) and finally Ca (0.394%), the highest components of Bigen powder HD was Pb (74.49%) followed by Cu (8%), Zn (6.47%), Si (3.45%), K (3.44%), Al (3%), Ca (0.83%) and finally Cr (0.32%) and the highest components of Bigen cream HD was Al (29.63%) followed by Cu (17.515%), Cl (11.53%), Si (10.76%), Zn (10.1%), Ca (9.2%), Pb (8.995%) and finally Cr (2.27%), as shown in table (2) and figures (5,6,7,8).

Table 2
Elemental components Percentage of SHD, Tancho HD, Bigen HD powder, and Bigen HD cream by Energy dispersive x-ray (E.D.X.) microanalysis.

Element	SHD	Tancho HD	Bigen HD powder	Bigen HD cream
Al	29.215	----	3	29.63
Pb	6.265	----	74.49	8.995
Cr	1.08	----	0.32	2.27
Mn	0.19	----	----	----
Zn	8.99	5.41	6.47	10.1
Cl	8.925	4.4	----	11.53
Ca	28.99	0.395	0.83	9.2
Sc	2.15	----	----	----
Fe	5.235	----	----	----
Cu	8.96	5.55	8	17.515
Mg	----	28.445	----	----
Si	----	55.8	3.45	10.76
K	----	----	3.44	----

5. Discussion:

PPD is found in over thousands hair dye formulations sold across the world. Epidemiologic researches revealed that workers within the textile dye and rubber industries, also hair colors users and barbers, were shown to have a significant risk of bladder cancer, non-hodgkin's lymphoma, multiple myeloma, and hematopoietic malignancies **(Saranya et al., 2014).**

In developed countries, standard hair dye formulations contain no more than 2% PPD in 100 ml dye solution making it less toxic if accidental poisoning occurs **(Hamdouk et al., 2011).** Due to lack of standard regulations in developing countries, these concentrations range from 2–90% **(**Jain et al., 2011).

A variety of analytical techniques were used to measure PPD as gas chromatography coupled mass spectrometry (GC/MS) **(Wang and Krynitsky., 2011)**, inductively coupled plasma mass spectrometry (ICP/MS) **(Ahmed et al., 2013)**, high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) **(Ko et al., 2019)**.

In previous studies, the determination of PPD was carried out by GC and many peaks were found in the GC chromatograms using a flame ionization detector **(**Coligan et al., 1996), but in our experiment, PPD

was detected as one peak using GC/MS in splitless mode. This method is simple, rapid, reproducible technique, sensitive and accurate analytical method and can be used to determine PPD in commercial hair dyes.

In our results, it was found that 99.706% of SHD and 99.80% of Tancho HD was PPD, and this means that SHD and tacho HD mainly PPD. This agreed with previous results done by Coligan et al., (1996) who reported that the conentration of PPD in SHD was 99.85%. Also, the result of our study was in accordance with the result of **Ahmed et al., (2013)** who reported that 99.66% of SHD was PPD.

The concentration of PPD of other commercial HDs in our results was shown in Table 1 as follow: 0.492% in bigen HD powder and 5.563% in bigen HD cream.

Heavy metals (HMs) are metals with a specific density of more than 5 g/cm3 that harm the environment and living beings **(Monisha et al., 2014).**Some of them may be harmful to human health even at extremely low concentration since they have prolonged biological half-lives and are non-biodegradable, allowing them to persist in the body for long time **(**Djahed et al., 2018).

Many cosmetic items contain heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn), and nickel (Ni) **(Abdul et al., 2017).** They can enter the body through ingestion or absorption through the skin. Because the majority of cosmetics are applied topically, dermal exposure is thought to be the most important route for hair dyes **(Arshad et al., 2020).** Individual metals absorption is influenced by variety of parameters, including physical and chemical properties of the mixtures **(Sani et al., 2016).**

Heavy metals as arsenic (As), Aluminium (Al), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu) and zinc (Zn) can build up within the body over time and have been linked to a wide range of health issues as headaches, vomiting, nausea, diarrhea, contact dermatitis, loss of hair, malignancies, reproductive and developmental disorders, amnesia, mood disturbances, neurological, skeletal, blood, immune and renal problems, Many of them are hormone disruptors and respiratory toxins (Palpandi and Kesavan, 2012**).**

There are currently no global limits for pollutants such as heavy metals in cosmetics, with the exception of 20 g/g for lead and 5 g/g for cadmium **(**Al-Dayel et al., 2011). While the regularity limits for certain metals in cosmetics in Canada are 10 g/g for Pb, 3 g/g for As, Cd, and Hg, and 5 g/g for Sb **(**Ullah et al., 2017).

The elemental composition of the sample is determined using Energy dispersive X-ray spectroscopy (E.D.X) **(Mujeeb and Zafar., 2017).**

In our study, we found that the amount of Aluminum (Al) in the hair dye samples studied varied greatly. Bigen cream HD had the greatest Al level (29.63%), followed by SHD (29.215%), and finally Bigen HD powder (3%), while absent in tancho H and this agreed with previous study done by **Ahmed et al., (2013)** who reported that Aluminum represented the highest component of stone hair dye (55.12 %). This could be due to a discrepancy in the dye's origin or a difference in the sensitivity of the devices.

Another study was done by **Shihata., (2018)** to assess the concentration of heavy metals in SHD and showed that the highest level in black stone hair dye were: Aluminum (45.44mg/l), Lead (45mg/l), zinc (32.2mg/l), iron (29.76mg/l), cupper (24.26mg/l) and Magnesium (52.24mg/l).

Sample of bigen HD powder had highest concentration percentage of lead (Pb) at (74.49%) whereas Bigen HD cream and SHD contain least amount of Pb (8.995%) and (6.265%) respectively and absent in Tancho HD.

This agreed with another study done by Benzeid et al., (2021) on Morroco who reported that thirteen samples of hair dyes out of total 16 samples, were with extremely high lead level ranged from 448.43 to 3617.02 ppm. Also, the result of our study was in accordance with the result of **Hussein., (2015)** carried out on the Baghdad market in Iraq who reported a maximum lead level of 0.92 ppm in hair dyes. Another study of Ozbek and Akman reported the same outcome **(Ozbek and Akman., 2016).**

Many researchers investigated the heavy metal content of cosmetic products from various countries. **(**Ullah et al., 2017, **Farrag et al., 2015 and** Iwegbue et al., 2016).

6. Conclusions:

Based upon the results, we determined that hair dye samples purchased from salons and pharmacies contained high levels of para phenylenediamine, which raises the risk of sensitization among those who use them, even though the color's label did not state so.

Furthermore, continued use of these hair dyes may result in an increase in heavy metal levels (aluminium, chromium, copper, zinc, iron, lead, and nickel) through dermal contact, oral ingestion, or other forms of human exposure, causing harmful effects to consumers over time.

The two methods described in this study (GC/MS and EDX) have been shown to be suitable with satisfactory accuracy and good reproducibility for the determination of PPD in hair dye samples and for detection of heavy metals elements present on them.

EDX microanalysis could represent a powerful tool in different biomedical fields and forensic science.

Abbreviations

PPD	Paraphenylene diamine
GC/MS	Gas chromatography/mass spectrometry
EDX	Energy dispersive x-ray
SHD	Stone hair dye
HMs	Heavy metals
HD	Hair dye
ICP/MS	Inductively coupled plasma mass spectrometry
HPLC/MS	High performance liquid chromatography mass spectrometry
CE	Capillary electrophoresis
Al	Aluminium
Pb	Lead
Cr	Chromium
Cu	Copper
Cd	Cadmium
Cl	Chlorine
Ca	Calcium
Zn	Zinc
Ni	Nickel
Mn	Manganese
As	Arsenic
Hg	Mercury
Sc	Scandium
Si	Silicone
Sb	Antimony
Mg	Magnesium
K	Potassium
Fe	Iron

Declarations

Ethical Approval:

All ethical approval was taken from the ethical committee in Faculty of medicine, Aswan University , no.

-Consent to Participate:

All ethical approval and consent were taken.

-Consent to Publish:

All authors approve to publish the paper

-Authors Contributions

Randa H AbdelHady

Idea of the research, share in writing and revision

Heba A Yasa

Idea of the research, Share in the statistics and writing of the research

Marwa M Mahmoud

Share in the analysis and the lab work, and writing of the research

Shaltout ES

Share in the statistics and lab analysis and writing of the paper.

-Funding:

No fund was taken for this research.

-Competing Interests

No conflict of interest.

-Availability of data and materials

All data are available when required.

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Figures

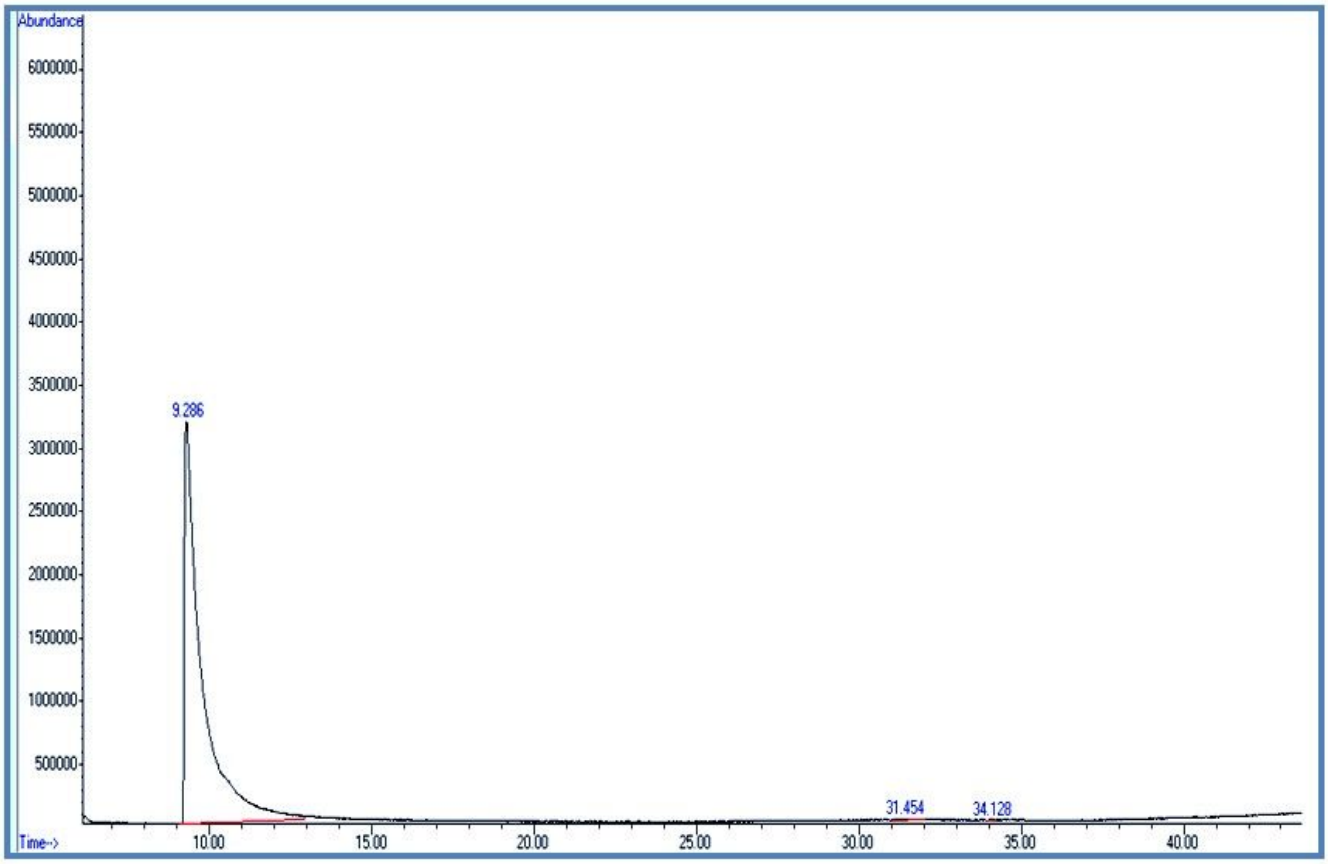

Figure.1: Chromatogram of PPD in stone hair dye. Retention time of SHD using GC/MS

Figure 1

See image above for figure legend

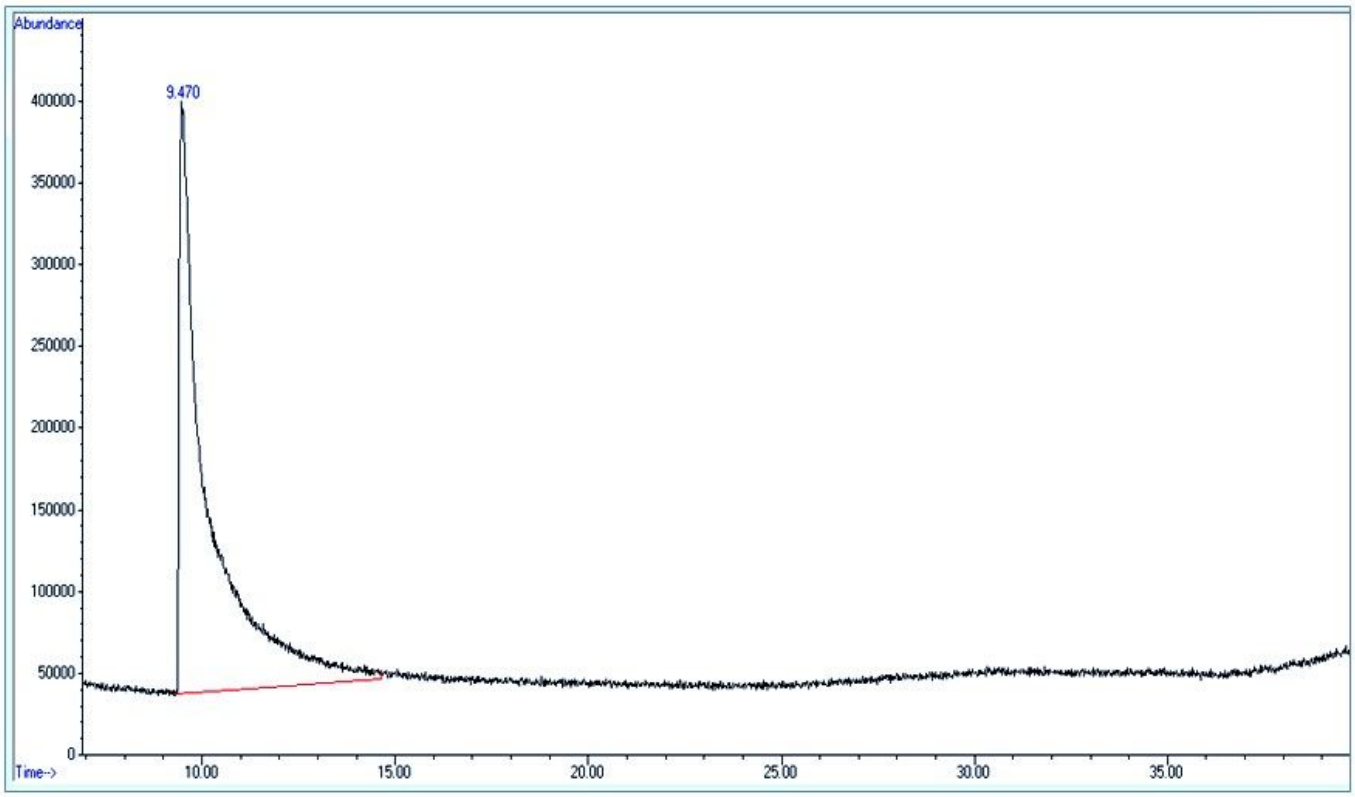

Figure 2: Chromatogram of PPD in Tancho hair dye. Retention time of tancho HD using GC/MS

Figure 2

See image above for figure legend

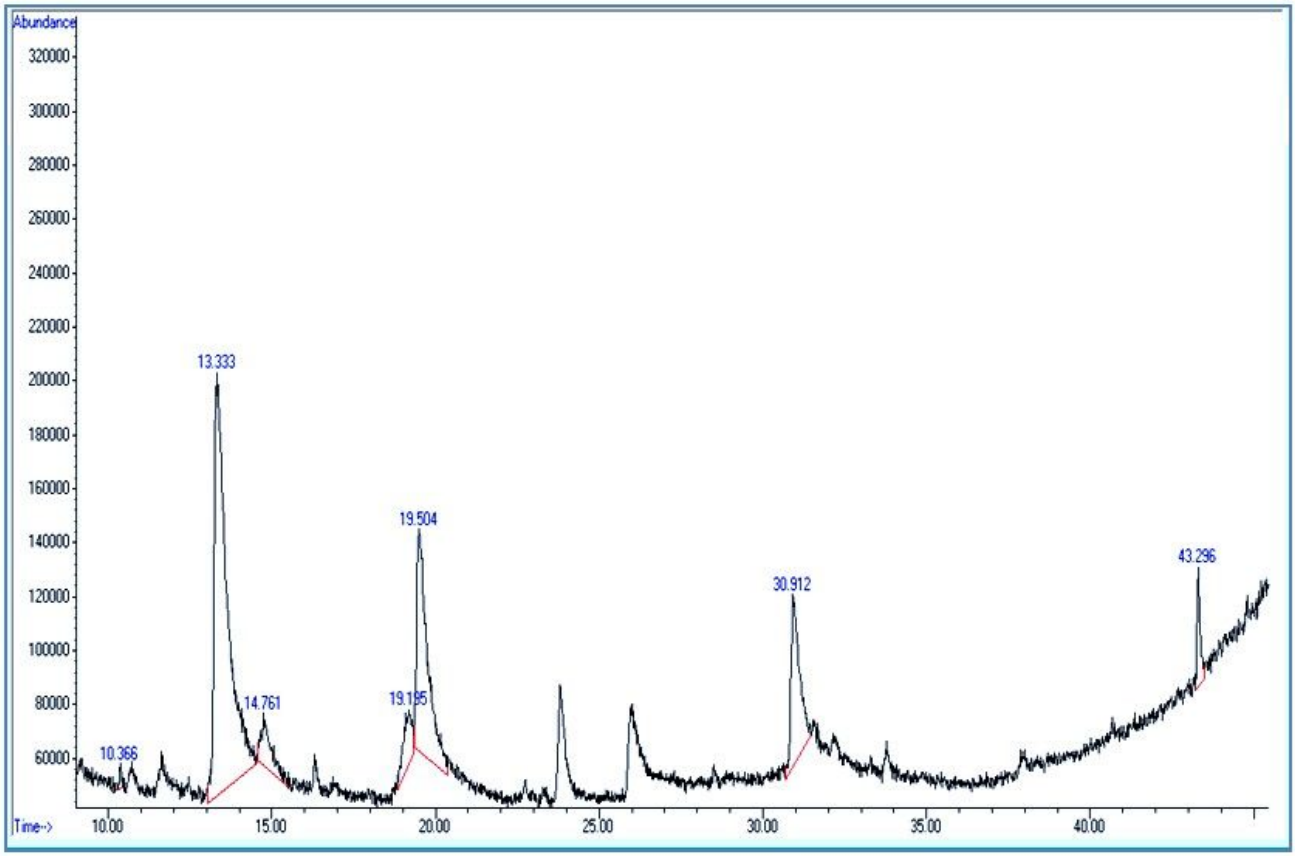

Figure 3: Chromatogram of PPD in Bigen powder hair dye. Retention time of bigen powder using GC/MS

Figure 3

See image above for figure legend

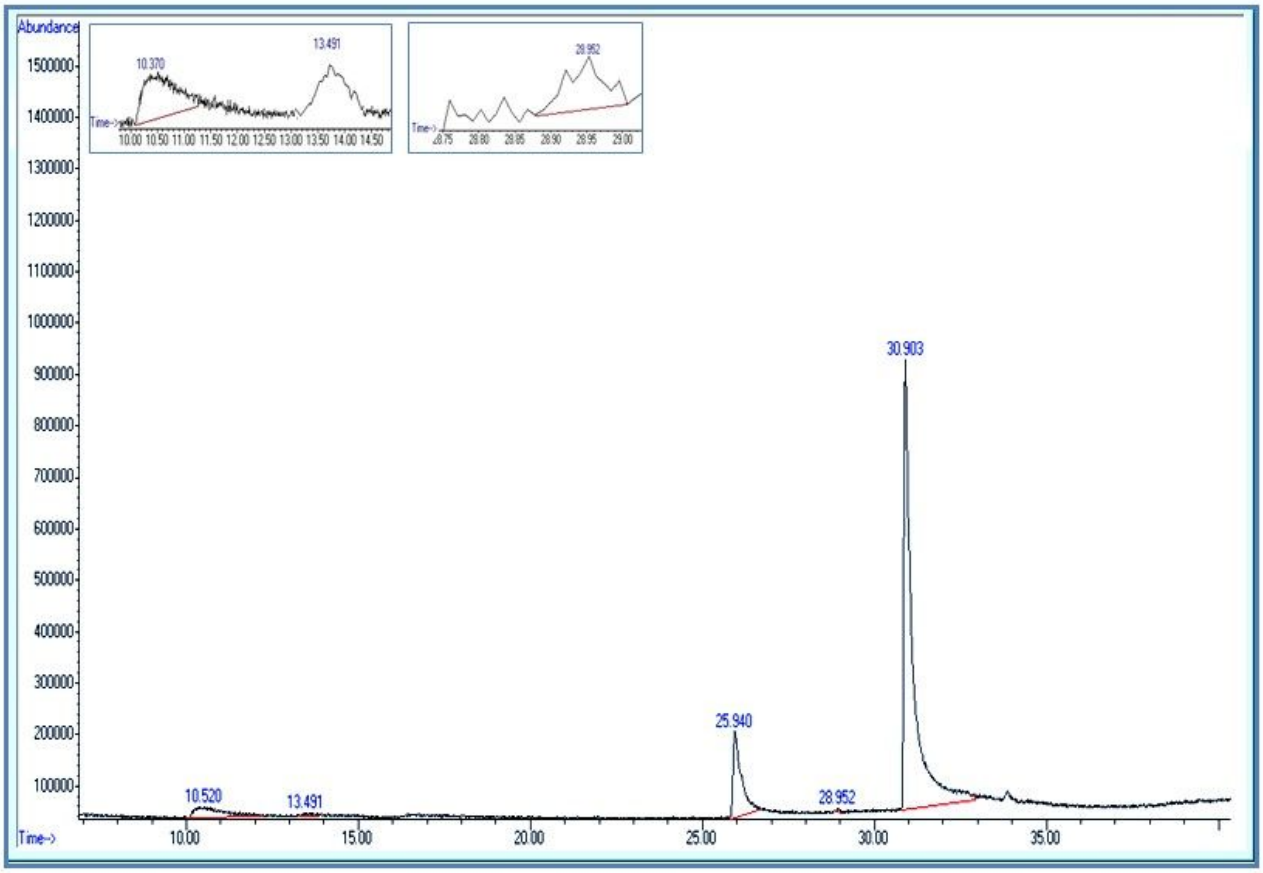

Figure 4: Chromatogram of PPD in Bigen cream hair dye. Retention time of bigen cream using GC/MS

Figure 4

See image above for figure legend

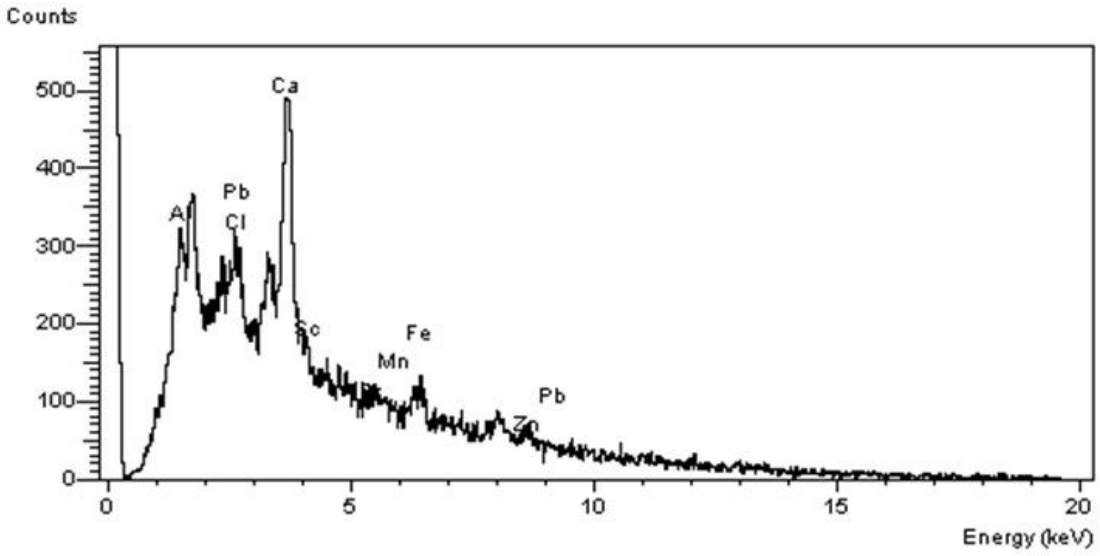

Figure 5: EDX spectrum in SHD.

Figure 5

See image above for figure legend

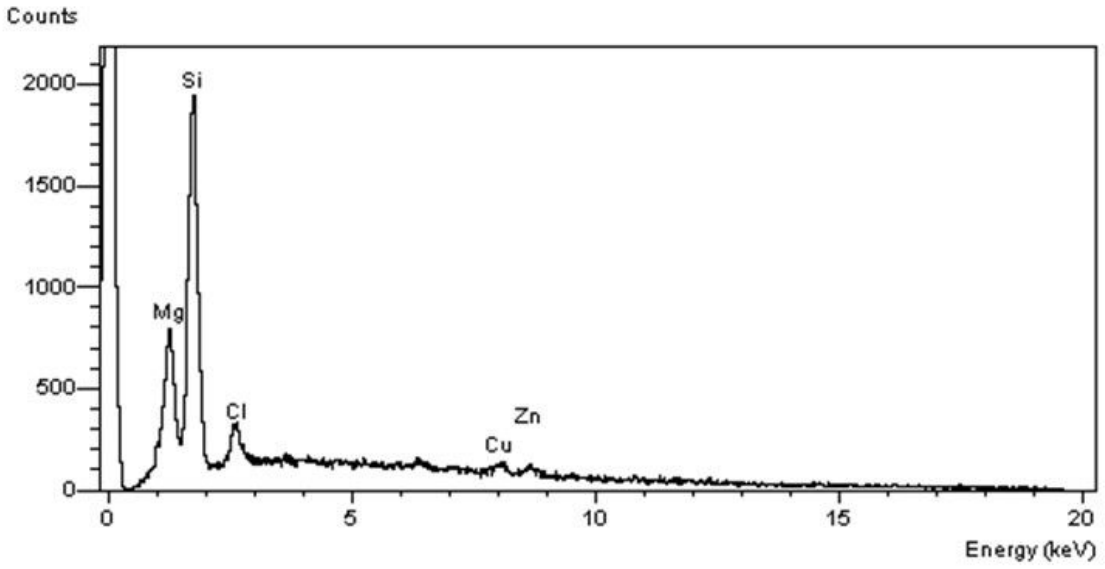

Figure 6: EDX spectrum in Tancho hair dye.

Figure 6

See image above for figure legend

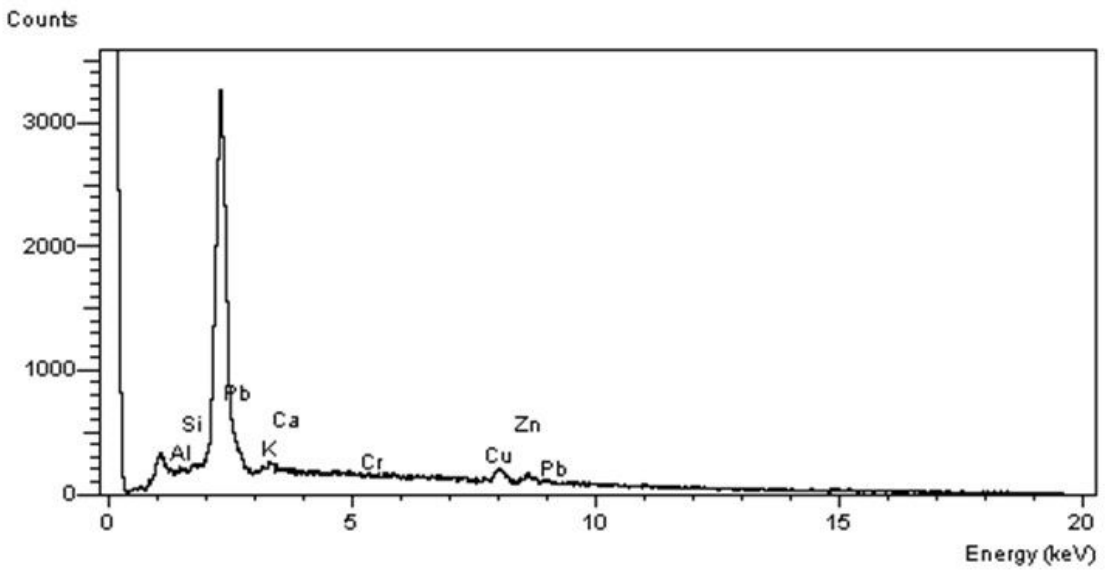

Figure 7: EDX spectrum in Bigen powder hair dye.

Figure 7

See image above for figure legend

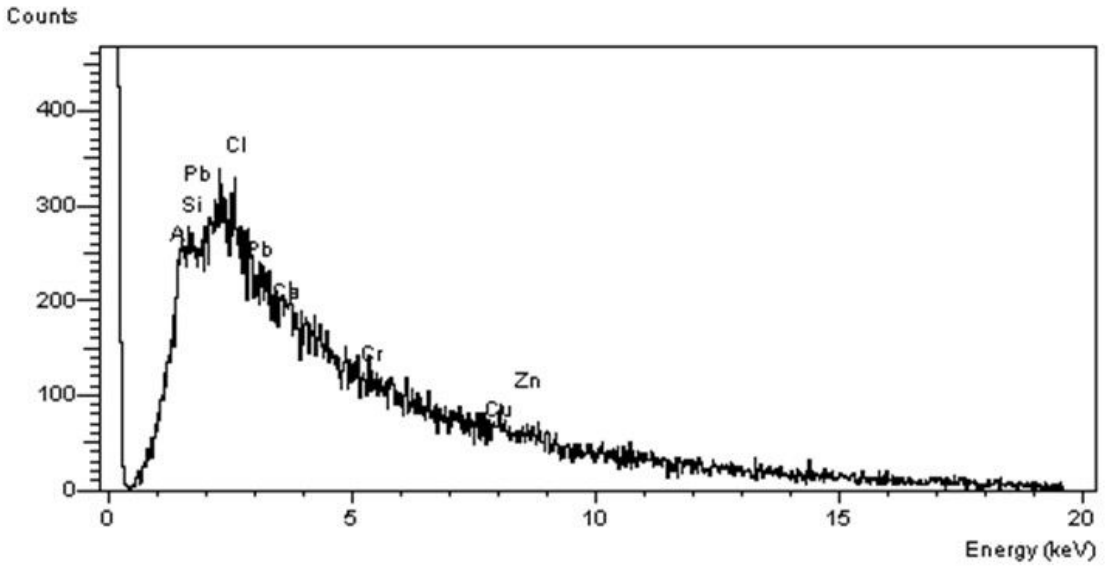

Figure 8: EDX spectrum in Bigen cream hair dye.

Figure 8

See image above for figure legend