

Toxicity of Black Stone Hair Dye: Forensic Analyses of P-Phenylenediamine (P-PPD)

Ahmed MA Shihata*

Forensic Medicine Authority – Assuit Chemical Lab-Ministry of Justice, Egypt

***Corresponding Author:** Ahmed MA Shihata, Forensic Medicine Authority – Assuit Chemical Lab-Ministry of Justice, Egypt, E-mail: ahmed_shihata_7@yahoo.com

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Abstract

Black stone hair dye is widely used in middle east countries and in Egypt specialized in South of Upper Egypt. Many cases of toxicity and mortality either due to accidental and deliberate ingestion of hair dyes were reported in Egypt and other countries as suicide and homicide. Black stone hair dye contains toxic metals, but main toxic compound in black stone hair dye is P- phenylenediamine (PPD). Many methods of PPD extraction to biological samples were used but efficiency method with 10% hydrochloric acid hydrolyses then extract PPD in alkaline medium 8-9.

Keywords: Black stone; PPD; Hydrolyses; Derivatisation

Objective

Studying extraction method and identify suitable detection and efficiency method analyse are scope of this research.

Introduction

Hair dye compounds are commonly classified according to color fastness, so it's can be divided into categories based on its chemical composition and mechanism of action; permanent dyes, temporary or semi-permanent dyes [1].

70% of hair dye formulations used at home and in hair salons are based on permanent dyes, which offer high diversity of coloration, long permanence effect, ease of application and versatility [2,3].

Black stone hair dye (fig. 1) is a traditional hair dye popularly used in North Africa and Middle East and in most of third world deep black coloration substance and cheap. Black stone hair dye imparts black color to hair dye, to make hair bright blacker. It is also used in tattoos and henna to produce black henna [4]. It's used because it is cheap, has high temperature stability.

Main toxic compound in black stone hair dye is P-Phenylenediamine (PPD) aromatic amine or 1, 4-phenylenediamine is primarily used as a dye intermediate and as a dye.



Figure 1: Chemical structure of is P-Phenylenediamine (PPD)

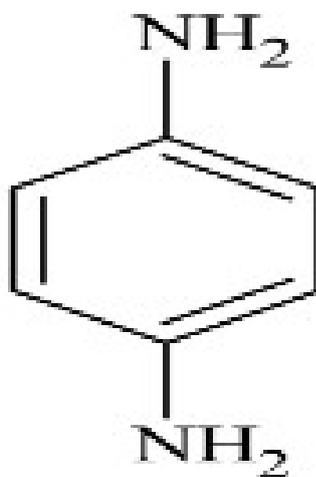


Figure 2: Black Stone Hair Dye

Paraphenylene diamine is a very toxic compound. It is dose dependent with estimated lethal dose of 0.5mg - 0.8mg/kg or 17-21gm orally, or if remains in contact with the skin for at least 08 - 12 hours [5]. Causes severe dermatitis, gastritis, renal failure, convulsions, coma and death [6, 7]. PPD and some nitro aromatic compounds the main toxic ingredient of black stone hair dye.

Asphyxia of PPD (autopsy photos):

Many cases are death suicidal and homicidal, through cases autopsy edema round and above trachea, vocal cords and larynx ex-

ternal and internal these photos of these organs explain edema as albumin white egg. [fig: 3, 4, 5, 6, 7].



Figure 3: Neck swelling



Figure 4: Edema round above Trachea and Larynx from external



Figure 5: Edema internal vocal cords and Larynx



Figure 6: Edema Trachea

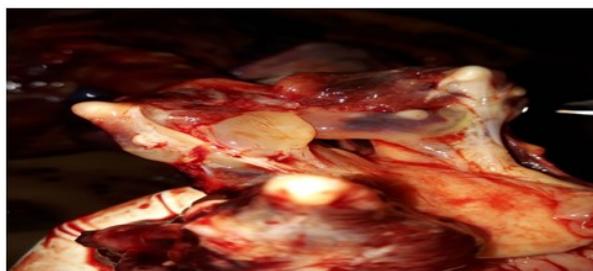


Figure 7: Edema round above Trachea from external

Hydrolyses

Hydrolyses a chemical reaction that uses water to break down a compound, a chemical bond is broken into two parts a hydrogen ion, and a hydroxide. Many types of compounds, including esters, amides, acid anhydrides, ethers, alkyl halides, nitriles, phosphates, carbohydrates, acetals, epoxides, and lactones. There are three types of hydrolysis reactions: salt, acid, and base reactions.

Derivatization

Derivatization is generally chemical modification of low volatility or have poor thermal stability compounds to make suitable for gas chromatography analysis [8, 9]. Four main methods for derivatization

1. Silylation increases volatility and thermal stability of the compound, in this method replacing active hydrogens (-OH, -NH, -COOH, -SH groups) are displaced by a silyl group, most often tetramethylsilane (TMS). Common Silylation reagents are N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA).
2. Acylation compounds with active hydrogens (OH, SH, NH) are reacted with carboxylic acid or derivatives of carboxylic acids to form esters, thioesters, and amides, respectively. Common Acylation reagents are trifluoroacetic anhydride (TFAA) and acetic anhydride.
3. Alkylation derivatization involves the replacement of active hydrogens with an aliphatic or aliphatic-aromatic such as benzyl group (esterification) to form esters from carboxylic acids. Common Alkylation reagent 2-propanol with 2M-HCl in esterification [10].
4. Chiral derivatization is a specific technique used to separation enantiomers by creating diastereomers (chiral compounds). It's very dangerous transdermal and some heavy toxic metals were determined in black stone hair dye, the concentration of Lead, Aluminum, chromium in lack stone hair dye.

Methods and Materials

Materials

P- phenylenediamine (PPD) free from Faculty of science Al-Azhar Univ. Assuit, Methanol HPLC grade and Ammonium hydroxide, and acetonitrile, HCl, chloroform from Algomhoria company Cairo - Egypt. N, O- Bis (trimethylsilyl) trifluoroacetamide (BSTFA) purchased from Sigma Aldrich, BSTFA.

Sample Preparations

Liver and kidney tissues samples were received from forensic medicine authority Assuit chemical lab department and these samples were taken from two cases of suicidal fourth and fifth decades. Method of extraction was including digestion 5 gm of liver and also kidney from each case by 10 N HCl and valued in water bath, over night residue of tissues was filtered. Extract was converted to alkaline medium [8-9] by ammonium hydroxide and using chloroform solvent. PPD was tested by TLC on silica gel plates and chloroform: methanol (4:1 v/v) and injection in GCMS after derivatisation.

Chromatographic Conditions

Instrumentation: GC-MS, (Agilent 6890) mass spectrometry detector (Agilent 5973), column Column of GC-MS is Agilent HP-5-MS; 0.25

mm× 60 m × 0.25 um capillary, 60 m × 250 um nominal.

Gas type is helium and pressure 39.8 psi, flow 1.5 ml/min and velocity 32 cm/sec. Oven temperature: initial temperature 100 °C and hold time 2 min, rat 25 °C/ min to 280 °C, hold time 20 min and run time 25 min. Vortex (FALC) vortex for 60 second then incubate at 70 Celsius for 20 min. Inject 1uL of each specimen into GC-MS.

Results and Discussions

The chosen method of extraction and suitable solvent is principle work of qualitative and quantitative analyses, chosen solvent should ideally extract as much of the target analyte as possible.. Many methods of protein precipitation extraction such as ethyl alc (stass otto), ammonium sulfate and hydrolyses by many mineral acids.

Suitable extraction of PPD from biological samples is 10% hydrochloric acid hydrolyses then extract PPD in alkaline medium (8-9) by ammonium hydroxide and using chloroform solvent. Qualitative analyses by TLC 4:1 (chloroform: methanol) and ferric chloride spray was used figs. [7-8].

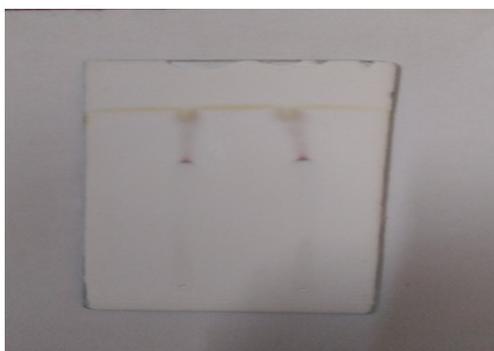


Figure 8: TLC of Black Stone Hair Dye

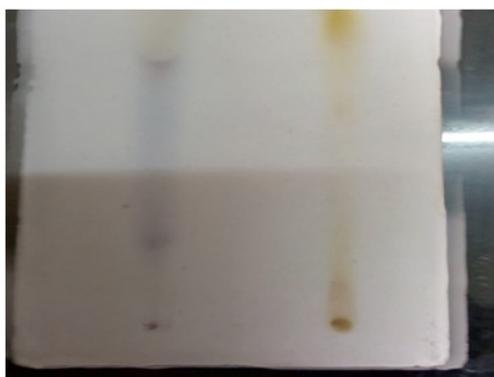


Figure 9: TLC of Black Stone Hair Dye with FeCl₃

Validation Study

Calibration Work

MassHunter creates a calibration curve performed by drawing the calibration curve at different concentrations of PPD stock solution was freshly prepared because degradation of PPD (oxidative). PPD quantifications were performed using the peak area. The peak area for each standard solution and sample was calculated.

Limit Of Detection (LOD): several calibration were injected in different concentrations (500, 200, 100, 50, 10 and 5 ng) a peak at 10 ng concentration fulfilled the criteria of acceptance for LOD of liquid- liquid extraction concentration as peak noise ratio

$\geq 3:1$ (determined by peak height).

Selectivity: 4 blank samples were prepared and injected and no significant signals were detected in the same retention time of analyte.

Sensitivity: many calibrations were injected in different concentration (500, 200, 100, 50, 10 and 5 ng) the response was found to be directly proportional to concentration.

Limitation: samples with concentration less than 5n/L extraction did not show signal with accepting the method.

Mortality has been reported old female and male 40 and 50 years in Upper Egypt at different months, families mentioned that were orally unknown materials and dead at reached to emergency of hospital. GCMS is not suitable but at using chemical derivatization with BSTAFA, P-Phenylenediamine 2TMS ions 252, 73, 111 and 45 figs. (10, 11). while ion fragmentation of 3TMS 309, 218, 73, 147, 105, 174 and 45 fig. (12). PPD at concentration more 5 ng/ml very good.

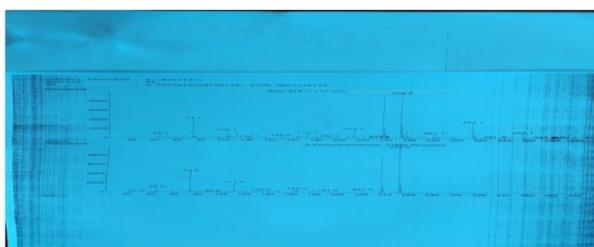


Figure 10: Ion fragmentation of PPD 2TMS

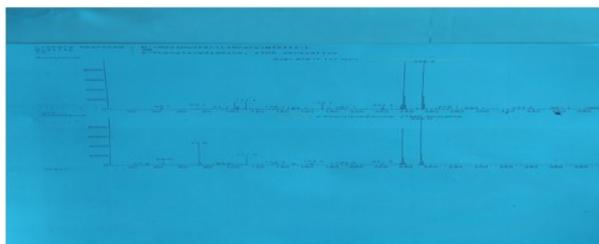


Figure 11: Ion fragmentation of PPD 2TMS

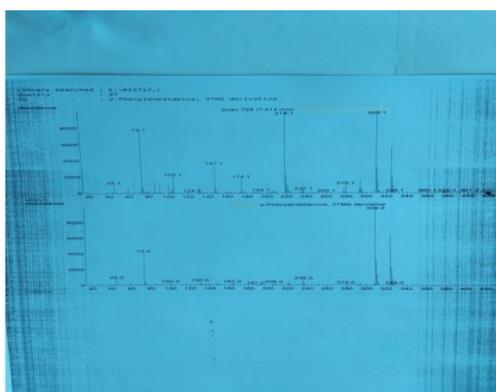


Figure 12: Ion fragmentation of PPD 3TMS

Conclusion

The proposed HCl hydrolyses extraction and TLC and GC-MS (derivatization) method a simple, accurate and reproducible method for routine analysis to biological analyses.

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