HPLC-UV and FTIR Analytical Techniques to Determination of Para-phenylenediamine in Some Black hair dyes

A. A. Saleh Salah[(1)]*
S. M Qasem Mofleh[(2)]

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[1] Department of Biology, Faculty of Toor Al-Baha, Lahej University
[2] Department of Chemistry, Faculty of Education, Aden University.
* Corresponding Author's Email: ahmedawadsalah.1974@gmail.com

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A. A. Saleh Salah
Department of Biology, Faculty of Toor Al-Baha, Lahej University Yemen
ahmedawadsalah.1974@gmail.com

S. M Qasem Mofleh
Department of Chemistry, Faculty of Education, Aden University, Yemen

Abstract— Para-phenylenediamine (PPD) is a chemical compound commonly, used in the dyeing industry, particularly in hair coloring products. PPD poses potential health risks to humans, cause respiratory problems, asthma, allergic reactions, leading to skin irritation, rashes and potential carcinogenic effects during dealing, using or exposure. The aim of the present study uses a rapid, develop. suitable sensitive analytical method for stability studies to investigates PPD by using two analytical methods: High-Performance Liquid Chromatography (HPLC) with UV detection and Fourier Transform Infrared (FTIR) spectrum to confirm the identification of its component PPD in some dying hair samples that use in Yemen. FTIR analysis was employed to identify characteristic functional groups associated with PPD in the dye matrix, providing qualitative information about its presence. The spectral data illustrated that N-H aromatic stretching various peaks in Black Stone Dye, Brand-1, Brand-2 and Brand-3 samples to different PPD concentrations or the presence of impurities, and additional materials in samples collected. HPLC utilized for qualitative and quantitative analysis of PPD in Black Henna samples, where the result HPLC analysis an appropriate method to achieve optimal separation of PPD from other components in the dye samples. Calibration curves were established using known concentrations of PPD, enabling accurate quantification of PPD in Black Henna samples at concentrations 1.9% for Brand-2, Brand-1 (6.98%), Brand-3 (14.33%) and Black Stone (70.35%) that make the three samples higher than permissible levels in black henna samples. The results demonstrate that the combined use of FTIR and HPLC offers a robust methodology for the detection and quantification of PPD in black henna samples that integrated approach can facilitate better monitoring of PPD levels, contributing to improved consumer safety and environmental protection.

Keywords — : Para-Phenylenediamine, hair dye, Blackstone dye, High-Performance Liquid Chromatography (HPLC), and Fourier Transform Infrared (FTIR).

I. Introduction

Natural henna, derived from the leaves of the Lawsonia inermis family Lythraceae plant, has been used for centuries for body art and hair coloring that cultivated in some countries in Asia (Yemen, Pakistan, India) and Africa Egypt, Sudan, Morocco) etc. The dye from putty of the powder henna leave's with water or oil are produces a rich reddish-brown color, which binds to the hair and skin, creating a natural coloration. In addition to its coloring properties, henna is known for its conditioning effects, helping to strengthen hair and promote scalp health, that is highly regarded for making it a popular choice for adornment women's bodies hair during celebrations of the wedding and social events particularly in Indian subcontinent and in the Arab world [1-3]. The composition the active dye molecule the active in leaves of henna which given the red color is 2-hydroxy-1,4-pnaphthoquinone (Lawsone) Fig.1a. Para-Phenylenediamine (PPD); its chemical formula is ($C_6H_8N_2$) (Fig.1b). This aromatic amine produced by many manufacturing companies, due to is not found in nature. It's a derivative of paranitroanaline a white and water-soluble solid, then due to air oxidation change darken color [4-9].

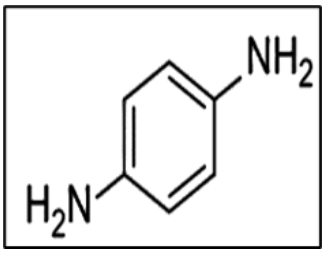

Fig. 1a. Structure of paraphenylenediamine

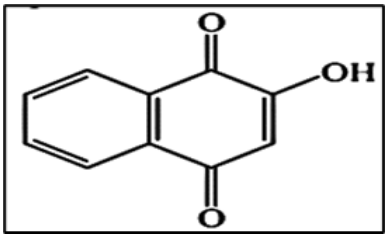

Fig. 1b. Structure of Lawsone

PPD is applied as a precursor to aramid polymers and fibers, and as a dye in fur and other textiles, printing, lithograph and photocopying, also is one of the major additive colore cosmetics compounds found in hair dye, temporary tattoos and other dyes [10-12]. PPD has been recognized as a potent contact allergen [13]. The exposure to PPD may be due to inhalation, ingestion skin and eye contact or through fabricating, dealing and using [1, 8]. Recently the research has found that worker in beauty salons who use and use hair dyes, workers in dyes factories and textile industries are more exposure to develop cancerous diseases such as leukemia, lymphoma, bladder cancer, hematopoietic cancer's [15-17]. This exposure to PPD whether short or long-term various health issues including, allergic reactions: in some individuals, leading to symptoms such as skin irritation, itching, and swelling dermatitis, may cause respiratory problems, asthma, gastritis, renal and potential carcinogenic effects. Due to its potential health risks, many countries regulate the use of PPD in cosmetic products. The permissible levels of PPD vary by region, in European Union (EU) and Scientific Committee on Consumer Products (SCCP) are low, the use of PPD in cream hair dyes is expected to increase and consequently increase the risk of skin sensitization, whereas in the United States has not approved PPD for use in hair dyes, and products containing it are required to carry warning labels [2, 8, 14]. The analytical techniques of the Capillary Electrophoresis (CE), Ultraviolet-visible spectrometry (UV-vis) Inductively Coupled Plasma Mass Spectrometry (ICP/MS), Thin Layer Chromatography (TLC), Gas Chromatography Coupled Mass Spectrometry (GC/MS), and High-Performance Liquid Chromatography (HPLC) are used to determined PPD [18, 19, 20]. The HPLC with UV detection is considered one of the best modern analysis techniques for characterization (separating, identification and determining) of PPD in hair dye samples, which by retention time and its spectrum, make sure it easy for the presence of pigment (PPD) in hair samples and determine its percentage [2, 21]. For all reasons and techniques that have been mentioned, the study aims to use of FTIR and HPLC for determination of the pigment (PPD) in commercial black henna hair.

II. MATERIALS AND METHODS

A. Samples

The samples more available and commonly used Black henna were collected four samples (may be containing PPD) from shops selling black henna from different areas randomly in Aden Governorate, then instead of the brand name, they were given the following names: Brand-1, Brand-2, Brand-3 and Black Stone Dye.

B. Materials

Para-Phenylenediamine 98 % used to as control sample was purchased (AZchem, Germany. The solvent used for HPLC: Acetic acid 99.8 % Ammonia, (Fisher, USA) and Methanol 99.9 % (Carlo erba, France), and all chemicals analytical used throughout this study were analytical grade and highly pure. The HPLC with UV/Vis Detector (from 190 to 1000 nm, The Series 200 Autosampler, Series 2000 Analytical Pump, Series 200 Column Oven.

C. Methods

1. Fourier Transform Infrared spectroscopy (FTIR):

To prepare the sample for Fourier Transform Infrared (FTIR) Pekinelmer, USA analysis of black henna dyes containing PPD, a small amount of the dry dye is first weighed and then placed directly onto the FTIR. Next, the FTIR instrument is turned on and set to the appropriate spectral range (typically from 4000 to 400 cm^{-1}). The crystal containing the sample is positioned correctly within the device, and the scanning process is initiated, where the instrument sends infrared light through the sample and measures absorption. The data is recorded and converted into a spectrum that shows the various peaks representing the functional groups in the sample, allowing for analysis based on their positions and intensities. The analysis in this study is focused on identifying of the functional groups, particularly those present in sample dyes hair. The samples are prepared and analyzed of FTIR in the quality control laboratories of the **High Board for Pharmaceuticals and Medicines and Medical Appliances (Aden).**

2. High Performance Liquid Chromatography (HPLC):

a) Standard Preparation: Para-Phenylenediamine 98 % used to as control sample was purchased (AZchem, Germany. The solvent used for HPLC: Acetic acid 99.8 % Ammonia, (Fisher, USA) and Methanol 99.9 % (Carlo erba, France), and all chemicals analytical used throughout this study were analytical grade and highly pure. The HPLC with UV/Vis Detector (from 190 to 1000 nm, The Series 200 Autosampler, Series 2000 Analytical Pump, Series 200 Column Oven.

b) Sample Preparation: The four samples study were used to isolation, identification and determination of PPD prepared by weigh1g in 50ml volumetric flask and dissolved with methanol 50% solution and sonication then filtered, these solutions taken from it 1ml in volumetric flask and diluted with methanol 50%. Before analyzing any sample was diluted 1 mL of the standard PPD with metha-

nol 50% solution to 5mlto determine its spectrum and its Retention Time (R_T).

c) HPLC operating conditions: Chromatography separation was achieved using reverse phase chromatography with isocratic elution at a flow rate of 1.5 ml/min and a run time 2.1 min /cycle. Chromatography analysis was performed on the Altech Prevail (C_{18}) column, 250 × 4.6 cm, 5µm particle size, the column temperature were 30^0, pressure 174 bar and a manual injector with a 20 µl loop and UV-Detector at a wavelength of 290 nm was used for the injection of the sample solution and mobile phase [1].

d) Statistical processing of the results: The analysis was done by using Origin 8 and excel statistical programs.

III. RESULTS AND DISCUSSION

A. Fourier Transform Infrared spectroscopy (FTIR):

Fourier Transform-Infrared (FTIR) spectroscopy is based on the principle that molecules absorb infrared light, the molecules within the sample absorb this light at characteristic frequencies, causing the bonds of functional groups molecules to vibrate and stretch, this absorption produces a characteristic spectrum that can be used to identify the functional groups in a sample [9, 22]. The results of the FTIR analysis of black henna dyes containing PPD provide a deep understanding of the chemical composition of these dyes. The peaks that observed in the spectrum reflect to presence of specific functional groups in dye samples, which resemblance with of the standard PPD. FTIR spectra of control and samples analyzed are shown in Fig. 2-6, respectively. The comparative values of IR peaks of the control with samples analyzed are given in Fig. 7 presents the FTIR spectra of PPD, compared with samples of study.

The amino group (–NH) is a key factor in determining the dye's properties, as evidenced by the peaks, which is more pronounced in the dye samples compared to the PPD standard sample. This group may be increase in hydrogen bonding, which enhances chemical interactions with other components, such as water or solvents [22, 23]. So, we will discussion these results according to appeared of the big or weak spectra peaks which indicated to the functional groups in of FTIR diagrams:

1. N-H vibrations: The N-H stretching primary amine 3300 and 3400 cm^{-1} [24] were observed at 3390-3306 cm^{-1} in control and in Black Stone dye at 3373-3317 cm^{-1}**,** while in brand-1 sample appeared a strong spectra with weak peaks at 3370 and 3290 cm^{-1} and in brand-3 was observed one peak at 3372 cm^{-1} [25] **,** also observed the peak at **367**6 cm^{-1} may be belonging to the asymmetric stretching vibrations of N–H bond of NH_2 group in brand-3, this result agreeing with report [26]**.** All these peaks absent in brand-2 but appearing a big board peak from 3400 to 3200 cm^{-1} in brand-2 and brand-1 samples, may be due to associated the N–H stretching vibration with the hydroxyl group (O–H) stretching of water. Both O-H and N-H stretches appear in spectra around 3350 cm^{-1}, therefor may not be able to distinguish between them, however, O-H stretching peaks are significantly broader than N-H stretching peaks [27, 28, 29].

2. C–N stretching: The C–N stretching was observed at 1321-1202 cm^{-1} in control and in Black Stone dye at 1320-1250cm^{-1} and in analyzed brand-1, brand-2 and brand-3 at 1255, 1260 and 1259 cm^{-1} respectively.

3. C-N-H bending: The C-N-H bending significant difference was observed, the peaks corresponding to appear at 1621, 1633, 1609, 1627 and 16309 cm^{-1} for the control sample, Black Stone dye, brand-1, brand-2 and brand-3 respectively.

4. Carbon-carbon stretching (aromatic): The C-C stretching vibrations were observed at 1517, 1515, 1516 cm^{-1}, 1518, and 1514 cm^{-1} in the control, black stone dye, brand-1, brand-2 and brans-3.

5. Carbon-hydrogen stretching (aromatic): The peak at 3007 cm^{-1} corresponds to C-H (aromatic) stretching, the peak were observed at 3010, 3009 and 3012 cm^{-1} for control sample, black stone dye and bran-3 respectively, while absent in bran-1 and bran-2 [30].

6. Ring vibration: The peak at 824 cm^{-1} is due to substituted benzene in control and brand-1, at 822 cm^{-1} in the black stone dye, whereas weak peaks at 830 cm^{-1} in brand-2 and brand-3 [31].

7. C-H in-plane bending: The peaks were observed at 1127cm^{-1} in control and black stone dye and at 1026 cm^{-1} in brand-1, whereas at 1014 and 1016 cm^{-1} in the brand-2 and brand-3 respectively [22]**.**

8. C-C deformation: The peaks C-C deformation due to were appeared at 514 cm^{-1}, 508, 513, 455 and 463 cm^{-1} in control, black stone dye, brand-1, brand-2 and brand-3 respectively [30] . The peaks at 667, 698, 604, 664 and 585 cm^{-1} in control sample, black stone dye, in the brand-1, brand-2 and brand-3 respectively, this may be attributed to the out-of-plane bending vibrations of benzene nuclei in the phenazine skeletons.

9. The aliphatic C-H stretching: The aliphatic C-H stretching was observed at 2919-2840 and 2920-2845 cm^{-1} in Brand-1 and Brand-2 samples respectively [31]. The different in the peaks like presence of strong board spectra in at 3350 cm^{-1} and

appear the aliphatic C-H stretching at 2919-2840 and 2920-2845 cm^{-1} in Brand-1 and Brand-2 where disappear in control and black stone dye may be due to different of the concentrations in PPD, and to presence of impurities or add other materials to henna dyes can significantly affect the properties of the dyes, our observations in this study are corresponds these results with previous studies [22, 23, 32].

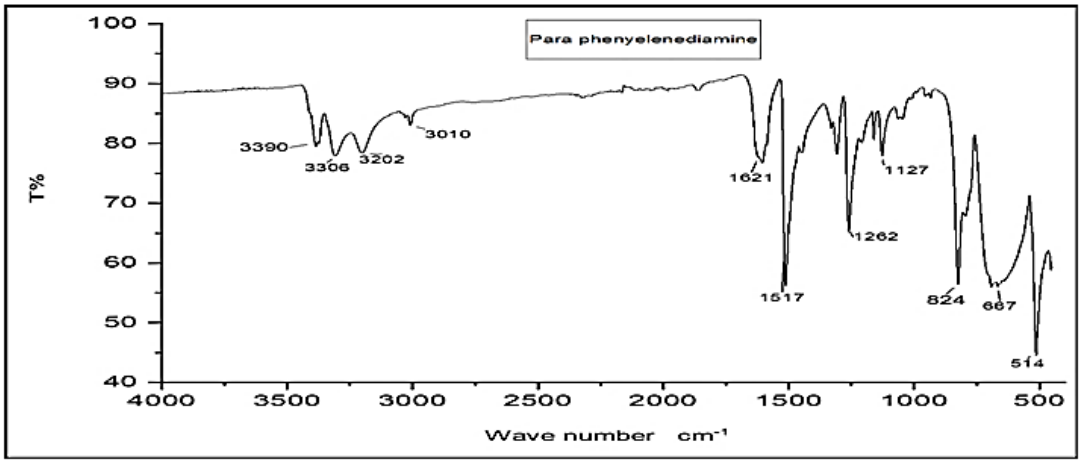

Fig. 2. FTIR spectra of control of p-phenylenediamine

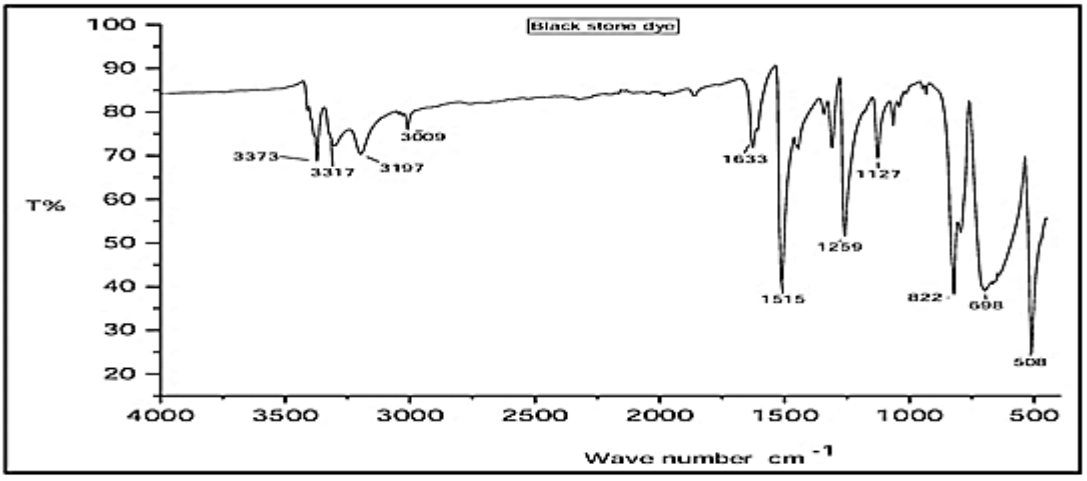

Fig. 3. FTIR spectra of Black stone dye

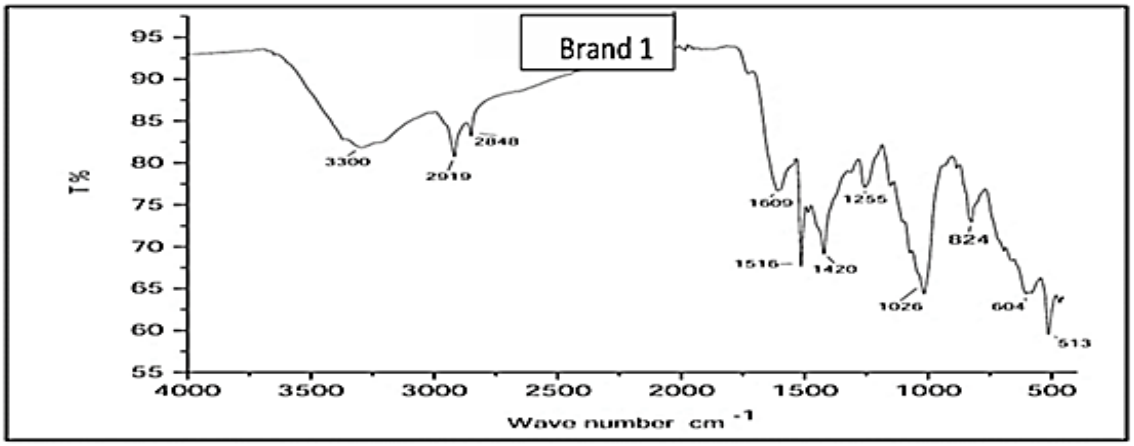

Fig. 4. FTIR spectra of (brand-1) Black Henna dye.

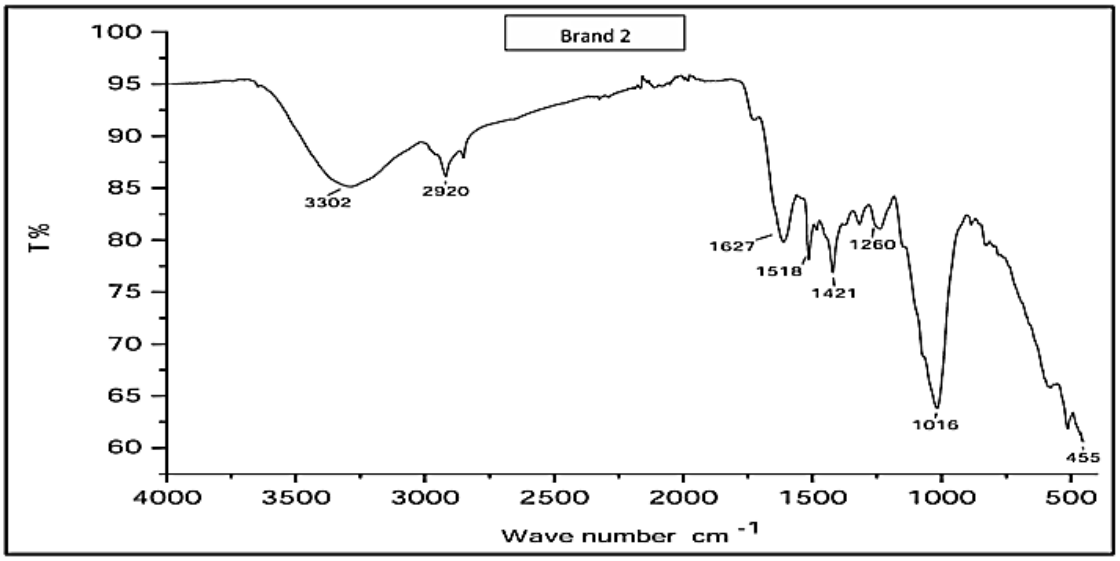

Fig. 5. FTIR spectra of (brand-2) Black Henna dye

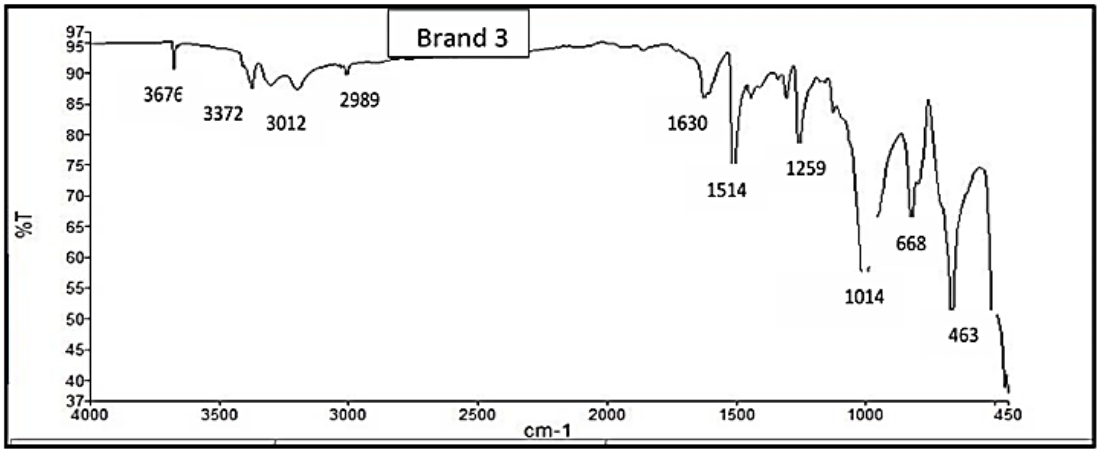

Fig. 6. FTIR spectra (brand-3) Black Henna dye

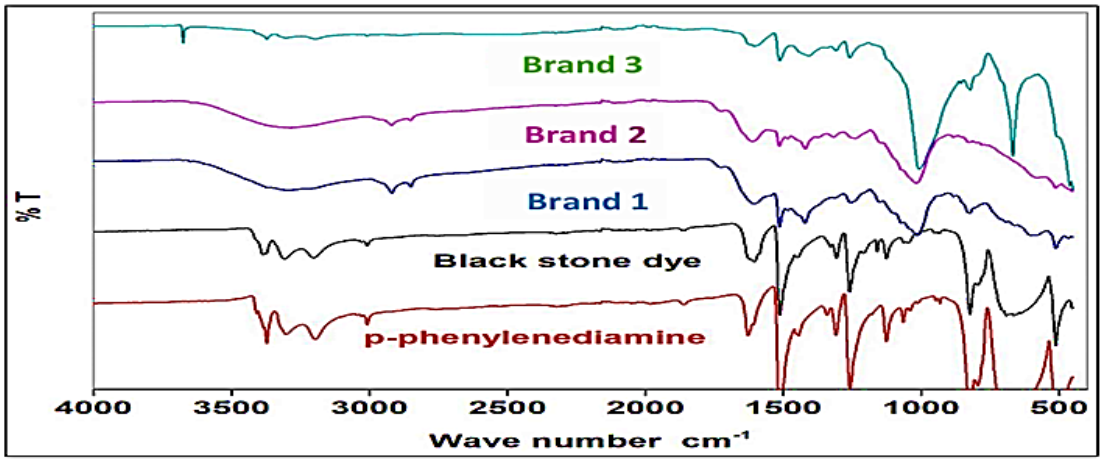

Fig. 7. Chromatograms of different concentrations of PPD by FTIR spectra

B. High Performance Liquid Chromatography (HPLC):

The HPLC Chromatogram of the standard PPD solution was prepared by different concentration: 5, 10, 15, 20, and 25 µg ml^{-1}, these peaks shown in Fig. 8 – 12, and explicates difference peaks area due to variation in concentration that has same of the retention time. All HPLC Chromatogram for the five different standard concentrations together in Fig.13.

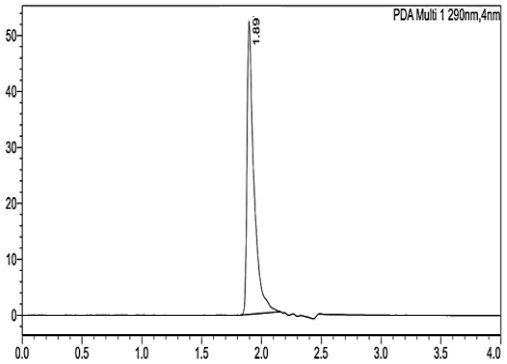

Fig. 8. HPLC Chromatogram of the for standard PPD 5 µg.mL^{-1}

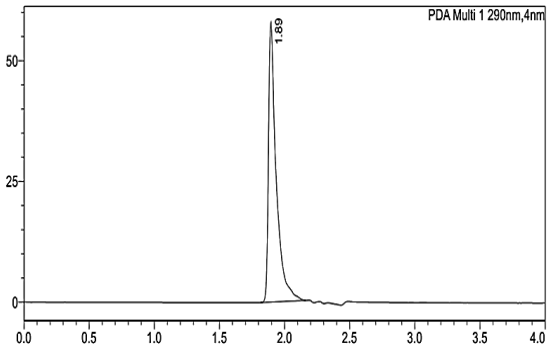

Fig. 9. HPLC Chromatogram of the for standard PPD 10 µg.mL^{-1}

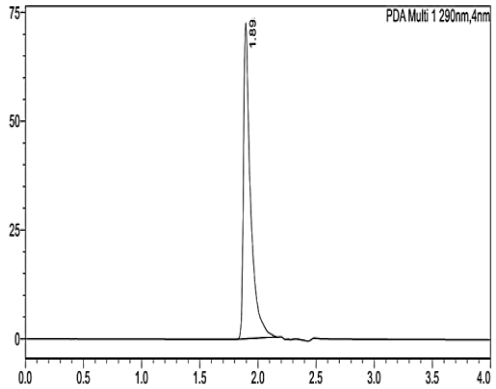

Fig. 10. HPLC Chromatogram of the for standard PPD 15 µg.mL^{-1}.

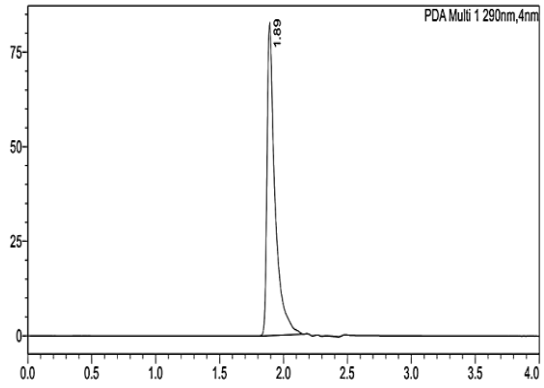

Fig. 11. HPLC Chromatogram of the for standard PPD 20 µg.mL^{-1}.

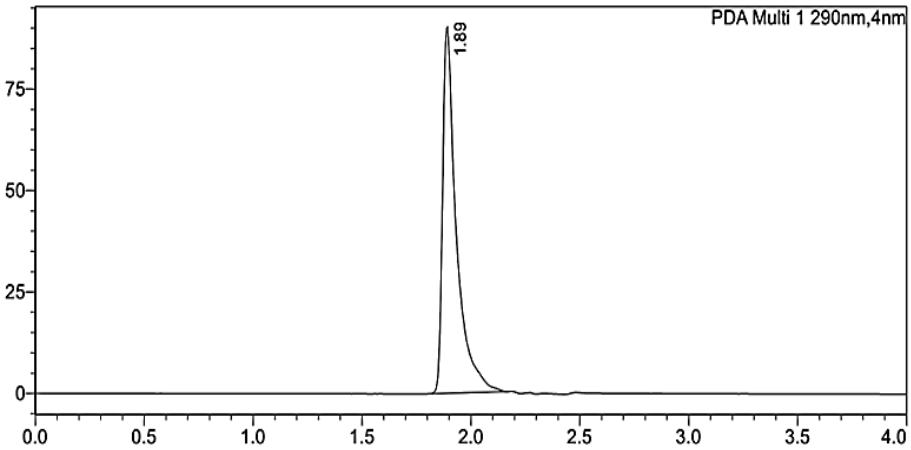

Fig. 12. HPLC Chromatogram of the for standard PPD 25 µg.mL^{-1}.

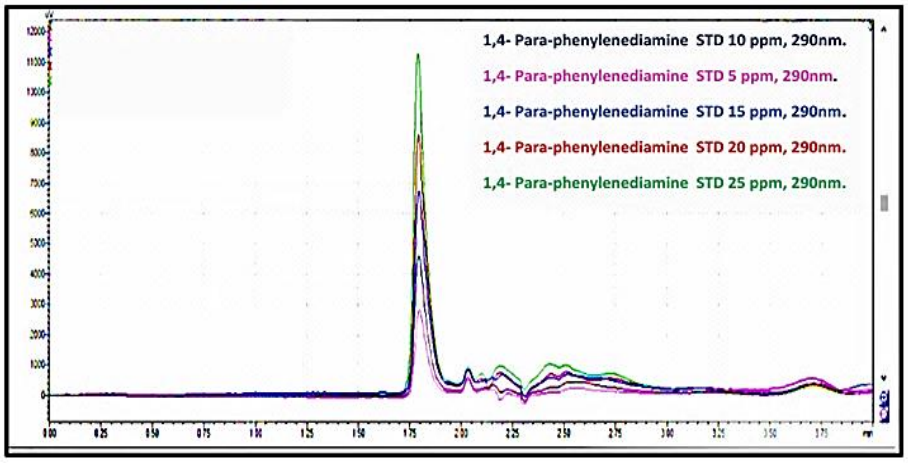

Fig. 13. HPLC of the chromatograms for different concentration of standard PPD

Table. 1. that illustrated the retention time, peak area and the concentrations of the five standards PPD

Name	No	R_T (min)	Peak Area (µV.S)	Conc. (µg ml^{-1})
Standard PPD	1	1.89 min	211714 µV.S	5 µg ml^{-1}
Standard PPD	2	1.89 min	257946 µV.S	10 µg ml^{-1}
Standard PPD	3	1.89 min	307970 µV.S	15 µg ml^{-1}
Standard PPD	4	1.89 min	359130 µV.S	20 µg ml^{-1}
Standard PPD	5	1.89 min	405696 µV.S	25 µg ml^{-1}

The table. 1 was illustrated the retention time, peak area and the concentrations of the five standards PPD.The result analysis showed in Fig.14 the calibration curve of PPD standard a good linear relationship between the concentrations of the standard solutions of PPD in axes (x) and the peak area in axes (y) which the chromatographic measurements were to find the concentration of PPD in the collected samples.

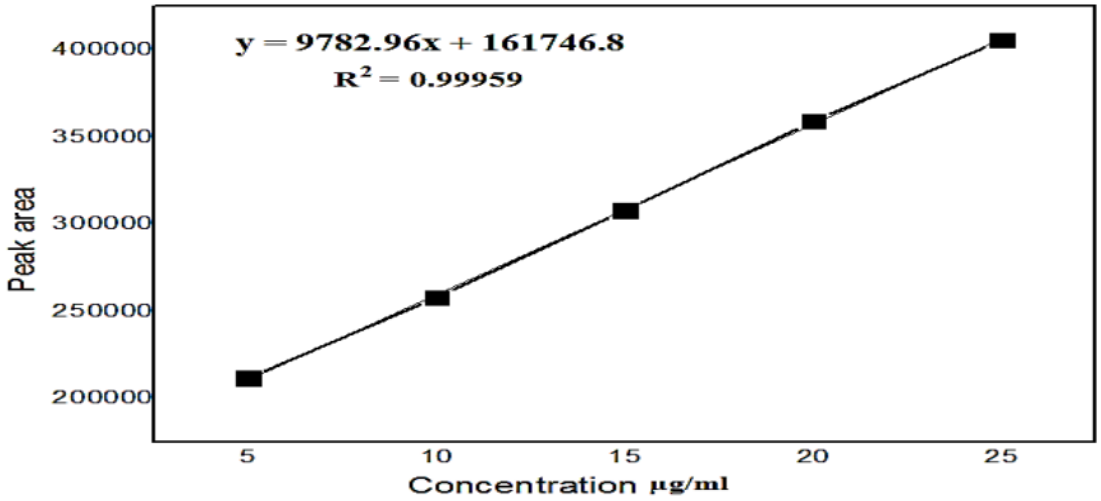

Fig. 14. Calibration curve for standard solutions of PPD, expressed on a linear scale

The result shown in Fig. 15, 16, 17, and 18 the chromatograms of the black Henna samples where all the Peaks appears clearly of R_T for of the Black stone dye, brand-1, brand-2, and brand-3 at 1.895, 1.891, 1.889 and 1.892 minutes, respectively under the same analytical condition. Moreover, there was a good match of the spectrum of each of our samples with the standard that is indicated that the peak and the retention time of the PPD in samples was the same as that of the PPD in standard which emphases of presence of PPD in all samples that's illustrated in Table.2. PPD concentrations of all samples were monitored from HPLC readings.

Table.2 illustrated the Retention Time (R_T) and peak area for of the Black stone dye, brand-1, brand-2, and brand-3

No	Name	R_T (min)	Peak Area (µV.S)
1	Blackstone dye	1.895 min	1538634 µV.S
2	brand-3	1.891 min	442216 µV.S
3	brand-1	1.889 min	298288 µV.S
4	brand-2	1.892 min	161185 µV.S

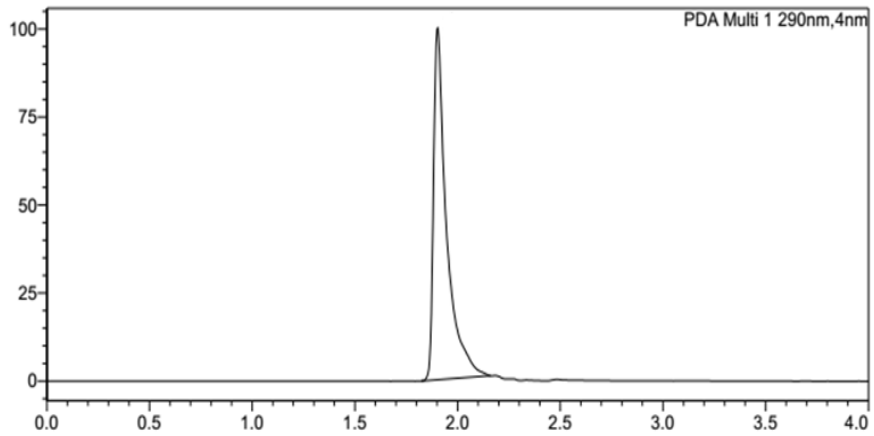

Fig. 16. chromatogram brand-3

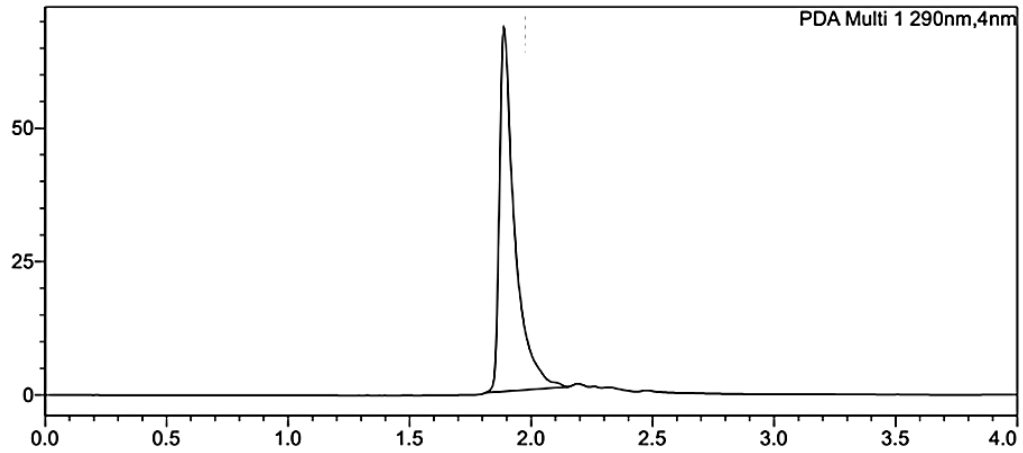

Fig. 17. chromatogram brand-1

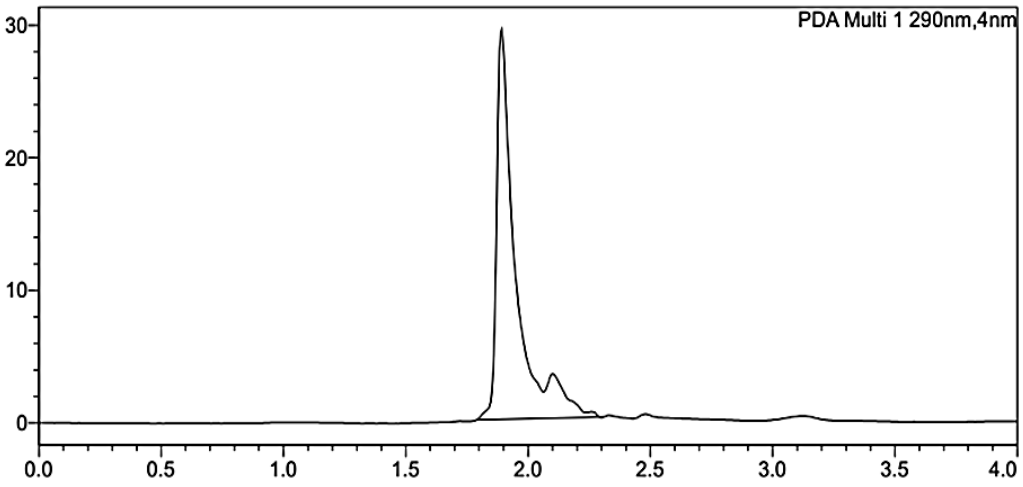

Fig. 18. chromatogram brand-2

As shown in Table3, the PPD was found in all the Henna samples (4 samples) with concentration 19, 69.79, 143.32, 703.52 µg ml^{-1} for Brand-2, Brand-1, Brand-3 and Black stone, respectively ranging between 19 to 703.52 µg ml^{-1}. The PPD was found in all the Henna samples (4 samples) with concentration (percentage) ranging between 1.9% to 70.35%. By the chromatographic spectrum and retention time. Thus, HPLC accurately quantify the PPD concentration**.** The permitted of the concentration PPD in hair dye products established by the European Union and specified by SCCP (Scientific Committee on Consumer Products) is from 2 to 6 % [1, 2, 16]. The analytical results of the concentration of PPD for brand-2 sample 1.9% which take placed within these permissible limits, and the concentration of PPD in brand-1 sample (6.98%) slightly exceeds the permissible limit. Moreover, the concentration of PPD for brand-3 sample reached (14.33%), exceeding the permissible limit, while the Black stone sample had a concentration of (70.35%), exceeding the permissible limit by a very high this percentage. The concentration of PPD in black henna samples in this study for brand-2 (1.9%) close from result that reported by Kang and Lee (2.35%) [33], also the result for brand-3 (14.33%) agree with result that reported by Brancaccio, et al in their studies (15.7%) [34, 35]. In our results of the containt of PPD in Black Stone (70.35%) which considers higher than that reported by Abouhadaf. Raed et al (37.04%) [8] and closer with previous results done from the higher concentration of PPD by Abdelhady et al (99.85%) [15]. Also, that closer from result of our study was in accordance with the result of Eissa et al, who reported that 95 .15 % of BSD was PPD [4], and this finding is consistent with other researchers who found the level of PPD in black henna dyes was much higher than that found in hair color [2, 14, 17].

Table. 3. The Concentration of para-Phenylenediamine in the four Henna Samples

No	Sample	Peak Area (µV.S)	Conc. Samples µg ml^{-1}	PPD%
1	Black Stone	1538634 µV.S	703.52 µg ml^{-1}	70.35
2	brand-3	442216 µV.S	143.32 µg ml^{-1}	14.33
3	brand-1	298288 µV.S	69.79 µg ml^{-1}	6.98
4	brand-2	161185 µV.S	19 µg ml^{-1}	1.9

In our results of the containt of PPD in Black Stone (70.35%) which considers higher than that reported by Abouhadaf. Raed et al (37.04%) [8] and closer with previous results done from the higher concentration of PPD by Abdelhady et al (99.85%) [15]. Also, that closer from result of our study was in accordance with the result of Eissa et al, who reported that 95 .15 % of BSD was PPD [4], and this finding is consistent with other researchers who found the level of PPD in black henna dyes was much higher than that found in hair color [2, 14, 17]. The result of HPLC indicate acorrelation with FTIR results. The analysis revealed that the black stone dye was high, aligning with the control results as well as with brand-1 and brand-2. In contrast, brand-3 exhibted alower concentration of PPD. Additional substances that may influence the spectral composition of the dye.

C. Validation of the used Method:

The validation of the analytical methods are use different factors that including: The limits of detection (LOD), quantification (LOQ), Linearity, RSD%, Precision, Accuracy, Linearity, Recovery, etc. All validation parameters for PPD, regression equations, correlation coefficients, the Relative Standard Deviation, quantification and limits of detection are given in Table.4 .The standard solutions of PPD were prepared at different concentration from 5 to 25 µg ml^{-1} which use the calibration curve to determination of the linearity of HPLC response from linear correlations between peak areas (axies y) and concentrations (axies x) were obtained a good linear correlations. The linearity of the The standards calibration curves was validated by the high value of correlation coefficients of the regression graph (R^2 =0.997). A Tailing Factor 1.59 is observed. The LOD and LOQ were determined to be 0.53 and 1.61 µg ml^{-1} for PPD respectively, which were the more sensitive than the previously reportes method in [1 and 36].

The precision and Accuracy by prapared series solutions afterthat, repeatable and expresse as the Relative Standard Deviation (RSD%), where the result of precision for solutions 5, 10, 15, 20, and 25 µg ml^{-1} 1.05, 1.39, 0.29, 0.98, and 0.79 % respectvily, indicating a good repeatability.

Table.4. are given the validation parameters for PPD, regression equations, correlation coefficients, the Relative Standard Deviation, quantification and limits of detection.

Parameter	PPD	Precision (RSD %) N=3	
R_T (min)	1.9 min	µg ml^{-1}	1.05
Tailing Factor	1.59	10 µg ml^{-1}	1.39
Coefficient of regression(R^2)	0.99959	15 µg ml^{-1}	0.29
Intercept	161746.8	20 µg ml^{-1}	0.98
Slope	9782.96	25 µg ml^{-1}	0.79
LOD	0.5298 µg ml^{-1}	LOQ	1.6054 µg ml^{-1}

IV. CONCLUSION

This study highlights of PPD in black henna dyes, providing important information about the properties of these materials and their effects, including the toxicity of this dye on skin and hair. Black henna containing PPD is a source of concern due to its health risks, as it can cause severe allergic reactions, leading to skin inflammation and scalp irritation. However, direct contact of PPD on the skin, eyelashes or eyebrows, is strictly prohibited in FDA has not permitted to use PPD directly on the skin. Using FTIR spectroscopy, that verify by the control to analyze the chemical structure and specific functional groups, particularly amino groups (–NH) which pres-

ence in all samples. The prominent peak observed in the range of 3300-3400 cm^{-1} indicates the presence of strong hydrogen bonding, enhancing the dye's stability and adhesion. By comparing our results with previous researches, we establish the credibility of using FTIR as analytical tool. Also, in this study using HPLC is simple, rapid, reproducible technique, sensitive and accurate analytical method and can be used to determine PPD in commercial hair dyes and the evaluation of PPD in samples Black henna that collected from Aden local markets using HPLC. Therefore, based on the findings it can be concluded that natural Henna adulteration by adding PPD should be closely monitored in Yemen since PPD poses toxicological health hazard.

V. RECOMMENDATIONS

The study recommended for improved monitoring and quality control of PPD in cosmetic products. The study recommended to analysis of black henna product, that natural Henna can be adulteration by adding PPD, so should be closely monitored in Yemen since PPD poses toxicological health hazard. Highlight the significance of the study's findings and their contribution to the field of cosmetic safety and quality assurance.

ETHICAL AND COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article. Ethical approval of carrying out this study was obtained from the Ethical Scientific Research Committee of the University of Aden (Rec-57-2019). (Annex 2)

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VI. REFERENCES

[1] M. G. Elmanfe, M. A. Ali, A. O. Abduljalil, L. O. Khreit, and H. A. Ali, "Evaluation of Paraphenylenediamine in Kohl and black Lipstick Samples Collected from Libyan Local Markets Using HPLC," *Al-Mukhtar Journal of Sciences*, vol. 37, no. 1, pp. 13–21, 2023.

[2] A. Saravanan *et al.*, "A comprehensive review on different approaches for CO2 utilization and conversion pathways," *Chemical Engineering Science*, vol. 236, p. 116515, Feb. 2021, doi:10.1016/j.ces.2021.116515.

[3] E. S. Jen, A. M. Adam, H. S. Garba, O. O. M. Attah, and I. N. Dibal, "Lawsonia inermis and para-phenylenediamine exerts an insidious effect on the epidermal, renal, and hepatic tissue with repeated application: a histological and morphometrical study," *European Journal of Anatomy*, vol. 27, p. 4, 2023.

[4] B. U. Eissa, F. Khogali, E. Elamin, E. Osman, M. O. Elmahi, and H. W. Ali, "The potential effect of henna (stone dye) extract paraphenylenediamine on human blood cells, liver and renal function," *Journal of Pharmaceutical Research International*, vol. 33, pp. 192–200, 2021.

[5] N. Puri and A. Puri, "A study on contact dermatitis to hair dye and henna," *Our Dermatology Online/Nasza Dermatologia Online*, vol. 4, no. 4, 2013.

[6] K. Raghu, "Hair Dye Poisoning with Refractory Arrhythmia & Interstitial Nephritis Rare Presentation."

[7] S. E. El-Amin, G. A. Ibrahim, A. A. GahElnab, M. A. Mohammed, and K. Eltahir, "Toxicity effects of hair dye application on liver function in experimental animals," *Group*, vol. 2, p. 10, 2014.

[8] R. Abouhadaf, M. Elderbi, A. Elburi, and A. Salih, "Determination of Para-Phenylenediamine in Henna Cosmetic Products in Benghazi," *GSJ*, vol. 12, no. 1, 2024.

[9] A. Kassem *et al.*, "Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives," *Frontiers in Microbiology*, vol. 14, p. 1304081, 2023.

[10] B. E. Durán, D. Romero-Pérez, and J. S. Salvador, "Allergic contact dermatitis due to paraphenylenediamine: an update," *Actas Dermo-Sifiliográficas (English Edition)*, vol. 109, no. 7, pp. 602–609, 2018.

[11] A. Filali, I. Semlali, V. Ottaviano, C. Furnari, D. Corradini, and R. Soulayman, "A retrospective study of acute systemic poisoning of paraphenylenediamine (Occidental Takawt) in Morocco," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 3, no. 1, pp. 142–149, 2006.

[12] M. K. Bharali and K. Dutta, "Testicular toxicity of paraphenylenediamine after subchronic topical application in rat," *International Journal of Environmental Health Research*, vol. 22, no. 3, pp. 270–278, 2012.

[13] A. Schnuch, H. Lessmann, P. J. Frosch, and W. Uter, "para-Phenylenediamine: the profile of an important allergen. Results of the IVDK1," *British Journal of Dermatology*, vol. 159, no. 2, pp. 379–386, 2008.

[14] M. A. Khattak, F. Nadeem, D. Hwar, A. Khosa, U. A. Haq, and R. Z. Ansari, "The Quantitative Analysis of Para Phenylene Diamine Compound in Henna (Mehndi) by using High Performance Liquid Chromatography," *Advances in Basic Medical Sciences*, vol. 3, no. 1, 2019.

[15] R. H. Abdelhady, M. M. Mahmoud, and E. S. Shaltout, "Heavy Metals and Para-Phenylenediamine in Hair Dye," 2021.

[16] N. M. Abd ElRahman and R. M. Alsourory, "Monitoring of para-Phenylenediamine Presents in Some Adulterated Henna Preparations," *Sciences*, vol. 8, no. 02, pp. 355–359, 2018.

[17] S. A. S. Mahmud *et al.*, "Para-Phenylenediamine (PPD) in commercially available Henna preparations in Bangladesh," *Bioresearch Communications (BRC)*, vol. 8, no. 1, pp. 1088–1092, 2022.

[18] P. G. Wang and A. J. Krynitsky, "Rapid determination of para-phenylenediamine by gas chromatography–mass spectrometry with selected ion monitoring in henna-containing cosmetic products," *Journal of Chromatography B*, vol. 879, no. 20, pp. 1795–1801, 2011.

[19] H. A. M. Ahmed, M. R. Abdel Maaboud, F. F. Abdul Latif, M. A. Kamal El-Dean, M. K. El-Shaieb, E. Vilanova,

and C. Estevan, "Different analytical methods of para-phenylenediamine based hair dye," 2013.
[20] H. Y. Ko, H. Y. Lin, J. C. Shih, and L. Y. Chen, "Determination of phenylenediamines in hair colors derivatizated with 5-(4, 6-dichlorotriazinyl) aminofluorescein via micellar electrokinetic chromatography," *Journal of Food and Drug Analysis*, vol. 27, no. 3, pp. 825–831, 2019.
[21] M. H. Al-Enezi and F. S. Aldawsari, "Study of P-phenylenediamine (PPD) concentrations after hair dye mixing: a call for safety reassessment," *Cosmetics*, vol. 9, no. 2, p. 41, 2022.
[22] K. M. Trived, A. Branton, D. Trivedi, G. Nayak, R. Singh, and S. Jana, "Characterization of physical, thermal and spectroscopic properties of biofield energy treated p-phenylenediamine and p-toluidine," *Environmental & Analytical Toxicology*, vol. 5, no. 6, 2015.
[23] M. Firdaus and M. D. Prameswari, "Synthesis of 2, 2, 4-trimethyl-2, 3-dihydro-1H-1, 5-benzodiazepine using treated natural zeolite catalyst," *Bulletin of Chemical Reaction Engineering & Catalysis*, vol. 14, no. 1, pp. 9–16, 2019.
[24] B. Lakard, G. Herlem, S. Lakard, and B. Fahys, "Ab initio study of the polymerization mechanism of poly (p-phenylenediamine)," *Journal of Molecular Structure: THEOCHEM*, vol. 638, no. 1–3, pp. 177–187, 2003.
[25] A. U. G. Gabbas, H. B. M. Ahmad, N. Zainuddin, and Z. N. Ibrahim, "Synthesis, characterization and thermal polymerization of new 3, 4-dihydro-2H-1, 3-naphthoxazine monomers," *Polimery*, vol. 62, no. 2, pp. 86–92, 2017.
[26] S. Archana and R. J. Shanthi, "Synthesis and characterization of poly (p-phenylenediamine) in the presence of sodium dodecyl sulfate," *Research Journal of Chemistry Sciences*, vol. 2231, no. 606X, 2014.
[27] A. K. Abdul-Salam, L. M. S. Abdul-Rahman, and A. G. Atiya, "Synthesis and characterization of p-phenylenediamine N, N-butan-3-one oxime ligand and its complexes with some transition metals ions Ni (II), Pd (II), Pt (II), Cu (II) and Co (II)," 2012.
[28] A. Khelifa, R. Belmokhtar, A. Berenguer, E. Benyoucef, and E. Morallon, "New poly (o-phenylenediamine)/modified-clay nanocomposites: a study on spectral, thermal, morphological and electrochemical characteristics," *Journal of Molecular Structure*, vol. 1178, pp. 327–332, 2019.
[29] B. Smith, *Infrared Spectral Interpretation: The Beginning I - The Meaning of Peak Positions, Heights, and Widths*, 2024.
[30] S. Maiti, S. Sinha, H. Suchowski, and M. Singh, "Process parameter optimization for the detoxification of emerging environmental pollutant, PPD," *Journal of Hazardous Materials Advances*, vol. 10, p. 100290, 2023.
[31] V. Rajeshwari and B. Mahalakshmi, "Synthesis and Characterization of Nanosize Poly (p-phenylenediamine) in the presence of Benzalkonium Chloride," *International Journal of Advanced Science Research Management*, 2019, pp. 94–99.
[32] F. Kolcu, "An investigation of the enzymatic oligomerization of nitro-substituted phenylene diamine: Thermal and fluorescence properties," *Journal of New Results in Science*, vol. 13, no. 2, pp. 153–164, 2024.
[33] I. J. Kang and M. H. Lee, "Quantification of para-phenylenediamine and heavy metals in henna dye," *Contact Dermatitis*, vol. 55, no. 1, pp. 26–29, 2006.
[34] S. Aktas, A. Sukuroglu, D. Battal, and S. Burgaz, "Monitoring of Lawsone, para-phenylenediamine and heavy metals in commercial temporary black henna tattoos sold in Turkey," *Contact Dermatitis*, vol. 76, no. 2, pp. 89–95, 2017.
[35] R. R. Brancaccio, L. H. Brown, T. Y. Chang, P. J. Fogelman, A. E. Mafong, and E. D. Cohen, "Identification and quantification of para-phenylenediamine in a temporary black henna tattoo," *American Journal of Contact Dermatitis*, vol. 13, no. 1, pp. 15–18, 2002.
[36] C. H. Mounika and M. Kinnera, "A RP-HPLC method development and validation of Para-Phenylenediamine in pure form and in marketed products," *International Journal of Applied Pharmaceutical Sciences (IJAPS)*, vol. 2, no. 1, pp. 20–29, 2015.