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VALIDATED VISIBLE SPECTROPHOTOMETRIC ESTIMATION OF PARA-PHENYLENEDIAMINE, A CARCINOGENIC INGREDIENT IN HENNA HAIR DYES

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ABSTRACT:

A new, sensitive and validated visible spectrophotometric method was developed for the quantification of p-phenylenediamine in henna hair dye formulation. This method is based on the diazotization reaction of p-phenylenediamine with sodium nitrite, hydrochloric acid and ammonium sulphamate in ice cold condition followed by coupling of the formed diazotized salt with N-(1-naphthyl) ethylenediamine resulting in the formation of a magenta colored chromogen, the absorbance of which is measured at 535 nm. The method was found to be linear within the concentrations ranging from 1 – 5 mcg/ml. The proposed method in this study was validated for accuracy, selectivity, sensitivity and precision and was found to satisfy the fore mentioned validation parameters. This facilitates the method to be directly and easily applied for the determination of p-phenylenediamine in henna hair dye preparations.

Key words: 1,4-diaminobenzene, henna hair dyes, NED, para-phenylenediamine, PPD, Visible spectrophotometry.

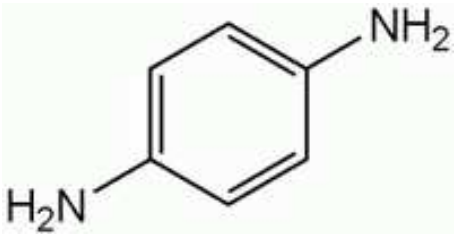
INTRODUCTION:

The presence of dangerous toxic adulterant para-phenylenediamine in commercial henna formulations, results in the frequent occurrences of cancer, anaemia, vertigo, gastritis, exfoliative dermatitis and ultimately death[(1)].

Toxicity associated with cosmetics is being taken seriously by regulatory authorities as well as the people. In 1933, many women were blinded and one died after using "Lush-Lure", a cosmetic containing para-phenylenediamine. This prompted the US Congress to pass a legislation to amend Food and Drugs Act to Food, Drugs and Cosmetics Act in 1938[(2)]. This scenario leads to the search for the better analytical methodologies in dealing with cosmetics.

p-Phenylenediamine (PPD), also called para-phenylenediamine, 1,4-diaminobenzene or 1,4-phenylenediamine is an aromatic amine used as a component of engineering polymers and composites, aramid fibers, hair dyes, rubber chemicals, textile dyes, and pigments. PPD is selected because of its high temperature stability, high strength, and chemical and electrical resistance. However, it is a strong potential carcinogen, which makes it a controversial chemical to be included in hair dyes. Its instability attributed to its oxidative decomposition to para-benzoquinone is a hurdle for the development of newer analytical methods. It has a molecular formula of $C_6H_8N_2$ and a molecular weight of 108.14. It has the structural formula (Figure 1). It is a white to light purple powder (Tan solid) with a slightly aromatic odour. It has a melting point of 143-145 °C and is soluble in 100 parts cold water, alcohol, chloroform and ether as cited in http://en.wikipedia.org/wiki/Wikipedia:Chemical_sources.

Figure 1: Chemical structure of PPD



The CDC lists p-phenylenediamine as being a contact allergen. The NIOSH Pocket Guide to Chemical Hazards lists exposure routes as being through inhalation, skin absorption, ingestion, and skin and/or eye contact; symptoms of exposure include throat irritation (pharynx and larynx), bronchial asthma, and sensitization

dermatitis. It is not official in any of the pharmacopoeia as cited in http://www.cdc.gov/niosh/ipcsneng/neng0805.html and http://www.cdc.gov/niosh/rtecs/ss7ad550.html.

Several methods have been reported for determination of PPD including AAS, mercury analyzer and ICP emission spectroscopy[3], RP-HPLC[4-9], GC-MS[10] in biological fluids and henna hair dye samples. In view of the above fact, a simple analytical method is in need for its quantitative estimation. The objective of the work is to develop new spectrophotometric method for the estimation of PPD in henna hair dye preparations with good accuracy, simplicity, precision and economy.

The proposed method is based on the formation of a magenta colored chromogen by diazotizing PPD with sodium nitrite and hydrochloric acid in ice cold condition and coupling of the resulting diazotized salt with N-(1-naphthyl) ethylenediamine and all optimization parameters were also considered. Also, the developed method was applied to commercial samples of cosmetic henna hair dye preparation.

MATERIALS AND METHODS:

Chemicals

Para-phenylenediamine (99.4 % purity) was obtained from S. d. fine chemicals (Mumbai, India). Henna powder hair dye (Indica) was obtained from the local market (Tamil Nadu, India). All the chemicals and solvents used were of A.R. grade procured from Merck and S. d. fine chemicals (Mumbai, India).

Instrument

A Shimadzu UV/VIS double beam spectrophotometer (model UV-1700(E) 320VCE) with 1 cm matched quartz cells, were used for all spectral measurements. In addition, an Electronic balance (model US-300g) and a digital photo colorimeter (model 312) were also used.

METHODOLOGY:

Preparation of standard solution:

100 mgs of PPD was dissolved in 100 ml standard flask with 20 ml of 0.5M hydrochloric acid and made up to the mark with water (Solution I). Solution I was suitably diluted after treatment with 1 ml each of 0.2 % sodium nitrite solution and 2 M hydrochloric acid in ice cold condition for 2 minutes for diazotisation. It was then swirled with 1 ml of 0.5% ammonium sulphamate solution for 2 minutes to destroy excess of nitrous acid liberated and the color was developed by coupling with 1 ml of 0.1% NED solution, allowed to stand for 5 minutes and then made up to the mark with water and the absorbances of the resulting solutions were measured at 535 nm against the reagent blank. Calibration curve was prepared by plotting concentration versus absorbance and was found to be linear over the concentration range 1-5 mcg/ml. The color was stable upto 10 min.

Preparation of test solution:

100 mgs of commercial herbal hair dye (triturated immediately before performing the assay) was taken in separate 100 ml volumetric flasks and p-phenylenediamine was extracted using 20 ml of 0.5 M hydrochloric acid and made up to mark with water and then filtered. 2 ml of the above solution was then pipetted out into a 25 ml volumetric flask and then made up to the mark with water. From the above solution, 1 ml was pipetted out into a 10 ml volumetric flask and the color was developed by the addition of suitable reagents and then made up to the mark with water and the absorbance was measured at 535 nm within 10 minutes.

RESULTS AND DISCUSSION:

From the absorption spectral analysis, the λ_{max} of diazotized and coupled derivative of PPD was found to be 535 nm. The calibration curve was obtained for a series of concentration in the range of 1-5 mcg/ml (Fig. 2). The method was found to be linear and hence, suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics are summarized in (Table 1). Regression analysis of Beer's law

plot revealed a good correlation. The effects of various excipients generally present in the hair dye were investigated. The results indicated that they did not interfere in the assay.

Figure 2: Calibration curve of PPD by the proposed method

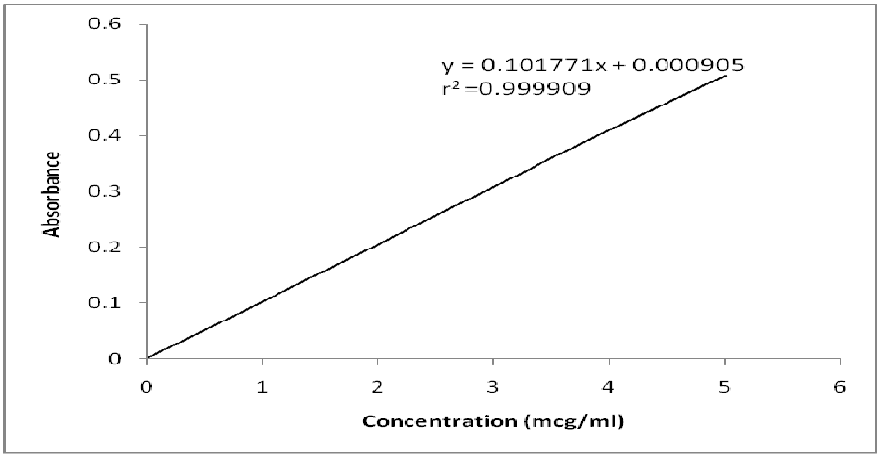


Table 1: Regression analysis of the calibration curve for the proposed method

Parameters	Values
Absorbance maximum (nm)	535
Linearity range (mcg/ml)	1-5
Correlation co-efficient	0.999955
Regression co-efficient	0.999909
Regression equation	Y=0.101771X + 0.000905
Slope	0.101771
y- intercept	0.000905

The proposed method was validated as per the ICH guidelines[11]. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying within the range of ± 2.0. This showed that the precision of the method is excellent. The recovery technique was performed to study the accuracy and reproducibility of the proposed method. For this, known quantities of the PPD solutions were mixed with definite amounts of pre-analyzed samples and the mixtures were

analyzed. The total amount of PPD was determined by using the proposed method and the amount of added drug was calculated by the difference. The % recovery was found to be within the range of 98 – 100 %. This showed that the recovery of PPD by the proposed method is satisfactory and the results are shown in (Table 2).

Table 2: Summary of validation parameters

Parameters	Values
% Content	20.43%
Accuracy (% Recovery)	101.27%
Precision(RSD)	0.629063

RSD indicates Relative Standard Deviation

Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of PPD in henna hair dye preparations.

CONCLUSION:

To conclude with, a simple, sensitive, selective visible spectrophotometric method was developed for determination of PPD in marketed cosmetic henna hair dyes. This proposed visible spectrophotometric method was found to be accurate, precise and economical. The apparatus and reagents used seem to be accessible even for the simple laboratories. Therefore, developed methods can be recommended for routine and quality control analysis of PPD in henna hair dyes preparations.

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