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DEVELOPMENT OF A VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF METANIL YELLOW IN DIFFERENT FOOD SAMPLES.

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ABSTRACT

The purpose of the investigation was to develop a simple spectrophotometric method for the determination of percentage level of occurrence of metanil yellow, a non permitted synthetic food color. This method was based on the reaction between metanil yellow and hydrochloric acid (under specific experimental conditions) to produce a color complex with an absorbance maximum of 450 nm. The complex obeyed Beer's law in 2 µg mL^{-1}- 20 µg mL^{-1} concentration range and exhibited a good correlation coefficient (R^2), a satisfactory Sandell's sensitivity and of recovery (R %) close to 100 %. The use of the described simple and sensitive spectrophotometric method for the routine quality control analysis of food stuffs by determination of metanil yellow overcomes any barriers to food safety evaluation occasioned by the need for costly analytical equipment. The statistical data support the accuracy and precision of the proposed method.

KEYWORDS: Metanil yellow, Visible spectrophotometer, New method, Quantification, Food analysis, Quality control analysis

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INTRODUCTION

From the organoleptic point of view, the visual aspect is an important factor for the preference of the products by the purchaser. Thus the synthetic food dyes occupy an important place in the class of essential additive for food industry in the conquest of markets. This synthetic chemical substance is used to enhance flavor or make food bright and attractive. This is mostly used for financial gain or due to improper conditions of processing. Metanil yellow is a non permitted synthetic azo dye (coal tar color), being used extensively for coloring different foodstuffs in many developing countries. It is found in laddu, papadum, and spices such as turmeric powder, orange or yellow colored sweets, ice cream etc. It is also found in prepared foods such as biryani. It is chemically designated as 3-(4-Anilinophenylazo) benzenesulfonic acid sodium salt**,** also known as acid yellow36, and its chemical formula is $C_{18}H_{14}N_3NaO_3S$, representingmolecular weight of 375.38. It is a yellow colored powder that is soluble in water under ordinary condition. Intake of metanil yellow is hazardous to the health and cause irreparable damage to the systems including lipid peroxidation in liver[1], alternation of absolute and relative weight of testis[2], alternation in hematological parameters, body weight, serum glucose, serum inorganic phosphorus, T_3, T_4, calcium, LDH and cholesterol of brain, liver and heart[3], decrease in total erythrocyte count and hemoglobin i.e. normochromic macrocytic anaemia[4] etc, when taken regular intervals over long period of time. The structural formula of metanil yellow is represented in Figure-1.

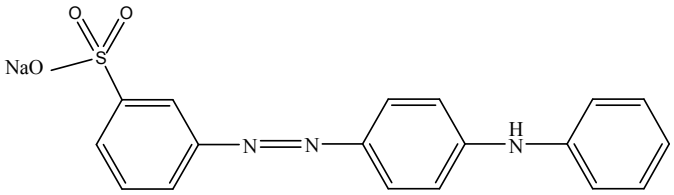

Figure 1
Chemical structure of metanil yellow.

Many analytical methods have been developed for the qualitative and quantitative analysis of food color, including Thin Layer Chromatography(TLC)[5],UV/VIS Spectrophotometry[6-7], Mass Spectrometry[8], Capillary Electrophoresis [9-10] and various combinations of this techniques like HPLC coupled with ultraviolet detection[11-12] etc. The fundamental methods derivative spectrophotometry has been described by Talsky[13] and Owen[14]. Several analytical methods have been developed for quantification of metanil yellow in foodstuffs, like 2-directional high-performance thin-layer chromatography[15],thin-layer chromatography[16], high performance liquid chromatography solid phase extraction [17], HPLC[18] etc. Even though there is no one visible spectrophotometric methods reported for the determination of metanil yellow in various food products. For routine analysis, simple, rapid and cost effective visible spectrophotometric methods are required and preferred. So, there is a need for development of sensitive, accurate and flexible visible spectophotometric methods for determination of metanil yellow in foodstuffs and quality control analysis. So, the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between metanil yellow and hydrochloric acid under specific experimental conditions.

MATERIALS AND METHODS

Apparatus and Chemicals

A Systronics 118 single beam UV-VIS spectrophotometer with 1.0 cm quartz cell was used for the measurement of absorbance. All the weights were taken on Citizen CY 120 electronic balance [Sl. No 9061515]. Metanil yellow dye were obtained from Sigma-Aldrich company (3050 Spruce Street, St. Louis, MO 63103 USA) was used for the preparation of standard curve. Hydrochloric acid of analytical reagent grade was supplied by Merck Specialties Private Limited and other chemicals used were of analytical grade for this method.

Procedures

Preparation of standard stock solution

The standard stock solution of metanil yellow was prepared by dissolving 0.04 mg of metanil yellow in 4 ml of distilled water. Then 0.03 ml of metanil yellow solution was mixed with distilled water to make the volume up to 3 ml to make stock solution. The prepared stock solution contains 100 µg mL^{-1} of metanil yellow stored at room temperature. A series of standards were freshly prepared during the time of analysis.

Preparation of sample solutions

The food samples evaluated have been collected and protecting them from any contamination. 1 gm of sample was measured and dissolved in 10 ml of distilled water and mixed with a vortex mixture. Then after 30 minutes the sample solution was filtered. The filtrate was then collected and 0.5 ml of filtrate was taken in a test tube and volume was made up to 3 ml by adding with distilled water. Finally 50 µl of 1(N) HCl was added to the sample for appearance of pink color.

Determination of optical wavelength (λ_{max}) of maximum absorption

0.3 ml of stock solution was taken in a test tube and the volume was made up to 3 ml with distilled water. Then 50 µl of 1(N) HCl was added to it. The solution was mixed well and kept at room temperature for 5 minutes to develop the color. From the UV-absorption spectra 450 nm was selected as λ_{max} obtaining by scanning the pure metanil yellow solutions in the range of 300-600 nm for measuring the absorbance of above solutions to prepare the calibration curve (Fig. 2).

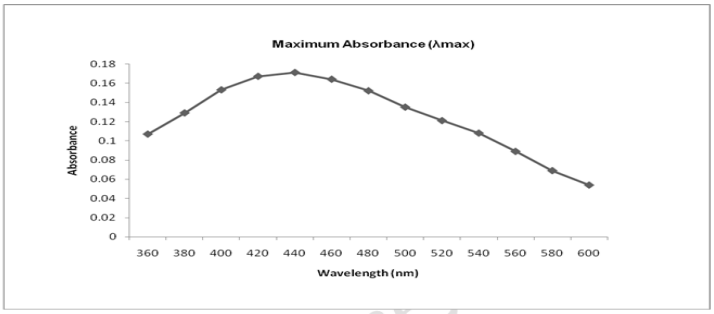

Figure 2

Determination of optical wavelength (λ_{max}) of maximum absorption of color compound formed between the reaction of metanil yellow and HCl in aqueous medium

Preparation of regression curve

A calibration curve was established to scrutinize the linearity of the technique. For this purpose, 3 ml of ten different concentrations of metanil yellow (which contains 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg mL^{-1} of metanil yellow) were prepared. Then 50 µl of 1(N) HCl solution was added to each and allowed to stand for 5 minutes for appearance of pink color. The absorbance was measured at 450 nm against a reagent blank prepared concurrently. The amount of additive was then calculated from the calibration curve (Fig.3).

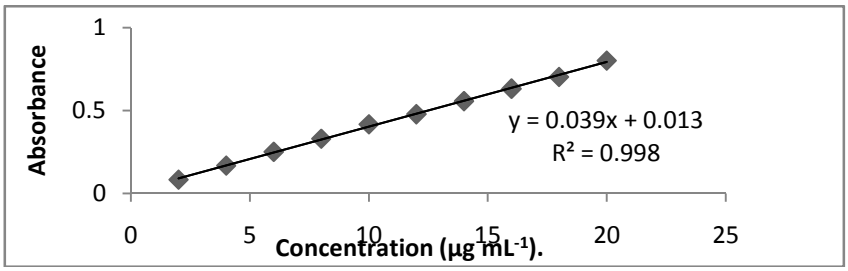

Figure 3
Calibration curve of metanil yellow concentration against absorption.

Determination of metanil yellow from foodstuffs

Sensitivity

Sensitivity of the proposed method for substance was determined by calculating Sandell's sensitivity (µg/cm^2/0.001/abs unit). Sandell's sensitivity can be defined as the smallest weight of the substance that can be detected in column of the solution of unit cross section. The weight of the sample can be expressed as µg and area cm^2. The Sandell's sensitivity was calculated from the formula- SS= M/ε, where M and ε indicates the molecular weight and molar absorptivity.

Accuracy

The accuracy of the proposed method was evaluated through recovery test by standard addition method. Pre analyzed turmeric powder (which contained no amount of metanil yellow) spiked with pure metanil yellow at three different levels (which was known) and the amount of metanil yellow was found by the proposed method (4, 6, and 8 µg mL^{-1}, n=3). Each determination was repeated three times and finally the percentage of recovery (R %) was calculated from the formula- Percentage of Recovery (R %) = 100 x C_f /C_r, Where, C_r= real concentration of metanil yellow in different samples and C_f=Concentration of metanil yellow obtained from the standard addition curve.

Precision

The precision of the method was determined by replicate analysis of three separate solutions of the working standards at three concentration levels of each additive. Intra-day precision was evaluated by measuring, in triplicate, three different samples at the same concentration under the same experimental condition on the same day, according to the sample preparation method described previously. The precision was calculated from the results obtained by the earliest analysis of samples with the same three concentrations (4, 6, and 8 µg mL^{-1}, n=3).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Three series of standard solutions of metanil yellow were prepared (ten different dilutions of metanil yellow- 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg mL^{-1}) and absorbances were measured in triplicate, at 450 nm. The limit of detection and limit of quantification were calculated directly from the calibration curve using the formula 3.3 σ/s and 10 σ/s respectively, where s is the slope of the calibration curve and σ is the standard deviation of the intercept[19-20].

RESULTS AND DISCUSSION

Identification of the complex

First the nitrogen atom attached to the benzene ring containing a -SO_3Na group at the meta position captured a H^+ ion to form compound 2. Then the lone pair of secondary nitrogen atom takes part in conjugation to form compound 3 and showed pink color (Fig.4).

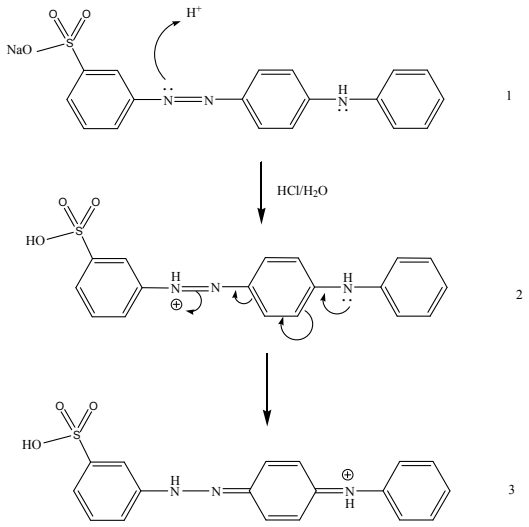

Figure 4
Proposed chemistry of the color species.

Method validation

The aim of the work was to establish a simple, rapid and less environmental toxic method to assay matanil yellow in foodstuffs by UV-spectrometry. Initially, a sample of pure metanil yellow was prepared and an UV-VIS spectroscopic scanning run allowed for selecting the wavelength of 450 nm as the best for the detection of metanil yellow in the standard solution as well as in sample solutions. Appropriate amount of the stock solution were diluted with distilled water, yields concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg mL^{-1}, and 50 µl of 1(N) HCl was added to each concentration. Absorbance of the standard solution was plotted against the theoretical concentration. The linearity was evaluated by calculating the correlation coefficient obtained from the linear regression analysis. A linear relationship was found between the absorbance at 450 nm at the concentration of metanil yellow in the range of 2 to 20 µg mL^{-1}. Different optical characteristics of the proposed method are summarized in theTable-1.

Table 1
Optical characteristics of the proposed method.

Parameters	Methods
Absorption maxima (nm)	450 nm
Beer's law limits (µg mL^{-1})	2-20
Sandell's sensitivity (µg cm^{-2} /0.001 abs. unit)	0.0240921
Molar absorptivity (L mol^{-1} cm^{-1})	1.55810×10^{4}
Regression equation (Y*=a+bx)	Y= 0.039+ 0.013
Intercept (a)	0.013
Slope (b)	0.039
% RSD	1.063
Correlation coefficient (R)	0.998
Limit of detection (µg mL^{-1})	0.048
Limit of quantification(µg mL^{-1})	0.147

Y=a+bx, where x is the concentration of metanil yellow in µg mL^{-1} and Y is the absorbance at the respective maximum absorbency.

Agreement with Beer's law was evident from the concentration range of the final dilution of 2-20 μg mL^{-1} with an apparent molar absorptivity and Sandell's sensitivity of 1.55810×10^4 $L.mol^{-1}cm^{-1}$ and 0.0240921 μg cm^{-2}/0.001A, respectively. The correlation coefficient (R^2) obtained for the line was 0.998 indicating very good linearity. The linear regression equation was calculated to be Y=0.039+0.013 where X and Y are concentration in μg mL^{-1} and absorbance respectively. The limit of detection (LOD) and the limit of quantification (LOQ) are calculated to be 0.048 and 0.147 μg mL^{-1}, respectively. The method had excellent reproducibility for standard solution of 10 μg mL^{-1}.The percent of recovery (R %) which is an index of accuracy were found to be nearly 100% (Table-2).

Table-2
Results of recovery study via standard-addition method.

Sample name			Spectrophotometric method		
Sample's Sl. No.	Amount of metanil yellow added (μg)*	Optical density	Total found by the proposed method (μg)	Percentage of recovery (R%)	R % ± SD
1	4	0.169±0.002	3.999±0.053	99.975±1.339	
2	6	0.247±0.002	6.010±0.053	100.167±0.885	99.927±0.970
3	8	0.324±0.003	7.971±0.079	99.64±0.990	

Average of three determinations, R%. Percentage of recovery.

The percent relative error (RE) and the relative standard deviation (RSD %) which is a measure of precision are summarized in Table 3 and reveal the high accuracy and precision of the method.

Table 3
Results of percent relative error and precision study.

Spectrophotometric method				
Metanil yellow taken (μgmL^{-1})	Metanil yellow found (μg mL^{-1})	(RE) %	*(RSD) %	Range of error
4	3.999	0.250	1.32	±0.030
6	6.010	0.166	0.88	±0.030
8	7.971	0.375	0.99	±0.045

Average of three determinations. %RE. Percent relative error, SD. Standard deviation, %RSD. Relative standard deviation

Application to real samples

The investigative technique developed in this paper was applied to determine the amount of metanil yellow present in different food samples like turmeric powder, chili powder and besan (Gram flour) etc. The linearity was evaluated by calculation of the correlation coefficient obtained from the linear regression analysis. Recovery and precision, Limit of Quantification and Limit of Detection obtained from this method was satisfactory which proved the successful development of the technique.

CONCLUSION

In conclusion, a simple derivative spectrophotometric method is developed for the quantitative determination of non permitted synthetic food color metanil yellow in different foodstuffs. The sensitivity offered by the proposed method surpasses that of the existing spectrophotometric methods in terms of linear range and quantification limits. Simplicity of pretreatment and measurement, and use of non-rigid experimental conditions could make the proposed method as effective tool to analyze accurately and precisely the metanil yellow in food samples using simple instrumentation and low-cost materials.

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