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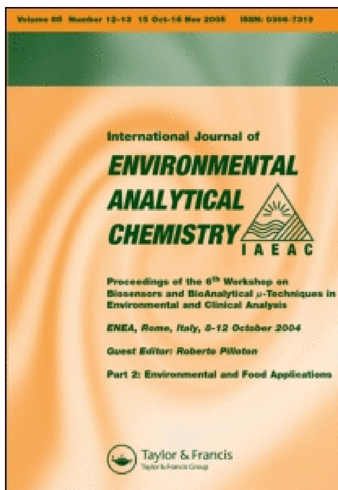

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Spectrophotometric Estimation of Metanil Yellow in Foodstuffs

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Metanil yellow, manufactured for textile and other purposes, is frequently used to colour foodstuffs. A simple and sensitive spectrophotometric method has been developed for the estimation of metanil yellow in foodstuffs and biological tissues. The recovery of metanil yellow is 86.5% to 96.9%. Curcumin, bile salts, riboflavin etc. do not interfere. The method is reliable, rapid and the lowest limit of detection is 1 μg. This method can also be applied as a simple colorimetric procedure by reading the colour value in any standard colorimeter using a green filter.

KEY WORDS: Metanil yellow, spectrophotometric estimation, foodstuffs, biological tissues, colorimetric procedure.

INTRODUCTION

Metanil yellow (monoazo; C.I. Acid yellow 36 (13065), the sodium or calcium salt of [m-(p-anilinophenyl) azo] benzene sulfonic acid has been toxicologically classified under category CII by the Joint FAO/WHO Expert Committee on Food additives. Category CII comprises colours for which the available data are inadequate for safety evaluation. According to U.S. Food and Drug Administration this colour has been allowed for external use only.

Metanil yellow, manufactured for textile and other purposes, is frequently used to colour foodstuffs in India.[1] As part of a long-term study on its toxicity, the dye has been found to produce testicular lesions in experimental animals.[2-3] During these studies the need was felt for a sensitive method for the quantitative estimation of metanil yellow. A simple and sensitive spectrophotometric method for the estimation of metanil yellow in foodstuffs and biological tissues is described in this communication.

EXPERIMENTAL

Urine, serum and body organs (brain, liver, kidney and testis) were collected

from normal adult male albino rats of I.T.R.C. colony. A mixture of wheat, rice, pulse (*Cajanus indicus*) constituted the foodstuff.

Reagents and chemicals

Metanil yellow (obtained from M/s Vesco Products Co., Calcutta, India).
1.6 N HCl in n-butanol.
0.8 N HCl in n-butanol.
Acidified n-butanol containing 0.001 g equivalent HCl/litre.
Solvent ether.
Ethanol.

General procedure

Principle—A differential solvent extraction technique was used for the quantitative extraction of Metanil yellow from tissues or food samples after removal of lipids. The estimation of metanil yellow is based on the sharp colour change from yellow to violet in presence of an acid.[4,5]

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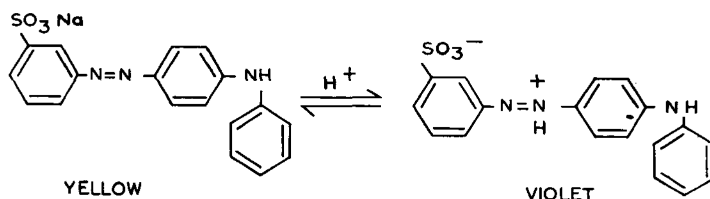

FIGURE 1 Acid-Base behaviour of metanil yellow.

Preparation of standard sample

Homogenates (10% W/V) of brain, liver, kidney and testis were prepared in distilled water. Serum and urine were taken as such.

Removal of lipids

The sample (1.0 ml) was thoroughly shaken with 5.0 ml of ethyl ether in a separating funnel. The aqueous and ether layers were separated. The etherial layer containing lipids was washed with 0.5 ml water and the washing was added to the aqueous layer and the etherial layer was discarded. The volume of the sample was made up to 1.5 ml.

Extraction of the dye

The above extract (1.5 ml) was mixed with 5.5 ml of absolute ethanol to give 80 % concentration of ethanol in the mixture. After about 20 to 30 minutes the suspension was centrifuged at 3,000 rpm for 15 minutes. The supernatant was recovered and kept aside. The sediment was washed with 80 % ethanol several times and centrifuged till the supernatant became colourless. The supernatants were pooled, added to the original supernatant and ethanol removed completely by evaporation. The remaining aqueous solution was diluted to 5.0 ml with distilled water.

Isolation of the dye

The extract as above was shaken with 1.0 ml of acidified n-butanol and the aqueous and n-butanol layers were separated. The aqueous layer was washed again with 1.0 ml acidified n-butanol and the wash was added to the solvent layer. The pooled butanol extract was approximately 1.5 ml (after accounting for n-butanol which was dissolved in water) and contained all the dye. This layer was yellow in colour as all the acid had been removed by aqueous washings.

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Estimation

To the extracted dye was added 1.5 ml of 1.6 N HCl in n-butanol to give a final concentration 0.8 N HCl and allowed to stand for 15 min. The absorbance of the solution was read at 520 nm. A standard solution of dye (20 μg/ml) was subjected to the same extraction procedure as above. The solution, if necessary, was diluted with 0.8 N HCl in n-butanol.

Improvements in the procedure

Removal of the lipids is essential to avoid interference during chromatography and the colorimetric estimation. Similarly if the fat content of the food is high, there would be difficulty in separating ether and water layer due to the formation of fat water emulsion. This difficulty can be overcome by the addition of NaOH and subsequent neutralization with HCl. Lipids could also be removed from dry samples of food by Soxhlet extraction with ethyl ether.

When two or more coloured substances are present along with metanil yellow, aliquots of alcohol extracts are chromatographed on paper using n-butanol : acetic acid : water (4 : 1 : 5)[6] or n-butanol : alcohol : 2 N NH_4OH (3 : 1 : 1).[7] Metanil yellow gives consistently an Rf value of 0.48 in these two solvent systems. The spot corresponding to metanil yellow is cut and eluted with known volume of 80 % alcohol and processed as described earlier for its estimation.

RESULTS AND DISCUSSION

The method developed as above was used to estimate the percent recovery of metanil yellow from various tissues, urine, serum and foodstuffs. This was done by adding 20 μg/ml of metanil yellow to homogenates or urine or serum. The recovery was from 86.5% to 96.9% (Table I).

TABLE I

Recovery of metanil yellow

Material	Volume of homogenate (ml)	Metanil yellow added (μg)	Metanil yellow recovered (μg)	Recovery percent
Brain (10% homogenate)	1.0	20.0	19.9	96.9
Liver (10% homogenate)	1.0	20.0	17.3	86.5
Kidney (10% homogenate)	1.0	20.0	18.9	94.7
Testis (10% homogenate)	1.0	20.0	18.0	90.1
Serum	1.0	20.0	18.5	92.4
Urine	1.0	20.0	18.6	93.2
Foodstuff (10% homogenate)	1.0	20.0	18.5	92.7

Absorption curve

The absorption spectra of metanil yellow in different solvents are shown in Figure 2. The dye absorbs at 420 nm to 440 nm in water, alcohol, alkaline and salt solutions. The absorption peak however shifts to 520 nm on acidification

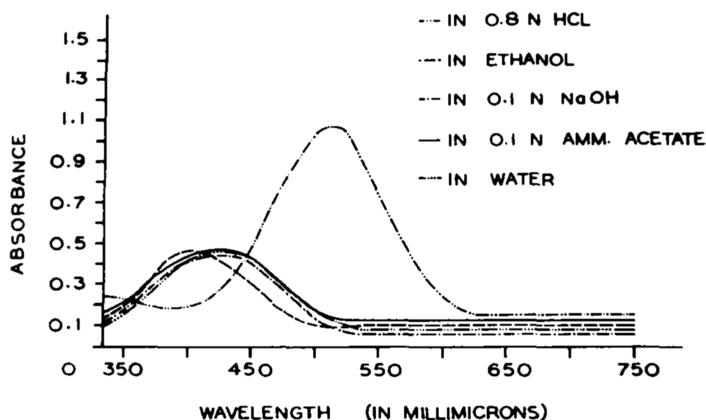

FIGURE 2 Visible absorption spectra of metanil yellow in various media.

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accompanied by a change in colour from yellow to violet. HCl was found to be the most suitable from the point of view of sensitivity and ease of handling in bringing about the change in colour. HCl (0.1 N to 2.0 N in n-butanol) has been used to develop the colour with 5 μg/ml of metanil yellow and maximum absorbance at 520 nm was found with 0.8 N HCl.

Stability of colour

The developed colour is stable up to 24 hrs. After this time a precipitate appeared and the colour faded. The colour is developed at room temperature (20–30°) and is stable to heat up to 100°C.

Conformity to Beer's Law

The absorbance of the dye in its acid form bears a linear relationship to concentration (up to 10 μg/ml) as evident from Figure 3.

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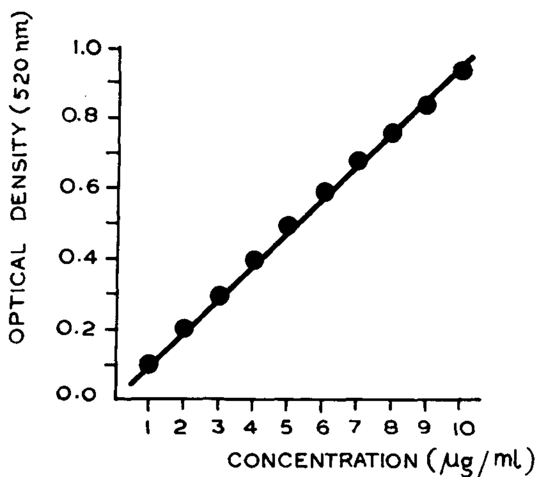

FIGURE 3 Relationship between optical density and concentration of metanil yellow in 0.8 N HCl.

PRECISION AND ACCURACY

The present method has given a recovery of 86.5 % to 96.9 % metanil yellow in various body organs and foodstuffs. Curcumin, bile salts, riboflavin etc. do not interfere. The method is reliable, rapid and sensitive and the lowest limit of

detection is 1 μg. This method can also be applied as a simple colorimetric procedure by reading the colour value in any standard colorimeter using a green filter.

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