Qualitative and Quantitative Detection of Rhodamine B Extracted from Different Food Items using Visible Spectrophotometry

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ABSTRACT: A study was done to detect the presence of Rhodamine-B dye quantitatively and qualitatively using Visible Spectrophotometry. Rhodamine B was found to be illegally used by sweet makers or bakers for colouring the different confectionery. The study involved the detection of Rhodamine-B quantitatively and qualitatively from street foods (Sweets and Confectioneries) collected from different cities in India. A total of 75 samples of different varieties of sweets and confectionery were collected from street food corners/ locations in Delhi, NCR region, Mumbai, Pune, Agra and Kerala. A standard control of the Rhodamine-B was used with a concentration of 20µg/ml. Samples were extracted based on organic solvent extraction using chloroform and isobutanol. The absorbance and concentration were measured using visible spectrophotometry at 503 nm. The concentrations of the extracted Rhodamine-ranged from 0.07 to 1.09 µg/mL and absorbance values were ranging from 0.08 to 1.27. Out of the total 75 sample tested, most of the 30 positive specimens were from Pune. The present study indicates that there is prevalence of Rhodamine-B use in street foods mostly in the Pune regions. The extraction method was found useful even in extracting Rhodamine-B with low concentrations. Further research is needed to determine the prevalence of Rhodamine-B from the street foods.

Keywords: Rhodamine-B , Visible Spectrophotometry, detection , street foods

Introduction

Food adulteration is defined as the process by which the quality or the nature of a given substance is reduced through the addition of a foreign or an inferior substance and the removal of a vital element [1]. More recent studies showed the use of nonpermitted colours like Rhodamine Orange II, and Auramine in street food like jilebi and coconut burfi (coconut based fudge) [2]. Another study in which 700 food items were analyzed from urban areas and 300 from rural areas showed that 93% and 95% respectively contained permitted colours viz. tartrazine, sunset yellow, ponceau 4R, carmoisine, erythrosine and brilliant blue while 7% of the food from urban and 5% from rural contained non-permitted colours [3]. The use of certain dyes has been banned, as they are found to be toxic in experimental animals.

With the liberalization of trade in India, there is a growing list of food additives and processing aids which require approval from the regulatory authorities for their use in different foodstuffs. There is an urgent need for the scientific community in India to evaluate whether these additives are necessities as they are hazardous to the Indian consumer. In India several independent studies carried out in various parts of the country have focused on adulteration of different food with added colours. The findings of most of these studies showed that a variety of food such as milk products, including ice cream, khoya (a dairy product which is made by and reducing the milk to a semi-solid stage), cottage cheese, non-milk products (sweets, savouries), legumes, miscellaneous (confectionery, soft drinks, spices, condiments, tea, flattened rice, fish, fresh vegetables and cut fruit) and spices (turmeric, chilli powder) were usually adulterated with non-permitted colours such as metanil yellow, auramine, rhodamine B, congo red, malachite green and orange II [4,5].

A market survey carried out in 236 outlets in urban areas showed that a variety of food from categories like breakfast accompaniments, beverages, sweetmeats, bakery food, savouries and confectionery contained added colours. However certain food such as spices, condiments, rusk, vegetables, savouries and a variety of cooked food preparations such as soups, noodles, gravy curries, starters, manchuria (starters made from chicken, or vegetables with cornflour and sauces), biryani (rice preparation made vegetables or chicken or lamb), ground legume flour used in preparation of savouries like sev, chegodi, boondi, finger fries, bajjis, all of which do not form a part of the PFA permitted list of specified food items, were found to contain added colours [6].

Several cases of adulteration of food with colours have been recorded. Mustard seeds were adulterated with non-permitted colour (not specified) but had conformed to the standards [7]. Metanil yellow, a non-permitted colour was a common adulterant in food like laddu, toor dal and turmeric, which could be due to its easy availability and reasonable cost [8]. Spices like chilli powder were found to contain non-permitted colours like sudan dyes [9]. Analysis of samples of sweets and confectionery collected during festivals showed the wide usage of nonpermitted colours like rhodamine to the extent of 10-95ppm, orange II (135-560ppm) and auramine (15-400 ppm) [10]. The use of permitted colours also evoked concern as they were used in excess of the statutory limit (100ppm) to an extent of 15157 mg/kg in sweetmeats and 9450µg/ml in beverages or they were used in food in which they were not permitted [11].

Rhodamine is used as a dye and as a dye laser gain medium.Rhodamine dyes are generally toxic, and are soluble in water, methanol and ethanol. Rhodamine is a banned dye as per PFA act (1954), by Government of India, because the same dye has found to be carcinogenic in human.

Different methods of detection of Rhodamine are in use in the laboratories like TLC,UV, HPLC, Paper Chromatography. Rhodamine is a banned dye as per PFA Act, 1954. Rhodamine is harmful to human as it is carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity. Rhodamine is used for food adulteration in Sweets and Confectionery.It causes health hazards in humans who consume it. Examination for this is been done for the detection of Rhodamine from Food Stuffs in Forensic Toxicology Laboratories and Food Adulteration Laboratories.

The objective of the present study was to detect the presence of Rhodamine-B dye quantitatively and qualitatively from street foods using visible spectrophotometry. Since Rhodamine B is a legally banned dye in India as per PFA Act 1954 and as per subsequent amendment in 2004, it has been used by sweet makers or bakers for coloring the different confectionery, the result of the present study would give enough scope to further explore the possibilities of extraction of Rhodamine and its detection through qualitative and quantitative analysis. The present study has given more emphasis on the detection of Rhodamine-B extracted from street foods (Sweets and Confectionery) collected from different regions in India as it has been found that the use of Rhodamine-B in sweets and confectionery were more predominant in the street foods available in different metro Cities in India.

Sample size

In total 75 samples of different varieties of sweets and confectionery were collected from street food corners/ locations from Delhi, NCR region, Mumbai.

Criteria for Sample Collection

Samples were collected on the basis of its color like pink, rose, and red. Only sweets, squash, syrups were collected. The samples were collected from different regions in India, Pune, Agra and Cochin. As control sample 2mg of Rhodamine B was used.

Materials and method

Samples of street foods (sweets, squash, syrups) control of Rhodamine-B, chloroform, Chloroform and Isobutanol, separating funnel, distilled water, water bath, visible spectrophotometer (UV-VIS Spectrophotometer(Elco-SL-150).

The standard control of Rhodamine-B was prepared by dissolving 2 mg of Rhodamine B in 100 ml of chloroform to obtain 20 µg/ml. The organic solvents used for the extraction of Rhodamine B were chloroform and Isobutanol. The solvents were mixed in a ratio 8:2 and mixed with water. The samples of sweets were ground or macerated to an aqueous slurry and was shaken with the extraction solvent i.e. Chloroform: isobutanol for 30-45 mins. The squash, syrups were shaken with the extraction solvent in the similar way. The contents were filtered and residual slurry were again extracted twice with extraction solvent mixture. The filtered fractions were combined and taken in to separating funnel and into the same 10-15 ml distilled water was added and shaken vigorously. Chloroform layer was collected and the process was repeated. The chloroform layers were combined and the same was concentrated to 10 ml by evaporating on water bath and the sample was subjected for visible spectrophotometric analysis.

Results and discussion

Out of 75 samples experimented 60 samples showed positive results and among 60 samples the maximum positive results were showed by the samples from Pune. Quantitative and qualitative estimation of Rhodamine was performed by comparing the absorbance value of Rhodamine at a particular wavelength of maximum absorption i.e. 503 nm. The absorbance of control sample formed the basis of comparison with the samples, which consisted of randomly sampled food substances. Absorbance values of control as well as 60 test samples shows that the concentration of Rhodamine-B in the spurious food samples

collected from street were differing. This finding substantiates the results of previous study Pune [12]. The present study established that there is a prevalence of Rhodamine in street foods mostly in the regions like Pune and the study is giving a scope for further research in same field. The absorbances and concentrations were measured using visible spectrophotometry at 503 nm. The concentration of the extracted Rhodamine B ranged from 0.071 to 1.09µg/ml and the absorbance ranged from 0.084 to 1.27.

Statistical analysis was done and the t value was found to be 8.08 and p value was found to be 0.01.

Table 1: Absorbance and Concentration of Control and samples

Samples no.	Absorbance	Sample Concentration (µg/ml)	Difference of absorbsnce	% difference of absorbance
1	0.973	0.837	0.131	11.7
2	1.104	0.95	0.009	0.808
3	0.783	0.67	0.33	29.6
4	0.972	0.833	0.141	74.8
5	Nil	Nil	Nil	Nil
6	1.104	0.935	0.178	100.8
7	0.0845	0.071	1.028	131.71
8	0.956	0.81	0.157	116.4
9	0.904	0.76	0.209	123.1
10	Nil	Nil	Nil	Nil
11	1.245	1.05	-0.132	-11.1
12	1.109	0.94	0.004	99.6
13	Nil	Nil	Nil	Nil
14	0.864	0.73	0.249	77.6
15	0.894	0.75	0.219	124.4
16	Nil	Nil	Nil	Nil
17	1.09	0.92	0.023	102.1
18	1.001	0.84	0.112	111.18
19	Nil	Nil	Nil	Nil
20	0.978	0.82	0.135	113.8
21	0.995	0.84	0.118	111.8
22	Nil	Nil	Nil	Nil
23	0.973	0.82	0.14	114.3
24	Nil	Nil	Nil	Nil
25	0.94	0.79	0.173	115.9
26	Nil	Nil	Nil	Nil
27	1.11	0.94	0.003	100.2
28	0.895	0.75	0.218	124.3
29	0.675	0.57	0.438	164.8
30	0.564	0.47	0.54	197.3
31	nil	Nil	Nil	Nil
32	0.76	0.64	0.353	146.44
33	0.756	0.64	0.357	147.2
34	nil	Nil	Nil	Nil
35	1.273	1.09	-0.16	114.3
36	nil	Nil	Nil	Nil
37	1.113	0.94	0	100
38	nil	Nil	Nil	Nil
39	nil	Nil	Nil	Nil
40	1.041	0.88	0.072	106.9
41	nil	Nil	Nil	Nil
42	0.864	0.73	0.249	128.8
43	1.00	0.84	0.113	89.84
44	Nil	Nil	Nil	Nil

45	1.113	0.943	0	100
46	0.97	0.95	0.143	12.8
47	1.1	0.99	0.123	11.05
48	0.78	1	0.33	29.6
49	0.97	0.91	0.143	12.8
50	1.1	1.67	0.013	1.16
51	0.08	1.2	1.046	93.9
52	0.95	0.96	0.163	14.6
53	0.9	0.99	0.213	19.13
54	1.24	1.57	-0.127	-11.4
55	1.1	1.43	0.013	1.16
56	0.86	1.32	0.253	22.7
57	0.89	1	0.223	20.03
58	1.09	1.3	0.023	2.066
59	0.97	0.98	0.143	12.8
60	0.99	1.45	0.123	11.05
61	0.94	1.32	0.173	15.54
62	1.11	1.57	0.003	0.26
63	0.89	0.96	0.223	20.03
64	0.67	1.42	0.443	39.8
65	0.56	1.2	0.553	49.6
66	0.76	1.02	0.353	31.7
67	0.75	0.99	0.363	32.6
68	1.23	1.07	-0.117	-10.5
69	1.11	1.6	0.003	0.269
70	1.04	1.64	0.073	6.558
71	0.86	1.32	0.253	22.7
72	1	1.52	0.113	10.15
73	0.86	1.43	0.253	22.7
74	1	1.07	0.113	10.15
75	1.11	1.09	0.003	0.269

Table 2: Mean,Standard deviation and t&p values

MEAN ABSORBANCE	STANDARD DEVIATION	t VALUE	P VALUE
0.85	0.23	8.08	Less than 0.01
1.25	0.25		

For the qualitative and the quantitative estimation of Rhodamine-B extracted from the food stuffs using the methodology adopted in this study , one has to consider a proper method of extraction as it has been found that the Rhodamine-B is found to be relatively less in concentration when conventional techniques in extraction were used which would definitely affect the results of Rhodamine detection.

The present Visible Spectrophotometric qualitative and quantitative detection of the presence of Rhodamine-B extracted from the food stuffs using the methodology adopted would definitely give an insight to the forensic scientific community to further explore the possibilities of detection of the same using the methodologies in similar lines.

Conclusion

For the detection of Rhodamine-B from suspected samples collected from street foods, one has to be considering the standardized methodology adopted to detect Rhodamine B. Rhodamine B is commonly and illegally used for coloring of food which cause a lot of health hazards. Absorbance value of control as well as 30 test sample shows that the concentration of Rhodamine in the spurious food Samples from street detected were differing. Rhodamine B is a carcinogenic substance, mutagen, tumorigen Continuous consumption of foods containing Rhodamine cause many ill effects and even damage body organs as well. The toxicity studies of Rhodamine shows that it is causing toxicity to

humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.

Since Rhodamine-B was detected from samples and hence the sample size was lesser ,it gives a scope for further research to explore the probability of detection of Rhodamine in such foods collected from street food corners considering more number of samples so that the prevalence of the use of Rhodamine in street foods in different regions in India could be assessed and also the types of food which are adulterated .The results of the present study is indicating and giving an alarming signal with regard to street food adulteration with Rhodamine, which are consumed by common people thus causing health hazards.

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