Inductively coupled plasma-mass spectrometric profiling of metal contamination in Holi colours

To welcome the arrival of spring, Holi, the festival of colours, is celebrated in India by smearing coloured powder or spraying coloured water on each other[1]. Following this celebration, a significant number of people suffers from dermatological complaints[2] and ocular problems[3]. Most Holi colours sold in the market contain toxic chemicals with potentially severe health impacts[4]. Various ocular adverse effects of these colours have been reported, including conjunctivitis and corneal abrasion[5,6]. These colours also cause temporary blindness, renal failure, skin cancer and pose significant health risk[7]. Studies have confirmed diagnosis of acquired methanoglobinemia developed in many cases in children after playing with Holi colours[8]. Methanoglobinemia can be life-threatening, but can be overcome if treated on time with intravenous methylene blue[9]. Public awareness and regulatory actions are needed to avoid such conditions. Lack of control over the quality and content of the Holi colours is a problem, as they are frequently sold by vendors who do not know their origin. Most consumers are not aware of the Package Product Act (PPA) that demands the contents, price and address of the manufacturer to be mentioned on the packet containing these colours. In case the customer has any complaints, there is hardly a chance for him getting redressal in any forum. The Prevention of Food adulteration Act (PFA) covers only edible foodstuff, but offers no protection to customers who suffer because of adulterated Holi colours[10].

Holi colours are often adulterated with heavy metals[11]. A myriad of colours – red, yellow, blue, green, magenta, turquoise blue/Prussian blue (bluish green), purple, orange, black, silver, etc. are available in paste, dry powder 'gulal' and water-soluble forms. Here we report the validation of a method for the determination of metal contaminants, using inductively coupled plasma-mass spectrometer (ICP-MS) in water-soluble and gulal colours. All colours were in the dry, solid powder form.

ICP-MS is a sensitive instrument for multielemental identification, quantification and analysis of more than 25 metals at parts per billion (ppb) levels in a single run. In the present study, 22 water-soluble Holi colours and 18 gulal colours were randomly sampled from various shops in Jaipur, India. While sampling, special attention was taken to procure colours manufactured from different parts of the country in sealed packets of various sizes as well as in loose form. The water-soluble colours and gulal were oven-dried at 60°C overnight and accurately weighed for further analysis. Sample (0.5 g) of each type of colours was digested in teflon tubes with nitric acid and hydrogen peroxide in the ratio 5 : 1. All samples underwent pressurized digestion in a microwave-heated system (SINEO microwave, China). The heating programme was conducted in three steps of different timescales [130°C for 10 min, 150°C for 5 min and 180°C for 25 min at pressure of 0.2 MPa; total time 40 min] and at constant power supply of 400 W. The final extract was filtered in 50 ml volumetric flasks and diluted to the mark with MilliQ water. The samples were digested along with the reagent blank in duplicates. Each sample was analysed thrice with ICP-MS (XSERIES 2, Thermo Scientific, USA), to detect and quantify the metals in the colour. ICP-MS was operated at radio frequency power supply of 1404 W, cool gas flow rate of 13 l/min, intermediate gas flow rate of 0.7 l/min, nebulizer gas flow rate of 0.98 l/min and dwell time of 10 ms/peak. For blank sample, a sample of colour was chosen in such a way that it contained minimal or negligible analyte (metal ion) concentration. The analyte concentration was deducted during every calculation so that the blank sample was only matrix without any analyte. This blank sample was marked as zero matrix sample. This blank sample was further spiked at six points covering the expected lower limit and now processed range, including LLOQ. For the calibration curves, these standard solution mixtures were diluted stepwise with 2% nitric acid and solutions for seven points, excluding the blank test solution were prepared so that the matrix included variation could be under control. Three replicates were prepared for each analyte concentration.

The content of heavy metals obtained from ICP-MS analysis (mg/l) was converted into mg/kg using Temminghoff and Houba's formula[12]

$$\frac{\{(a-b)\times v\}}{w},$$

where a is the concentration of the heavy-metal in the sample digest (mg/kg), b the concentration of the heavy metal in the blank digest (mg/l), v the total volume of the digest at the end of the digestion process (ml) and w is the weight of the colour samples (g). Counts were recorded and analyte concentration was calculated with Plasma lab software. Analysis of variance was carried out using SPSS (version 20) by one-way analysis of variance with Turkey's posthoc tests.

Several parameters were taken into consideration for the validation of Holi colours using ICP-MS, namely linearity, the limit of detection (LOD), limit of quantification (LOQ), minimum detection limit (MDL), accuracy, precision (inter-day repeatability at LOQ, 2XLOQ and 4XLOQ) and uncertainty. There are no stringent guidelines for validation of metals in Holi colours. Method validation using ICP-MS for wine and alcoholic beverages[13], tattooing pigments[14] and textiles[15] has been reported. Based on this background, a validation schedule was designed, according to International Conference on Harmonisation (ICH) guidelines[16]. The instrumental detection limit (DL) is the lowest concentration or the quantity of the analyte which can be measured by the instrument. DL was calculated by aspirating the reagent blank ten times and multiplying it by three times the standard deviation (SD) of the replicated measurements. MDL or LOD is the lowest concentration of the analytes in the matrix blank that can be measured with reasonable statistical certainty. LOD was calculated on the ten digested blanks and expressed as 3.3 times the SD of the replicated measurements divided by the slope of the calibration curve. LOQ was calculated on ten digested blanks and expressed as ten fold the SD of the replicated measurement divided by the slope of the calibration curve. Accuracy was evaluated by

Table 1. Method of validation for metals in Holi colours

Atomic mass of metals	Range (µg/l)	Calibration curve equation	R^2	DL (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)	RSD (%)	UM (%)
27Al	5–100	$y = 0.9971x + 0.192$	0.999	0.01	0.50	1.50	100.66	652.19	9.20
52Cr	0.5–60	$y = 1x + 0.001$	0.999	0.001	0.10	0.30	98.89	2.31	12.52
55Mn	0.5–60	$y = 1x - 0.0019$	0.999	0.001	0.10	0.30	99.57	7.62	7.53
57Fe	5–100	$y = 0.9936x + 0.4262$	0.999	0.005	0.50	1.50	99.97	837.97	11.76
59Co	0.5–60	$y = 1x + 0.0002$	0.999	0.001	0.10	0.30	99.57	0.76	10.28
60Ni	5–100	$y = 0.9959x + 0.2713$	0.999	0.001	0.50	1.50	99.83	0.19	4.14
65Cu	5–100	$y = 0.996x + 0.2698$	0.999	0.005	0.50	1.50	101.58	2.40	3.46
66Zn	5–100	$y = 1.0003x - 0.0198$	0.998	0.005	0.50	1.50	100.78	2.07	3.84
75As	0.5–100	$y = 1.0002x - 0.0136$	0.999	0.001	0.10	0.30	111.26	0.17	40.51
107Ag	5–100	$y = 0.9999x + 0.0047$	0.999	0.005	0.50	1.50	97.27	0.04	21.08
111Cd	0.5–100	$y = 1x - 0.0001$	0.999	0.001	0.10	0.30	99.83	0.01	47.50
202Hg	0.5–60	$y = 1x - 0.0002$	0.999	0.001	0.10	0.30	94.60	1.07	9.01
208Pb	0.5–60	$y = 1x + 0.0001$	0.999	0.001	0.10	0.30	103.39	4.77	3.10

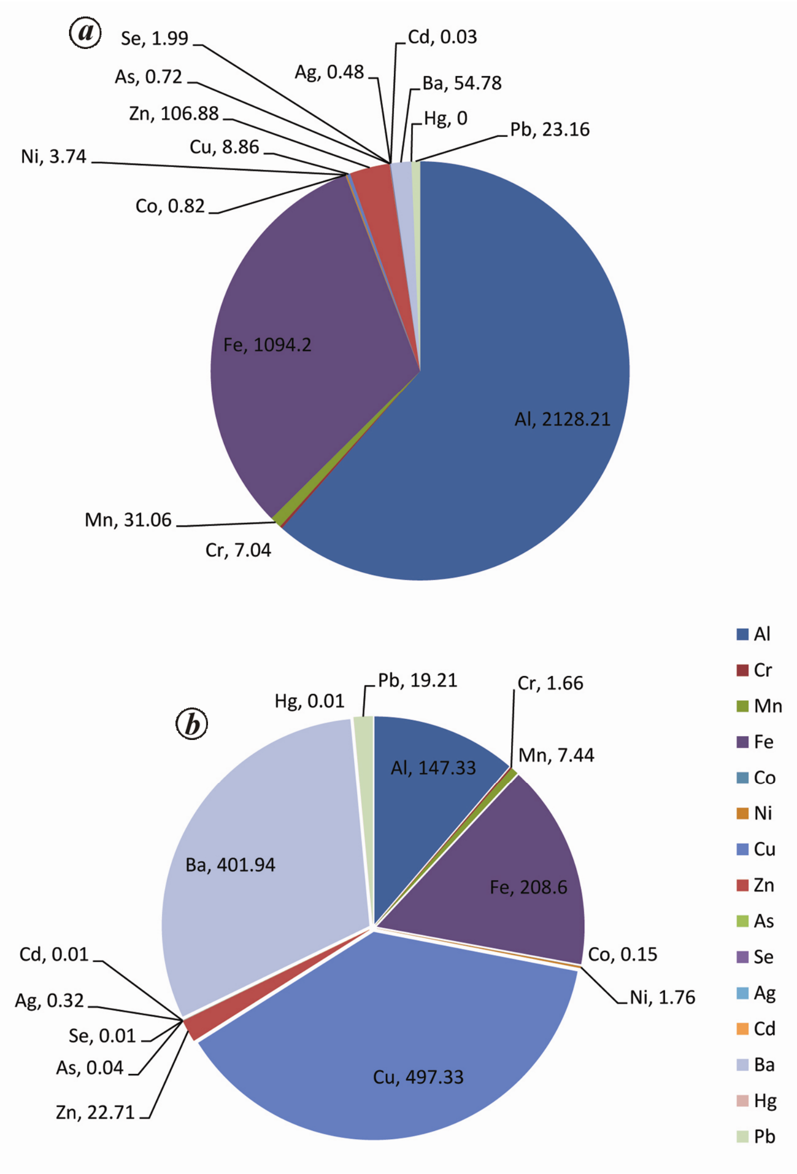

Figure 1. Diagramatic representation of heavy metal content (mg/kg) in (***a***) water-soluble Holi colour and (***b***) Gulal Holi colour.

conducting a recovery study on nine digested blanks at three spiked levels (three concentrations/three replicates each of the total analytical procedure) containing all the elements under study. Accuracy is reported as percentage recovery by the assay of known amount of the analyte in the sample. The acceptable criteria for the percentage recovery is between 70 and 120. Inter-day repeatablity was determined by analysing the digested colour sample ten times on three different days over a period of one week. Relative standard deviation (RSD) was calculated for the analysis of precision. The acceptable criterion for RSD is $\leq 20\%$.

The validation data for the analysis for colours by ICP-MS are summarized in Table 1. Using the calibration solution, a linearity curve, based on $y = ax + b$, was determined, where y is the signal intensity, and x the known concentration of the given analyte, b the intercept and a the slope. The linearity of the calibration curve was considered acceptable when the correlation factor $R > 0.999$ (Table 1). The DL ranged from 0.001 to 0.01 ug/l for various metals. The method showed the ability to distinguish between analytes measured and other substances. There was no matrix interference in the region of interest where the target analyte was expected to elute. Accuracy as percentage recovery ranged from 98.95 to 103.37, which was within the range 70–120. When inter-day repeatability was determined, RSD was found to be in the range 0.01–4.77%, except for aluminium and iron. All possible sources of uncertainty were taken into consideration. The extended uncertainty, UM ($k = 2$)

Table 2. Heavy metal content in the various colours of gulal and water-soluble Holi colours

Metals (mg/kg)	Gulal Holi colours									Water-soluble colour			
	Green	Light green	Purple	Orange	Red	Magenta	Yellow	Blue	Light Pink	Magenta	Bluish green	Orange	Purple
Al	74.4 ± 6.55	22.53 ± 0.21	57.61 ± 0.03	45.55 ± 2.28	43.01 ± 1.81	1.62 ± 1.17	2.72 ± 1.49	9.74 ± 0.21	28.34 ± 0.002	31.45 ± 6.09	1276.96 ± 5.30	839.74 ± 1.45	10.16 ± 0.022
Cr	0.6 ± 0.08	0.75 ± 0.93	0.84 ± 0.02	0.56 ± 0.46	0.60 ± 0.21	27.96 ± 1.25	0.03 ± 0.59	0.32 ± 0.024	0.3 ± 0.0034	0.36 ± 0.30	5.25 ± 1.54	2.48 ± 0.28	0
Mn	2.8 ± 2.01	1.09 ± 0.76	7.44 ± 0.066	3.51 ± 3.43	4.79 ± 2.29	0.41 ± 0.19	1.59 ± 1.26	1.03 ± 0.042	2.04 ± 0.024	1.3 ± 1.79	24.25 ± 1.51	8.06 ± 0.55	0.8 ± 0.001
Fe	85.4 ± 2.62	112.89 ± 0.01	126.99 ± 0.2	88.17 ± 5.48	90.21 ± 3.57	60.49 ± 2.55	5.2 ± 8.34	109.02 ± 0.6	85.18 ± 0.0045	52.87 ± 2.71	749.47 ± 3.30	781.98 ± 4.41	52.15 ± 0.023
Co	0.1 ± 0.02	0.12 ± 0.003	0.05 ± 0.07	0.06 ± 0.05	0.05 ± 0.06	0.03 ± 0.02	0	0.07 ± 0.03	0.33 ± 0.0032	0.02 ± 0.04	0.59 ± 0.34	0.915 ± 0.20	0
Ni	0.3 ± 0.15	1.76 ± 0.87	0.31 ± 0.011	0.74 ± 0.89	0.64 ± 0.29	0.61 ± 0.29	0	0.28 ± 0.001	0.6 ± 0.034	0.05 ± 0.15	2.38 ± 1.47	0.23 ± 0.05	0
Cu	167.1 ± 2.97	2.4 ± 0.04	11.53 ± 0.05	99.97 ± 1.61	90.88 ± 0.75	109.08 ± 4.56	0.06 ± 0.9	0.13 ± 0.012	0.28 ± 0.032	1.52 ± 0.81	6.51 ± 3.57	2.32 ± 0.08	1.86 ± 0.031
Zn	1.9 ± 1.21	22.71 ± 0.043	0.84 ± 0.043	8.25 ± 1.52	7.20 ± 5.9	8.5 ± 3.5	0.07 ± 0.61	1.76 ± 0.067	0.54 ± 0.056	53.81 ± 2.96	4.21 ± 2.43	2.125 ± 0.12	35.77 ± 0.0043
As	0	0 ± 0.055	0.01 ± 0.055	0.01 ± 0.01	0.006 ± 0.02	0.004 ± 0.001	0	0	0	0.35 ± 0.25	0.32 ± 0.19	0.18 ± 0.14	0.6 ± 0.0021
Ag	0.1 ± 0.06	0.32 ± 0.023	0.03 ± 0.022	0.14 ± 0.16	0.10 ± 0.069	0.11 ± 0.004	0	0.01 ± 0.01	0.03 ± 0.023	0.14 ± 0.26	0.13 ± 0.19	0.08 ± 0.05	0.08 ± 0.0072
Hg	0	0	0 ± 0.034	0	0.01 ± 0.01	0	0	0	0	0	0	0	0
Cd	0	0	0.01 ± 0.67	0.01 ± 0.01	0.006 ± 0.03	0	0	0.01 ± 0.01	0	0	0.02 ± 0.01	0.02 ± 0.014	0.03 ± 0.0082
Pb	9.8 ± 9.60	0.05 ± 1.53	1.53 ± 0.032	0.79 ± 3.73	3.46 ± 1.81	3.4 ± 1.6	0	0.02 ± 0.001	0.08 ± 0.004	0.11 ± 0.09	20.28 ± 14.40	5.72 ± 1.29	0.2 ± 0.71

represents an interval of 95% to the true value and is between 3.10% and 47.50% (Table 1).

This digestion method is suitable for the determination of volatile elements because the digestion of all powdered forms of colour is completed in a double-closed system. The results of the analysis revealed the presence of many toxic metals in various proportions in both water-soluble colours and gulal (Figure 1). A high concentration of aluminium was found in water-soluble colours in comparison to gulal. Aluminium is carcinogenic and responsible for skin allergies or discolouration, dermatitis and irritation of the mucous membrane[17,18]. Chromium, responsible for bronchial asthma and pneumonia[11,19], was more in water-soluble colours in comparison to gulal (Table 2). A significant amount of manganese, cobalt, iron, zinc, arsenic and selenium was noted in water-soluble colours compared to gulal. Nickel, reported to be responsible for causing dermatitis pneumonia[11], was found to be more predominant in water-soluble colours than in gulal. On quantification of cadmium in water-soluble colours, it was found to be slightly more in trace amounts in comparison to gulal. It has been reported that on accumulation of this toxic heavy metal in the body, weak and brittle bones may be formed[20]. Mercury was detected in trace amount in only gulal (0.01 mg/kg), whereas lead was found more in water-soluble colours (20.28 mg/kg) in comparison to gulal (1.53 mg/kg). Copper was found to be in high concentration in gulal (497.33 mg/kg) compared to water-soluble forms (8.86 mg/kg). On the basis of the comparative assessment of heavy metal content, it is evident that except for copper, silver and mercury, water-soluble colours contain more heavy metals in comparison to gulal (Figure 1 *a* and *b*).

In Holi colours, chemicals are introduced to get different shades. Both the dry and the soluable forms of Holi colours were used in the present study. Most of the water-soluble colours in their dry state were either slimy green or orange, but after dissolving in water, these were visibly catagorized into four types, magenta, bluish-green, orange and purple. On the basis of the solubility in water and new colour identification, 14 samples turned magenta, 4 turned bluish-green/Prussian blue, 2 turned orange and 2 samples turned purple after adding water. Among them, the bluish-green water-soluble sample colour showed maximum heavy metals content (Table 2). A great variability in the Al and Fe content existed among all the bluish-green, magenta and orange water-soluble samples and also among the green, orange and red-coloured gulal samples. The orange, red, magenta and green-coloured gulal samples showed high copper content and also great variability in the content existed within various samples of the same colours. As copper is responsible for eye allergy and temporary blindness[19], it is advisable that while playing with gulal, care should be taken to avoid the colour going into the eye.

The colourants in gulal contain heavy metals and are known as systemic toxins as they are not only deposited in the kidney, liver and bones, but are also capable of disrupting the metabolic functions[17]. Among all the colours tested (Table 2), it was concluded that gentian violet Holi colour showed maximum content for Al, Cr, Mn, Fe, As, Se, Cd and Pd. This colour has also been reported to cause skin discolouration, dermatitis and allergy or irritation of the mucous membrane[11,20]. Light-green Holi colour was tested and found to contain maximum concentration of cobalt, nickel, zinc and silver, whereas the green colour gulal showed maximum content of copper. Only red gulal colour showed presence of traces of mercury. It has been reported that this toxic metal can enter human body through the skin and by inhalation[11,19,20]. Compared to gentian violet and green Holi colour, less heavy metal content was quantified by ICP-MS in orange, red, magenta, blue and light pink gulal colours. There was a significant difference among the various colours ($P \geq 0.05$) of gulal and water-soluble Holi colours.

The present study is an approach to create consciousness and awareness on the impact of chemical colours. It could provide evidence to identify and quantify the presence of high concentration and also define trace amounts of metal contaminants in both gulal and water-based Holi colours. To maintain the colourful gaiety and fervour of Holi, an eco-friendly approach with organic Holi colours from vegetable-based dyes should be encouraged.

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