Inter-laboratory Comparison Programme on Sudan Dyes in Chilli Powder

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Summary

An inter-laboratory comparison programme (HKGL0904) for the determination of seven Sudan dyes in chilli powder was organized by the Government Laboratory of Hong Kong in 2009. The programme involved the quantitative determination of Sudan I and Sudan IV, and the identification of Sudan II, Sudan III, Sudan orange G, Sudan red 7B and dimethyl yellow in two test samples. HKGL0904 was one of the ad hoc studies offered to local testing laboratories to respond to some "hot issue" food contaminants. The primary objectives of the programme were to examine the capability of participants for the analysis of Sudan dyes in food matrices and to assist their preparation for an accreditation application. In total 11 local laboratories registered in the programme and 10 of them returned results. No unsatisfactory z-score for Sudan I or IV was detected on the basis of consensus values and Horwitz standard deviation in the assessment. Identification of the other five Sudan dyes was satisfactory although there was one false negative result. It was concluded that the overall performance of the participants was good.

Introduction

Reliable measurement is one of the essential prerequisites in establishing the global mutual recognition of analytical results that underpins international trade, commerce and regulation. To ensure good quality of data in chemical measurement and its application (or metrology in chemistry) the infrastructure involves the dissemination of measurement traceability and comparability to field laboratories by national metrology institutes (NMI) or designated institutes (DI) within the International Bureau of Weights & Measures (BIPM) system[1]. Furthermore, NMI's or DI's are responsible for devoting resources to strengthen the analytical capability of field laboratories in their respective regions. These aims can be achieved by the provision of advisory services, and co-ordination of technical seminars and inter-laboratory comparison programmes[2]. Being a DI since 2005 under the International Committee of Weights and Measures mutual recognition arrangement (CIPM-MRA), the Government Laboratory (GL) of Hong Kong has been upholding its roles to promote the important concept of chemical metrology to the testing communities in the territory[3]. Seminars and workshops on food analysis are regularly held as an effective mean of information exchange and sharing. For instance, a technical meeting in early September 2011 on phthalates in foodstuffs was organized for field laboratories and counterparts in academia and industry to address a recent incident originating in Taiwan.

In addition, international and local inter-laboratory comparison programmes such as malachite green in eels[4] and melamine in milk[5] have been organized. In view of the versatile function of inter-laboratory comparison programmes for validating testing methods[6], GL is dedicated to keeping this activity running in the future. However, inter-laboratory comparison schemes should be carefully planned in order to make good use of the limited available resources. As a consequence, local stakeholders are often consulted on what they really need and want from time to time. Sudan dyes, especially Sudan I and IV, were found to be priority target food contaminants during a survey conducted in 2008-2009. Most of the local interest in Sudan dyes mainly arose because of serious food incidents concerning Sudan dye contamination over the past decade and to further the intention for gaining third party accreditation.

Sudan dyes are classified as carcinogens by the International Agency for Research on Cancer (IARC) and should not be used in or added to foods. The first Sudan dye incident began with information reported by France through the Rapid Alert System for Food and Feed (RASFF) in May 2003, in which Sudan I was discovered in dried chilli products originating from India. A Commission Decision[7] was issued a month later and EU Member States were requested to test commodities for Sudan dyes so as to evaluate the extent of the problem. By the end

of 2003, a total of 119 cases were notified through RASFF[8]. In February 2005 Sudan I was detected in Worcester sauce manufactured in the United Kingdom and a nationwide recall of related food products was initiated. In November 2006 illegal use of Sudan IV in duck feeds intended to produce a high market value red egg yolk in many provinces in China was disclosed by the media. Consumers in Hong Kong were concerned as the majority of eggs and egg products are imported from China. The event was followed by extensive food monitoring and laboratory testing in the territory.

A variety of analytical techniques for Sudan dyes have been recently reviewed in the literature[9]. Common methodologies include HPLC with ultra violet detection[10], enzyme-linked immunosorbent assay[11] and LC-MS/MS[12] and are widely used by laboratories worldwide. This paper presents the results obtained from Hong Kong food laboratories in an inter-laboratory comparison programme (HKGL0904) for the determination of seven Sudan dyes in chilli powder.

Preparation of Sudan Dye Working Standards

Appropriate quantities of certified reference materials of Sudan I and IV (BW3524 and BW3527, National Institute of Metrology, China) were dissolved in acetonitrile. Four working standard solutions (WS) were prepared by diluting various aliquots of the solution with acetonitrile in 100 mL volumetric flasks. The final concentrations were 38.1 mg/L in WS1 and 46.9 mg/L in WS2 for Sudan I, and 50.3 mg/L in WS3 and 47.0 mg/L in WS4 for Sudan IV respectively. The same preparation procedure was applied to high purity chemicals of Sudan II, Sudan III, Sudan Orange G, dimethyl yellow (purchased from Sigma-Aldrich) and Sudan Red 7B (purchased from Riedel de Haen). The concentrations of Sudan II, and Sudan Red 7B were approximately 110 mg/L in WS5 and Sudan III, Sudan Red 7B and dimethyl yellow were 100 to 110 mg/L in WS6. From these six WS 20 to 50 mL aliquots of each were mixed together in different combinations and placed in two separate beakers containing about 2 L acetone to which were added the blank (uncontaminated) chilli powder.

Preparation of Test Materials

About 3kg of chilli powder was purchased from a local market and was confirmed to be free of target Sudan dyes using an accredited LC-MS/MS method. The bulk material was oven dried at 60°C overnight and divided into two equal portions, labeled as Sample I (dried weight = 1483.716 g) and Sample II (dried weight = 1333.220 g). Each portion of sample was independently mixed with known quantities of different mixed WS of Sudan dyes as described above. (The final nominal concentrations of Sudan dyes in Sample I and II are shown in Table 1.)

Table 1: Nominal of Sudan dyes in Chilli Samples

Analyte	Nominal Concentration (mg/kg)	
	Sample I	Sample II
Sudan I	0.987	0.580
Sudan IV	0.977	1.41
Sudan II	1.87	0
Sudan III	0	1.13
Sudan orange G	0	0
Sudan red 7B	1.89	1.25
Dimethyl yellow	0	1.15

The two slurries were individually thoroughly mixed in blenders for four hours with gentle stirring. The organic solvent in the mixtures were initially evaporated in a fume hood. The air dried samples were subject to further oven drying at about 80°C overnight before being loaded into a three dimensional rotating drum and mixed. Chilli powder, in about 50 g aliquots, was dispensed into fifteen clean amber glass bottles, gently purged with nitrogen, and capped immediately. The remaining bulk was bottled in 30 g aliquots. Thirty five bottles for each sample batch were eventually prepared, labelled and recorded. All sample bottles were stored at room temperature in a secure area.

Programme Protocol

Before the commencement of the programme a technical seminar on the history of the illegal use of Sudan dyes in food and the latest analytical methods, in particular LC-MS/MS, in foodstuffs was arranged for local testing laboratories in October 2009. The objective was to establish a forum to communicate with attendees the practical difficulties in Sudan dye analysis and to reinforce their analytical skills. Laboratories who were interested in participating in HKGL0904 were registered before 30 November 2009 and test materials were collected from the organiser in the first week of December 2009. Participating laboratories were each given a 50g sample of each of the trial chilli powders: Sample I (blue label) and Sample II (orange label). The approximate concentrations of the seven Sudan dyes were revealed to be in the range of 0.05 to 5 mg/kg. Participants were required to determine (i) the concentration of Sudan I and Sudan IV and (ii) qualitatively analyse for the presence of Sudan II, Sudan III, Sudan Orange G, Sudan Red 7B, and dimethyl yellow in the two test materials. Ten of the eleven laboratories (91%) returned results on or before the submission deadline of 10 January 2010

A standard result *proforma* (Appendix 1) was provided for data and information input. Analytical results for Sudan I and IV were requested in mg/kg to three significant figures or three decimal places, with the expanded measurement uncertainty and coverage factor stated. Identification of the other targets dyes was requested as, either positive, negative or below detection limit (specified). Technical details of methods used were also requested to be reported. Participating laboratories were allowed to use any validated test method normally used by them for the measurement of Sudan dyes for their customers.

High purity Sudan dye standards were also provided to participants if any of the standards were claimed to be unavailable to them.

Performance Assessment for Participants

Performance was evaluated using a common performance index, z-score, which was calculated as $(x_i - x) / \sigma_p$, where x_i was the mean value of the individual participant, x was the consensus mean obtained from participants' data using robust test procedures and σ_p was the proficiency standard deviation (in mg//kg) estimated from the Horwitz equation.

In general, those achieving $|z| \leq 2$ were interpreted as having produced results that are satisfactory and those having a z-score in the range $2 < |z| < 3$ are considered to have produced questionable results. Results are regarded as unsatisfactory if $|z| \geq 3$ and participants were requested to investigate the cause and take necessary follow up actions.

LC-MS/MS Method for Homogeneity and Stability Tests

Sample Treatment

About 5 g of well-mixed samples were weighed accurately into a 50 mL centrifuge tube. A 200 μL aliquot of mixed deuterated internal standard solution (Sudan I-d_5 and Sudan IV-d_6 from Sigma-Aldrich) at 200 mg/L and 40 mL of acetonitrile were added into the centrifuge tube and stoppered. The analytes were extracted by a 30-minute vigorous shaking of the tube and the organic solution was filtered through anhydrous Na_2SO_4 into a 100 mL volumetric flask. The extraction procedure was repeated using another 40 mL of acetonitrile with 60 minute sonication. The second portion of acetonitrile was filtered through anhydrous Na_2SO_4 into the same volumetric flask and the combined filtrate was made up to the mark with acetonitrile. An aliquot of 20 mL was taken into a 50 mL round bottom flask and the organic solvent was removed by rotary evaporation. The residue was reconstituted with 1 mL of 0.2% (v/v) formic acid in acetonitrile and was ready for LC-MS/MS analysis. Blank

samples and spiked samples used for quality control (QC) were prepared using the same procedures. All samples were kept in the dark before injection.

LC-MS/MS Conditions

Chromatographic separation was on a Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm) with a 25-minute gradient elution programme. Mobile phase consisted of 0.2% formic acid in water (A) and 0.2% formic acid in acetonitrile (B), and which varied from 30% of A and 70% of B to 5% of A and 95% of B, was delivered at a flow rate of 0.6 mL/min. Injection volume was 20 μL. Ionization voltage at 5500V, source temperature at 450°C, nebulizing gas, auxiliary gas and collision gas of the mass spectrometer (ABI 3200 Q-Trap) were optimized. Mass detections for the analytes were based on two multiple reaction monitoring (MRM) daughter ions in positive electrospray ionization mode. Operating conditions of MRM were obtained using a flow injection of 0.1 mg/L of respective standard solution to produce the optimal signal (Table 2).

Table 2: Setting of MRM's for Sudan Dyes

Analyte	Parent Ion (m/z)	Daughter Ion (m/z)	DP (V)	EP (V)	CE (V)
Sudan I	249	231	35	7	38
		93*	35	7	40
Sudan I-d_5	254	236	30	7	20
Sudan IV	381	156	50	7	40
		106*	50	7	50
Sudan IV-d_6	387	224	50	9	30
Sudan II	277	128	30	7	43
		121*	30	7	25
Sudan III	353	197	50	11	30
		128*	43	11	50
Sudan orange G	215	122	33	7	21
		93*	33	7	37
Sudan red 7B	380	183*	30	6	25
		169	30	5	35
Dimethyl yellow	226	133	40	7	40
		77*	40	7	33

* represents diagnostic ion
DP: Declustering Potential; EP: Entrance Potential and CE: Collision Energy

Measurement uncertainty for the determination of Sudan I and IV was estimated from the claimed uncertainty of the certified reference materials, variability (between-bottle) in the homogeneity test and recovery of spiked sample. Expanded uncertainties of the LC-MS/MS method at a confidence level of 95% were ±16.2% for Sudan I and ±16.1% for Sudan IV.

Homogeneity Test

Twelve bottles were randomly selected from both Sample I and II before the commencement of the programme in November 2009. Two sub-samples (#1 and #2) of 1g aliquots were taken from each bottle and determination of Sudan dyes was carried out using the above LC-MS/MS method in a randomized order. Overall results are presented in Table 3.

Table 3: Results (mg/kg) of Duplicate Analysis in Homogeneity Test

Sample No.	Sample I				Sample No.	Sample II			
	Sudan I		Sudan IV			Sudan I		Sudan IV	
	#1	#2	#1	#2		#1	#2	#1	#2
1	0.823	0.769	0.827	0.795	1	0.469	0.446	1.40	1.24
4	0.749	0.738	0.794	0.748	4	0.467	0.461	1.36	1.32
7	0.757	0.723	0.735	0.770	10	0.476	0.438	1.25	1.38
9	0.756	0.766	0.729	0.734	13	0.473	0.470	1.36	1.43
12	0.755	0.747	0.888	0.754	17	0.495	0.479	1.23	1.22
14	0.772	0.740	0.796	0.740	18	0.465	0.458	1.25	1.33
17	0.736	0.755	0.778	0.801	19	0.450	0.487	1.44	1.36
19	0.756	0.826	0.772	0.835	20	0.482	0.469	1.31	1.34
21	0.763	0.802	0.770	0.723	22	0.476	0.481	1.37	1.28
23	0.748	0.731	0.781	0.733	24	0.450	0.474	1.27	1.23
26	0.754	0.747	0.734	0.776	28	0.473	0.490	1.30	1.32
29	0.756	0.746	0.780	0.665	30	0.487	0.473	1.22	1.30
Mean	0.759		0.769		Mean	0.470		1.31	
SD, S_x	0.0255		0.0448		SD, S_x	0.0144		0.0665	
RSD (%)	3.6		5.8		RSD (%)	3.1		5.1	

Sudan II, Sudan III, Sudan Red 7B and dimethyl yellow were positively identified (S/N > 100) in all the duplicates in Sample I and II.

The homogeneity status was evaluated in according with the requirement stipulated in ISO/IEC 13528:2005[13]. The between-sample standard deviation (S_s) should not be greater than $0.3\sigma_R$ where σ_R is the performance standard deviation which was estimated using Horwitz equation of 2 x $C^{-0.15}$ where C is the mean concentration in mass fraction of Sudan I or IV from homogeneity test. S_s was derived from the standard deviation of sample average (S_x) and within-sample standard deviation (S_w) using Equation 1:

$$S_s = \sqrt{S_x^{\,2} - (S_w^{\,2}/2)} \qquad \text{Equation 1}$$

S_w was calculated from the absolute difference of the duplicates (w_t) and number of tested samples (n) as in Equation 2. Evaluation of the statistical parameters for homogeneity testing are summarized in Table 4.

$$S_w = \sqrt{\sum w_t^2}/(2n) \qquad \text{Equation 2}$$

Table 4: Statistical Evaluation of Homogeneity for Sudan I and IV

Statistical Parameter	Sample I		Sample II	
	Sudan I	Sudan IV	Sudan I	Sudan IV
C (mg/kg)	0.759	0.769	0.470	1.313
σ_R (%)	16.56	16.52	17.79	15.25
$0.3\sigma_R$ (%)	4.97	4.96	5.34	4.58
$0.3\sigma_R$ (mg/kg)	0.038	0.038	0.025	0.060
$\sum w_t^2$ (mg^2/kg^2)	0.0127	0.0492	0.00495	0.0805
S_w (mg/kg)	0.023	0.0453	0.0144	0.0579
S_x (mg/kg)	0.025	0.0448	0.0144	0.0665
S_s (mg/kg)	0.020	0.0314	0.0102	0.052

Since S_s of Sudan I and IV in Sample I and II were less than those of the corresponding value of $0.3\sigma_R$, the test materials were considered homogeneous and adequate for the inter-laboratory comparison programme.

Stability Test

The stability of Sudan dyes in Sample I and II at room temperature (about 25°C) and at an elevated temperature of 37°C was monitored on 14 January 2010, some days after receiving results from participants. One sample was randomly taken from each of the test materials and analysed in triplicate under the same operational conditions as those in the homogeneity test. The stability of Sudan I and IV was evaluated from the absolute difference between the mean value (C) obtained in the homogeneity test and the mean value (y_i) of each stability test. The absolute difference should not be more than $0.3\sigma_R$ (ie. $|C - y_i| \leq 0.3\sigma_R$). Results are tabulated in Tables 5 and 6.

Table 5: Stability Test for Sudan Dyes in Sample I at 25 and 37°C

Analyte	Concentration (mg/kg)					$\|C-y_i\|$	$0.3\sigma_R$	Status
	#1	#2	#3	y_i	C			
Sudan I	0.738	0.755	0.711	0.735	0.759	0.024	0.038	Pass
	0.725	0.747	0.718	0.730	0.759	0.029	0.038	Pass
Sudan IV	0.749	0.786	0.757	0.764	0.769	0.005	0.038	Pass
	1.25	1.35	1.30	1.30	0.769	0.010	0.038	Pass
Sudan II	+	+	+	NA	NA	NA	NA	NA
	+	+	+	NA	NA	NA	NA	NA
Sudan III	-	-	-	NA	NA	NA	NA	NA
	-	-	-	NA	NA	NA	NA	NA
Sudan orange G	-	-	-	NA	NA	NA	NA	NA
	-	-	-	NA	NA	NA	NA	NA
Sudan red 7B	+	+	+	NA	NA	NA	NA	NA
	+	+	+	NA	NA	NA	NA	NA
Dimethyl yellow	-	-	-	NA	NA	NA	NA	NA
	-	-	-	NA	NA	NA	NA	NA

Shaded regions represent results at 37 °C
+: positive identification, -: negative identification and NA: not applicable

Table 6: Stability Test for Sudan Dyes in Sample II at 25 and 37°C

Analyte	Concentration (mg/kg)					$\|C-y_i\|$	$0.3\sigma_R$	Status
	#1	#2	#3	y_i	C			
Sudan I	0.446	0.497	0.474	0.472	0.470	0.002	0.025	Pass
	0.481	0.479	0.487	0.482	0.470	0.012	0.025	Pass
Sudan IV	1.26	1.27	1.25	1.26	1.31	0.05	0.06	Pass
	1.26	1.26	1.26	1.26	1.31	0.05	0.06	Pass
Sudan II	-	-	-	NA	NA	NA	NA	NA
	-	-	-	NA	NA	NA	NA	NA
Sudan III	+	+	+	NA	NA	NA	NA	NA
	+	+	+	NA	NA	NA	NA	NA
Sudan orange G	-	-	-	NA	NA	NA	NA	NA
	-	-	-	NA	NA	NA	NA	NA
Sudan red 7B	+	+	+	NA	NA	NA	NA	NA
	+	+	+	NA	NA	NA	NA	NA
Dimethyl yellow	+	+	+	NA	NA	NA	NA	NA
	+	+	+	NA	NA	NA	NA	NA

Shaded regions represent results at 37 °C
+: positive identification, -: negative identification and NA: not applicable

Stability of Sudan I and Sudan IV in the test samples were found to meet the set requirement. Furthermore, the presence of Sudan II, III, Sudan Red 7B and dimethyl yellow in all samples indicated these dyes showed satisfactory stability covering the entire period of the programme from early October 2009 to mid-January 2010.

Results and Discussion

Participants' Performance

All the 10 participants submitted quantitative results on Sudan I and IV and qualitative results for other dyes in the two test materials (Table 7). Consensus robust mean values of Sudan I and IV were found to be respectively 1.048 mg kg^{-1} and 0.890 mg kg^{-1} in Sample I; and 0.626 mg/kg and 1.387 mg/kg in Sample II. The consensus values showed good agreement with the nominal values (Table 1), with deviations of -8.9% to 6.2% for Sudan I and -1.4% to 5.3% for Sudan IV respectively. The results demonstrated that the majority of the participants achieved good accuracy (based on the assumption that the nominal values were close to the "true" values) in the analysis of two Sudan dyes in chilli powder. Expanded measurement uncertainty of the consensus values was calculated in accordance with ISO/IEC13528[13] using Equation 3, where SDr is the standard deviation of the robust mean and p is the number of participants.

$$Mu = 2 \times (1.25 \times \frac{SDr}{\sqrt{p}}) \qquad \text{Equation 3}$$

The expanded uncertainty of the consensus values for Sudan I and IV were within 18% in this programme. These values only served as reference and would not be used in the performance assessments. Between-laboratory variation is a tool often used to express the actual dispersion or variability of data within a population. It was found to have a range from 15.2 to 23.0%. Based on these values, the precision of participants' data can be assessed using the Horrat value[14]. A Horrat value is expressed as the ratio of between-laboratory variation and the standard deviation estimated by the Hortwitz equation. In general, a Horrat value of 1 indicates the overall precision of the inter-laboratory comparison is satisfactory, but unsatisfactory for any value > 2. As shown in Table 7, the Horrat values for Sudan I and IV were respectively at 1.0 to 1.5 and 0.94 to 1.3, were considered to be reasonable.

Table 7: Participants' Results for Sudan Dyes in the Two Test Materials

Lab No.	Sample I (mg/kg)							Sample II (mg/kg)						
	S2	S3	OG	7B	DY	S1	S4	S1	S4	S2	S3	OG	7B	DY
1	+	-	ND	ND	-	0.68	0.88	0.54	1.46	-	+	ND	ND	+
2	+	-	-	+	-	0.90	0.90	0.53	1.32	-	+	-	+	+
3	+	-	-	-	-	1.25	0.810	0.745	1.13	-	+	-	+	+
4	+	-	ND	ND	ND	0.975	0.558	0.546	0.816	-	+	ND	ND	ND
5	+	-	-	+	-	1.08	0.995	0.602	1.53	-	+	-	+	+
6	+	-	-	+	-	1.127	0.838	0.705	1.243	-	+	-	+	+
7	+	-	-	+	-	0.88	0.76	0.62	1.90	-	+	-	+	+
8	+	-	-	+	-	1.27	1.04	0.674	1.25	-	+	-	+	+
9	+	-	-	+	-	1.512	1.066	0.812	1.601	-	+	-	+	+
10	+	-	-	+	-	0.90	0.91	0.52	1.56	-	+	-	+	+
Robust Mean (mg/kg)						1.03	0.89	0.61	1.39					
Expanded uncertainty (mg/kg)						0.19	0.11	0.085	0.21					
Between-lab Variation (%)						23.0	15.2	17.2	19.0					
Horwitz SD (%)						15.8	16.2	17.0	15.1					
Horrat value						1.5	0.94	1.0	1.3					

S1 to S4: Sudan I to IV, OG: Sudan orange G, 7B: Sudan red 7B, DY: Dimethyl yellow
+: positive identification, -: negative identification and ND: Not determined.

In the qualitative analysis, Lab. #2 did not report for Sudan Orange G and Sudan Red 7B; and Lab. #4 did not report for Sudan Orange G, Sudan Red 7B and dimethyl yellow. Lab. #3 gave one false negative result for Sudan Red 7B in Sample I. Apart from that, the identification for these five Sudan dyes was acceptable (Table 8).

Table 8: Qualitative analysis of Sudan Dyes

Analyte	Correct Identification	
	Sample I	Sample II
Sudan II	10 out of 10	10 out of 10
Sudan III	10 out of 10	10 out of 10
Sudan orange G	8 out of 8	8 out of 8
Sudan red 7B	7 out of 8	8 out of 8
Dimethyl yellow	9 out of 9	9 out of 9

Analytical Methods Used by Local Testing Laboratories

Two participants (# 1 and 3) used LC-UV, seven used LC-MS/MS, and one (#4) used both techniques for the quantitative and qualitative analyses. All the LC systems were quite standard with reversed-phase C_8 or C_{18} analytical columns using acidic mobile phase of high polarity (acetonitrile, methanol or acetone in formic/acetic acids) under gradient elution programmes. Quantification was reported to rely on external calibration or standard addition. Five participants (#2, 5, 6, 8 and 10) employed isotopically-labelled internal standards such as Sudan I-d_5 and Sudan IV-d_6 for recovery correction.

Owing to high solubility of Sudan dyes in common organic solvents, extraction performed by participants mainly involved simple shaking or sonication of the samples with acetonitrile, acetone or n-hexane. Extraction duration varied from 10 to 90 minutes and the extraction efficiency was reported ranging from 50 to 120%, which was independent of the extraction time. Two participants (#1 and 4) used solid phase extraction (SPE) as an additional clean-up procedure.

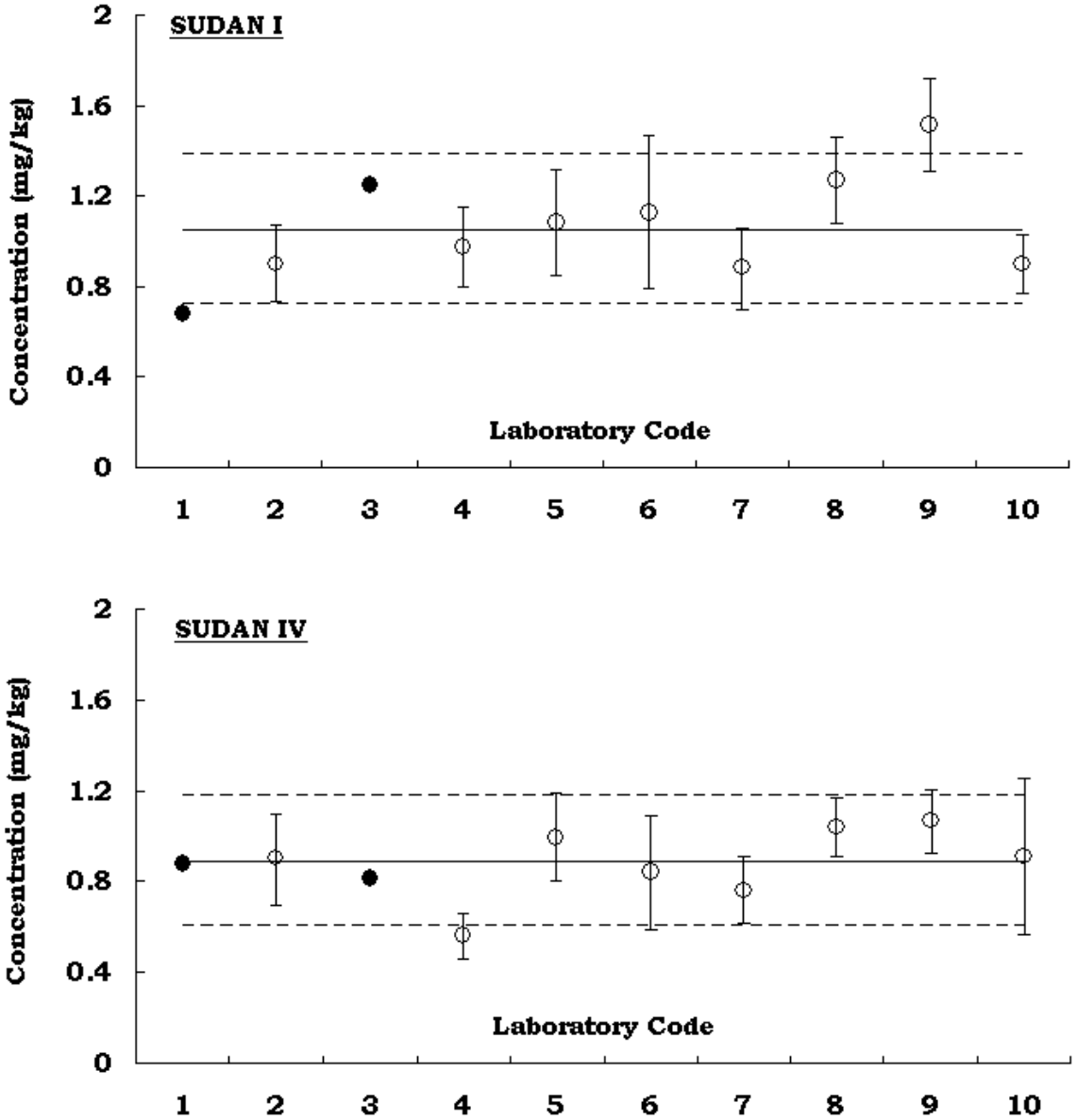
Accreditation status was claimed only by participants #2 and #10 for qualitative analysis of the seven Sudan dyes; none of the quantification methods had been accredited at the time of the programme. Since an uncertainty estimation is one of the vital criteria for validated testing methods under ISO/IEC 17025[15], participants were required to submit their uncertainty budgets for Sudan I and IV in order to prepare for future accreditation. The expanded uncertainties at a coverage factor (k) of 2 reported were mostly in the range of 10 to 30%, which agreed with the LC-MS/MS method for Sudan dyes developed in our laboratory. However, the two LC-UV participants (participants #1 and #3) did not provide such information in this programme.

Participants' z-Scores

Distribution of participants' data with the associated uncertainty are shown in Figures 1 and 2. The z-scores for Sudan I and IV were evaluated as a mean to assess the performance of participants from the data pool. In brief, there were 5 questionable results identified from the four participants (Lab #1, 4, 7 and 9) but no unsatisfactory results ($|z| \geq 3$) were returned (Figure 3 and 4).

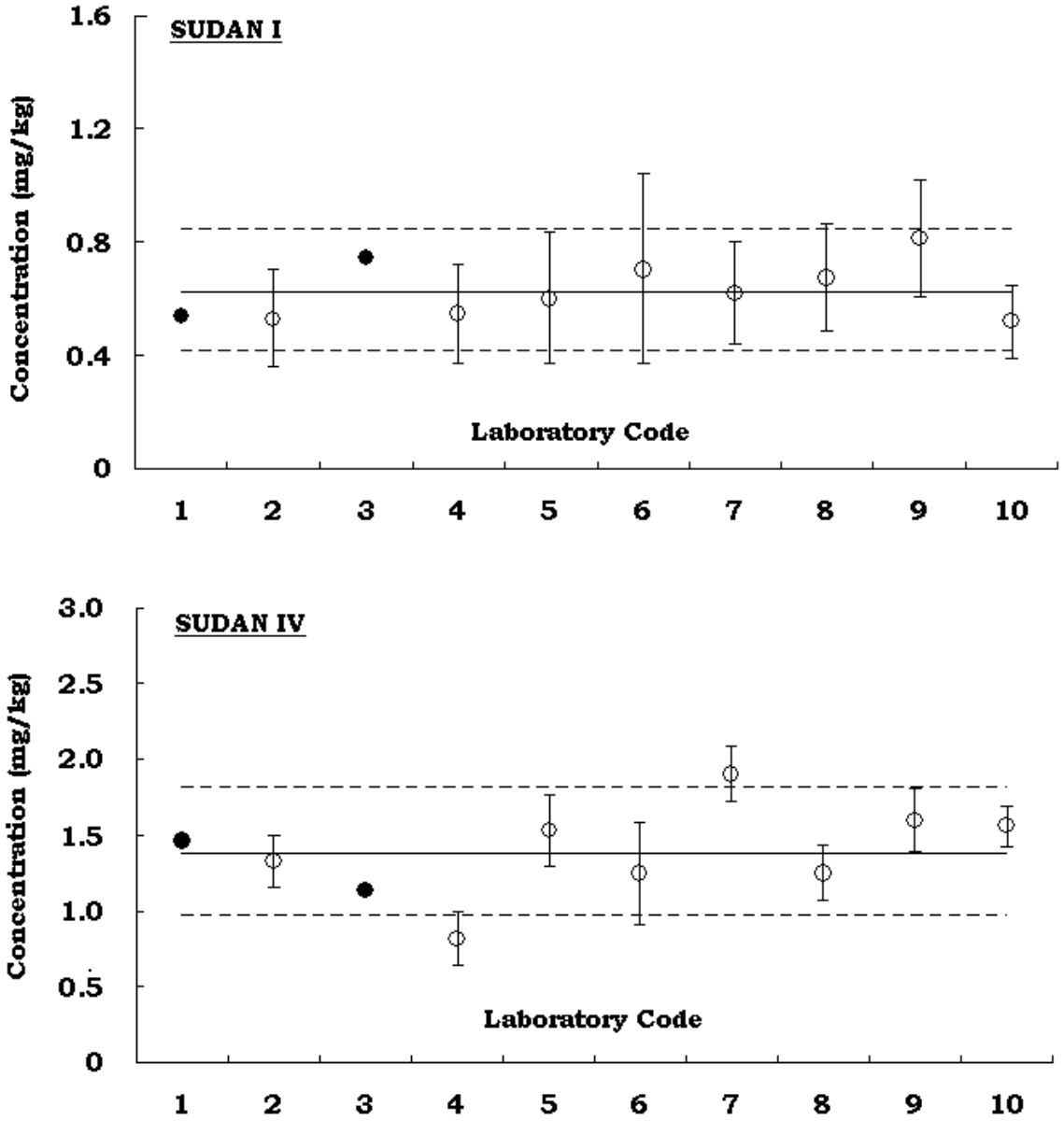
Judging from the Horrat and z-score assessments, the overall performance of the quantitative analysis of Sudan dyes in chilli powder from the ten local testing laboratories was satisfactory. The consensus mean values were in good agreement with the nominal gravimetric values and no unsatisfactory z-scores were identified. There was no significant bias between the results obtained by LC-UV and LC-MS/MS methods. In the qualitative tests, one participant gave a false negative result for Sudan Red 7B and was required to investigate the problem and to find out the cause before providing the service to their customers. The present inter-laboratory comparison programme, HKGL0904, successfully provided an external quality control mechanism to improve the testing capability of local testing laboratories in food analysis.

Figure 1: Reported concentration of Sudan I and IV in Sample I



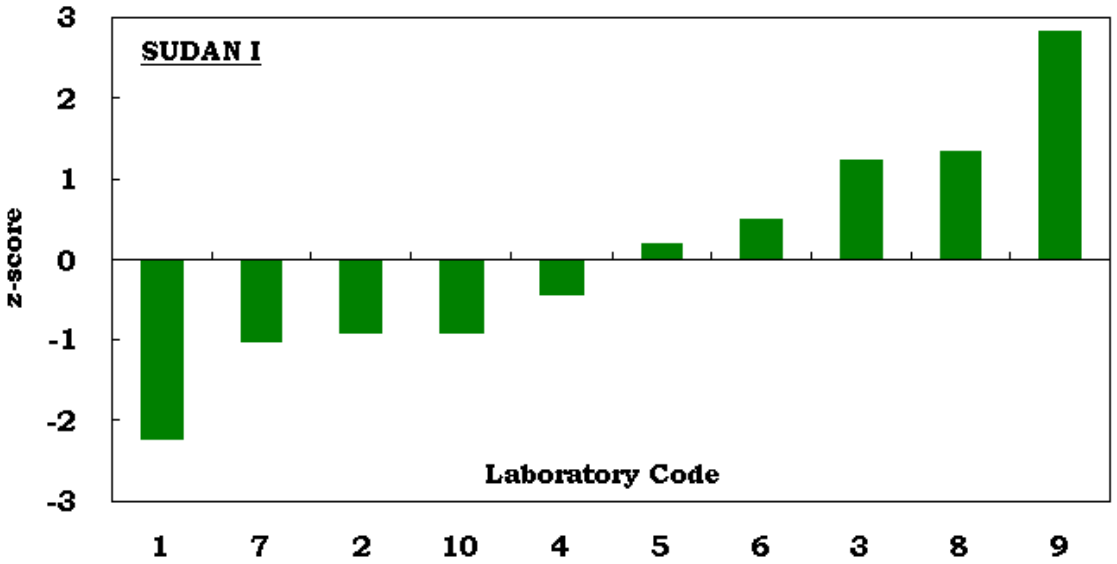
Solid lines represent the consensus values, dotted lines represent |z|= 2 and error bars represent reported expanded uncertainty. Solid circles indicated laboratories did not provide measurement uncertainty information.

Figure 2: Reported concentration of Sudan I and IV in Sample II



Solid lines represent the consensus values, dotted lines represent |z|= 2 and error bars represent reported expanded uncertainty. Solid circles indicated laboratories did not provide measurement uncertainty information

Figure 3: Participants' z-scores for Sudan I and IV in Sample I



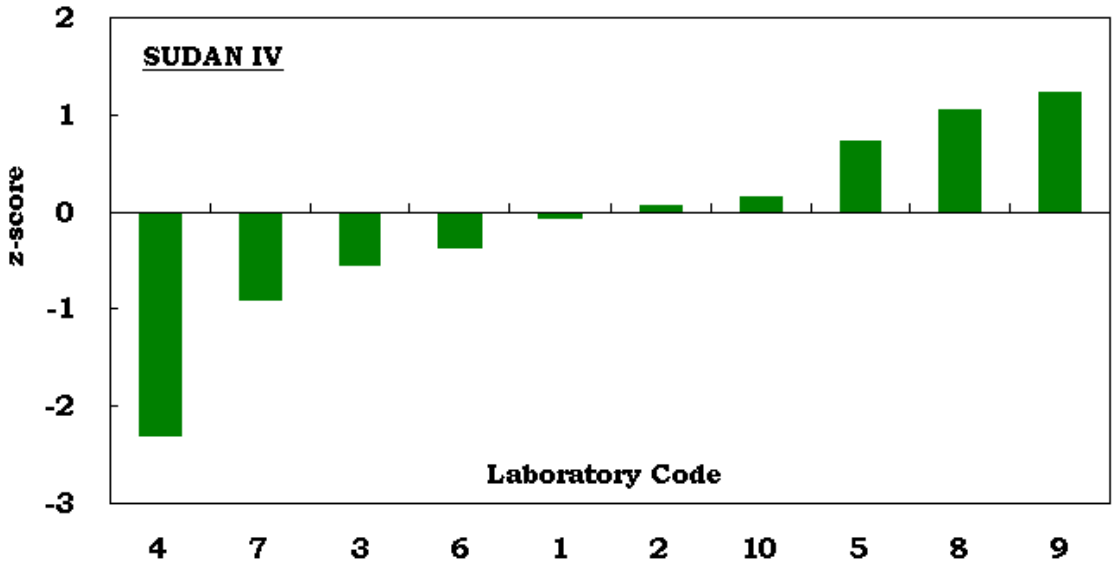
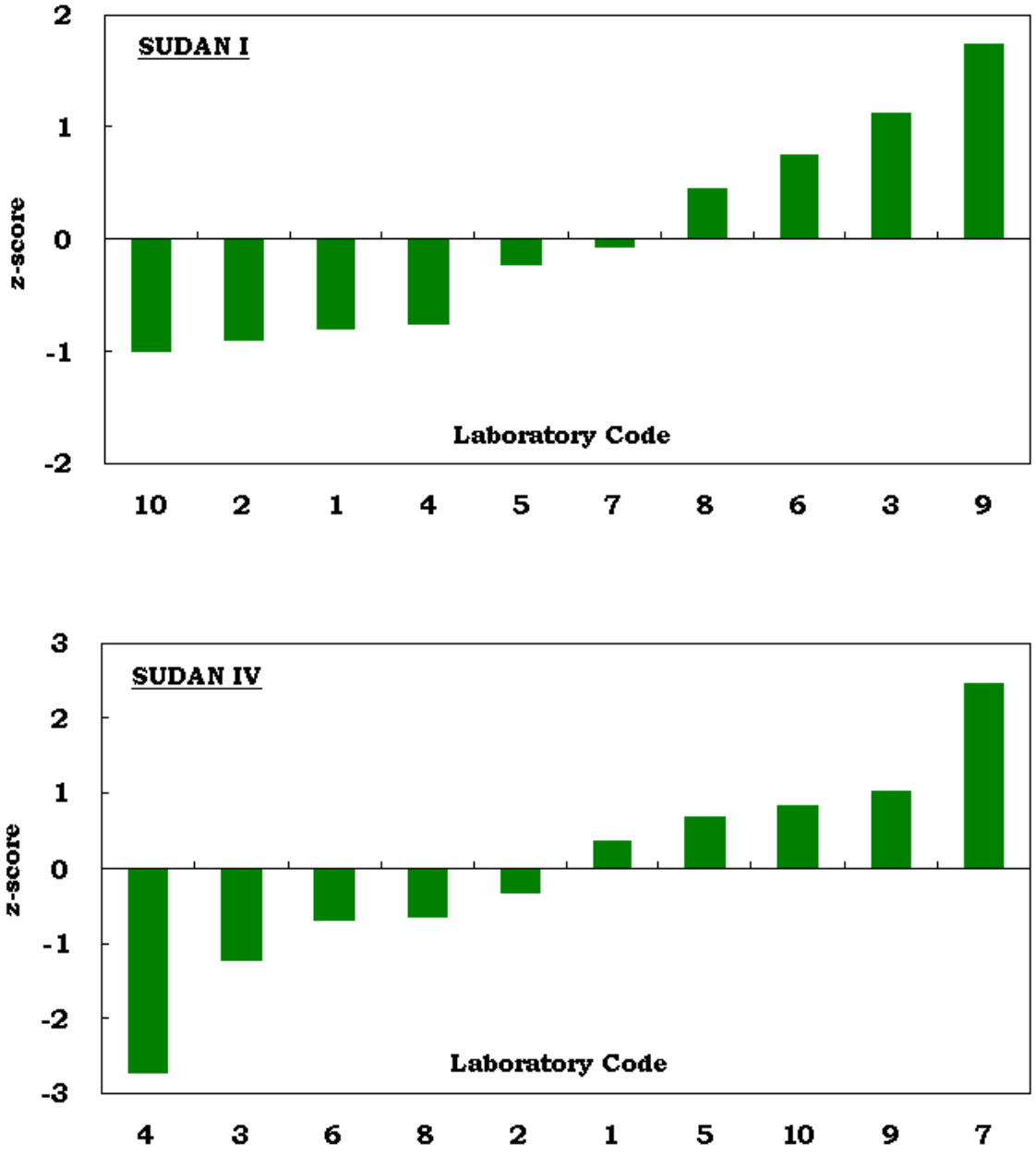


Figure 4: Participants' z-scores for Sudan I and IV in Sample II



Conclusion

An inter-laboratory comparison programme is well recognized as serving a dual instructional/policing role in measurement science. Therefore, it is an invaluable mechanism to improve testing competency as well as to identify problems. HKGL0904 is a fit-for-purpose example that successfully demonstrates this tool in assisting local participating laboratories.

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Appendix 1: Result Proforma in HKGL0904

Result Proforma

<Both Part I & II MUST be completed by Participating Laboratory>

Laboratory Code: (official use)
Name of Participating Laboratory:

_____

Contact Person / Email: _____

Signature: _____

Part I: Analytical Results

	Quantitative Analysis of Sudan Dyes (in mg/kg)					Measurement Uncertainty	
	Dye	**#1**	**#2**	**#3**	**Mean**	**MU (mg/kg)**	**Coverage Factor (*k*)**
Sample I	**Sudan I**						
	Sudan IV						
Sample II	**Sudan I**						
	Sudan IV						

Notes:

- If value determined is less than the limit of quantification (LOQ), please specify, eg. < 0.1 mg/kg
- Report values to a maximum of 3 significant figures or 3 decimal places, whichever is appropriate
- It is the responsibility of the participants to avoid collusion and falsification of result so as to ensure a reliable assessment to be made in this interlaboratory programme. Information on the identities and results of the participating laboratories will be kept confidential and will not be disclosed to unauthorized parties by the organizer.

Dyes	**Qualitative Analysis of Sudan Dyes**	
	Sample I	**Sample II**
Sudan II		
Sudan III		
Sudan Orange G		
Sudan Red 7B		
Dimethyl Yellow		

+ for Detected, " – " for not Detected or < MDL (please specify)
Please submit this Result Proforma electronically to the organizer at GL0904@govtlab.gov.hk

Part II: Method Information

1. *Analytical Instrument: LC-UV / LC-MS / LC-MS/MS / GC-MS / ELISA / CE
Others (pls. specify):

*Derivatization: YES ;NO
(pls. specify)

2. Chromatographic column: *eg. C_{18} (150mm x 2.1mm, 5□m) or DB 5 (30m x 0.25mm x 0.25□m)*

3. Mobile phase(s):
or Carrier gas

4. *Calibration: External cal. curve; One point cal.; Standard addition;
Others (pls. specify):

5. *Internal standard(s) YES (pls. specify)
; NO

6. Extraction solvent(s):

7. *Extraction technique: Agitation; Shaking; Ultrasonic; Soxhlet; A SE; SPE

Others:
(please specify)

8. Extraction duration: hrs.
min.

9. Clean-up procedure

10. Recovery (%)

11. *Correction for recovery YES; NO

12. *Method accredited: YES; NO

13. Other additional info:

* Please circle as appropriate