



Analytical Method Development and Validation of Iron, Arsenic, Cadmium, Mercury and Lead Content in Sunset Yellow by Inductively Coupled Plasma Mass Spectrometry (Icp-MS)

Ravi Parimi^{1*}, T Rattaiah Gupta¹, K. E. Pravallika¹, Saravanan Ravindran²

¹University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur -522510, A.P, India.

²Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Selaiyur, Chennai 600073, Tamil Nadu, India.

*Corresponding author's E-mail: parimiv@gmail.com

Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 23 Nov 2023	A method for determining the iron, arsenic, cadmium, mercury, and lead content in Sunset Yellow by ICP-MS was developed, verified, and implemented for routine ICP-MS analysis. Inductively coupled plasma mass spectrometry (ICP-MS) is a good instrument for determining the basic composition of numerous substances. Iron, arsenic, cadmium, mercury, and lead metal as defined in USP (233) linearity, method precision, accuracy, robustness, and ruggedness factors were taken into account and analysed for method validation in this work. The recovery rates of iron, arsenic, cadmium, mercury, and lead metals ranged between 92.0% and 101.4%. The detection limits of all elements investigated demonstrated the procedure's suitability for routine analyses. To summarize, the provided analytical processes can be used to measure the mass fractions of iron, arsenic, cadmium, mercury, and lead components in Sunset Yellow samples.
CC License CC-BY-NC-SA 4.0	Keywords: ICP-MS, Element analysis, Method validation, Lead, Sunset Yellow

1. Introduction

Sunset yellow is seen in foods, contraceptives, cosmetics, and pharmaceutical drugs. Sunset yellow FCF is a yellow-orange dye or orange colour. It is used in a variety of products, including candies, confectionery, snacks, sauces, and preserved fruits¹. To create a brown colouring in chocolates and caramel, E123, amaranth, and sunset yellow are frequently used in combined form.

Instruments and Materials

Table 1 Instruments/Apparatus used for study

S. No	Instruments and Equipments	Company	Model	Software
1.	Weighing balance	Shimadzu	AY 200	-
2.	ICP-MS	Agilent	7800	Mass Hunter
3.	Glass ware	Borosilicate	Class A	-

Table 2 Chemicals used for study

S. No	Chemicals and Reagents	Grade	Manufacturer
1.	Iron, arsenic, cadmium, mercury and Lead	NIST	Agilent Technologies
2.	Iron	NIST	Agilent Technologies
3.	Arsenic	NIST	Agilent Technologies
4.	Cadmium	NIST	Agilent Technologies
5.	Mercury	NIST	Agilent Technologies
6.	Water	LC/MS Grade	Fluka
7.	Nitric Acid	Trace metals	Merck
8.	Hydrochloric Acid	Trace metals	Merck

Instrumental conditions:

Plasma gas flow 15.00 l/min

Mode: He Gas
Plasma voltage: 1550 watts
No of sweeps: 100
No of replicates: 3
Energy discrimination 3 volts
Acid used: Nitric Acid and Hydrochloric Acid

Preparation of standard Solutions²

Preparation of internal standard solution

Transferred 0.4 ml of 100 ppm Bismuth, germanium, indium, scandium, terbium and yttrium solution into 20 ml volumetric flask added 3.2 ml of nitric acid, and make up to volume with water.

Preparation of intermediate stock solution (ISS):

Transferred 10 ppm of each 0.260 ML of Arsenic, Cadmium, mercury, 0.5ml of Iron, arsenic, cadmium, mercury and Lead stock solution and 0.250 ml of iron 1000 ppm stock solution into 20 ml volumetric flask added 3.2ml of nitric acid and 0.8 ml of hydrochloric acid then diluted to 20 ml with water.

Preparation of calibration curve standards (linearity Standard solutions):

Preparation of calibration blank:

Accurately transferred 4 ml of nitric acid, 1 ml of hydrochloric Acid and 0.5 ml of internal standard solution into 25 ml volumetric flask and make upto volume with water.

Preparation of 30% Solution:

Transferred 0.3 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 50% Solution:

Transferred 0.5 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 100% Solution:

Transferred 1.0 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 150% Solution:

Transferred 1.5 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 200% Solution:

Transferred 2.0 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Sample Preparation:

Weighed and transferred 125 mg of sample into 25 ml volumetric flask and added 4ml of nitric acid 1 ml of hydrochloric acid and 0.5 ml of internal standard solution and make up to volume with water.

Procedure for estimation of Iron, arsenic, cadmium, mercury and Lead content in Sunset yellow:

Injected six replicates 100% standard solution into ICP-MS system for system suitability and for estimation of Iron, arsenic, cadmium, mercury and Lead content in Sunset yellow was injecte calibration curve standards and sample solution

Calculation ppm with respect to test concentration:

Calculation ppm of alanyte = $\frac{\text{instrument calculated Conc (in ppb)} \times \text{make up Volume (mL)}}{\text{Sample weight in mg}}$

Method Validation

The proposed method for the estimation of Iron, arsenic, cadmium, mercury and Iron, arsenic, cadmium, mercury and Lead content in Sunset yellow was subjected to validation to ensure their suitability for regular analysis³.

System Suitability

Many analytical processes depend on the quality of the System suitability assessment once the efficiency of an instrumental condition has been maximized. The idea behind the testing is that the tools, electronics, processes involved in the analysis, and the materials to be examined all work together as a cohesive system that is capable of being assessed as such⁴. In all cases, the relative standard deviation (R.S.D) for the target analyte was not more than 15% and sample was injected 40 sec with constant speed of peristaltic pump 3.5 revolutions per minute. Recorded the system suitability test was performed using six replicate injections of standards before analysis of samples. System suitability parameters were shown in Table: 3.

Linearity:

In order to show linearity for the target analyte using a single plot, a number of drug material standard solutions were produced in the concentration range of 25% to 200% of test concentration and injected into the system⁵. The relationship between concentration level (ppb) and counts per second is represented on a calibration graph.

Preparation of calibration curve standards (linearity Standard solutions):

Preparation of calibration blank:

Accurately transfer the 4 ml of nitric acid, 1 ml of hydrochloric Acid and 0.5 ml of internal standard solution into 25 ml of volumetric flask and make up to volume with water.

Preparation of 30% Solution (150ppb of Iron, 1.56 ppb of Arsenic, Cadmium, Mercury and 3.0 ppb of Iron, arsenic, cadmium, mercury and Lead):

Transferred 0.3 ml of intermediate stock solution into 25 ml volumetric flask added 4ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 50% Solution (250ppb of Iron, 2.6 ppb of Arsenic, Cadmium, Mercury and 5.0 ppb of Iron, arsenic, cadmium, mercury and Lead):

Transferred 0.5 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 100% Solution (500ppb of Iron, 5.2 ppb of Arsenic, Cadmium, Mercury and 10.0 ppb of Iron, arsenic, cadmium, mercury and Lead):

Transferred 1.0 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 150% Solution (750ppb of Iron, 7.8ppb of Arsenic, Cadmium, Mercury and 15.0 ppb of Iron, arsenic, cadmium, mercury and Lead):

Transferred 1.5 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Preparation of 200% Solution (1000ppb of Iron, 10.4 ppb of Arsenic, Cadmium, Mercury and 20.0 ppb of Iron, arsenic, cadmium, mercury and Lead):

Transferred 2.0 ml of intermediate stock solution into 25 ml volumetric flask added 4 ml of nitric acid and 0.5 ml of internal standard solution then diluted to 25 ml with water.

Precision:

System precision:

The system precision was established by injecting six replicate injections of 100 % standard solution in to the system by maintaining the optimized instrumental conditions⁶. Results of system precision studies were shown in Table: 3.

Method precision:

Six samples of drug product spike at 100% level of the working sample concentration were prepared and injected into the system⁷. Results of method precision studies were shown in Table: 4

Accuracy:

Accuracy was performed by following standard addition method. In this standard was added to pre-analyzed sample solution.

Preparation of 50% of standard addition sample:

Weighed and transferred 125 mg of sample into 25 ml volumetric flask and added 4ml of nitric acid, 1 ml of hydrochloric Acid 0.5 ml of intermediate stock solution and 0.5 ml of internal standard solution and make up to volume with water.

Preparation of 100% of standard addition sample:

Weighed and transferred 125 mg of sample into 25 ml volumetric flask and added 4ml of nitric acid, 1 ml of hydrochloric Acid, 1.0 ml of intermediate stock solution and 0.5 ml of internal standard solution and make up to volume with water.

Preparation of 150% of standard addition sample:

Weighed and transferred 125 mg of sample into 25 ml volumetric flask and added 4ml of nitric acid, 1 ml of hydrochloric Acid 1.5 ml of intermediate stock solution and 0.5 ml of internal standard solution and make upto volume with water.

Procedure:

Sample solutions prepared separately by addition of standard stock at 50% and 150% of working sample concentration were injected three times and sample solution prepared separately by addition of standard stock at 100% was injected three times into the chromatographic system⁸.

$$\% \text{ Recovery} = [(C_t - C_{pa}) / C_s] \times 100$$

Where C_t = Total concentration of analyte

C_{pa} = Concentration of pre-analysed sample

C_s = Concentration of standard added to pre-analysed sample.

Recovery studies were shown in Table: 6

Limit of detection and limit of quantitation:

The LOD and LOQ were calculated based on the Linearity of the elements. The LOD and LOQ were calculated as per the $LOD = 3.3 \times SD/S$ & $LOQ = 10 \times SD/S$

Where SD = standard deviation,

S= slope of the calibration curve, were given in Table: 4.

Robustness:

The robustness study was performed to evaluate the influence of small but deliberate variation in the instrumental condition. The Robustness was performed at plasma gas flow rates and peristaltic pump Rotations per minute by using linearity solutions of Iron, arsenic, cadmium, mercury and Lead⁹.

Robustness values for change in plasma gas flow rates and peristaltic pump Rotations per minute were given in Table: 7.

Table: 3. System Suitability Results

S No	CPS of Iron	CPS of Arsenic	CPS of Cadmium	CPS of Mercury	CPS of Lead
1	113296.845	1037.653	1047.953	1039.053	2010.823
2	113256.674	1039.691	1043.197	1043.691	2009.731
3	114987.786	1037.742	1047.653	1047.738	2012.423
4	111566.842	1038.731	1042.031	1042.731	2011.323
5	112167.412	1038.912	1049.112	1040.912	2008.312
6	114701.651	1038.751	1043.751	1039.751	2010.632
Mean	113329.535	1038.580	1045.616	1042.312	2010.540
% RSD	1.19	0.07	0.28	0.31	0.07

Acceptance Criteria: The % RSD for the Counts per seconds of six injections results should not be more than 15.0 %.

Table: 4. Calibration curve/Linearity:

S. No.	Con of Iron	CPS of Iron	Con of Arsenic	CPS of Arsenic	Con of Mercury	CPS of Mercury	Con of Cadmium	CPS of Cadmium	Con of Lead	CPS of Lead
1	150	33652.325	1.56	310.256	1.56	315.235	1.56	321.568	3	556.238
2	250	58083.712	2.6	519.456	2.6	520.456	2.6	521.456	5	1004.57
3	500	112167.41	5.2	1038.91	5.2	1049.11	5.2	1040.91	10	2008.31
4	750	165251.13	7.8	1558.37	7.8	1561.24	7.8	1560.37	15	3012.57
5	1000	221334.82	10.4	2098.22	10.4	2080.24	10.4	2080.82	20	4018.24
Correlation		0.9999		0.9999		1.000		1.000	-	0.9999
Slope		223.2		201.7		199.7		199.4		202.6
Intercept		1003.31		7.090		4.19		5.65		28.43
LOD		34.83		0.09		0.09		0.23		1.13
LOQ		114.94		0.30		0.3		0.76		3.73

Acceptance Criteria: Correlation coefficient should be not less than 0.99

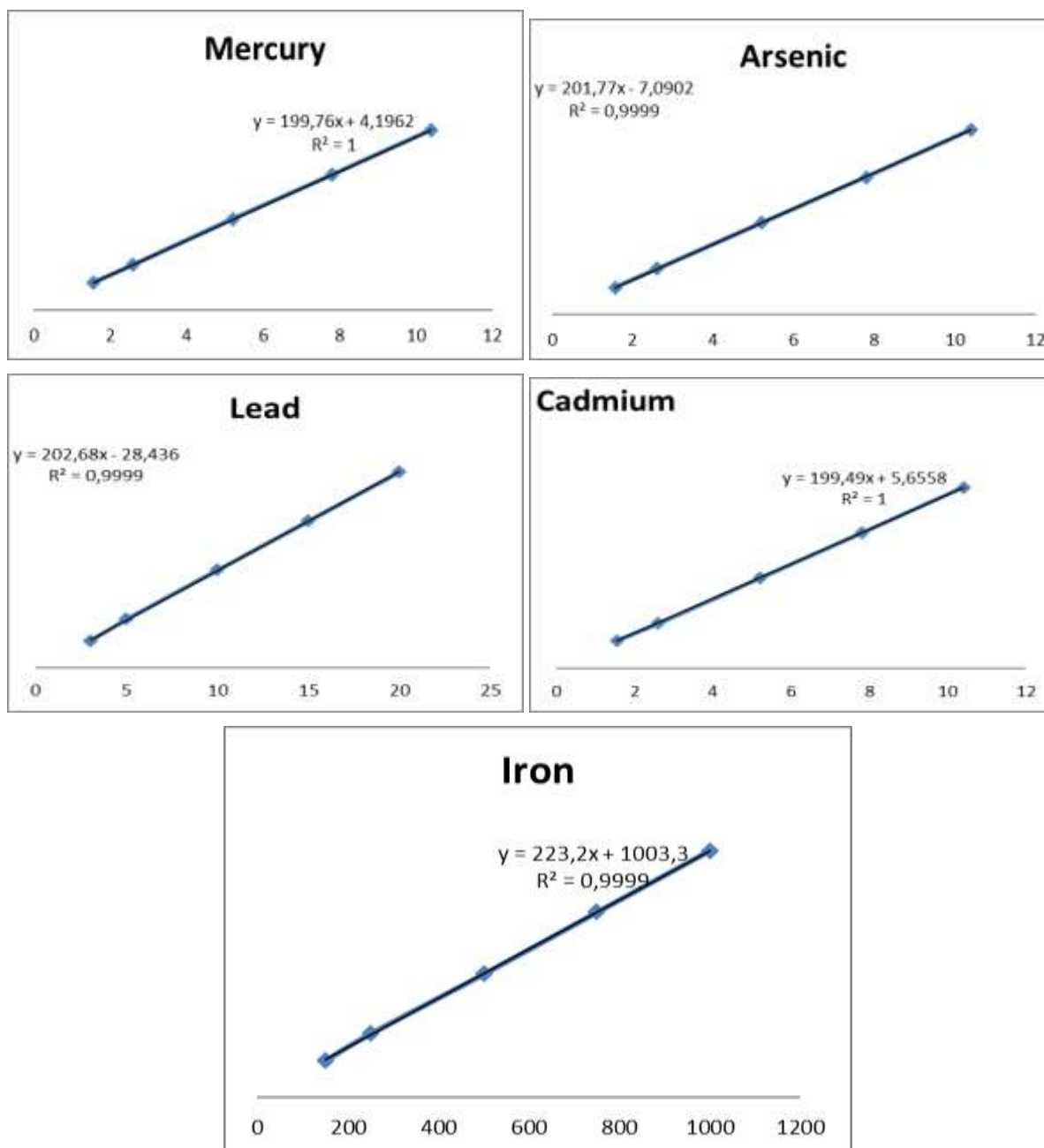


Table 5: Method Precision

S. No.	Con of Iron	% recovery	Con of Arsenic	% recovery	Con of Mercury	% recovery	Con of Cadmium	% recovery	Con of Lead	% recovery
1	103701.781	91.7	1382.587	133.1	1161.250	111.1	1037.490	99.5	1981.045	98.5
2	101352.872	89.3	1369.839	131.9	1121.970	107.3	1027.196	98.6	1963.126	97.6
3	100098.125	88.1	1380.597	132.9	1122.239	107.3	1050.207	100.8	1942.051	96.6
4	100830.554	89.2	1388.583	133.7	1131.519	108.3	1042.459	100.0	1945.056	96.7
5	101504.767	89.8	1386.888	133.5	1111.773	106.3	1024.924	98.4	1978.324	98.4
6	100915.965	89.2	1381.110	132.9	1108.813	106.0	1017.123	97.6	1951.686	97.1
mean	101400.677	89.5	1381.601	133.0	1126.261	107.7	1033.233	99.1	1960.215	97.5
%RSD	1.2	1.3	0.5	0.5	1.7	1.7	1.2	1.2	0.9	0.8

Table 6: Accuracy

	CPS of Iron	% recovery	CPS of Arsenic	% recovery	CPS of Mercury	% recovery	CPS of Cadmium	% recovery	CPS of Lead	% recovery
50%	53321.546	94.1	678.712	130.7	655.601	125.4	506.043	97.1	979.133	97.4
	51848.262	91.5	679.751	130.9	643.577	123.1	499.268	95.8	992.202	98.7
	51961.592	91.7	692.214	133.3	646.714	123.7	497.183	95.4	984.160	97.9
100%	103923.184	91.7	1382.350	133.1	1161.680	111.1	1037.101	99.5	1980.383	98.5
	101203.275	89.3	1369.887	131.9	1121.946	107.3	1027.720	98.6	1962.288	97.6
	99843.320	88.1	1380.273	132.9	1121.946	107.3	1050.651	100.8	1942.182	96.6
150%	150784.946	88.7	2037.694	130.8	1665.667	106.2	1515.001	96.9	2892.163	95.9
	150784.946	88.7	2059.504	132.2	1657.824	105.7	1522.819	97.4	2892.163	95.9
	151124.935	88.9	2023.673	129.9	1690.761	107.8	1518.128	97.1	2913.273	96.6

Acceptance Criteria: The % recovery for each level should be 70% to 150%

Table: 7. Robustness:

S. No.	Element	Correlation coefficient Flow @4.1	Correlation coefficient Flow @4.5
1	Iron	0.9997	0.9999
2	arsenic	0.9996	0.9998
3	cadmium	0.9999	0.9997
4	mercury	0.9998	0.9999
5	Lead	0.9997	0.9998

Acceptance Criteria: Correlation coefficient should be not less than 0.99

Table: 8. Solution stability

S. No.	Con of Iron	CPS of Iron	Con of Arsenic	CPS of Arsenic	Con of Mercury	CPS of Mercury	Con of Cadmium	CPS of Cadmium	Con of Lead	CPS of Lead
1	150	32552.320	1.56	298.235	1.56	289.652	1.56	302.235	3	554.235
2	250	54783.725	2.6	516.325	2.6	512.365	2.6	502.325	5	991.265
3	500	111167.412	5.2	1029.356	5.2	1032.236	5.2	1021.265	10	1995.368
4	750	162151.127	7.8	1549.235	7.8	1558.236	7.8	1512.125	15	3011.249
5	1000	215334.822	10.4	2058.365	10.4	2045.568	10.4	2052.248	20	4001.256
		0.9998		1.0000		0.9998		0.9999		0.9999

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

4. Conclusion

The objective of the present study was to develop a estimation of Iron, arsenic, cadmium, mercury and Iron, arsenic, cadmium, mercury and Lead content in Sunset yellow. The need for new development of the analytical method was identified the existing methods such that the present method developed was much simple, precise and accurate when compared to the earlier works.

The method was validated by evaluating linearity, accuracy and precision. The results conclude that the method was suitable for its intended use for the estimation of Iron, arsenic, cadmium, mercury and Lead content in Sunset yellow.

Acknowledgements

We would like to thank Dr. P. RAVI, Assistant professor Acharya Nagarjuna University, Andhra Pradesh, India, for giving me a good support.

References:

1. https://en.wikipedia.org/wiki/Sunset_yellow
2. Campbell MK, Farrell SO. 2011. *Biochemistry. Seventh edition* Pacific Grove: Brooks /Cole. ISBN 978-1-111-42564-7.
3. Hayes C. The effect of non-cariogenic sweeteners on the prevention of dental caries: a review of the evidence. *Journal of dental education*. 2001 Oct;65(10):1106-9.
4. Nicolas GG, Lavoie MC. Streptococcus mutans and oral streptococci in dental plaque. *Canadian journal of microbiology*. 2011 Jan 1;57(1):1-20.
5. Nelson DL. Oxidative phosphorylation and photophosphorylation. *Principles of biochemistry*. 2005.
6. Ich IC. Topic Q2 (R1) validation of analytical Procedures: text and methodology. In *Int. Conf. Harmon* 2005 (Vol. 1994, p. 17).
7. Chudzinska M, Debska A, Baralkiewicz D. Method validation for determination of 13 elements in honey samples by ICP-MS. *Accreditation and Quality Assurance*. 2012 Feb;17:65-73.
8. Elder D. ICH Q4: Pharmacopeial Harmonization and Evaluation and Recommendation of Pharmacopeial Texts for Use in the ICH Regions. *ICH Quality Guidelines: An Implementation Guide*. 2017 Sep 27:281-310.