Analytical Method Validation for Quantitation of Multi-Toxic Metals using Microwave-assisted Digestion with ICP-MS analysis for *Mangifera indica* Matrix

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Abstract

The aim of this research work is to develop and validate the analytical method followed by the microwave-assisted digestion method for the quantitative analysis of the toxic metals in Mangifera indica matrix by using ICP-MS, which will be used for field sample analysis. The ICP-MS technique has several applications such as the determination of single elements, and multi-element analysis in different matrices. The use of pesticides may generate residue in fruit which makes them risky to be administered. Thus, it is essential that these residues must be detected and quantified. ICP-MS plays a significant function in the quantitation of elemental residues. To determine toxic and essential elements in the Mangifera indica matrix at the different maturity levels by using Inductively coupled plasma mass spectrometry (ICP-MS) equipped with microwave digestion is considered an excellent tool for the digestion of many samples analysis. The analytical method developed and validated for the quantitative analysis for the determination of toxic metals (Chromium (Cr), Manganese (Mn), Nickel (Ni), Copper (Cu), Arsenic (As), Cadmium (Cd), Lead (Pb). Numerous parameters have been evaluated for the validation of the method, namely: matrix effect, linearity, the minimum detection limit (LOD), the limit of quantification (LOQ), precision, accuracy, extraction solution stability, and standard solution stability. Matrix effects (± 20.0%) were not considered significant. The linearity concentrations range (0.1 to 32.0 µg/L) were drawn and the value of correlation coefficient (r) in linearity was ≥ 0.99. The precision % RSD was 10.0% and accuracy (% recovery) was 70 to 110% of the fortified concentration at LOQ level and 10 times LOQ Level. The final extraction stability was within the limit of 70 to 120% of the test item concentration with % RSD (20%). The standard solution stability % Difference was ± 10, it is concluded that the standard solution was stable throughout the experiment phase. The validated analytical method will be used for the quantitation of the toxic metals in Mangifera indica matrix for monitoring and further evaluation purpose at the lower residual level.

keywords: ICP-MS, Matrix Effect (% ME), Mangifera indica, Microwave-assisted Digestion, SANTE/2020/12830

I. INTRODUCTION

The growth rate of national consumption of pesticides over different decades has shown wide fluctuations, though the overall trend is negative (-2.48%) while analyzing the trend of pesticide consumption in India for the time duration of 2000 to 2013, a growth trend has observed in 17 states/UTs.

Maharashtra, Uttar Pradesh, Haryana, Punjab, and Andhra Pradesh are the states that accounted for 70% of total pesticide consumption. The growth has been observed highest in Andaman & Nicobar Islands, Jammu & Kashmir, and Tripura. The research work evaluated the need for a critical review of the mismatch in policy and CQDs in the pesticide sector and discussed the retail sale pattern of pesticides in the respective region of India [1]. The pesticide has been a necessary part of agriculture to protect crops and livestock from pest infestations and yield reduction. Besides their usage, pesticides could pose potential risks to food

safety, the environment, and all living things. The utilization of pesticides increasing concern about the environmental impact of repeated pesticide use has prompted research into the environmental fate of these agents, which can spread from treated fields to air, other lands, and water bodies [2]. Pesticides influence the host's energy metabolism, and nervous, cardiovascular, and endocrine systems, either directly or indirectly. They cause many diseases, including metabolic syndrome, malnutrition, atherosclerosis, inflammation, pathogen invasion, nerve injury, and infectious disease susceptibility [3].

Food safety efforts and monitoring are required by international standards set by the joint Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Codex Alimentarius Commission. Hence, efforts have been made to harmonize maximum residue limits worldwide under the aegis of the World Trade Organization (WTO) and the Codex Alimentarius, MRLs still vary from one geographical location to another. MRLs in a particular animal product may differ from one country to another depending on the local food safety regulatory agencies and drug usage patterns [4]. The Food Safety and Standard Authority of India (FSSAI) under the Ministry of Health and Family Welfare utilizes the Good Agricultural Practice (GAP) data for fixation of MRL, keeping in view the dietary exposure and risk assessment after approval of the same pesticide by the Registration Committee (RC) [5]. The subject is becoming a greater concern for obtaining precise and accurate results, the sampling and sample processing in pesticide residue analysis of food and soil must be important. Briefly evaluate the often neglected but crucial part of sample collection and processing for the pesticide residue analysis and the strength of their sample processing [6]. Pesticides can be classified in many ways based on use, toxicity, mode of entry, mode of action, chemistry, and formulations. The pesticides are classified based on their chemical properties into inorganic pesticides and organic pesticides. Synthetic pesticide is classified into organophosphate (OPs), organochlorine (OCs), carbamate, cyclodiene, synthetic pyrethroids, triazole, and nicotinoid, which are commonly used based on the requirement of the crops and specific characteristics.

The research article described the validation process for the simultaneous analysis of 21 elements in food samples by inductively coupled plasma-mass spectrometry (ICP-MS) after closed-vessel microwave digestion. This validation was realized in parallel with the analysis of the 1322 food samples of the second French Total Diet Study (TDS) by the National Reference Laboratory (NRL) of the French Food Safety Agency (AFSSA). Furthermore, the method was supervised by several internal and external quality controls (IQC and EQC) [65]. Evaluation of trace level elements from the fruit sample and total free amino acid composition in the blueberry and strawberry was determined by an Amino Acid Analyzer. Eleven free amino acids were found in both berries. The trace elements in each dried fruit sample were determined by ICP-MS with microwave digestion [66].

Specific element determination was also developed by an analytical procedure using ICP-MS for the determination of Arsenic in commercialized the Tetra Pack® package of fruit juice. The microwave-assisted acid digestion of juice was carried out for the sample preparation. ICP-MS/MS, an effective instrumental strategy for overcoming spectral interferences and give accurate results of Arsenic content at trace levels [67]. The method was developed for the utilized product for fruit ripening, calcium carbide (CaC_2) is widely used as a low-price artificial ripening agent of fruits. It may be toxic for human consumption. This study aimed to assess heavy metals present in commercial-grade CaC_2 and subsequently their quantification in mango peels for the identification of possible marker elements as an indicator of CaC_2 ripening. Samples were prepared by using microwave-assisted digestion followed by multi-elemental quantitative analysis using ICP-MS [68].

A simple method for the determination of elements in food samples by using ICP-MS. The samples were processed by using concentrated nitric acid and hydrogen peroxide with a known quantity of samples. The decomposition of samples was carried out in a microwave digestion

Step	Time	T2	Power
1	0:15:00	180 °C	1800 W*
2	0:10:00	220 °C	1800 W*
3	0:15:00	220 °C	1800 W*

system and analytical method validation was performed with achieving acceptance criteria.48 The evaluation of the concentrations of 23 minor and trace elements in aromatic spices by ICP-MS by the wet digestion (microwave system). The analytical method validation was performed, and all results were within the acceptance criteria. The analysis of samples of aromatic spices indicated

the presence of variable amounts of both minor and trace elements [69]. A comparison study was conducted for the concentrations of elements determined in different tomato species (Khaki, Cherry, and Italy) at different stages of maturation by ICP-OES and ICP-MS following microwave-assisted acid digestion. The digestion efficiency

Instrument	Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) [Agilent Technologies, 7800 with Mass Hunter 4.4 Workstation software]
Element	Chromium (Cr), Manganese (Mn), Nickel (Ni), Copper (Cu), Arsenic (As), Cadmium (Cd), Lead (Pb)
Mode of Analysis	Helium [He]
RF power	1550 W
RF Matching	1.60 V
Peak Pattern	3 points
Spray chamber temperature	2 °C
Gas Flow Rate	
Plasma	15.0 L/minute
Nebulizer	1.05 L/minute
Auxiliary	0.90 L/minute
Note: The mode of analysis for ions with a Mass above 38 was evaluated with He Mode.	

was evaluated by residual carbon content and trace element recoveries. The nitric acid was used for the digestion procedure sample and standard procedures [70].

Consequently, the design of this research article aims to discuss the determination of metal residue in the Mangifera indica matrix by the accessible digestion, and detection methods. The digestion and detection techniques would be useful for the evaluation and monitoring of metal residue for field samples and fruit samples that are available in the market.

II. MATERIALS AND REAGENTS

A. Mangifera indica material

Mango samples were acquired from the farmer's farm in the industrial region of Valsad district, Gujarat. The sample matrix was used for the analytical method validation was performed by using microwave-assisted acid digestion followed by ICP-MS analysis.

B. Reagents and standards

The ICP-MS analysis employed high-purity grade compounds. The microwave acid digestion of samples was done using extra pure grade HNO3 (69%) and HCl (36%) that were purchased from J.T. Baker, USA. To create calibration standards, a ready-made elements stock solution from Inorganic Venture in the USA was acquired. This solution had 1000 g/mL of each element. Standard solutions for each target analyte were created by diluting the stock solution of the standard for external calibration to the desired working range of 0.1-32 g/L. In order to make up the volume and dilution of calibration standards,

ICP-MS external containing Chromium (Cr), Manganese (Mn), Nickel (Ni), Copper (Cu), Arsenic (As), Cadmium (Cd), and Lead (Pb) were created in 2% (v/v) HNO3 in ultrapure water (Fisher Scientific). For the digesting process, Fisher Scientific's hydrogen peroxide (H2O2) was used.

III. EXPERIMENT PHASE

Microwave-assisted digestion an ETHOS UP sample preparation system (Milestone, Germany) equipped with a rotor (SK-15 high-pressure) and high-performance reaction vessels with pressure-activated-venting was used for the closed vessel microwave-assisted acid digestion of samples. About 5 g of sample matrix was accurately weighted in the dried PTFE digestion vessels and 5 mL of HNO3 and 1 mL of Hydrogen peroxide were immediately added. The tightly closed vessels were placed into a microwave digestion system and followed the below-mentioned program in TABLE 1.
Key: T2 = Temperature sensor.

TABLE 1: Digestion Program of Micro-assisted digestion Technique

The digested solutions were cooled at room temperature and transferred to the 50 mL volumetric flasks and diluted with water. The reagent blank solution was also prepared by following the same procedure just by omitting the sample. All the prepared samples and blank samples were transferred to the Tarson centrifuge tube for ICP-MS analysis.

ICP-MS analysis, the multi-elemental analysis was performed on 7800 ICP-MS (Agilent Technologies, USA) with an advanced sample introduction system. The instrumental parameters under optimized conditions are given in Table 2. ICP-MS Mass Hunter Work Station software version was used for data handling and interpretation.

TABLE 2: ICP-MS Instrumental Method Parameters

IV. EVALUATION OF VALIDATION PARAMETERS

In the research article, the analytical method validation parameters were covered with consideration as per the requirement of SANTE guidelines. The analytical method for the determination of multi-elements in the matrix (Alphonso) covered the aspects namely:

i) Matrix Effects (% ME),
ii) Linearity Curve,
iii) Limit of Detection (LOD),
iv) Limit of Quantification (LOQ),
v) Precision (% RSD) and Accuracy (% Recovery),
vi) Stability (standard solution stability at the initial time   point and at the end of the experiment), and
vii) Final extraction stability up to 7 days in refrigerator (2 to 8 °C) condition.

The analysis was performed on the ICP-MS Instrument and analysed the mass Spectrograms (m/z) of toxic elements to get the precise and accurate results (see Fig. 1).

Figure 1: Mass Spectra of Multi-elements in *Mangifera indica* Matrix

All the above-mentioned analytical method parameters were performed and the results were found within the acceptable criteria as per the SANTE/12830 guideline requirements.

## V. RESULT AND DISCUSSION

A. Matrix Effect

Matrix matched standard was prepared to determine the matrix effects. Interference-free blank matrix was fortified with the analyte and compared with the standard solution prepared in a solvent. Three injections of each standard solution were injected. Matrix effects are considered significant if they exceed ± 20%. Matrix effects will be calculated using the following equation:

Matrix Effect %

$$= \frac{\text{CPS of matrix match standard} - \text{CPS of standard in 2\%HNO3 solution}}{\text{CPS of standard in 2\% HNO3 solution}} \times 100$$

Linearity, Limit of Detection (LOD), and Limit of Quantification (LOQ)

7 different concentrations of reference standards solutions were prepared in extracts of interference-free blank matrix and injected onto ICP-MS. Linearity was extended over a range from 30% of the LOQ and 20% above the highest level. The linear calibration curve was established
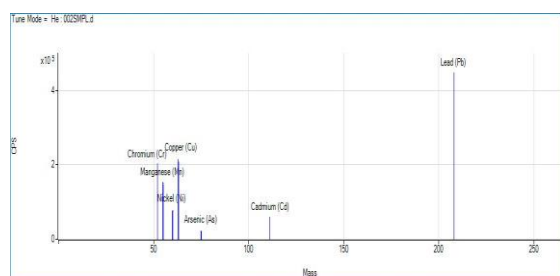
by plotting the peak area against concentration (µg/L). The intercept (a), slope (b), and regression equation were calculated. The correlation coefficient (r) was ≥ 0.99. The regression residual "di" was calculated using the following formula.

$$di = Yi - Yyi$$

Where,
Yi is the measured value i;
Yyi is the estimated value that corresponds to yi and is derived from the calibration function.

The lowest concentration of the linearity standards was considered as a limit of detection (LOD). The LOQ of the method was the minimum concentration/quantity of an analyte in the matrix (lowest validated level) with acceptable recovery and precision

B. Precision (% RSD) and Accuracy (% Recovery)

Precision and accuracy of the analytical method was reported at two fortification levels i.e., LOQ and 10 × LOQ level.

Recovery of the analytical method was determined by the method of test item addition to the matrix (Mangifera indica).

Recovery and repeatability (as precision, % RSD) data were reported for the following fortification levels:

• LOQ 5 samples

• 10 × LOQ Level 5 samples

Additionally, for unfortified samples, that have undergone the same sample preparation process as the fortified samples, residue levels were reported as follows:

• Blank matrix 2 samples

The test item concentration in each replicate, mean of test item concentration, SD and % RSD were calculated and reported. A single injection was performed for each replicate of the sample solution. Mean recoveries at each fortification were in the range of 70% to 120% and % RSD at each fortification level did not exceed 20%.

The % RSD was calculated using the following formula:

$$\text{Precision} (\% RSD) = \frac{\text{Standard Deviation}}{\text{Mean Recovered Concentration}} \times 100$$

The Accuracy (% Recovery) was calculated using the following formula:

$$\% \ Recovery = \frac{Recovered \ Concentration}{Fortified \ Concentration} \ x \ 100$$

C. Standard Solution Stability

During the end of the experiment phase, a new reference standard solution at the lowest and highest linearity level will be prepared. The newly prepared reference standard solution and old prepared reference standard solution (stored in the refrigerator) will be injected five times onto HPLC. The % difference between the old and newly prepared reference standard solution will be calculated using the following formula:

$$\% \ Stability = \frac{Mean \ intensity \ of \ old \ prepared \ solution/concentration \ of \ old \ prepared \ solution}{Mean \ intensity \ of \ new \ prepared \ solution \ /concentration \ of \ new \ prepared \ solution} \ x \ 100$$

$$\% \ Difference = \% \ Stability - 100$$

The means from at least 5 replicate measurements for each of the two solutions were not differed by more than 10%.

D. Final Extract Stability

The final extracted sample solutions were stored in refrigerator condition (2 to 8 °C) for 7 days. The same sample solutions were injected against freshly prepared standards. A single injection was performed for the same sample solutions. The test item concentration, mean of test item concentration, SD and % RSD were calculated and reported. Mean recoveries of the final extracted sample solution were in the range of 70% to 120% and % RSD was not exceeded 20%.

VI. DECLARATION OF COMPETING INTEREST

The authors report no declarations of interest.

VII. ACKNOWLEDGMENTS

Conclusion

From the results of the validation, it is concluded that the method is sensitive, precise, and accurate for the analysis of the multi-elements concentration, and homogeneity, in the mango matrix. The extracted sample solution stability was established for up to 7 days, the multi-elements were found stable in extracted solution for up to 7 days at refrigerator conditions (2 to 8 °C).

The results of the validated analytical method of ICP-MS analysis criteria are within the guideline SANTE/2020/12830, Rev.1 (February 24, 2021) specified limit.

REFERENCES

[1] Agricultural and Processed Food Products Export Development Authority (APEDA), Ministry of commerce and Industry, Government of India, (http://apeda.gov.in/apedawebsite/SubHead _Products/Mango.htm)

[2] Food and Agriculture Organization of the United Nations, (http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/glossary/jp/)

[3] Human Health Risk Assessment, United States Environmental Protection Agency, (https://www.epa.gov/risk/human-health-risk-assessment)

[4] Citation, S. (1993). Sensory and Physicochemical Property Relationships in Food That Define and Predict End-Use Quality - Agricultural Research Service. https://doi.org/10.17226/2126.

[5] https://images.app.goo.gl/tMLSC42TYkvEw PnR9.

[6] European Commission. (2000). SANCO/3029/99 rev. 4 (11/07/2000) Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. European Commission, 26. Retrieved from https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_res_pre-reg-cont-monitor.pdf.

[7] Millour, S., Noël, L., Kadar, A., Chekri, R., Vastel, C., &Guérin, T. (2011). Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation. Journal of Food Composition and Analysis, 24(1), 111–120.https://doi.org/10.1016/j.jfca.2010.04.002

[8] Zhang, H., Wang, Z. Y., Yang, X., Zhao, H. T., Zhang, Y. C., Dong, A. J., Wang, J. (2014). Determination of free amino acids and 18 elements in freeze-dried strawberry and blueberry fruit using an Amino Acid Analyzer and ICP-MS with micro-wave digestion. Food Chemistry, 147, 189–194. https://doi.org/10.1016/j.foodchem.2013.09.118

[9] Shaheen, N., Irfan, N. M., Khan, I. N., Islam, S., Islam, M. S., & Ahmed, M. K. (2016). Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh. Chemosphere, 152, 431–438. https://doi.org/10.1016/j.chemosphere.2016.02.060

[10] Pinheiro, F. C., Amaral, C. D. B., Schiavo, D., &Nóbrega, J. A. (2017). Determination of Arsenic in Fruit Juices Using Inductively Coupled Plasma Tandem Mass Spectrometry (ICP-MS/MS). Food Analytical Methods, 10(4), 992–998. https://doi.org/10.1007/s12161-016-0663-7

[11] Dehelean, A., &Magdas, D. A. (2013). Analysis of mineral and heavy metal content of some commercial fruit juices by inductively coupled plasma mass spectrometry. The Scientific World Journal, 2013. https://doi.org/10.1155/2013/215423

[12] Sim, L. P., Wang, J., Tong, B. M. K., Shin, R. Y. C., & Lee, T. K. (2016). Development of an apple juice certified reference material for cadmium, lead, total arsenic and arsenic species. International Journal of Mass Spectrometry, 411, 14–20. https://doi.org/10.1016/j.ijms.2016.11.001

[13] Hassan, S., Mazhar, W., Farooq, S., Ali, A., & Musharraf, S. G. (2019). Assessment of heavy metals in calcium carbide treated mangoes by inductively coupled plasma-mass spectrometry (ICP-MS). Food Additives & Contaminants: Part A, 00(00), 1–8. https://doi.org/10.1080/19440049.2019.1671990

[14] Bressy, F. C., Brito, G. B., Barbosa, I. S., Teixeira, L. S. G., & Korn, M. G. A. (2013). Determination of trace element concentrations in tomato samples at different stages of maturation by ICP OES and ICP-MS following microwave-assisted digestion. Microchemical Journal, 109, 145–149. https://doi.org/10.1016/j.microc.2012.03.010

[15] Nardi, E. P., Evangelista, F. S., Tormen, L., SaintPierre, T. D., Curtius, A. J., Souza, S. S. d., & Barbosa, F. (2009). The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. Food Chemistry, 112(3), 727–732. https://doi.org/10.1016/j.foodchem.2008.06.010

[16] Islam, M. S., Ahmed, M. K., Habibullah-Al-Mamun, M., & Raknuzzaman, M. (2015). The concentration, source and potential human health risk of heavy metals in the commonly consumed foods in Bangladesh. Ecotoxicology and Environmental Safety, 122, 462–469. https://doi.org/10.1016/j.ecoenv.2015.09.022.

[17] Khan, N., Choi, J. Y., Nho, E. Y., Jamila, N., Habte, G., Hong, J. H., … Kim, K. S. (2014). Determination of minor and toxic elements in aromatic spices by micro-wave assisted digestion and inductively coupled plasma-mass spectrometry. Food Chemistry, 158, 200–206. https://doi.org/10.1016/j.foodchem.2014.02.103.

[18] The Online e-Pesticide Manual published by BCPC UK.

[19] Vaishnavi Nair. Market Potential of Agrochemicals used in Mango and Okra from Valsad and Surat Districts. IMPACT Int J Res

Bus Manag (IMPACT IJRBM).2018;6(7):37-42.

http://www.impactjournals.us/journals.php?id=78&jtype=2&page=33.

[20] Bian, Y., Wang, B., Liu, F., Chen, F., & Peng, Q. (2019). Residue behaviour and dietary risk assessment of emamectin benzoate in mango under field condition using modified QuEChERS method combined with HPLC-MS / MS. International Journal of Environmental Analytical Chemistry, 00(00), 1–13. https://doi.org/10.1080/03067319.2019.1637427.

[21] Table, O. (2015). Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. European Commission, Directorate of General Health and Consumer Protection. Document nº. SANTE/2015/11945.

[22] SANTE. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-Approval Control and Monitoring Purposes. SANTE/2020/12830. 2021;(February):1–50.