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Research Article

ICP-MS Analysis and Validation by Microwave Digestion System for Determination of Heavy Metals in Allergy and Cancer Drugs Taken Orally

Elif KÖSE [a, b], Aslihan DALMAZ [c], Sezen SİVRİKAYA ÖZAK [d, *]

[a] *Department of Chemistry, Graduate Education Institute, Düzce University, 81620, Düzce, Türkiye*
[b] *Nobel Drug Pharmaceutical R&D Center (Nobel-Ilac Ar-Ge Merkezi), 81100 Düzce, Turkiye*
[c] *Department of Natural and Herbal Products/Cosmetic Products, Graduate Education Institute, Düzce University, 81620, Düzce, Türkiye*
[d] *Department of Chemistry, Faculty of Art and Science, Düzce University, 81620, Düzce, Türkiye*
* *Corresponding author's e-mail address: sezensivrikaya@duzce.edu.tr*
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ABSTRACT

The drug, one of the most important products that cannot be replaced in the field of health, prevents all threats to human health when used in place and dosage. Therefore, it has an important place in public health. Determination of the amounts and impurities of various elements used in drug production is an important parameter. According to the ICH Q3D(R1) international compliance conference guide, methods by which limit concentrations can be determined for elemental impurities by applying inductively coupled plasma (ICP) analysis to drugs are specified. In this study, the sample preparation step in the analysis of 23 elements in cancer and allergy drugs in tablet form was optimized by microwave digestion, and method development and validation studies were carried out for these drugs with inductively coupled plasma-mass spectrometry (ICP-MS). When the analysis results were evaluated, it was seen that the data obtained were by the limit values specified in the ICH. Detection and quantification limits of the developed method, and relative standard deviation values were determined, and recovery studies were carried out by adding standards at 4 different concentrations to determine the method's precision. The correlation coefficients obtained for heavy metals in this study ranged from 0.9993 to 1.000, while the detection limit was found to be between 0.001 and 1.756 µg/L. Thus, the reliability and precision of the validation study developed has been determined and it has been shown that this method can be used in similar drug samples.

Keywords: *Validation, Elemental impurity, Microwave digestion, Inductively coupled plasma-mass spectrometry, Drug*

Oral Yol ile Alınan Alerji ve Kanser İlaçlarında Ağır Metallerin Tayini için Mikrodalga Çözünürleştirme Sistemi ile ICP-MS Analizi ve Validasyonu

Öz

Sağlık alanında yeri doldurulamayacak ürünlerin en önemlilerinden biri olan ilaç yerinde ve dozunda kullanıldığında insan sağlığı için oluşan tüm tehdit unsurlarına engel olmaktadır. Toplum sağlığı açısından değerlendirildiğinde önemi oldukça büyüktür. İlaç üretiminde kullanılan çeşitli elementlerin miktarlarının ve safsızlıklarının belirlenmesi çok önemli bir parametredir. İlaçlar için indüktif eşleşmiş plazma (ICP) analizi, Uluslararası uyum konferansı kılavuzu ICH Q3D(R1) referans alınarak elementel safsızlıkların eşik konsantrasyonlarının belirlenmesinde kullanılan bir yöntemdir. Bu çalışmada tablet formundaki kanser ve alerji

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ilaçlarındaki 23 elementin analizinde örnek hazırlama basamağı mikrodalga çözünürleştirme işlemi ile optimize edilmiş ve indüktif eşleşmiş plazma-kütle spektrometresi (ICP-MS) ile bu ilaçlar için metot geliştirme ve validasyon çalışmaları gerçekleştirilmiştir. Yapılan analiz sonuçları değerlendirildiğinde elde edilen verilerin ICH'de belirtilen sınır değerlere uygun olduğu görülmüştür. Geliştirilen yöntemin belirleme ve tayin limitleri, rölatif standart sapma değerleri belirlenmiş ve ayrıca yöntemin kesinliğini tespit etmek amacıyla test örneklerine 4 farklı konsantrasyonda standart eklemesi yapılarak geri kazanım çalışmaları yapılmıştır. Bu çalışmada ağır metaller için elde edilen korelasyon katsayıları 0,9993 ile 1,000 arasında değişirken dedeksiyon limitleri ise 0,001 ile 1,756 µg/L arasında bulunmuştur. Böylelikle geliştirilen validasyon çalışmasının güvenilirliği ve kesinliği belirlenmiş olup, benzer ilaç örneklerinde bu yöntemin kullanılabilir olduğu gösterilmiştir.

Anahtar Kelimeler: *Validasyon, Elementel safsızlık, Mikrodalga çözünürleştirme, İndüktif eşleşmiş plasma-kütle spektrometrisi, İlaç*

I. INTRODUCTION

While there are positive developments in many areas with the advancement of technology day by day, the increase in the use of metallic compounds and heavy metals also brings environmental pollution. This threatens the health of living beings through the consumption of heavy metals used in food, drugs, and beverages [1-2]. Trace elements are defined as impurities that are released to nature and the environment as a result of their use in some industries, and that accumulate in living systems in many ways and pose a threat. With these aspects, trace elements have been accepted as pollutants affecting human health since ancient times and will continue to be [3-5].

When examined from another aspect, the metabolism of living things also needs trace elements. Although the amount of these elements varies, it causes various discomforts in case of deficiency or excess [6]. At this point, the safety of pharmaceuticals used during drug treatments is of great importance. Pharmacological and toxicological evaluation constitute the main parameters for determining the safety of a drug. In addition, the problems caused by the impurities in the dosage forms affect the drug safety. In terms of drug safety, the damage to be caused by the pharmacological and toxicological effects on the living organism will be much greater. All these reasons reveal the necessity of performing the characterization of the products, especially pharmaceutical products, produced for human consumption without errors. The efficacy, quality, and safety of a drug will thus be assured by controlling and monitoring the impurities it contains. Thus, studies on the impurities of drugs have become popular lately [7-8].

The toxicological aspect of drugs is also important because of the elemental impurities they contain as well as the benefits it provide to the living metabolism. For this reason, various analytical methods such as inductively coupled plasma-optical emission spectroscopy (ICP-OES) [9-13], inductively coupled plasma-mass spectroscopy (ICP-MS) [14-20], atomic absorption spectroscopy (AAS) [21, 22], graphite furnace atomic absorption spectroscopy (GFAAS) [23] and flame atomic absorption spectroscopy (FAAS) [24], which are extremely important in the determination of elemental impurities in drugs, have been developed. When these analytical methods in the literature are examined from a scientific point of view, ICP-MS and microwave-assisted digestion, which is one of the sample pre-processing methods, come to the fore when compared to other methods mentioned. ICP-MS is a versatile analysis method with many outstanding advantages. It is one of the most sensitive techniques that help to perform the analysis of multiple elements by providing the simultaneous detection of more than one element in an analysis [25-27]. On the other hand, the microwave-assisted digestion process increases performance and provides higher yields [28-31]. ICP-MS provides great support to the pharmaceutical industry in the analysis of heavy metals in pharmaceuticals.

This study aims to optimize the determination of 23 elements in orally administered allergy and cancer drugs using a microwave-assisted solubilization pretreatment process. In addition, the development of a new method for these drugs and the validation studies of this developed method are to be carried out

with the ICP-MS instrument. Then, the results of the analysis will be determined by taking into account the ICH criteria. Afterwards, the optimization parameters of the method will be determined the precision and reliability values will be determined and the evaluation of the method will be carried out.

II. MATERIAL and METHODS

A. APPARATUS

Agilent brand 7850 model ICP-MS was used to analyze elemental impurities in drugs. Mass Hunter software was used for device control and data analysis. Sartorius Stedim Biotech brand atrium 611UV model ultrapure water device was used as the ultrapure water source for the preparation of the solutions. Mettler Toledo brand electronic device balance was used during sample weighing. CEM brand Mars model microwave digestion system was used during sample preparation in the study. Heidolph brand MR Hei-Standard model magnetic stirrer heater was used during sample preparation. Method parameters and operating conditions in the ICP-MS instrument are given in Table 1.

Table 1. *ICP-MS operating conditions and method parameters.*

ICP-MS conditions	**Value**
Radio frequency power	1550 W
Radio frequency matching	1.9 V
Uptake speed (Nebulizer pump)	0.3 rps
Nebulizer gas flow rate	1.07 L/min
Plasma gas	Axial
Plasma gas flow rate	15 L/min
Spray chamber temperature	2 ^{o}C
Analyzer pressure	8.47- 10-5 Pa

B. REAGENTS AND CHEMICALS

All of the chemicals used in this study were not subjected to any purification process. ICH Q3D/USP<233>Orals impurity Kits were purchased from Agilent. Suprapure nitric acid (67%) was purchased from VWR Chemicals and suprapure hydrochloric acid (30%) was purchased from Merck and used in microwave-assisted digestion of allergy and cancer drugs. Pharma internal standard 1, standard A, standard B, standard C, standard D standard mixtures were diluted in 2% nitric acid and prepared at a concentration of 100 ppb, and a stock standard was created. These prepared stock solutions and the samples in the study were stored at 4 °C. High purity argon gas (plasma gas) and nitrogen (cooling gas) were used in ICP-MS systems. The gases used in the analysis systems were obtained from a gas supplier (Düzce, Türkiye). All drugs used in the study were supplied from the pharmacies in Düzce province.

C. MICROWAVE-ASSISTED DIGESTION PROCEDURE OF DRUG SAMPLES

Determination of heavy metal amounts in drugs under optimum conditions is possible by taking them into the solution completely. With the proper disintegrating of the samples, they will be completely taken into solution. In this study, the microwave-assisted digestion process was preferred because it is fast and short in time, and it is the last developed digestion method with superior properties. Microwave-assisted digestion procedures for allergy and cancer drugs are shown in Figure 1. After the drug sample

was weighed and 6 mL of suprapure nitric acid and 1 mL of suprapure HCl acid were added to the Teflon containers of the microwave digestion system, digestion was carried out with the appropriate microwave method indicated in Table 2. After the microwave digestion process was completed, the sample solutions were taken into a 25 mL flask. Then, 2.5 mL of this solution was taken and diluted to 10 mL in test tubes and the samples were given to the ICP-MS instrument for analysis.

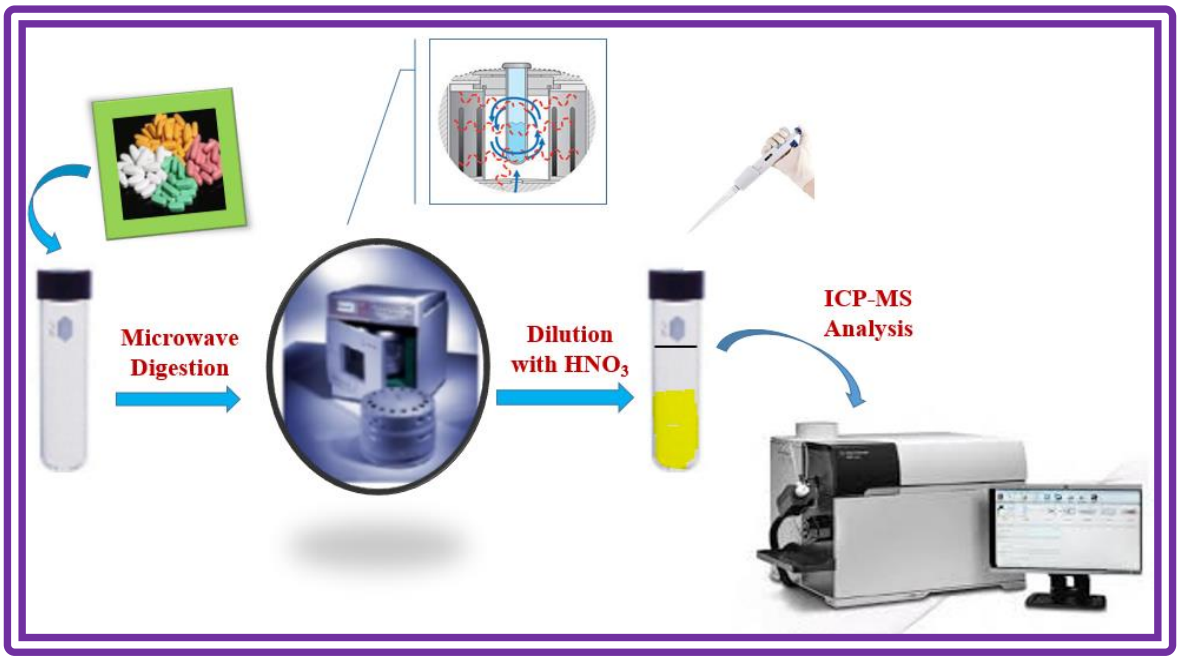

Figure 1. *ICP-MS Analysis by microwave digestion process allergy and cancer drugs.*

D. METHOD VALIDATION

In order to determine the suitability of the methods used in the analysis of heavy metals, validation parameters such as the method's calibration equations, determination limits, calibration coefficients, repeatability values, recovery values, and detection limits were calculated. In addition, the reproducibility values of the analysis results performed on drugs, which are real samples, were calculated and the results were recorded. The calculation techniques and parameters used for the accuracy of the method used in the study are given in order.

Table 2. *Microwave-assisted digestion procedure parameters.*

Energy	**Power** *(W)*	**Ramp Time** *(min.)*	**Temperature** *(°C)*	**Hold Time** *(min.)*
1600	90	8.00	140	15.00
1600	90	15.00	210	20.00

D. 1. Linear Range

In this study, standards with different concentrations were prepared and injected into the ICP-MS instrument in triplicate. Calibration charts in the study were created by evaluating the results obtained as a result of injection.

D. 2. Calibration Equation

It corresponds to the equation created through linear regression analysis of the data obtained as a result of the analysis for the linear range study.

D. 3. Calibration Coefficient

It corresponds to the R^2 value of the obtained calibration equation. In this study, the correlation coefficient varies between 0.9993 and 1.000.

D. 4. Repeatability

The repeatability value is expressed as the relative standard deviation value (%RSD) of the data obtained from the recovery studies.

D. 5. Detection and Quantification Limit

The limit of detection (LOD) is the lowest concentration of analyte at which the analyte can be detected in the sample under laboratory conditions, but cannot measure its exact amount. The limit of quantification (LOQ) is expressed as the lowest concentration of analyte at which the presence of analyte in the sample can be detected under laboratory conditions. By using these calibration lines, the detection limits (LOD) and the quantification limits (LOQ) of each element were calculated. Calculated using LOD = 3xSDxC/S-B and LOQ = 10xSDxC/S-B; where SD is the standard deviation of the 15blank measurement, C is the concentration of the standard, S is the signal values of the standard, B is the mean of the signal values of the blank.

D. 6. Recovery

Standard solutions of 4 different concentrations containing the analytes were prepared by adding them to the recovery study samples. Recovery values were given to the ICP-MS instrument repeatedly after the samples were prepared, and the results were determined.

III. RESULTS AND DISCUSSION

A. ANALYSIS RESULTS AND CONFORMITY

With the help of the allowable daily exposure (PDE) limit of the elements in the oral drugs, the target limits of the drugs were determined according to the daily intake doses. During the evaluation of elemental impurities, there is a control threshold limit to see if additional control equipment is needed. The limit is the 30% level of the allowable daily exposure (PDE) of the specific elemental impurity. When the results of the analysis are examined, they are evaluated according to these limits, and the conformity values are given in Table 3 and Table 4. In addition, in this study, when the analysis results

of the elements analyzed in allergy and cancer drugs were calculated, it was seen that they were below the 30% limit and therefore conformity was given.

Table 3. *Cancer drug analysis result and conformity.*

Element		Limits				
Symbol	**Class**	**Oral PDE *(µg/day)***	**Option Target Limit *(µg/g)* 0.229 g/day drug product**	**30% limit *(µg/g)***	**Analysis results *(µg/g)***	**Action**
Cd	1	5	21.83	6.55	0.039	Valid
Pb	1	5	21.83	6.55	2.541	Valid
As	1	15	65.50	19.65	0.003	Valid
Hg	1	30	131.00	39.30	0.013	Valid
Co	2A	50	218.34	65.50	0.981	Valid
V	2A	100	436.68	131.00	0.001	Valid
Ni	2A	200	873.36	262.01	0.030	Valid
Tl	2B	8	34.93	10.48	0.001	Valid
Au	2B	100	436.68	131.00	0.030	Valid
Pd	2B	100	436.68	131.00	0.024	Valid
Ir	2B	100	436.68	131.00	0.040	Valid
Os	2B	100	436.68	131.00	0.113	Valid
Rh	2B	100	436.68	131.00	0.008	Valid
Ru	2B	100	436.68	131.00	0.001	Valid
Se	2B	150	655.02	196.51	0.019	Valid
Pt	2B	100	436.68	131.00	0.000	Valid
Li	3	550	2401.75	720.52	0.021	Valid
Sb	3	1200	5240.17	1572.05	0.037	Valid
Ba	3	1400	6113.54	1834.06	0.101	Valid
Mo	3	3000	13100.44	3930.13	0.258	Valid
Cu	3	3000	13100.44	3930.13	0.488	Valid
Sn	3	6000	26200.87	7860.26	0.315	Valid
Cr	3	11000	48034.93	14410.48	0.864	Valid

Table 4. *Allergy drug analysis result and conformity.*

Element		Limits				
Symbol	**Class**	**Oral PDE *(µg/day)***	**Option Target Limit *(µg/g)* 0.205 g/day drug product**	**30% limit *(µg/g)***	**Analysis results *(µg/g)***	**Action**
Cd	1	5	24.39	7.32	0.021	Valid
Pb	1	5	24.39	7.32	2.464	Valid
As	1	15	73.17	21.95	0.011	Valid
Hg	1	30	146.34	43.90	0.138	Valid
Co	2A	50	243.90	73.17	0.238	Valid
V	2A	100	487.80	146.34	0.023	Valid
Ni	2A	200	975.61	292.68	0.901	Valid
Tl	2B	8	39.02	11.71	0.002	Valid
Au	2B	100	487.80	146.34	0.014	Valid
Pd	2B	100	487.80	146.34	0.013	Valid
Ir	2B	100	487.80	146.34	0.021	Valid
Os	2B	100	487.80	146.34	0.186	Valid

Rh	2B	100	487.80	146.34	0.004	Valid
Ru	2B	100	487.80	146.34	0.000	Valid
Se	2B	150	731.71	219.51	0.009	Valid
Pt	2B	100	487.80	146.34	0.000	Valid
Li	3	550	2682.93	804.88	1.611	Valid
Sb	3	1200	5853.66	1756.10	0.051	Valid
Ba	3	1400	6829.27	2048.78	0.165	Valid
Mo	3	3000	14634.15	4390.24	0.479	Valid
Cu	3	3000	14634.15	4390.24	0.205	Valid
Sn	3	6000	29268.29	8780.49	0.315	Valid
Cr	3	11000	53658.54	16097.56	0.320	Valid

B. ANALYTICAL PARAMETERS OF THIS METHOD

The analytical parameters of the validation studies for cancer and allergy drugs are presented in Table 5. According to the results, the calibration curves obtained from the calibration graphs prepared at different linear measurement intervals have high regression coefficients. By using these calibration lines, the detection limits (LOD) and the quantification limits (LOQ) of each element were calculated. Calculated using LOD = 3xSDxC/S-B and LOQ = 10xSDxC/S-B; where SD is the standard deviation of the 15 blank measurements, C is the concentration of the standard, S is the signal values of the standard, B is the mean of the signal values of the blank. Here, while Cd and Tl elements have the lowest detection limit, the Cr element has the highest value. In addition, the equations of each calibration curve are given in Table 5.

Table 5. *The analytical parameters of the validation studies.*

Element	**R^2**	**Calibration Curves**	**Linear Range**	**LOD** ***(µg/L)***	**LOQ** ***(µg/L)***
Cd	0.9998	y = 0.1141x + 2.0797E-004	0.1-40	0.004	0.013
Pb	0.9987	y = 0.0212x + 0.0038	0.1-40	0.035	0.117
As	0.9998	y = 0.0390x + 1.1666E-004	0.3-120	0.007	0.025
Hg	0.9999	y = 0.0050x + 1.1308E-004	0.6-240	0.079	0.263
Co	0.9998	y = 0.7136x + 0.0109	1-400	0.009	0.031
V	0.9999	y = 0.1205x + 0.0011	2-800	0.013	0.043
Ni	1.0000	y = 0.2341x + 0.0184	4-1600	0.034	0.115
Tl	0.9993	y = 0.0572x + 3.4351E-005	0.160-64	0.001	0.004
Au	1.0000	y = 0.0605x + 0.0018	2-800	0.027	0.090
Pd	0.9997	y = 0.5320x + 0.0048	2-800	0.018	0.059
Ir	0.9999	y = 0.0771x + 0.0013	2-800	0.020	0.067
Os	0.9999	y = 0.0189x + 0.0035	2-800	0.059	0.197
Rh	0.9997	y = 2.4843x + 0.0148	2-800	0.017	0.055
Ru	0.9998	y = 0.4092x + 0.0016	2-800	0.015	0.050
Se	0.9999	y = 0.0012x + 1.6606E-004	3-1200	0.071	0.238
Pt	1.0000	y = 0.0358x + 4.9195E-004	2-800	0.017	0.057
Li	0.9998	y = 0.0058x + 0.0090	11-4400	0.280	0.935
Sb	1.0000	y = 0.6104x + 0.0602	24-9600	0.170	0.568
Ba	0.9999	y = 0.1720x + 0.4695	28-11200	0.312	1.041
Mo	0.9999	y = 0.1378x + 0.0915	60-24000	0.436	1.453
Cu	0.9998	y = 0.6515x + 0.8605	60-24000	0.489	1.630
Sn	0.9998	y = 0.1622x + 0.0980	120-48000	0.931	3.102
Cr	1.0000	y = 0.1749x + 0.1063	220-88000	1.756	5.854

C. PRECISION OF THIS METHOD

In order to calculate the precision of the method developed for cancer and allergy drugs, the relative standard deviation (RSD) values were calculated by analyzing the drug solutions at two different concentrations (25% and 100%) for both drugs 7 times. The results are given in Table 6. As a result of

the analysis, it is seen that the RSDs are quite low. Obtained RSD values vary between 0.46 and 11.52. While the V element has the lowest RSD value with 0.46%, it has the Au element with 11.52%. The low RSD values indicate the reproducibility, precision of the validation study, and reliability of the method.

Table 6. *The precision results of this method.*

Element	Cancer Drug		Allergy Drug	
	%RSD			
	25%	**100%**	**25%**	**100%**
Cd	2.91	2.62	1.18	3.39
Pb	4.61	7.96	5.16	7.93
As	2.39	2.11	2.45	2.51
Hg	2.44	1.80	1.12	2.29
Co	3.46	2.48	4.16	2.65
V	2.16	0.81	1.06	0.46
Ni	1.50	2.19	4.53	2.71
Tl	2.50	0.53	0.96	1.37
Au	4.38	3.99	11.52	2.41
Pd	2.14	2.21	2.63	5.00
Ir	2.54	1.26	0.91	1.91
Os	2.82	0.66	1.32	4.00
Rh	3.13	2.53	1.97	2.97
Ru	2.30	2.18	2.17	2.86
Se	2.78	1.80	2.82	3.57
Pt	9.29	1.79	0.47	1.31
Li	3.31	0.68	0.95	1.03
Sb	1.53	3.95	3.73	4.79
Ba	1.40	4.12	4.23	5.15
Mo	1.75	1.44	1.34	1.07
Cu	1.88	2.14	1.98	2.53
Sn	1.86	2.34	2.05	2.99
Cr	2.02	0.92	0.83	0.49

D. RECOVERY

In this study, a recovery study was carried out to determine the accuracy of the analysis method. Recovery studies of drugs are performed by adding known concentrations of analyte to the sample. The samples selected from the intermediate stock solution were added at 4 different concentrations and the recovery values were calculated. Table 7 and Table 8 show that heavy metals can be detected in allergy and cancer drugs since the recovery values found are within acceptable limits. When the results obtained were examined, it was determined that the highest recovery in the cancer drug was 112.24% for Pb, 100.53% for Sb, and 102.49% for Ba. On the other hand, the lowest recovery was found in Au with 81.09%. In the allergy drug, the metals with the highest recovery values are 105.59% for Co, 105.25% for Ir, 102.52% for Os, 107.75% for Sb, and 105.58% for Ba. The lowest recovery values in the allergy drug were obtained in Ru metal at 87.74%, Pb metal at 87.78%, and Au metal at 86.37%. When the recovery values are evaluated in general for both drugs, it is seen that even the values specified as the lowest recovery value for the elements analyzed in the study are at a high level.

Table 7. *Application of the developed ICP-MS analysis procedure to the cancer drug sample.*

Element	Spike *(ppb)*	Found *(ppb)*	Recovery *(%)*
Cd	1.25	1.14	87.33
	2.5	2.26	88.37
	5	4.48	88.63

	7.5	6.85	90.62
Pb	1.25	4.50	88.96
	2.5	6.90	109.27
	5	9.20	104.36
	7.5	12.70	112.24
As	3.75	3.47	92.40
	7.5	6.93	92.37
	15	13.86	92.36
	22.5	20.82	92.53
Hg	7.5	6.68	88.82
	15	13.57	90.37
	30	27.25	90.76
	45	41.07	91.23
Co	12.5	12.42	95.17
	25	24.73	96.81
	50	48.85	96.65
	75	73.18	96.87
V	25	21.82	87.07
	50	44.00	87.90
	100	87.72	87.66
	150	131.83	87.85
Ni	50	46.25	89.85
	100	92.03	90.70
	200	182.24	90.45
	300	289.97	96.18
Tl	2	1.70	84.82
	4	3.43	85.72
	8	6.83	85.35
	12	10.24	85.32
Au	25	20.77	82.94
	50	40.58	81.09
	100	86.34	86.30
	150	133.13	88.73
Pd	25	21.29	85.03
	50	43.10	86.14
	100	86.39	86.36
	150	141.65	94.41
Ir	25	23.59	94.15
	50	48.07	96.03
	100	96.92	96.86
	150	145.04	96.65
Os	25	20.59	81.82
	50	41.58	82.88
	100	83.57	83.42
	150	140.07	93.27
Rh	25	22.22	88.86
	50	47.05	94.09
	100	94.17	94.16
	150	142.74	95.15
Ru	25	21.39	85.57
	50	42.87	85.73
	100	86.02	86.01
	150	131.09	87.39

Se	37.5	35.22	93.85
	75	70.63	94.14
	150	139.12	92.73
	225	207.09	92.03
Pt	25	20.64	82.58
	50	41.91	83.81
	100	91.93	91.93
	150	139.90	93.26
Li	137.5	129.74	94.34
	275	268.52	97.63
	550	545.91	99.25
	825	802.60	97.28
Sb	300	269.51	89.82
	600	603.26	100.53
	1200	1186.17	98.84
	1800	1803.87	100.21
Ba	350	319,39	91.21
	700	648.02	92.55
	1400	1392.90	99.48
	2100	2152.55	102.49
Mo	750	652.79	86.99
	1500	1302.93	86.84
	3000	2614.46	87.14
	4500	4015.96	89.23
Cu	750	706.09	94.05
	1500	1412.18	94.10
	3000	2819.85	93.97
	4500	4254.05	94.52
Sn	1500	1419.13	94.58
	3000	2811.45	93.70
	6000	5593.19	93.21
	9000	8483.41	94.25
Cr	2750	2496.54	90.74
	5500	5014.29	91.15
	11000	10060.79	91.45
	16500	15201.84	92.13

Table 8. *Application of the developed ICP-MS analysis procedure to the allergy drug sample*.

Element	**Spike** ***(ppb)***	**Found** ***(ppb)***	**Recovery** ***(%)***
Cd	1.25	1.20	92.13
	2.5	2.39	93.32
	5	4.55	90.01
	7.5	6.87	90.94
Pb	1.25	4.46	90.18
	2.5	5.79	93.37
	5	7.93	91.24
	7.5	9.83	87.78
As	3.75	3.76	99.85
	7.5	7.47	99.38
	15	14.26	94.99
	22.5	21.21	94.18
Hg	7.5	7.45	96.65

	15	14.69	96.61
	30	28.48	94.29
	45	42.79	94.65
Co	12.5	13.58	105.59
	25	26.08	102.87
	50	49.23	97.75
	75	73.61	97.68
V	25	23.77	94.95
	50	47.34	94.61
	100	91.29	91.26
	150	135.24	90.14
Ni	50	49.72	96.82
	100	96.77	95.48
	200	184.43	91.60
	300	293.80	97.50
Tl	2	1.87	93.18
	4	3.68	92.05
	8	7.11	88.81
	12	10.64	88.64
Au	25	22.21	88.77
	50	43.21	86.37
	100	92.06	92.04
	150	142.57	95.04
Pd	25	22.37	89.40
	50	44.73	89.42
	100	88.88	88.86
	150	130.48	86.97
Ir	25	26.17	104.55
	50	52.66	105.25
	100	101.05	101.02
	150	151.22	100.79
Os	25	23.18	91.71
	50	45.53	90.56
	100	91.51	91.26
	150	154.07	102.52
Rh	25	24.84	99.34
	50	49.91	99.82
	100	96.00	95.99
	150	144.53	96.35
Ru	25	22.80	91.20
	50	45.49	90.98
	100	87.74	87.74
	150	132.88	88.58
Se	37.5	37.84	100.88
	75	73.59	98.10
	150	141.31	94.20
	225	209.51	93.11
Pt	25	22.82	91.26
	50	46.19	92.38
	100	96.08	96.08
	150	146.10	97.40
Li	137.5	136.23	97.44
	275	263.34	94.96
	550	515.65	93.36
	825	777.84	94.02
Sb	300	320.10	106.68
	600	646.55	107.75
	1200	1256.94	104.74

	1800	1869.17	103.84
Ba	350	345.77	98.72
	700	683.94	97.67
	1400	1474.78	105.32
	2100	2217.41	105.58
Mo	750	715.38	95.29
	1500	1417.09	94.43
	3000	2766.77	92.20
	4500	4172.21	92.70
Cu	750	759.10	101.17
	1500	1500.70	100.03
	3000	2878.63	95.94
	4500	4308.39	95.74
Sn	1500	1519.43	101.27
	3000	2965.20	98.83
	6000	5667.84	94.46
	9000	8572.75	95.25
Cr	2750	2721.86	98.96
	5500	5427.02	98.67
	11000	10528.82	95.71
	16500	15580.84	94.43

IV. CONCLUSION

In this study, a microwave-assisted digestion process and ICP-MS analysis and validation were performed for the determination of heavy metals in orally administered allergy and cancer drugs. In order to determine the reliability and accuracy of the results obtained, the microwave-assisted digestion process, which is one of the most suitable processes for sample preparation, was preferred. Being a fast method, realizing the digestion process in a much shorter time compared to other methods, and with its superior properties, it increased the performance of the solubilization process and provided higher efficiency. The ICP-MS method, which was chosen for the analysis of 23 elements in cancer and allergy drugs in tablet form with the proposed method, has many advantages compared to other methods when the method was developed in this study. When the analysis results were examined, it was determined that the microwave-assisted solubilization process and the ICP-MS analysis performed were more acceptable and appropriate due to their features such as the lower amount of chemicals needed in this study, the low loss of volatile elements, and the high reproducibility values. The correlation coefficients obtained for heavy metals in this study ranged from 0.9993 to 1.000, while the detection limit was found to be between 0.001 and 1.756 μg/L. In addition, the RSD values obtained in the study ranged from 0.46 to 11.52, while the lowest RSD value of 0.46% was obtained in the V element. The low RSD values obtained prove the repeatability and reliability of the validation study. The developed method has been successfully applied for the determination of heavy metals in cancer and allergy drugs.

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V. REFERENCES

[1] E. Yilmaz, and M. Soylak, "Assessment of Concentration of Trace Metals in Traditional Bread Samples from Turkey," *Fresenius Environmental. Bulletin*, vol. 23, no. 3, pp. 782-786, 2014.

[2] A. Vural, I. Narin, M. E. Erkan, and M. Soylak, "Trace Metal Levels and Some Chemical Parameters in Herby Cheese Collected from South Eastern Anatolia-Turkey," *Environmental Monitoring Assessment*, vol. 139, pp. 27–33. 2008.

[3] H. Altundag, S. Albayrak, S. M. Dundar, M. Tuzen, and M. Soylak, "Investigation of The Influence of Selected Soil and Plant Properties from Sakarya, Turkey, on the Bioavailability of Trace Elements by Applying an *in vitro* Digestion Model," *Biological Trace Element Research*, vol. 168, pp. 276–285, 2015.

[4] U. P. Onyedima, N. E. C. Siddhant, P. O. Chukwu, A. B. Ajong, and F. Dehmchi, "Evaluation of Risk Caused by Intake of Trace Metal Through Consumption of *Pleurotus* Tuber-Regium Collected Around Automobile Village in ABIA State," *Bulletin of the Chemical Society of Ethiopia*, vol. 35, pp. 229– 241, 2021.

[5] M. Herrero, J. Rovira, M. Nadal, and J. L. Domingo, "Risk Assessment Due to Dermal Exposure of Trace Elements and Indigo Dye in Jeans: Migration to Artificial Sweat," *Environmental Research*, vol. 172, pp. 310–318, 2019.

[6] S. K. Taneja, and R. Mandal, "Mineral Factors Controlling Essential Hypertension—a Study in the Chandigarh, India Population," *Biological Trace Element Research*, vol. 120, pp. 61–73, 2007.

[7] S. Ródenas, F. J. Sánchez-Muniz, S. Bastida, M. I. Sevillano, T. Larrea Marín, and M. J. González-Muñoz, "Blood Pressure of Omnivorous and Semi-Vegetarian Postmenopausal Women and Their Relationship with Dietary and Hair Concentrations of Essential and Toxic Metals," *Nutricion Hospitalaria*, vol. 26, no. 4, pp. 874–883, 2011.

[8] S. Yalcın, S. Sezer, R. Apak, "Characterization and Lead(II), Cadmium(II), Nickel(II) Biosorption of Dried Marine Brown Macro Algae *Cystoseira barbata*," *Environmental Science Pollution Research*, vol. 19, pp. 3118–3125, 2012.

[9] S. Farahani, N. Eshghi, A. Abbasi, F. Karimi, F. E. S. Malekabad, and M. Rezaei, "Determination of Heavy Metals in Albumen of Hen Eggs from the Markazi Province (Iran) Using ICP-OES Technique," *Toxin Reviews*, vol. 3, no. 2, pp. 96–100, 2015.

[10] N. Khan, I. S. Jeong, I. M. Hwang, J. S. Kim, S. H. Choi, and E. Y. Nho, "Method Validation for Simultaneous Determination of Chromium, Molybdenum and Selenium in Infant Formulas by ICP-OES and ICP-MS," *Food Chemistry*, vol. 141, no. 4, pp. 3566–3570, 2013.

[11] S. Karasakal, "Determination of Trace and Major Elements in Vegan Milk and Oils by ICP-OES After Microwave Digestion," *Biological Trace Element Research*, vol. 197, pp. 683–693, 2020.

[12] S. Paz, C. Rubio, A.J. Gutiérrez, D. González-Weller, and A. Hardisson, "Dietary Intake of Essential Elements (Na, K, Mg, Ca, Mn, Zn, Fe, Cu, Mo, Co) from Tofu Consumption," *Biological Trace Element Research*, vol. 199, pp. 382–388, 2021.

[13] N. H. Bings, A. Bogaerts, and J. A. Broekaert, "Atomic Spectroscopy," *Analytical Chemistry*, vol.78, no. 12, pp. 3917-3946, 2006.

[14] D. Beauchemin, "Inductively Coupled Plasma Mass Spectrometry," *Analytical chemistry*, vol. 80, no. 12, pp. 4455-4486, 2008.

[15] I. Mazurova, A. Khvaschevskaya, and N. Guseva, "The Choice of Conditions for the Determination of Vanadium, Chromium and Arsenic Concentration in Waters by ICP-MS Using Collision Mode," *Procedia Chemistry*, vol. 15, pp. 201–205, 2015.

[16] C. Moor, T. Lymberopoulou, and V. J. Dietrich, “Determination of Heavy Metals in Soils, Sediments and Geological Materials by ICP-AES and ICP-MS,” *Microchimica Acta*, vol. 136, no. 3–4, pp. 123–128, 2001.

[17] P. Pohl, A. Bielawska-Pohl, A. Dzimitrowicz, P. Jamroz, M. Welna, A. Lesniewicz, and A. Szymczycha-Madeja, “Recent Achievements in Element Analysis of Bee Honeys by Atomic and Mass Spectrometry Methods,” *Trends Analytical Chem*istry, vol. 93, pp. 67–77, 2017.

[18] M. L. Astolfi, M. E. Conti, E. Marconi, L. Massimi, and S. Canepari, “Effectiveness of Different Sample Treatments for the Elemental Characterization of Bees and Beehive Products, *Molecules*,”, vol. 25, no. 18, pp. 4263, 2020.

[19] G. Li, and F. Zhu, “Physicochemical Properties of Quinoa Four as Affected by Starch Interactions,” *Food Chemistry*, vol. 221, pp. 1560–1568, 2017.

[20] A. O. Mehder, E. Yilmaz, A. Sungur, M. Soylak, and Z. A. Alothman, “Assessment of Cr, Mn, Fe Co, Ni, Cu, Zn, As, Cd, and Pb Concentrations Found in Gluten-Free Foods from Saudi Arabia by Inductively Coupled Plasma Mass Spectrometry,” *Atomic Spectroscopy*, vol. 36, pp. 254–260, 2015.

[21] M. Yoshida, N. Ogi, and Y. Iwashita, “Estimation of Mineral and Trace Element Intake in Vegans Living in Japan by Chemical Analysis of Duplicate Diets,” *Health*, vol. 3, pp. 672–676, 2011.

[22] Y. A. Hannon, M. J. Al-Obaidi, S.S. Al-Khafaji, A.I. Jassim, “Heavy Metals Content in Some Chips Products in Iraqi Markets,” *Iraqi Journal of Science*, vol. 57, pp. 2818–2828, 2016.

[23] M. Schneider, É. R. Pereira, D. P. C. de Quadros, B. Welz, E. Carasek, J. B. de Andrade, J. C. Menoyo, and J. Feldmann, “Investigation of Chemical Modifiers for the Determination of Cadmium and Chromium in Fish Oil and Lipoid Matrices Using HR-CS GF AAS and a Simple ‘Dilute-And-Shoot’ Approach,” *Microchemical Journal*, vol. 133, pp. 175–181, 2017.

[24] S. Sel, F. A. Erulaş, F. Turak, and S. Bakırdere, “Simultaneous Determination of Chromium Species in Water and Plant Samples at Trace Levels by Ion Chromatography–Inductively Coupled Plasma-Mass Spectrometry,” *Analytical Letters*, vol. 52, no. 5, pp. 761–771, 2019.

[25] Y. Li, G. Peng, Q. He, H. Zhu, and S. M. Z. F. Al-Hamadani, “Dispersive Liquid-Liquid Microextraction Based on the Solidification of Floating Organic Drop Followed by ICP-MS for the Simultaneous Determination of Heavy Metals in Wastewaters,” *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*, vol. 140, pp. 156–61, 2015.

[26] S. A. Drivelos, and C. A. Georgiou, “Multi-Element and Multi-Isotope-Ratio Analysis to Determine the Geographical Origin of Foods in the European Union,” *TrAC Trends in Analytical Chemistry*, vol. 40, pp. 38–51, 2012.

[27] P. Pohl, A. Bielawska-Pohl, A. Dzimitrowicz, P. Jamroz, M. Welna, A. Lesniewicz, and A. Szymczycha-Madeja, “Recent Achievements in Element Analysis of Bee Honeys by Atomic and Mass Spectrometry Methods,” *TrAC Trends in Analytical Chemistry*, vol. 93, pp. 67–77, 2017.

[28] M. Sadowska, W. Hyk, A. Ruszczy´nska, A. Roszak, A. Mycka, and B. Krasnodebska-Ostrega, “Statistical Evaluation of the Effect of Sample Preparation Procedure on the Results of Determinations of Selected Elements in Environmental Samples. Honey Bees as a Case Study,” *Chemosphere*, vol. 279, pp. 130572, 2021.

[29] E. I. Muller, C. C. Müller, J. P. de Souza, A. L. Muller, M. S. Enders, M. Doneda, A. C. Frohlich, G. D. Iop, and K. F. Anschau, “Green Microwave-Assisted Wet Digestion Method of Carbohydrate-Rich Foods with Hydrogen Peroxide Using Single Reaction Chamber and Further Elemental Determination Using ICP-OES and ICP-MS,” *Microchemical Journal*, vol. 134, pp. 257–261, 2017.

[30] A. B. P. Leme, S. Bianchi, R. L. Carneiro, and A. R. A. Nogueira, “Optimization of Sample Preparation in the Determination of Minerals and Trace Elements in Honey by ICP-MS,” *Food Analytical Methods*, vol. 7, pp. 1009–1015, 2013.

[31] S. Oliveira, C. N. Alves, E. S. B. Morte, A. D. F. S. Junior, R. G. O. Araujo, and D. C. M. B. Santos, “Determination of Essential and Potentially Toxic Elements and Their Estimation of Bioaccessibility in honeys,” *Microchemical Journal*, vol. 151, pp. 104221, 2019.