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Factorial Design Optimization of Microwave Digestion for Macro and Microelements in Pregnant Women and Diabetics Multivitamin/Multielement Preparations by Inductive Coupled Plasma Mass Spectrometry (ICPMS)

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Abstract

In the present work, the microwave digestion procedure for the determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn in multivitamin/multielement (MVM) preparations used for pregnant women and diabetics employing ICP-MS, was optimized by factorial design 3^3 (27 runs). The microwave digestion conditions temperature (160, 180 and 200ºC), acid mixture volume (3.9, 5.2 and 6.5 ml) and radiation period (10, 15 and 20 min) were selected as factors. Moreover, a multiple response (MR) was built to establish a compromise conditions between the elements. The optimum conditions were found to be 160ºC, 6.5 ml, and 20 min for temperature, acid mixture volume and radiation period, respectively. The procedure was validated using standard reference material (SRM3280) and elements recoveries were found to be in the range 88 - 107% and was applied to determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn in commercial MVM samples obtained from the local market.

Keywords: Microwave digestion; MVM; Factorial design; ICP-MS.

1. Introduction

Multivitamin/Multielement (MVM) preparations are the most commonly used dietary supplements in the United States [1, 2], thy are available in numerous forms. According to the National Health and Nutrition Examination Survey III, nearly half of the U.S. population (44% of men, 53% of women) is using MVM [2, 3]. Many previous clinical studies have reported that using of a dietary supplement helped in prevention and mitigate of the diseases [4-6], In particular, it is recommended to provide MVM such as iron and folic acid to pregnant women for improving birth outcome, reduction of low birth weight as well as lesser rates of miscarriage [7, 8]. It is also, reported that MVM is very beneficial it maintaining the blood glucose levels in a normal range. Therefore, MVM is considered to be very helpful in reducing the risk for diabetes complications, as well as management of Type 2 Diabetes (T2D) [9]. However, the use of MVM is common in the general population and it is use has also increased over time [10].

Microwave-assisted digestion in closed vessels under pressure is an attractive method, especially for less acid consumption, decreased risk of sample contamination, loss of volatile elements, simple, fast, small sample, safety, and the possibility for programmed microwave energy and temperature that will ensure the complete decomposition of organic matter and it provides clear solution [11-14]. A variety of oxidizing and non- oxidizing acids, as well as concentrated or diluted acids have been used to digest MVM. Hydrochloric acid (HCl) or it is mixture with HNO_3 is useful for salts like metallic carbonates, phosphates, some oxides and some sulfides [15-17]. Nitric acid (HNO_3) very commonly used because of it is high boiling point (122°C) this propriety makes it capable to do oxidizing attack on many samples which are not dissolved by HCl [18]. Likewise, Hydrogen peroxide (H_2O_2) also have a higher boiling point (150°C) so H_2O_2 is also a strong oxidizer agent. H_2O_2

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is used together with HNO_3 to dissolve substances that are not fully dissolved by HNO_3 alone [12, 19].

Several previous studies have focused on optimization of microwave assistant digestion using multivariate techniques to assess the relationship between experimental condition factors and obtain statistically acceptable results for optimization method for quantification analysis of organic and inorganic species [20]. The factorial design is a good tool for finding the critical conditions of the factors (maximum and minimum) with lower consumption of reagents, decreased time spent during the optimization steps [20], and the pareto chart shows the statistical significance of the factors [21-23]. Anjos and colleagues[22] optimized a sample preparation procedure using microwave-assisted digestion for the determination of nickel and vanadium in crude oil employing inductively coupled plasma optical emission spectrometry (ICP OES). The optimization step was performed utilizing a two-level full factorial design (2^3). Khajeh [23] optimized an easy and fast microwave-assisted digestion method combined with flame atomic absorption spectrometry (FAAS) for zinc and copper determinations in milk samples, and this method was performed by 2-level full factorial (2^4) design based on analysis of variance (ANOVA). In addition to these advantages, the limited works had been done reporting the use of factorial techniques for elemental determination in multivitamin/multielement.

ICP-MS is a reliable and effective method for the determination of multi-element at trace level with higher sensitivities as compared with atomic absorption common methods. A sample travels through some main steps during analysis, it is converted into a suitable form for introduction into the plasma and ionized in the plasma. Ions are extracted from the plasma, focused and transported to the mass spectrometer. The mass spectrometer is use to separate the ions based on mass-to-charge ratio (m/z) and ions are counted to quantify the amount of each in the original sample [27, 28]. Further, it is a simple and fast technique having all the elements which can be analyzed by atomic absorption [24, 25]. In fact, inductively coupled plasma mass spectrometry (ICP-MS) is one of the spectroscopic methods which are included in the general chapter USP <233> for determination of elementals in pharmaceuticals [29].

The aim of this study was to develop an easy and fast procedure for determination of thirteen elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) in multivitamin/ multielement (MVM) pharmaceutical preparation that are being used for pregnant women and diabetics. The determination of elements will be performed by ICPMS after optimization microwave digestion conditions temperature, acid mixture volume, and radiation period with Design of Experiments (DOE) using factorial design 3^3 with 27 runs. In addition, current study aims to evaluate the method accuracy by using a certificated reference material as SRM3280.

2. Experimental and Material

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2.1. Standard reference material and reagents

For all analytical procedures, purified water (0.055 µS/cm) was obtained by using the Barnstead water purification system ASTM Type II (Thermo Electron LED GmbH, Germany). HNO_3 65%, HCl 36% analytical grade concentrated acid, and H_2O_2 6%w/v Scharlau (Gota Perez, Spain). ICP-MS stock tuning solution contains Ce, Co, Li, Tl and Y (10 mg/L). Multi-element calibration standard solution (10 µg/ml) containing the minerals of interest, were obtained from Agilent Technologies (Palo Alto, CA, USA), which were used for construction of calibration curves. Standard reference material multivitamin/multielement tablets (SRM 3280) obtained from National Institute of Standard and Technology (NIST, Gaithersburg, MD, USA), and was used for validation method.

In order to demonstrate the applicability of the developed methodology in the quality control, six pregnant women and diabetes preparations MVM products varied in the types, and amount of vitamins/minerals samples purchased from the local market. It was applied to the determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn.

2.2. Instrumentation and apparatus

Agilent ICP-MS 7800 (Model G8421A, **www.agilent.com**, USA) consists of per pump (ISIS) for sample introduction, aqueous solution plasma ignition mode and x-lens ion lenses model (Micromist Nebulizer). It is used for determination of selected element. The conditions for working of this equipment are shown in Table 1.

TABLE 1. Operation conditions for the ICPMS was used for determination of selected element.

ICP-MS condition	Value
RF power (W)	1550
Sample Depth (mm)	8.0
Sample up-take (sec)	30
Nebulizer type	Micro - Mist
Carrier gas (l/min)	1.05
Make up gas (l/min)	0.15

Plasma gas (l/min)	15	
Nebulizer Pump (rps)	0.1	
S/C temp (ºC)	2	
He gas (ml/min)	No gas Mode	He Mode
	0	5
Extract 1 (V)	0	0
Extract 2 (V)	160	160
Omega Bias (V)	-90	-90
Omega Lens (V)	10	10
Cell Enrance (V)	30	30
Cell Exit (V)	-50	-60
Oct P RF (V)	160	160
Oct P Basic (V)	-8	-18
Energy Discrimination (V)	5	3
Points/peak	3	
Repetitions	3	
Integration time /mass (sec)	0.3	

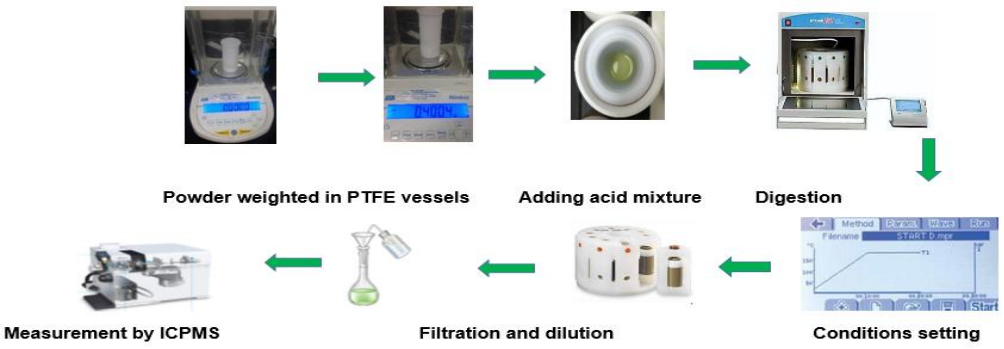
Milestone microwave digestion system (A START D, **www.milestonesys.com**, Germany), consists of reaction sensors for pressure, temperature control, and PTFE digestion vessels. The maximum power and temperature for this microwave are 1200 W and 300°C, respectively. It is noteworthy that A START D is equipped with non-contact temperature monitoring and infrared control in all containers, as well as direct monitoring of pressure in the reference vessel.

2.3. Statistical Analysis

Statistical process including relative standard deviation**,** recoveries**,** standard deviation, and multiple response (MR) were calculated using the Excel Software, windows version 2010. The factorial design analysis, and analysis of variance (ANOVA) were processed using MINTAB 19.

2.4. *Samples and reference material preparation procedures*

Prior to analysis, a set of 20 capsules were taken from MVM reference material standard (SRM 3280) bottles (four tablets from each of five bottles) and were manually crushed with an agate mortar and pestle to obtain the homogenous powder. The homogenous powder was sieved through a plastic sieve with a pore diameter of 1 mm. Later, 0.1 g tablets powder were weighted in PTFE vessels for 27 factorial runs and the conditions including acid mixture (5 ml HNO_3: 0.5 ml HCl :1 ml H_2O_2) volume, microwave temperature and radiation periods were set according to factorial design run. The radiation power was 600 W. The digested sample were filtrated and was diluted to 100 ml using deionized water, as shown in Scheme (1).



Scheme 1: Samples and reference material preparation procedures.

A set of 20 capsules solid MVM sample for each commercial pregnant women and diabetes preparation products were homogenized out in same way of reference material standard, and were pretreated and measured by ICPMS after optimization conditions including temperature (160ºC), acid mixture volume (6.5 ml), and radiation period (20 min), and validation methodology.

2.5. *Optimization strategy*

In our previous study, we have already reported that acid mixture (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2), sample weight 0.1 g, and radiation power 600 W were effective enough to extract MVM samples [30]. Therefore, full factorial design (3^3) with 27 runs was performed in the current study. Three factors including acid mixture volume (Acid Vol.), radiation period (Radiation Per.) and microwave temperature (Temp.), were chosen as the variables to obtain the influence of the factors, interaction of factors, and optimum conditions to extract elements simultaneously. The factorial levels are given in **Table 2**, and the experimental were carried on a reference standard SRM 3280.

TABLE 2. Variables and levels for the factorial design 3^3.

Factor	Low	Central point	High
Acid mixture volume (Acid Vol.) ml	3.9	5.2	6.5

Microwave temperature (Temp.)°C	160	180	200
Radiation period (Radiation Per.) min	10	15	20

3. Results and discussion

The microwave digestion conditions temperature (temp.), acid volume (Acid Vol.), and radiation period (Radiation Per.) were optimized to maximize multiple determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. In addition, a factorial experiment was carried out by Design of Experiments (DOE) using Minitab19. A number of 27 measurements were performed (3^3), as shown in Table 3. It was clear from our study that concentration values for Ca, Fe, Mg, and Na were always at maximum concentration in experiment run 7. However, other elements including Co, Mn, Ni, and Zn showed maximum concentration values in experiment run 9. On the other hand, elements Cr, Cu, K, Se, and V had maximum concentration values in runs 26, 6, 12, 14, and 10, respectively. Considering these points, a factorial design was framed using the multiple response (MR) which was based on the normalization of the concentrations by the highest values to find a single condition that lets the multi-element determination. This assumption has also been Reported by other authors [22, 31]. As shown in Table 3, the greatest value of MR was observed in experiment run 9 at a digestion temperature of 160ºC, acid volume of 6.5 ml, and at a radiation period of 20 min.

Two-way analysis of variance (ANOVA) was used to study the significant of the variance. According to ANOVA, the results as shown in Table 4, the percentage of contributions (PC) values reflected multiple element digestion, and were significantly affected (87.83%) by design mode conditions. The highest effect was observed in the case of interaction of temperature with radiation period (33.47%), acid volume with radiation period (23.96%), and the lowest value is the interaction of temperature with acid volume (11.49%). Besides, ANOVA results show the *p*-value (probability value) which indicates the significant differences in multiple responses with each factor. When the *p*-value is lower than 0.05, it demonstrates that the model is statistically significant [23]. Additionally, the interactions of acid volume with radiation period model, and interaction of temperature with radiation period model are statistical significant with p-value 0.047 and 0.02, respectively, as shown in Table 4.

Pareto chart (Fig. 1.), was used to study the major factors, and effect values in digestion obtained from 3^3 factorial designs, including temperature (A), acid volume (B) and radiation period (C), and their interactions (AC, BC, AC). The results show that two interaction factors temperature with radiation period (AC), and acid volume with radiation period (BC) bars exceed a vertical reference line (95% confidence interval) indicating statistically significance of AC, and BC at the level. However, the major effects (A, B, C) and interaction of temperature and acid volume (AB) are not statistically significant. The results reflect that these variables have a synergistic effect on MR response. This means that, the use of BC at higher levels, and temperature at the lower levels may result in a better analytical response. The same result was obtained by Mketo and coworker [32] in their observations. The interaction plot for MR (Fig. 2.) approve that higher acid volume (6.5 ml), and higher radiation period (20 min) with lower temperature (160ºC) which lead to better analytical response.

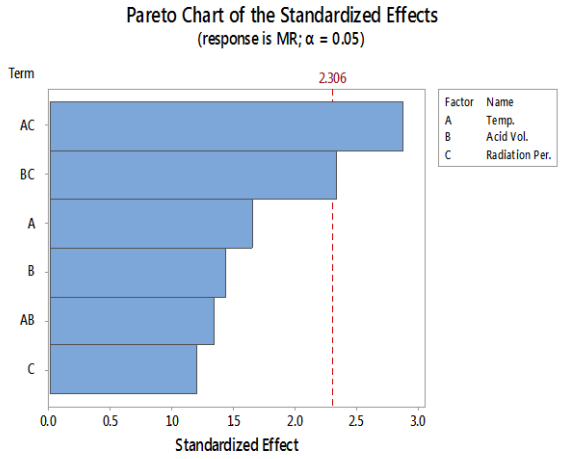


Fig. 1. Pareto chart of major effects and interaction obtained from 3^3 factorial designs. The vertical line defines the 95% confidence interval (A: Temperature, B: Acid Volume, C: Radiation period).

TABLE 3. Design condition microwave digestion in DOE factorial 3^3, and multiple response (MR).

Std Order	Run Order	Temp.	Acid Vo.	Radiation Per.	Ca conc. mg/g	Co conc. µg/g	Cr conc. µg/g	Cu conc. mg/g	Fe conc. mg/g	K conc. mg/g	Mg conc. mg/g	Mn conc. mg/g	Na conc. µg/g	Ni conc. µg/g	Se conc. µg/g	V conc. µg/g	Zn conc. mg/g	MR
1	1	160	3.9	10	104.28	0.81	92.66	1.44	12.44	56.97	66.5	1.48	323.7	8.28	17.12	7.83	10	11.60
2	2	160	3.9	15	96.01	0.71	88.43	1.36	10.89	49.05	63.58	1.3	309.44	7.15	7.87	7.25	8.64	10.25
3	3	160	3.9	20	106.04	0.83	71.77	1.52	12.76	58.8	66.21	1.4	322.24	8.34	16.6	7.3	10.07	11.40
4	4	160	5.2	10	103.17	0.73	89.78	1.55	11.33	52.34	64.31	1.28	312.99	8.62	8.98	8.12	10.41	11.02
5	5	160	5.2	15	98.13	0.74	73.28	1.4	11.41	56.24	65.76	1.49	320.08	8.03	17.9	7.02	9.7	11.03
6	6	160	5.2	20	115.98	0.81	70.31	1.67	12.57	62.03	65.67	1.45	319.64	7.77	16.72	7.3	9.39	11.46
7	7	160	6.5	10	121.66	0.97	80.15	1.48	13.19	58.63	70.33	1.46	342.33	8.61	18.06	7.3	10.4	11.87
8	8	160	6.5	15	102.19	0.74	83.8	1.6	11.4	53	65.01	1.37	316.44	8.36	16.56	8.31	10.11	11.31
9	9	160	6.5	20	118.36	1.00	82.11	1.41	12.58	56.16	70.24	1.49	341.89	8.89	15.79	8.34	10.74	11.90
10	10	180	3.9	10	119.03	0.8	97.27	1.56	12.27	52.14	70.05	1.42	340.97	7.62	17.08	9.1	9.2	11.80
11	11	180	3.9	15	100.94	0.75	87.9	1.52	11.57	59.83	66.84	1.48	325.34	7.07	17.92	6.52	8.55	11.11
12	12	180	3.9	20	97.76	0.72	59.78	1.29	11.06	66.16	64.9	1.37	315.89	6.59	8.47	7.21	7.96	10.20
13	13	180	5.2	10	112.52	0.79	80.94	1.41	12.22	53.25	68.71	1.37	334.42	7.78	4.4	8.79	9.4	11.00
14	14	180	5.2	15	99.65	0.69	80.43	1.46	10.69	64.52	64.85	1.41	315.62	6.68	29.72	6.99	8.08	11.17
15	15	180	5.2	20	108.13	0.77	78.48	1.58	11.95	58.4	66.54	1.37	323.88	7.08	17.34	8.05	8.56	11.21
16	16	180	6.5	10	101.77	0.84	94.8	1.5	12.99	56.17	64.26	1.36	312.76	7.71	17.06	7.26	9.31	11.37
17	17	180	6.5	15	97.15	0.75	92.72	1.45	11.55	55.3	65.57	1.33	319.16	7.48	17.19	6.5	9.04	10.94
18	18	180	6.5	20	113.11	0.77	83.09	1.49	11.85	54.66	67.8	1.43	330	7.4	4.24	8	8.94	10.87
19	19	200	3.9	10	120.12	0.83	76.8	1.52	12.85	52.24	67.42	1.48	328.14	7.34	17.15	7.49	8.86	11.38
20	20	200	3.9	15	105.26	0.76	89.34	1.36	11.73	55.69	69.57	1.47	338.59	7.19	17.78	7.3	8.69	11.20
21	21	200	3.9	20	105.98	0.8	75.79	1.46	12.28	50.04	69.33	1.46	337.47	6.71	17.58	6.75	8.11	10.94
22	22	200	5.2	10	94.12	0.7	76.67	1.36	10.78	55.02	63.68	1.32	309.95	6.77	17.24	8.05	8.18	10.53
23	23	200	5.2	15	104.11	0.8	81.5	1.52	12.29	61.59	66.01	1.4	321.3	7.51	17.56	8.95	9.07	11.47
24	24	200	5.2	20	96.75	0.72	97.49	1.41	11.17	52.56	64.29	1.33	312.92	6.32	17.69	6.36	7.63	10.57
25	25	200	6.5	10	96.41	0.74	76.88	1.51	11.5	55.79	67.58	1.22	328.91	7.29	17.83	7.86	8.81	10.95
26	26	200	6.5	15	111.29	0.78	82.16	1.37	11.99	55.57	67.81	1.46	330.07	6.99	17.85	8.37	8.45	11.28
27	27	200	6.5	20	104.17	0.75	93.12	1.41	11.62	59.4	67.76	1.37	329.82	7.4	16.52	6.82	8.94	11.18

TABLE 4. Result of ANOVA using MR values, considering temperature (temp.), acid volume (Acid Vol.), and radiation period (Radiation Per.).

Source	DF	Seq SS	PC	Adj SS	Adj MS	F-Value	*p*-value
Model	18	4.2431	87.83%	4.2431	0.23573	3.21	0.048
Linear	6	0.9137	18.91%	0.9137	0.15229	2.07	0.168
Temp.	2	0.4128	8.55%	0.4128	0.20642	2.81	0.119
Acid Vol.	2	0.2701	5.59%	0.2701	0.13507	1.84	0.22
Radiation Per.	2	0.2308	4.78%	0.2308	0.11538	1.57	0.266
2-Way Interactions	12	3.3294	68.92%	3.3294	0.27745	3.78	0.034
Temp.*Acid Vol.	4	0.5549	11.49%	0.5549	0.13872	1.89	0.206
Temp.*Radiation Per.	4	1.617	33.47%	1.617	0.40425	5.5	0.02
Acid Vol.*Radiation Per.	4	1.1575	23.96%	1.1575	0.28938	3.94	0.047
Error	8	0.5877	12.17%	0.5877	0.07346		
Total	26	4.8308	100.00%				

DF: Degree of freedom, Seq SS: sequential sum of squares, PC: percentage of contributions, Adj SS: Adjusted sums of squares, Adj MS: Adjusted mean squares, F-Value: F distribution, p-value: probability value.

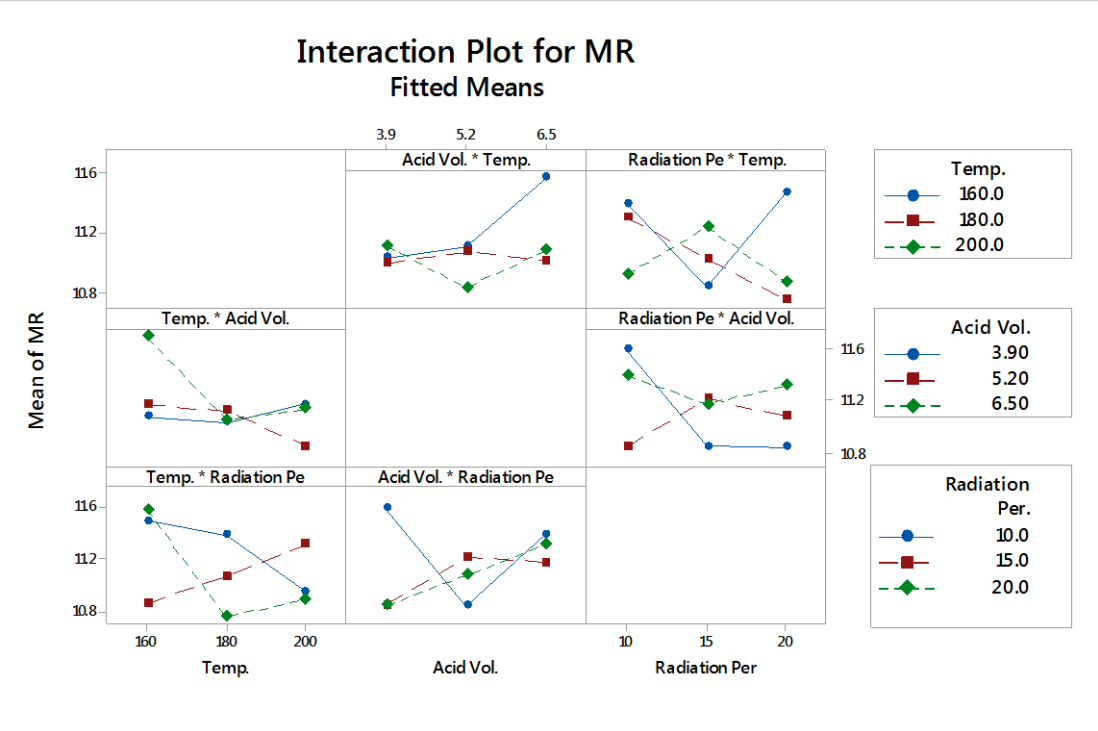


Fig. 2. Interaction plot for MR responses and factor levels effect of the factorial design 3^3.

From all of these observations by MR value and statistical analysis, acid volume (6.5 ml), and radiation period (20 min) with temperature (160ºC) were chosen as optimum conditions for the determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.

3.1. Validation of the method

3.1.1. Calibration curves

For quantitative analysis of the interesting elements in MVM samples, linear seven-point calibration curves (1 - 150 ng/ml) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn were built from multi-element standard solution. The data of calibration curves including correlation coefficient (R^2) detection limit (DL), and background equivalent concentration (BEC) of detector response are presented in Table 5. As it is shown in the table, the correlation coefficient is satisfactory in all cases with correlation coefficients (R^2) in range from 0.985 to 0.999.

TABLE 5. Calibration curves data (correlation coefficient (R^2), detection limits (DL), and background equivalent concentration (BEC)) for the interesting elements.

Element	Mode	Mass	R^2	DL (ng/ml)	BEC (ng/ml)
Ca	He	44	0.9952	0.5591	4.6767
Co	He	59	0.9989	0.0019	0.0047
Cr	He	52	0.9992	0.0088	0.0089
Cu	He	63	0.9988	0.0029	0.0276
Fe	He	56	0.9976	0.0476	0.1642
K	He	39	0.9928	0.9114	37.5306
Mg	He	24	0.9977	0.0938	0.3885
Mn	He	55	0.9991	0.0213	0.0123
Na	He	23	0.9849	1.7069	26.1724
Ni	He	60	0.9990	0.0119	0.0082
Se	He	78	0.9982	0.3838	0.1279
V	He	51	0.9990	0.0029	0.0011
Zn	He	66	0.9989	0.7245	1.1913

3.1.2. Accuracy

The accuracy of the procedure was confirmed by analysis of MVM standard reference materials SRM 3280 after digested under the optimum microwave conditions (acid volume (6.5 ml), and radiation (20 min) with temperature (160ºC)) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. Accuracy was expressed as the recovery percentage (R%) of each element. The element concentration values were found and compared with their concentration values in certificate of SRM 3280. According to the results present in Table 6, the recoveries of all element were founded in range of 88 - 107.5%. The agreement of the results shows that both the proposed mineralization process of samples and the quantitative determination of elements are correct.

3.1.3. Precision

As relative standard deviation (RSD %), the precision of the method expressed was evaluated as repeatability and reproducibility. The repeatability was calculated after analyzing a reference material SRM 3280 ten times in one day. The range of repeatability values were from 0.68 to 5.87% for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. furthermore, the reference material SRM 3280 was analyzed in order to study the reproducibility during three consecutive days. However, the reproducibility values ranged from 0.91 to 4.15% for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. Table 6 shows the lowest precision values that reflect imprecision of total procedure.

TABLE 6. The method recoveries values of selected elements in SRM 3280, and precision (repeatability and reproducibility) under the optimum conditions.

Element	Element value in SRM 3820	Element value found ± RSD	Recovery% (R %)	Repeatability RSD% (n=10)	Reproducibility RSD% (n=12)
Mg conc.mg/g	67.8 ± 4.0	70.24 ± 0.68	103.60	2.47	3.56
Cr conc. µg/g	93.7 ± 2.7	82.11 ± 1.13	87.63	2.45	3.10
Mn conc.mg/g	1.44 ± 0.11	1.49 ± 0.14	103.47	1.64	3.96
Cu conc.mg/g	1.4 ± 0.17	1.41 ± 0.27	100.71	0.68	0.91
Se conc. µg/g	17.42 ± 0.45	15.79 ± 0.91	90.64	1.24	1.29
Ni conc. µg/g	8.4 ± 0.30	8.89 ± 0.76	105.83	1.43	1.43
Co conc. µg/g	0.8 ± 0.01	0.86 ± 0.1	107.5	0.97	1.17
Na conc. µg/g	330± 20.0	341.9 ± 1.90	103.60	1.54	2.61
K conc.mg/g	53.1 ± 7.0	56.16 ± 1.47	105.76	1.72	1.89
Zn conc.mg/g	10.15 ± 0.81	10.74 ± 0.59	105.81	2.95	3.35
Fe conc.mg/g	12.35 ± 0.91	12.58 ± 1.03	101.86	4.72	4.15
V conc. µg/g	8 ± 2.0	8.34 ± 1.87	104.25	5.87	3.42
Ca conc.mg/g	110.7 ± 5.3	118.4 ± 2.99	106.92	1.67	1.96

3.2. Application

The optimum conditions were applied to microwave digestion for the determination of selected elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.) in six commercial pregnant women, and diabetics multivitamin/multielement pharmaceutical preparations products after the validation procedure. Table 7 shows the results of this experimental process. The concentration values were listed with relative stander deviation (RSD) using analysis of samples that were run in triplicate. Moreover, the values were compared with commercial labels of each product as shown in Table 7. In general, the concentration of the determined elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.) were in the range corresponding to the manufacturer labels.

TABLE 7. Results determination of selected elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.) in six commercial pregnant women and diabetes preparation products and three successive measurements (n = 3), by ICP-MS compared to labelled contents.

Preparation		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM1	**Labelled mg/caps**	168	-	0.04	0.5	10	40	50	1	0.05	-	0.03	5	-
	Found mg ± RSD %	167.2858 ±0.846	N/D	0.0409± 0.0020	0.5534 ±0.0399	10.3130 ±0.0350	40.0122 ±0.4819	50.0239 ±0.4874	1.0142 ±0.0661	0.0520 ±0.0129	N/D	0.0307 ±0.0032	5.1497 ±0.0199	N/D
MVM2	**Labelled mg/caps**	160	-	0.025	1.1	-	-	100	2	-	-	0.025	14	-
	Found mg ± RSD %	158.9463 ±0.9546	N/D	0.0250 ±0.0014	1.2885 ±0.0425	3.7398 ±0.5364	***3.4092 ±1.3177***	100.4042 ±0.2038	2.0717 ±0.0839	***9.70181 ±1.23165***	N/D	0.0250 ±0.0256	14.4981 ±0.0543	N/D
MVM3	**Labelled mg/caps**	168	-	0.04	0.5	10	40	50	1	0.05	-	0.03	5	-
	Found mg ± RSD %	168.1438 ±1.0194	N/D	0.0396 ±0.0018	0.5073 ±0.0017	10.1052 ±0.2932	40.3511 ±0.2476	53.0880 ±1.1875	1.11917 ±0.0472	0.0493 ±0.0066	N/D	0.0304 ±0.0015	5.2596 ±0.0990	***0.0117 ±0.0025***
MVM4	**Labelled mg/caps**	-	-	0.2	1	8	-	100	2	-	-	0.1	15	-
	Found mg ± RSD %	***4.7268 ±0.7009***	N/D	0.2075± 0.0057	1.0588 ±0.0238	8.0520 ±0.1790	***2.3900 ±0.7945***	96.8274 ±1.6728	2.0568 ±0.0305	***3.7674 ±0.****6786*	N/D	0.1019 ±0.0154	15.2289 ±0.0223	N/D
MVM5	**Labelled mg/caps**	120		0.025	1.5	8	40.5	50	3.5	-	-	0.05	15	-
	Found mg ± RSD %	120.0708 ±0.3810	N/D	0.0261 ±0.0009	1.5669 ±0.0333	8.01378 ±1.7244	40.5056 ±0.5028	50.5589 ±0.2228	3.5132 ±0.0605	***0.0377 ±0.0068***	N/D	0.0505 ±0.0130	15.4886 ±0.0125	N/D
MVM6	**Labelled mg/caps**	162	-	0.065	2	18	80	100	3.5	-	-	0.02	15	0.01
	Found mg ± RSD %	160.8123 ±1.2134	N/D	0.0660 ±0.0021	2.1915 ±0.0798	18.7374 ±0.1841	78.2441 ±1.1408	100.4622 ±0.3115	3.6743 ±0.1312	***7.5560 ±1.0701***	***0.0427 ±0.0131***	0.02192 ±0.0052	14.6164 ±0.6745	0.01062 ±0.0013

N/D: Not detected

However, the exception was found in the situation of Cu in sample (MVM2), Mn, and Zn in sample (MVM3), and the contents were found which were much higher than labelled. Some samples had Ca, K, Na, Ni, and V, and these samples did not mention their presence in label. These situations are marked as **bold** *italic* as shown in Table 7, since they could come from excipients and their salts with some microelements.

4. Conclusions

In the current study, we carried out the optimization, and validation of a factorial design of microwave digestion conditions including temperature, acid mixture volume, and radiation period for multiple determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn in the multivitamin/multielement preparations of pregnant women and diabetics using ICP-MS. Our validation data correlation coefficient (R^2), detection limits (DL), elements recoveries values, and precision show its fitness for the determination of selected elements in the multivitamin/multielement pharmaceutical preparations for pregnant women and diabetics. The analysis has shown that some MVM contains higher concentrations than the leaflets value and some contains elements which leaflets did not mention their presence.

5. References

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