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Optimized Microwave Digestion Method for Some Micro and Macro Elements in Pregnant Women and Diabetics Multivitamin/Multielement Preparations

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ABSTRACT

The goal of the current study was to obtain an optimum microwave decomposition conditions allowing the determination of several minerals (Fe, Mg, Zn, Cu and Mn by FAAs, and Cr and Se by GFAAs) in pregnant women and diabetic Multivitamin/Multielement (MVM) preparations. Optimum conditions were found to be 0.1 g of sample powder, and digested samples with 5 ml of HNO3, 0.5 ml of HCl and 1 ml of H2O2 and subjected to radiant microwave heating. A pretreatment, using a three-step heating program, irradiation from 10 minutes to 500W; irradiation from 16 minutes to 600W; and cooling. And a reactant of the oxidizing acid mixture was suitable for determining the seven metals studied without subsequent manipulation of the digestion product. The accuracy of the procedure was verified using three spiked samples at three different levels, and recoveries of Fe, Zn, Mn, Mg, Cu, Cr and Se were found to be in the range 98.59 ± 0.36-100.91 ± 0.61%, 99.05 ± 0.46-101.19 ± 0.56%, 97.30 ± 0.1-100.57 ± 0.09%, 97.50 ± 5.63-99.10 ± 3.62%, 98.93 ± 0.05-100.12 ± 0.057%, 99.136 ± 0.01-100.13 ± 0.02%, and 98.54 ± 0.07-99.85 ± 0.03%, respectively. The optimized method was validated and applied to the determination of Fe, Mg, Zn, Cu, Mn, Cr and Se in eight available commercially MVM capsules obtained from local market.

Keywords: Optimized; Microwave digestion; MVM; Pregnant women; Diabetics

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INTRODUCTION

Multi-vitamin/Multi-element (MVM) supplements contain at least three vitamins and/or minerals without herbs, hormones or drugs [1-8]. Macro-elements are the natural elements that the body needs more than any other minerals. Micro-elements are needed in minute amounts to maintain a healthy body. They are mainly needed as components of enzymes and hormones or are involved in the activation of enzymes [9]. Nowadays, the use of MVM preparations is widely spread to increase the daily intake of essential micronutrients. In the United States of America (USA) about 40% of the population consumes MV with or without minerals preparations [10]. They are strongly recommended before, during and after pregnancy [11,12]. They are consumed to support our body with the necessary vitamins, and the minerals which support the growth of the fetus, placenta and maternal tissues, and thus contribute to the success of the pregnancy [13-15]. Micro/macro-minerals play an important role in glucose metabolism, prevention and management of diabetes mellitus. The electrolyte imbalance in diabetes is mainly due to high blood glucose [9]. The safety of MVM depends on a variety of factors, including the manufacturing process, the purity and the origin of the raw ingredients. The manufacturer is responsible for the safety of MVM preparations prior to marketing and the fact that the information on the product label is true and not misleading. Several studies have shown that MVM can contain high levels of certain elements [16,17].

Sample treatment is still the most sensitive and time-consuming step in most analytical method [18,19]. The MVM samples were generally analyzed after complete digestion of the samples [19,20]. Several previous studies have focused on treatments based on microwave heating, mainly using home microwaves, which do not allow for monitoring of digestion conditions and can create dangerous conditions and less control of decomposition [18-22]. Different digestion acids and several programming steps are often required, sometimes including cooling steps between heating steps, which greatly improve the total processing time of the digestion process [23]. A generalized method for different matrices has been optimized, using combinations of high power and long digestion times that exceed the needs of many matrices [19-25]. In addition, most digestion processes have been carried out in some vessels applying high values of microwave power capability [26,27] as it is not possible to extrapolate the conditions to the simultaneous processing of a larger number of samples. Microwave and closed-container digestion of solid samples with concentrated acids or acid mixtures have also been used in several studies for the measurement of minerals in complex matrices [28,29]. The main advantages of microwave assisted digestion in closed containers are the high relative speed, the low possibility of contamination, the minimal loss of volatile elements and the possibility of simultaneously processing a large number of samples. Microwave also has been applied in the treatment of samples for the measurement of chrome and other minerals in MVM supplements [30]. In addition, microwave radiation was used as a source of energy and HNO_3 for the decomposition of 95 dietary supplements and reference materials (NIST) for the measurement of minerals; cadmium, mercury and lead [10,31]. Despite these advantages, only few studies have report the use of extraction approaches for minerals measurement in MVM formulation [10,32,33].

The current work is focusing on optimization of the oxidation mixture, radiation power, radiation period, and sample weight for extraction of Fe, Mg, Zn, Cu, Mn, Cr, and Se, from multi-vitamin/multi-mineral (MVM) preparations of pregnant women and diabetics using HNO_3, HCl and H_2O_2, followed by their AAS determination.

EXPERMINTAL SECTION

Reagents and Solutions

For all analytical procedures, purified water (0.055 µS/cm) was obtained using the Barnstead water purification system ASTM Type II (Thermo Electron LED GmbH, Germany). 65% HNO_3, 36% HCl concentrated analytical grade acid, and 6% H_2O_2 w/v Scharlau (Gota Perez, Spain), were used for MVM digestion and dilution of standard solutions for construction of calibration curves. Individual solutions containing the minerals of interest were prepared by suitable dilution of 1000 mg/L standard solution of each element (Scharlau, Gota Perez, Spain).

Apparatus

Atomic Absorption Spectrometer (Varian, Mulgrave, Australia) consist of AA240FS flame (fast sequential), GTA 120 (graphite tube atomizer) and single hollow cathode lamps were used for Fe, Mg, Zn, Cu, Mn, Cr and Se. The equipment has been used under the standard conditions recommended by the manufacturer. A START D digestion microwave system from Milestone (Germany) equipped with reaction sensors for pressure and temperature control and 12 high-pressure digestion vessels, 100 ml PTFE was used for total digestion of samples, power up to 1200W controls up to 300°C in the reference vessel, non-contact temperature monitoring and infrared control up to 300°C in all containers, direct monitoring of pressure and control up to 100 bar in the reference vessel.

Sample Preparation Procedures

Due to the lack of reference materials, MVM solid preparations were purchased from Vitabiotics (London, England) which were used for the home Control (CS) Samples. Prior to analysis, a set of 20 capsules were manually crushed with an agate mortar and pestle, homogenized and sieved through a plastic sieve with a pore diameter of 1 mm.

Acid Mixtures

Total digestion of the samples was performed by mixing about 0.4 g of the sample powder with various mixtures of HNO_3, HCl and H_2O_2 see Table 1, in order to find the most effective mixture for metal extraction from MVM. The containers were closed, fitted into the oven cavity and subjected to the following three-step heating program: (1) irradiation for 10 minutes at a power of 500 W; and (2) irradiating for 10 minutes at a power of 700 W; (3) After cooling the containers were opened and their contents were filtered and quantitatively transferred into 100 ml volumetric flasks. The volumes were made up to the mark with purified water. The concentrations of the metals of interest were determined in the solution after appropriate dilutions to the corresponding linear calibration curves ranges.

Microwave Digestion

The objective of the optimization of the experimental conditions of the digestion procedure is to examine the effect of the microwave power and the duration of radiation variation of the mass ratio of the sample to the oxidant mixture volume. In six separate runs, the digestion powers of the second stage of the microwave oven were 400, 500, 600, 700, 800 and 900 W respectively, and all other parameters were kept constant. In different vessels, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 g of samples were measured and dissolved in 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2 using the same digestion program power and the same radiation period. Finally, the radiation period of the second stage of the microwave were changed to 10 minutes, 13 minutes, 16 minutes, 19 minutes, 22 minutes and 25 minutes, respectively. All other parameters were kept constant. Each sample was replicated three times and a blank digestion was performed in the same manner (digestion conditions).

Table 1. Eleven combination mixtures of concentrated HNO_3 65%, HCl 37%, and H_2O_2 6% were used to obtain the most efficient condition for the extraction of metal from MVM

Mixture Number	HNO_3 ml	HCl ml	H_2O_2 ml
1	3	0	0
2	4	0	0
3	5	0	0
4	5	0.3	0
5	5	0.5	0
6	5	0.7	0
7	5	0.5	1
8	5	0.5	2
9	5	0.5	3
10	5	0	2
11	5	0	3

RESULTS AND DISCUSSION

Optimization of the Conditions for Microwave Digestion

When examining the efficiency of the oxidant mixture using HNO_3 alone for digestion a yellow color and white solid residue were observed. However, on increasing the volume of HNO_3 the color partially disappeared and the residue diminished. But on using a mixture of HNO_3, HCl, and H_2O_2, the yellow color completely disappeared. Some studies have reported the same observation [34]. Figure 1 shows recoveries under different oxidant mixtures. Less recoveries were observed when HNO_3 (3ml) was used alone that they were between (67-91%), and increase in HNO_3 volume (5 ml to 7 ml) did not affect recoveries of elements except for Fe, Zn, and Cu (R%>94%). An obvious increase in recoveries was observed for some elements Se, Cu, and Cr after using a mixture of HNO_3 (5 ml), HCl (0.3 ml) and gradual increased by adding more HCl (0.5 to 0.7 ml) (R% between 86-96%) because chlorides are in general terms soluble [33]. An improved extraction efficiency of elements was observed when an oxidant mixture HNO_3, HCl, and H_2O_2 was used. But excellent recoveries were observed by using an oxidant mixture combination of 5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2 that were between (94-101%). To ensure that the complete release of remaining elements bound to white solid residue, any increased in the H_2O_2 volume give negative effect in recovery of most elements. Some studies agree with our results reported HNO_3 can also be used together with HCl and H_2O_2 to improve the performance of digestion, and avoid interference with the residual [21,33]. Poor recoveries were observed when a mixture of HNO_3, and H_2O_2 was used R% between (45-93%). This experiment demonstrated that the oxidant mixture No.7 5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2 in microwave digestion of MVM were significant for almost all the elements. Therefore it was chosen as the best overall combination for the microwave digestion process.

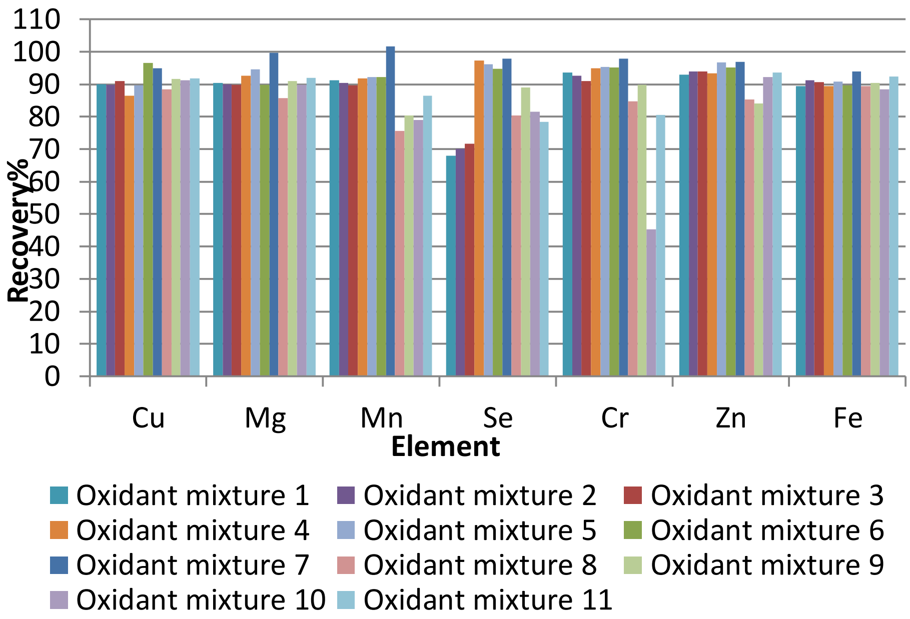

Figure 1. Effect of 11 oxidant mixtures on recoveries% of elements (Fe, Mg, Zn, Cu, Mn, Cr, and Se), weight 0.4 g MVM, radiation power 700 W at the second step of microwave program, and radiation period 10 min.

Radiation power is known to be an attractive parameter of affect in microwave based sample digestion procedures. For the optimization of the extraction radiation power in the current study, the impact of six radiation powers (400, 500, 600, 700, 800 and 900 W) were studied, at the second stage of the microwave program, and elements recoveries (Fe, Mg, Zn, Cu, Mn, Cr, and Se) were calculated under constant other factors (0.4 g MVM sample, 5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2, and 10 min radiation period). From Figure 2 It can be seen that modified radiation power from 400 to 600 W shows gradual improved extraction efficiency for all tested elements (R% 94-101%). However, elements Fe, Zn, and Cu did not show much effect between the six investigated radiation power levels. Therefore, the radiation power 600 W selected as the optimum radiation power in the second step. From the previous study [17] radiation power 700 W was selected, and authors did not examine other radiation power.

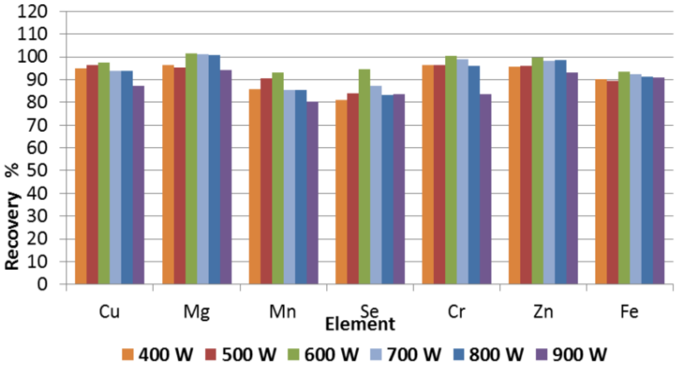

Figure 2. Effect of radiation power on recoveries% of elements (Fe, Mg, Zn, Cu, Mn, Cr, and Se), weight 0.4g MVM, radiation period 10min, oxidant mixture (5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2), and six radiation power 400, 500, 600, 700, 800, and 900 W.

Radiation period is one of the important features of microwave sample digestion methods its rapidness compared to the conventional methods. Therefore, radiation period is one of the factors that needed to be evaluated for the microwave digestion of MVM. The factor of radiation period was examined from 10 to 25 min and the recoveries against radiation periods illustrated in Figure 3. From this Figure it can be seen that recoveries of elements were not affected by change of radiation period from 10 to 25 min (R% 97-102%), less effect was observed for Se, Zn, and Cu, and good recoveries were obtained when samples were radiated for 16 min at second step microwave program. Therefore, this radiation period was selected as an optimum radiation period.

Under optimum radiation period, and radiation power, the effect of MVM weight was studied with a mixture of 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2, and it was evaluated in a range of 0.05 to 0.5 g as it is shown in Figure 4 From this Figure it can be seen that the change in the sample amount from 0.05 to 0.1 g had an effect on extracting recoveries of Se and Cr and it had less effect on recoveries efficiency for Fe, Zn, Cu and Mn, and did not affect Mg recovery. However, an increase in the sample amount from 0.1 to 0.5 g showed a drastic decrease in extraction efficiency for all elements studied. Similar observations have also been reported in the literature [35-37]. The weight of 0.1 g sample was chosen as the best overall weight for the microwave digestion process. The optimum conditions of oxidant mixture is 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2, 0.1 g MVM sample, microwave program (1) 10 min irradiation at 500 W power; and (2) 16 min irradiation at 600W power; (3) cooling. As discussed earlier optimum conditions were utilized in all subsequent experiments in order to complete validation (precision, sensitivity, accuracy, LOD and LOQ) of the proposed method.

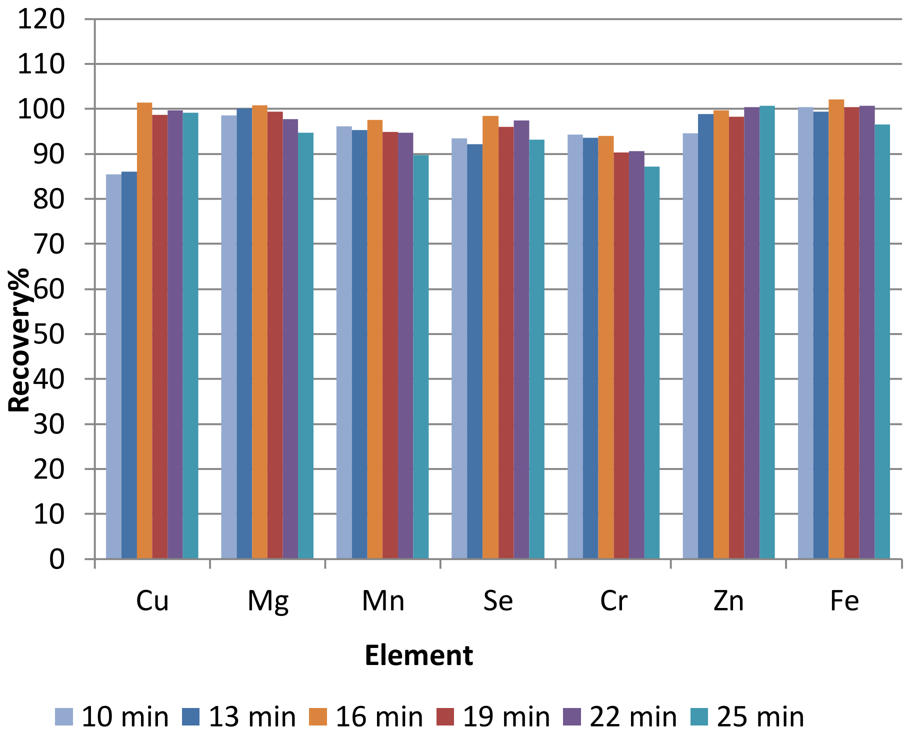

Figure 3: Effect of radiation period on recoveries% of elements (Fe, Mg, Zn, Cu, Mn, Cr, and Se), weight 0.4 g MVM, oxidant mixture (5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2), and radiation power 600 W for six different period 10, 13, 16, 19, 22, and 25min.

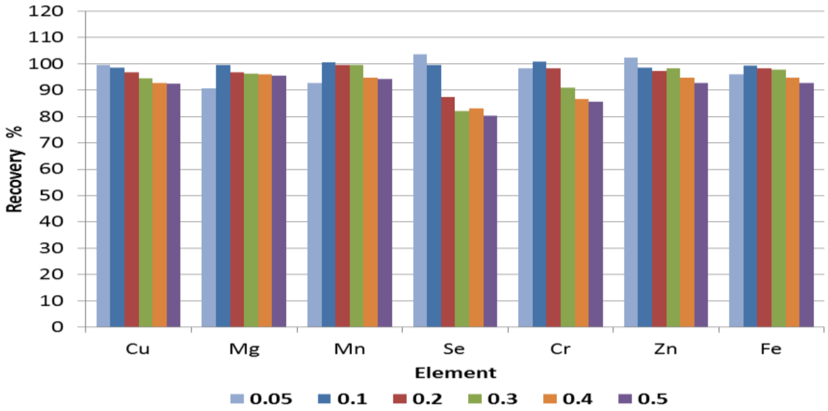

Figure 4. Effect of MVM weight on recoveries% of elements (Fe, Mg, Zn, Cu, Mn, Cr, and Se), at optimum radiation period, radiation power, and Oxidant mixture (5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2) for six different weights 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 g.

VALIDATION OF THE METHOD

Calibration Curve

For quantitative analysis of the elements in MVM samples, calibration curves were built for each element at different concentration levels. The data of calibration curve including linearity range, slope, and correlation coefficient, are presented in Table 2. As it is shown in the Table 2, the linearity is satisfactory in all cases with correlation coefficients (R^2) ranging from 0.9998 to 0.9851.

Precision

Precision (intra-day repeatability and inter-day reproducibility) is expressed as the Relative Standard Deviation (RSD%) of ten independent analyses of the spiked samples of MVM product. Repeatability values ranged from 0.0018 to 0.0329% for Fe, Mg, Zn, Cu, Mn, Cr, and Se. In order to study the intra-day repeatability, furthermore, the MVM samples were analyzed ten times during three consecutive days. In order to study the inter-day reproducibility values ranged from 0.0028 to 0.0626% for Fe, Mg, Zn, Cu, Mn, Cr, and Se as shown in Table 3. The lowest precision values reflect imprecision of total procedure.

Table 2. The data of calibration curve data, including linearity range, intercept, and correlation coefficient

Element	Unit	External Calibration linear Range	Slope	Intercept	R^2
Fe	µg/ml	1.2-5.0	0.0416	0.0097	0.9983
Zn	µg/ml	0.2-0.8	0.3212	0.0067	0.9988
Mg	µg/ml	0.1-0.4	0.5433	0.0047	0.9992
Mn	µg/ml	0.5-2.0	0.1103	0.0029	0.9995
Cu	µg/ml	1.0-4.0	0.0573	0.0043	0.9998
Se	µg/l	30.0-80.0	0.0003	0.0035	0.9851
Cr	µg/l	4.0-12.0	0.0109	0.0074	0.9971

Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for all elements were calculated as 3 and 10 times, respectively. The standard deviation of the response estimated by the standard deviation of *y*-intercepts of regression lines divided by the slope of the calibration curve as suggested by the International Conference on Harmonization (**ICH**) [38,39]. LOQs and LODs were expressed as µg of metal per mL of solution, and µg of metal per gm of MVM, taking into consideration the average sample weight and sample dilution in the process of preparation as shown in Table 3. LOQs and LODs value reflect the lowest amount of elements which can be determined accurately by the procedure.

Table 3. Wavelength (nm), precision, limit of detection (LOD), and limits of quantification (LOQ) for analyzed Fe, Mg, Zn, Cu, Mn, Cr, and Se elements in MVM.

Element	Wavelength (nm)	LOD		LOQ		Precision	
		µg/ml	µg/g	µg/ml	µg/g	Intra-day (RSD%)	Inter-day (RSD%)
Fe	248.3	0.539752	0.221297	1.79917	0.73765	0.030982	0.062624
Zn	213.9	0.030813	0.252665	0.102709	0.842208	0.003289	0.006483
Mg	285.2	0.013344	0.729467	0.044482	2.431667	0.001801	0.008789
Mn	279.5	0.0467	0.019147	0.155668	0.063823	0.011635	0.017223
Cu	324.8	0.068446	0.003939	0.228152	0.013129	0.02807	0.035696
Se	196	0.00995	0.003264	0.033166	0.010878	0.004819	0.006028
Cr	357.9	0.000795	0.000652	0.002649	0.002173	0.002277	0.0028

Accuracy

In this study, due to the lack of sufficient MVM certified reference material [17], the sample was spiked with the analyte in order to determine a possible proportional bias derived from the sample pretreatment and matrix interference; accuracy was expressed as the recovery percentage of the analyte [38]. A solution with known analyte concentration, depending on the actual concentration of elements in a sample, was added to the samples of MVM prepared in triplicate according to three different concentration levels. In order to determine the recovery (R) for each mineral, the following formula was used:

$$R = \frac{C_{obs} - C_{native}}{C_{spiked}} \times 100$$

Where $\mathbf{C_{native}}$ is the analyte concentration in the unspiked control sample.

$\mathbf{C_{obs}}$ is the analyte concentration of an element in the spiked sample.

$\mathbf{C_{spiked}}$ is the analyte concentration in the solution added to sample [38-40].

According to the results present in Table 4, the recoveries for spiked samples were in range of 97-101%. The agreement of the results shows that both the proposed mineralization process of samples and the quantitative determination of elements are correct.

Table 4. Recovery% ± RSD (%) of Fe, Mg, Zn, Cu, Mn, Cr, and Se in spiked MVM samples of three different concentration levels.

Element	**Spike Concentration 1**		**Spike Concentration 2**		**Spike Concentration 3**	
	Added µg/mg	**Recovery (%) ± RSD (%)**	**Added µg/mg**	**Recovery (%) ± RSD (%)**	**Added µg/mg**	**Recovery (%) ± RSD (%)**
Fe	0.02	98.59 ± 0.36	0.04	100.91 ± 0.61	0.08	99.72 ± 0.14
Zn	0.07	101.19 ± 0.56	0.125	99.05 ± 0.46	0.25	100.27 ± 0.47
Mn	0.05	97.30 ± 0.11	0.1	100.57 ± 0.09	0.2	100.55 ± 0.10
Mg	0.7	99.10 ± 3.62	1.25	97.50 ± 5.63	2.5	98.36 ± 2.92
Cu	0.03	98.93 ± 0.05	0.06	99.10 ± 0.04	0.120	100.12 ± 0.057
Cr	0.02	99.79 ± 0.01	0.04	99.136 ± 0.01	0.08	100.13 ± 0.02
Se	0.002	98.54 ± 0.07	0.004	99.85 ± 0.03	0.008	99.64 ± 0.05

APPLICATION

In order to demonstrate the applicability of the developed methodology in the quality control of pregnant women and diabetic's preparations MVM capsules, it was applied to the determination of Cu, Fe, Mn, Mg, Zn, Se and Cr in different samples purchased from the local market. The results for this experiment are shown in Table 5. The average concentration of the determined elements corresponded generally to the labelled content showed that an average recovery in the range 96-104% with the relative standard deviation of the repeatability of 1 to 0.0003%. The best results were obtained for Fe, Mg, Zn, Cu, and Mn. Recoveries were also acceptable for Se and Cr, but in two preparations were found much higher than labelled, these situations are marked as **bold** *italic* in Table 5. And two preparation samples (MVM 3 and MVM 8) contain Fe and Mn unlabelled see Table 5.

Table 5. Results obtained in the analysis of Fe, Zn, Mg, Mn, Cu, Cr and Se in commercial pregnant women and diabetics preparations MVM, (mean ± RSD) eight capsules product and three successive measurements (n=3) by AAs compared to labelled contents

Preparation		**Fe**	**Zn**	**Mg**	**Mn**	**Cu**	**Cr**	**Se**
MVM1	Labelled mg/caps	8	15	100	2	1	0.02	0.01
	Found mg ± RSD%	8.03 ± 0.12	15.70 ± 0.41	100.18 ± 0.66	2.02 ± 0.095	1.00 ± 0.04	0.023 ± 0.0009	0.095 ± 0.003
MVM 2	Labelled mg/caps	6	15	50	2	1.5	0.05	0.015

	Found mg ± RSD%	5.92 ± 0.08	15.07 ± 0.22	50.62 ± 0.47	2.02 ± 0.12	1.49 ± 0.03	0.044 ± 0.002	0.016 ± 0.009
MVM 3	Labelled mg/caps	___	14	100	2	1.1	0.025	0.025
	Found mg ± RSD%	1.91 ± 0.2	14.47 ± 0.25	103.19 ± 0.85	1.99 ± 0.13	1.08 ± 0.48	0.026 ± 0.0004	0.030 ± 0.004
MVM 4	Labelled mg/caps	10	5	50	1	0.5	0.04	0.03
	Found mg ± RSD%	9.90 ± 0.16	4.95 ± 0.09	51.57 ± 0.90	0.98 ± 0.03	0.48 ± 0.02	0.037 ± 0.0006	0.03 ± 0.007
MVM 5	Labelled mg/caps	10	5	50	1	0.5	0.04	0.03
	Found mg ± RSD%	10.01 ± 0.12	4.89 ± 0.07	50.10 ± 1.15	1.00 ± 0.03	0.49 ± 0.01	0.039 ± 0.0008	0.03 ± 0.005
MVM 6	Labelled mg/caps	8	15	50	3.5	1.5	0.025	0.05
	Found mg ± RSD%	7.96 ± 0.11	14.97 ± 0.75	50.50 ± 1.08	3.46 ± 0.25	1.51 ± 0.06	0.025 ± 0.0003	0.044 ± 0.002
MVM 7	Labelled mg/caps	12	12	100	2.5	1.5	0.05	0.1
	Found mg ± RSD%	12.26 ± 0.09	11.78 ± 0.19	99.94 ± 0.78	2.52 ± 0.03	1.47 ± 0.06	*0.06* ± 0.002	0.11 ± 0.011
MVM 8	Labelled mg/caps	10	1	10	2.5	2	_	0.05
	Found mg ± RSD%	10.08 ± 0.21	0.98 ± 0.03	9.73 ± 0.44	2.54 ± 0.04	1.98 ± 0.05	0.01 ± 0.001	*0.06* ± 0.005

CONCLUSION

Fast and accurate method for sample preparation followed with AAs for multi-element analysis of pregnant women and diabetics preparations MVM was optimized and validated. The method presented satisfactory linearity, LOD, LOQ, accuracy, repeatability, and reproducibility for total seven elements (Cu, Fe, Mn, Mg, Zn, Se and Cr). It was used for determination of the element contents in pregnant women and diabetics preparations MVM purchased from the local market.

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