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THE DETERMINATION OF MANGANESE IN PHARMACEUTICAL PRODUCTS OF VETERINARY USE

Mihaela SCRIPCARIU,[a]* Ion Gh. TĂNASE,[b] Şerban FLESCHIN[b] and Vasile MAGEARU[b]

[a]Institute for Control of Biological Products and Veterinary Drugs, Bucharest, 37 Sos. Dudului, Bucharest – 6, 060603, Roumania
[b] Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, 92-96 Sos. Panduri, Bucharest – 5, 0610644, Roumania

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The scope of the present study is to quantify manganese in pharmaceutical products of veterinary use, using flame atomic absorption spectrometry (f_AAS). The determination is made after sample mineralization in an HCl: HNO_3 (20:1, v: v) mixture followed by the instrument quantification and the validation of the result. The validation supposes to evidence that the interferences from the whole is meat analytical system and their measurement do not influence the results and ensure that there are no other effects which have not been considered.

INTRODUCTION

The animals' health and performance depend on a lot of factors such as vitamins, vitamins with minerals or only minerals. The last have a special importance in an efficient and quick growing. The diet has an essential role in maintaining the animals' health and in diseases' prevention.

This concept is based on understanding the contribution of minerals in reducing the negative effects of free radicals and toxic metabolites towards immune processes of animals' organism. The key elements are: Cu, Zn, Mn, Fe and Se, [1-4] which are added in animals' food through premixes and nutritive supplements.

The metals are administrated in food, so the organism can synthesize itself the enzymes, but in cases of their deficiency, oxidative stress and lesions of molecules and membranes could appear.

In Romania, the market of pharmaceutical products for veterinary use (premixes and nutritive supplements with vitamins, vitamins with minerals or only minerals) has increased in the last years. If in 1999 were proposed only 19 new products for authorization, in 2006 – the number of authorizations was 95. As a conclusion from the total new 514 pharmaceutical products authorized, 196 of them contain one of the trace elements (Fe, Cu, Zn and Mn named oligo-elements).

Manganese is an essential microelement for animal health and it is very important in the growth of the skeleton, monopolysaccharides synthesis, lipid metabolism, in the growth and function of the genital apparatus. Mn, like coenzyme A, is an important element in redox natural reactions.

EXPERIMENTAL

Reagents and apparatus

Manganese standard stock solution (1000 µg Mn/mL), hydrochloric acid concentrate solution 12 mol/L (c = 1,19 g/mL), nitric acid 65% solution, (c = 1,42 g/mL) and hydrogen peroxide (c = 30%), (MERCK, Germany), ultra pure water (PURITE NEPTUNE ANALYTICAL – PURITE, England), hydrochloric acid solution 6 mol/L (prepared by us in our laboratory), "ECONOMIX 80011", powder of vitamins with minerals for veterinary use (AGRAVIS RAIFFEISEN AG – Germany), which contains manganese under sulphate form (0.5% Mn) and "CANIFORT" – tablets of vitamins with minerals for veterinary use (S.C. ROMVAC COMPANY S.A.) that contain manganese under oxide form (1.2% Mn). A spectrometer GBC – AVANTA (flame atomization) (GBC – Australia) was used for determination at wavelength 279.5 nm.

Analytical procedure

About 1.0 g powder of "ECONOMIX 80011", exactly weighed, m_0, into chine crucible is calcined at $450^0 \pm 20^0$C in a thermo adjustable oven (NABER THERM – GmbH Germany). The cold ash is moistened with care adding a few drops of hydrogen peroxide and keeping it on electric mantle,

* Corresponding author: mihaelascripcariu@yahoo.com

until H_2O_2 is decomposed. Over the ash add 15 mL of HCl solution 6 mol/L, evaporate the acid on electric mantle and then add 1 mL of HNO_3 concentrated solution. Add then 5 mL HCl solution 6 mol/L and continue the evaporation to dry. After cooling, the residue is filling to mark quantitative with ultra pure water in a 100 mL volumetric flask.

The Mn concentration is determined f_AAS using the following equation:

$$\text{Total Mn } (\mu g/g) = c \times F_{dil} / m_0 \quad (1)$$

where: c = concentration of standard solution measured before sample (μg/mL), F_{dil} = dilution factor, m_0 = working quantity (g).

In the case of premixes and nutritive supplements, studied by us, the mixture HCl: HNO3 (20:1, v:v) gives the best results to determine Mn using f_AAS.

RESULTS AND DISCUSSION

The purpose of this paper was to propose a new flame atomic absorption spectrometric (f_AAS) method for manganese determination in pharmaceutical preparations, such as premixes and nutritive supplements, where the matrix is very complicated. For quantification the sample is first calcinated and then the ash is solubilised by HCl,[5] HF: H_2SO_4 : HCl (10:1:5, v:v:v),[6] HF:$HClO_4$ (20:25, v:v)[7] or HCl:HNO_3 (5:20, v:v).[8] In the case of premixes and nutritive supplements, studied by us, the mixture HCl: HNO_3 (20:1, v:v) gives the best results to determine Mn using f_AAS.

For choosing the acid mixture we took into consideration the results obtained after the mineralization and the cost of the acid we used. We worked with different acid solutions for different samples (Table 1) with different Mn concentrations and different matrices.

The results depend on the matrix and are presented in Fig.1. It is observed that the best results are obtained when treating the ash with a few drops of hydrogen peroxide and then with the mixture HCl: HNO_3 (20 mL (6 mol/L): 1 mL (conc 65%)).

Evaluation of analytical characteristics of method

The validation method for manganese determination in pharmaceutical powders for veterinary use (premixes and nutritive supplements) through f_AAS must prove that the equation includes all the interferences that could affect the final result.

For the validation procedure the following parameters are evaluated: linearity, precision and accuracy. The precision represents the degree of concordance between the result and the reference value.[9] The systematical and happened errors are quantified. The precision give us indications about the utility and applicability of this method to real samples.

Linearity is evaluated through graphical representation of the measured absorbance at λ = 279.5 nm. Fig. 2 shows the calibration curve and the regression line in the prediction range, as well as the trend line equation. Linearity shows the direct proportionality of the absorbance with Mn concentration solution into the given range (0.50 – 3.50 μg/mL).

Table 1

Samples used for the determination of an optimal ratio of acids and the optimization of the mineralization

Series no.	Name of product	Manufacturer	Composition	Conc.Mn mg/g
1.	ROVIMIX 31 COW powder	Roche Vitamins Hellas L.T.D. - Greece	• Vitam. liposoluble • mineral salts of Fe, Cu, Zn, Mn and Mg	30
2.	ROVIMIX 22+22M PIG powder	Roche Vitamins Hellas L.T.D. - Greece	• Vitam. lipo and hydrosoluble • mineral salts of Fe, Cu, Zn, Mn and Mg	20
3.	LOVIT VT solution	LOHMAN - ANIMAL HEALTH -Germany	• Vitam. lipo and hydrosoluble • mineral salts of Fe, Cu, Zn, Mn and Co	25
4.	3-211 powder	ISV PREMIXGYAR-Hungary	• mineral salts of Fe, Cu, Co, Mn, Zn, Ca	64
5.	ECONOMIX 80011 powder	AGRAVIS RAIFFEISEN AG – Germany	• Vitam lipo and hydro. • mineral salts of Cu, Co, Mn, Zn, Ca	5

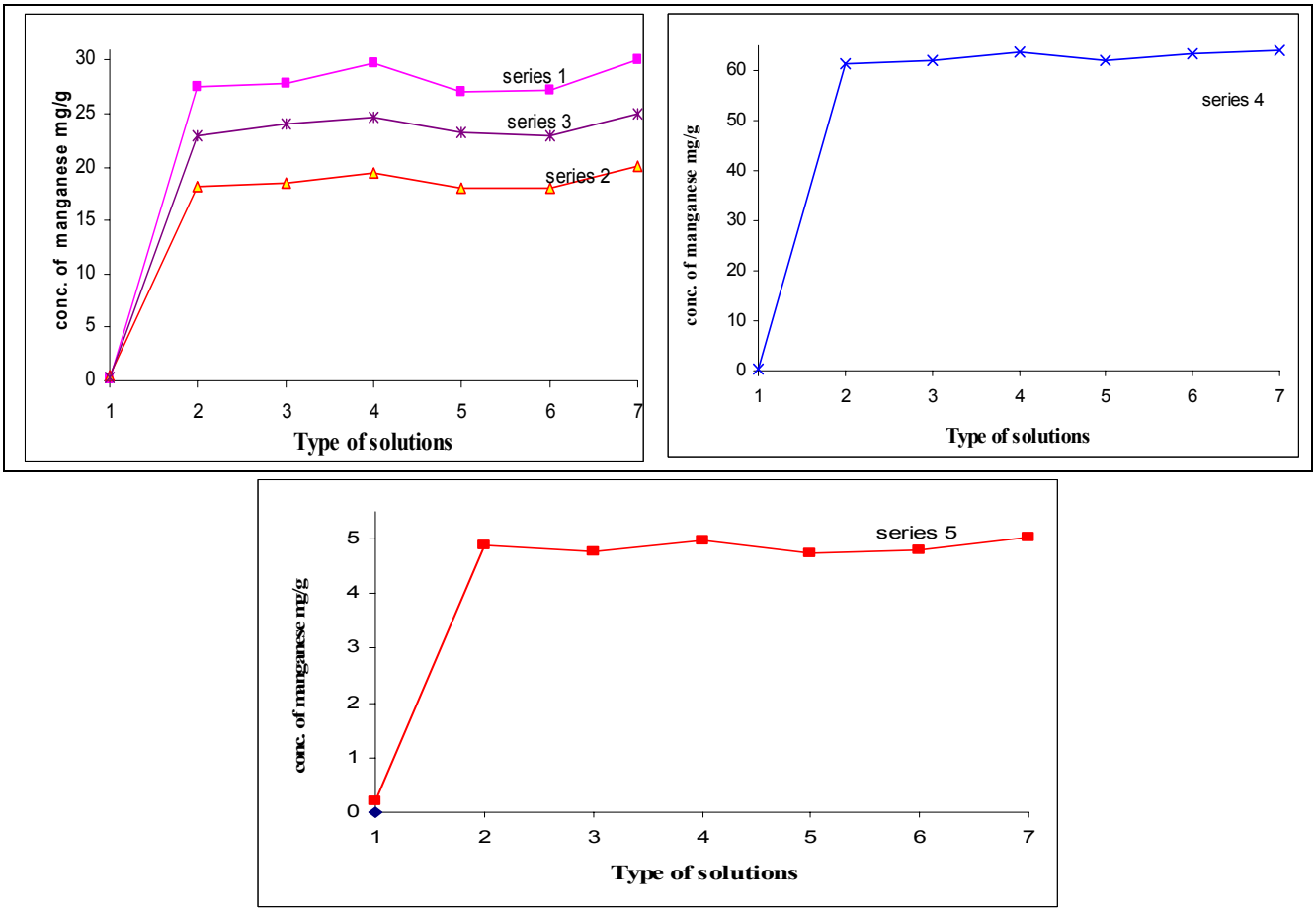

Fig. 1 – Mineralization of samples with different solutions: H_2O (1), HNO_3 conc. (2), HCl 6 M (3), HNO_3 conc. and HCl 6 M (4), HNO_3 conc. and H_2O_2 (5), HCl 6M and H_2O_2 (6), HCl 6 M, HNO_3 conc. and H_2O_2 (7).

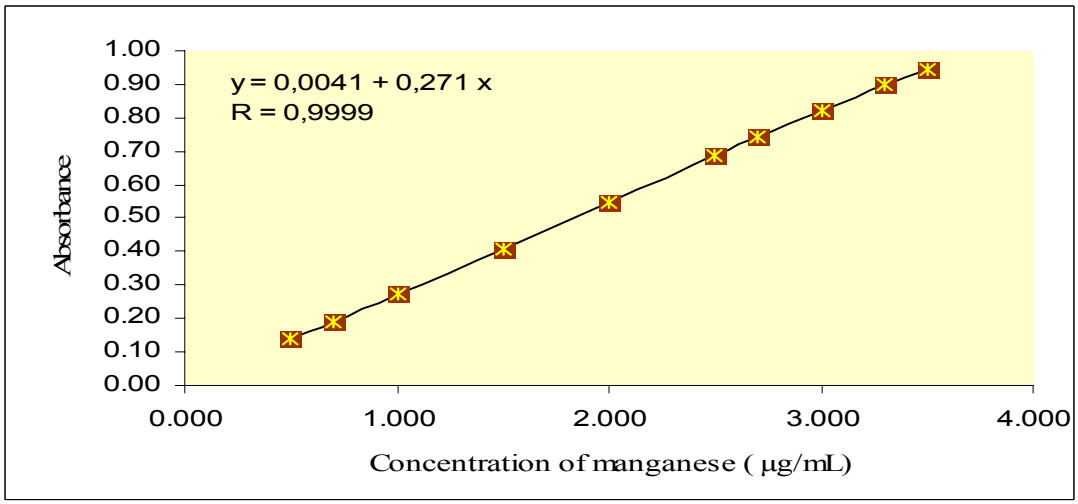

Fig. 2 – The calibration curve for Mn determination through f_AAS.

The linear regression data are sometimes not sufficient for evaluation. An alternative approach is the ratio between the analytic response and its respective concentration to the manganese content of the standard solution used. A graphical representation on a logarithmic scale can be seen in Fig. 3. The obtained line must be horizontal on the whole range, with a positive deviation at lower concentrations and a negative deviation at higher concentrations.[10] The deviations must not pass ± 5%,[11] and in our study was -1.5 and +1.9%.

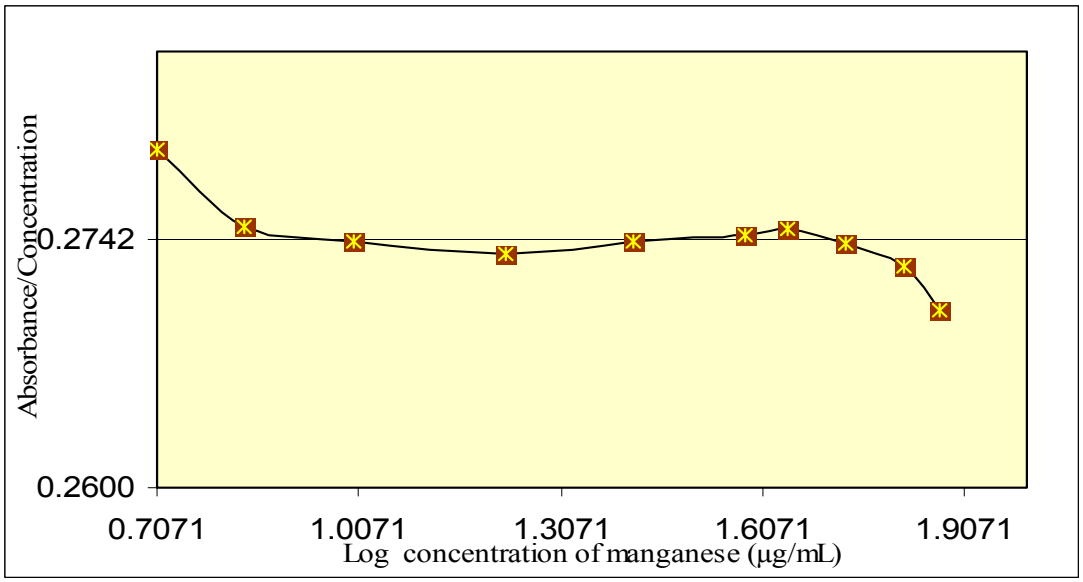

Fig. 3 – The graphical representation of the relative answers.

Another way of approaching is represented by statistic test of linearity[12] that presumes the calculation using standard data of a linear calibration as well as a non-linear function, too. The difference of dispersions DS^2 is calculated with the equation:

$$DS^2 = (N-2)\, s_{y1}^2 - (N-3)\, s_{y2}^2$$

where N represents the number of levels of concentration, s_{y1} is the standard residual deviation of one linearity regression ($y = 0{,}0041 + 0{,}271\ x$, $s_{y1}^2 = 0{,}0000168$) and respectively s_{y2} is the standard residual deviation of one non-linearity regression ($y = -0{,}0038 + 0{,}2825x - 0{,}0028x^2$, $s_{y2}^2 = 0{,}0000121$).

Necessary PG value for F test is calculated with equation: $PG = DS^2 / s_{y2}^2$

Comparing the obtained value, PG = 4.107, with theoretical F one (F= 5.35, from the table of Fisher–Snedecor's law) we can see that PG<F, so the non-linear function does not offer an improvement adjustment for the calibration and we can use the linear calibration curve.

ACCURACY is expressed by the analyte's recovery degree in the sample.[13] Because in the pharmaceutical products we are studying it is impossible to obtain blanks that contain all components, for a better optimization of the results, after determination of the Mn content in "ECONOMIX 80011" samples, we add 1 mL, 2 mL and respectively 3 mL standard solution of Mn (1mg/mL) to the sample, and re-evaluate the manganese content.

The precision of method is described by the recovery of Mn content[14] in the sample. In this way we verify the efficiency of mineralization. The results obtained are presented in Table 2.

Table 2

Results obtained for the verification of precision of method

Sample	Mn taken (mg/g)	Mn added (mg)	Mn found (mg/g)	Recovery (%)
I	5.075	1	6.059	98.400
		2	7.053	98.900
		3	8.055	99.333
II	5.050	1	6.046	99.600
		2	7.056	100.300
		3	8.042	99.733
III	5.046	1	6.032	98.600
		2	7.041	99.750
		3	8.028	99.400

The acceptance criterion for the analyst's concentration of 0.5% Mn must be between 95% and 105%.

PRECISION is presented as standard deviation, relative standard deviation (RSD %) and confidentiality interval. Manganese determination from the samples „ECONOMIX 80011" was realized on 6 determinations and the results are presented in Table 3.

Interval of confidence for individual values, (CIIv), (for 5 degrees of freedom and a probability of 95%) is determined with the formula: $\bar{x} \pm t^{n-1}{}_{5\%} \times s = 5.044 \pm 0.0136$ mg/g.

Interval of confidence for average values, (CIAv), (for 5 degrees of freedom and a probability of level of 95%) is determined with the formula: $\bar{x} \pm \frac{t^{n-1}{}_{5\%} \times s}{\sqrt{n}} = 5.044 \pm 0.0056$ mg/g.

Acceptance criterion of the method must be %RSD ≤ 3.7%.

INTERMEDIATE PRECISION: The analysis was made according to the described working procedure,[15, 16] by 3 analysts in 3 days. Table 4 presents the statistic results.

Table 3

Experimental results obtained for "repeatability"

Sample	Mn found (mg/g)	Average (mg/g)	Standard deviation	RSD %
1.	5.047			
2.	5.039			
3.	5.041			
4.	5.050	5.044	0.0053	0.11
5.	5.038			
6.	5.049			

Table 4

Experimental results obtained for intermediate precision

Parameter	Analyst I Average of 6 detns	Analyst II Average of 6 detns	Analyst III Average of 6 detns	Average of 12 detns	Average of 18 detns
Average Mn (mg/g)	5.0380	5.0542	5.0812	5.046	5.058
Standard deviation (mg/g)	0.0056	0.0052	0.0070	0.0097	0.0189
CIIv(mg/g)	5.0240 - 5.0529	5.0406 - 0.0677	5.0631- 5.0992	5.025 - 5.0676	5.0178 -5.0980
RSD%	0.111	0.104	0.138	0.192	0.375
CIAv(mg/g)	5.0326 - 5.0443	5.0486 - 5.0596	5.0738 - 5.0885	5.0401 - 5.0525	5.0485 - 5.0674
Student "t" factor	2.57	2.57	2.57	2.20	2.11

Acceptance criterion for acceptance of the method must be %RSD ≤ 3.7%.

The confidence interval is narrower as the standard deviation estimation is made based on a smaller number of determinations. Also, the confidence interval for medium values is narrower than for individual values.

The determination method for Mn using f_AAS was verified on different pharmaceutical veterinary samples with different matrices and that contain different forms of Mn. The followed parameters are: accuracy and precision under the aspect of repeatability and intermediate precision.

Thus, there was the same work procedure for a sample shaped as tablets as for powders, the only modification being the advance of the tablets for their transformation in powder.

The analyses were performed for "CANIFORT" (S.C. ROMVAC COMPANY S.A). (1.2 %Mn) tablets of vitamins with minerals for veterinary use.

ACCURACY: The acceptance criterion for the analyte's concentration of 1.2% Mn must be between 97% and 103%. Results: 97.50 %< 99.037 %< 100.50%,

PRECISION is presented as standard deviation, relative standard deviation (RSD %) and confidentiality interval. Acceptance criterion for acceptance of the method must be %RSD ≤ 2.7%.

REPEATABILITY: The results obtained are presented in Table 5.

INTERMEDIATE PRECISION: The results obtained are presented in Table 6.

Table 5

Experimental results obtained for "repeatability"

average Mn of 6 detns (mg/g)	12,510
Standard deviation (mg/g)	0,014
RSD%	0,11
r (mg/g)	0,0351
CIIv (mg/g)	12,4756-12,5457
CIAv (mg/g)	12,4963-12,5250

Table 6

Experimental results obtained for intermediate precision

Parameter	Analyst I Average of 6 detns	Analyst II Average of 6 detns	Analyst III Average of 6 detns	Average of 12 detns	Average of 18 detns
Average (mg/g)	12.334	12.271	12.350	12.303	12.318
Standard deviation (mg/g)	0.0183	0.0127	0.0213	0.0365	0.0389
RSD%	0,148	0,102	0,173	0,297	0,316
CIIv (mg/g)	12.2877-12.3818	12.2387-12.3033	12.2950-12.4049	12.2225-12.3833	12.2363-12.4008
CIAv (mg/g)	12.3156-12.3540	12.2578-12.2842	12.3276-12.3724	12.2797-12.3261	12.2992-12.3380

CONCLUSIONS

The analytical results and the statistical evaluation of this step led to the following conclusion: the method is LINEAR in the range 0.5 – 3.5µg/ml, PRECISE as can be seen from the RSD value (0.11%) and EXACT, resulting from recovery value. Once established this method it can be applied for all the pharmaceutical powders or tablets that contain manganese.

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