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Spectrophotometric determination of Zn in pharmaceutical preparations: Antioxidant and Zincuprin Forte by azo-dyes derivatives of thriazolodiazophenols

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To determine the miligram amounts of Zn(II) in phamaceutical multimineral (Zincuprin Forte) and multivitamin containing microelements (Antioxidant) preparations there have been used 3-(2′-hydroxynaftylazo-1′)-5-mercapto-1,2,4-triazol (Metrian) and 3-(5′-mercapto-1′,2′,4′-triazolo-3′-azo)-2,6-dihydroxybenzoas acid (Matriarez-γ). Durability of Zn(II) complexes expressed with lg K with pH 9.23 with Metrian is 10.311 and with Metriarez-γ with pH 7.35 it is 11.224. Zinc ions of the preparations, after mineralisation (30% H_2O_2 with the use of condenced H_2SO_4 with d = 1,84 g · cm^{-1}) were put into a soluble compound ion $[Zn(NH_3)_4]^{2+}$ by adding 5 ml of 25 % of ammonia solution ,at the same time eliminating, which was achieved by filtrating, hydroxide sediments of other microelements. Spectrophotometric determination was performed in the water-methanol solution (1:1) with permanent ion power μ=0.1, pH 7.35 (Metriarez-γ) and $\lambda_{anal.}$ 480 nm; pH 9.23, $\lambda_{anal.}$ 530 nm (Metrian). The outcomes were executed statistically and compared with the outcomes of AAS marking method. The main features of the proposed method are selectivity,precision and the possibility to repeat it. The outcomes of markings show lesser defect than the markings achieved with the use of AAS method.The marking method is simple and its feature is high level of sensitivness.

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1. INTRODUCTION

The basis to keep man's organism haealthy is proper nutrition, which should provide sufficient amounts of energy, carbohydrates, proteins, fats, microelements in the form of provitamins or vitamins and mineral salts. The sufficient amount of microelements in a diet enables to use the supplied remaining elements of food to build cells and to supply it with energy. The concentration of mineral substances must be on a proper level. Both too low and too high concentration, as well as exceeding the maximum level distempers organism's functioning thus leading to various illnesses.

In recent years there has been a considerable growth in the use of multimineral and multivitamin preparations with microelements. The wide use of preparations containing microelements requires quick and precise methods of marking ions, especially those of heavy metals.The most common element in multimineral and multivitamin preparations with microelements is zinc. In biological material, food and pharmaceutical preparations it is marked with the use of spectrofluorometric [1,2], spectrometric atomic absorpcion /AAS/ [3,4], capillary electrophoresis [5,6], electrochemicals [7], thin layer chromatography /TLC/ [8], high performance liquid chromatography /HPLC/ [9], ions chromatographic [10] methods.

In pharmaceutical preparations Zn II has been marked spectrophotometrically, mostly with the use of azo derivatives [11-13], natrium salt of werfarin [14], 1-(phenylo-2-pirydylo)-karbylideno-5-salicylidenotiocarbohydranon-(PPST). To mark the miligram amounts of Zn(II) in pharmaceutical mineral preparations (Zincuprin Forte) and multivitamin with microelements preparations (Antioxydant) there have been used: 3-(2′-hydroxynaftylazo-1′)-5-mercapto-1,2,4-triazol (Metrian) and 3-(5′-mercapto-1′,2′,4′-triazolo-3′-azo)-2,6-dihydroxybenzoas acid (Metriarez-γ) [15]. The durability of Zn(II) complexes expressed with lg K with pH 9.23 with Metrian is 10.311 and with Metriarez-γ with pH 7.35 is 11.224. Zinc ions after mineralisation (80 % H_2O_2 with adding conc. H_2SO_4 d = 1.84 g · cm^{-3}) of preaparations was put into a soluble compound ion $[Zn(NH_3)_4]^{+2}$ by adding 25% ammonia solution, at the same time eliminating the precipitate of other microelements by draining them off. Spectrophotometric marking was carried out in methanol-water solution (1:1) with permanent ionic energy μ=0,1.

2. EXPERIMENTAL PART

2.1. Apparatus and Reagents

Spectrophotometer UV-VIS produced by Cecil (England), type CE 6600 with 10 mm quartz tray.

Atomic Absorption Spectrometer produced by Perkin – Elmer (Germany), type 3300.

Pehameter produced by Meratronik (Poland), type N 5111 with glass-calomel plate (combined).

Basic solutions of dyes with the concentration $c = 2\times10^{-4}$ mol $\cdot$ l^{-1} was obtained by diluting 0.00543 g of Metrian, 0.00563 g of Metriarez-γ in 1 ml of DMF (one drop of piperidine was added to Metrian to make diluting easier) and supplimenting with methanol to 100 ml. The basic zinc sulphate (VI) water solution with $c = 2\times10^{-4}$ mol $\cdot$ l^{-1}.

Borate buffer solutions with pH 9.23 and 7.35.

Zinc was marked in pharmaceutical preparations:

Antioxident – tablets produced by Legosan AB No 7173

Zincuprin Forte – tablets produced by Farmpol (Chemical-Pharmaceutical Establishment in Poznań)

2.2. Procedure

Determination of standard curve

12 ml of dye (Metrian or Metriarez-γ) in methanol of the concentration $c = 2\times10^{-4}$ mol $\cdot$ l^{-1} was put into measuring soldering tools , 0.5 to 6.5 ml of water salt solution of Zn (II), single mole water solution of KNO_3 (in the amount as the ion force =1) and was supplemented with boran buffer of pH equal 9.232 (Metrian) and 7.35 (Metriarez-γ). The measurements of absorbation were carried out with analitical wavelength $\lambda_{anal.}$ for Metrian 530nm and 480nm. For Metriarez-γ in accordance with the reference (properly buffered dye solutions with the addition of 1M KNO_3 in the amount μ=0.1; excluding metal salts). There was observed a perpendicular course of calibration curves (comformability with Lambert-Beer Law), concentration ranging from 0.05 to 2.320 (Metrian) and from 0.05 to 2.250 μg ml^{-1}(Metriarez-γ). Corelation coefficient (r) was repectively 0.9956 (Metrian) and 0.9990 (Metriarez-γ) and also favourable values of regression equation ($y = a\ x \pm b$) : $y = 0.1371 \cdot x + 0.0404$ Metrian and $y = 0.2495\ x - 0.0395$ Metriarez-γ.

2.3. Preparation of solution of Antioxidant and Zincuprin Forte for Zn(II) determination

2.3.1. Mineralisation of tablets

One tablet of Antioxidant or Zincuprin Forte preparation was placed in a cone soldering tool and 1 ml of concentrated H_2SO_4 (d = 1.84 g · ml^{-1}) was added,which was later heated with drops of 10 ml 30% H_2O_2 until H_2O_2 (perhydrol) was disposed, and yet it was vaporised to 5 ml. It was again added to 10 ml 30 % H_2O_2, which was then repeated three times. After mineralisation had been completed, 5 ml of 25% solution of ammonia was added and left for about 60 minutes. The solutions were filtrated into 100 ml soldering measuring tools and (flasks) little soldering tools as well as filters wrer washed four times with 10 ml of water, then filtered; filetrings were later put together with the filterings in soldering tools and supplemented with water up to 100 ml.

Getting the solution ready for Zn(II) markings:

Either 5 ml of Antixiodant or 4 ml of Zincuprin Forte were transferred into 100 ml volume soldering tools and then supplemented with water up to the marking line (solution for determination).

2.3.2. Determination of Zinc (II) contents

12 ml methanol solutions of Metrian and Metriarez-γ of the concentration of 2×10^{-4} mol · l^{-1} were measured into 25 ml measuring soldering tools, 2.0 ml of mineralisates, for marking Antioxidant or Zincuprin Forte , single moled KNO_3 in the amount to make equal 0.1 and was supplemented to the volume of 25 ml with the buffer solution with pH 9.23 (Metrian) and 7.35 (Metrairez-γ). Absorbtion was measured after 30 minutes with analytic wavelength $\lambda_{anal.}$ 530 (Metrian) and 480 (Metriarez-γ) in relation to the reference (12 ml of corresponding dye with c = 2×10^{-4} mol · l^{-1}, single moled KNO_3 to make μ=0.1 and with the addition of proper buffer to the volume of 25 ml).

The outcome of 10 series of the contents of Zn(II) measurements (n=10) were worked out statistically, later compared with the outcomes of Zn (II) marking carried out with the help of AAS method (Perkin – Elmer λ=213.9, acetylene – air) and presented in Table 1.

Tab. 1. The outcomes of Zn(II) markings, together with the statistic mark for 10 measurings (n=10) with 95 % probability

Preparation	The amount of Zn(II) as declared in 1 tablet	The amount of Zn(II) marked with the use of AAS method	Dye	Zn(II) marked with the help of Metrian (Mn) and Metriatrez-γ (M-γ)							
				$\bar{x}$	SD	CV	SD_x	RSD in %	$\pm t \cdot SD_x$	% of mistake when referred to the amount	
										Marked with AAS method	Declared
Antioxidant	12.0510	12.3643	Mn	12.0643	0.1751	0.01451	0.05537	1.45	0.1357	-2.60	+0.11
			M-γ	12.1920	0.1503	0.01315	0.0507	1.31	0.1242	-1.39	+1.17 +2.60
Zincuprin Forte	32.7354	32.8500	Mn	32.78582	0.181001	0.00552	0.057240	0.52	0.140232	-0.20	+0.15
			M-γ	32.74998	0.4958	0.01514	0.1568	1.51	0.38414	-0.31	+0.05 +0.35

3. OUTCOME DISCUSSION

Zn(II) ions constitute, in the water-methanol environment with pH 9.23 for Metrian and 7.35 for Metriarez-γ, chelate complexes α:Zn(II) 2:1 amalgamation with Metrian of the following composition, red in colour (the colour of ligand in these conditons is orange), and with Metriarez-γ – orange and red (the colour of ligand with this pH value is yellow). The reaction of creating complexes is hasty, created last 72 hours, and the number value of durability expressed with lg K is 10.311 for $(Metrian)_2Zn(II)$ and 11.224 $(Metriarez\text{-}\gamma)_2Zn(II)$. These properties were used in the spectrophotometric analysis of mg amount of zinc in Antioxidant and Zincuprin Forte. The output precision expressed , among others, with section trust established at 95 % probability level is from ±0.1357 to ±0.1902 with the use of Metrian or ±0.1242 to ±0.3841 with the use of Metriarez-γ. Standard deviation of marking Zn(II) with the help of Metrian is from -0.2334 to +0.2579 (Antioxidant) and from -0.5475 to +0.8853 (Zincuprin Forte), and with the use of Metriarez- γ from -0.3221 to +0.7634 (Antioxidant) and from -0.5126 to +0.8363 (Zincuprin Forte). Proportional mistake of Zn(II) markings when referred to the declared amount is on average +0.13% (Metrian) and +0,60% (Metriarez- γ), when carried out with the help of AAS method +1.5% and regarading AAS method -1.10%.

4. CONCLUSION

The advantage of the worked out method of marking Zn(II) in multimineral preparations (Zincuprin Forte) and multivitamin containing microelements (Antioxidant) is high precision and the possibility to repeat the outcomes. The outcomes of markings are of lesser defect than the outcomes of markings with the use of AAS method. The marking method is simple and economical. Moreover, it´s feature is high level of sensitiveness.

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CURRICULA VITAE

Stanisław Zaręba (prof. of Medical University from 2000) was born in Potok Wyszomirski (province Lublin) in 1941. Graduated from Faculty of Pharmacy, Medical University of Lublin in 1964. Since 1964 worked in the Department of Medicinal Chemistry at this University. He received Ph.D. in 1972 ("Analytical analysis create of complexes azo-dyes of tiadiazole"), second degree post-graduate course (Analysis of drugs) in 1976 and habilitation in 1996 ("New derivatives of heterodiazoliloazophenols and their use in analysis of drugs").
He is the author of eight monographs to FP V and FP VI.
During his work Stanisław Zaręba has done the analysis of:

1. Phytochemistry – isolation and chromatography of sterol and triterpen fraction (Chelidonium majus, Berberys vulgaris, Sambucus ebulus).

2. Synthesis and properties of heterodiazoliloazophenols.
3. Determination of microelements in pharmaceutical preparations with microelements with use heterodiazoliloazophenols (derivatives: tiadiazole, triazole, oxodiazole, imidazole and benzimidazole).
4. Analysis of drugs.
5. Analysis of food.

Head of the Department of Food and Nutrition, Faculty of Pharmacy (since 1999). In the Department of Food and Nutrition Stanisław Zaręba is working on the analysis of: composition of food, contents of xenobiotic in food, analysis of biotope pollution, pollution of food and health of people.
Stanisław Zaręba published 75 articles and 31 reports of conferences.

Arkadiusz Pomykalski was born in Lublin (Poland) in 1973. Studies of pharmacy in the Faculty of Pharmacy, Medical University of Lublin in 1993-1998. At present he is an assistant in the Department of Medical Chemistry at this University. Actually, he is working on the analytical analysis of non-steroidal anti-inflammatory drugs.