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Short communication

Spectrophotometric determination of Fe(II) in pharmaceutical multivitamin preparations by azo dye derivatives of pyrocatechol[1]

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1. Introduction

The common use of pharmaceutical multivitamin preparations containing trace elements (K, Ca, Mg, Co, Mn, Cu, Zn, Fe, Cr, Mo, F, I, P and Se) in cases of physical and psychological strain, convalescence, deficiency of vitamins and trace elements requires quick and precise methods for the determination of the various ions, particularly those of heavy metals.

In recent years Fe(II) has been determined spectrophotometrically in pharmaceutical preparations using 1,10-phenanthroline [1], dipyridylketoxine [2]; mandelic-hydroxamic acid (MHA) [3], chlorpromazine hydrochloride [4], 2,4,6-tri-(2-pyridylazo)-1,3,5-triazine [5], thiosalicylic acid [6], and trifluoracetylacetone [7]. However, these sensitive reagents are not specific and require proper pre-treatment in the presence of other metal ions. In a search for new, selective and sensitive reagents for the spectrophotometric determination of biologically active metal ions, derivatives of pyrocatechol were studied: 2-(3′,4′-dihydroxyphenylazo-1′)-benzimidazole (BIAP) [8]; 3-mercapto-5-(3′,4′-dihydroxyphenylazo-1′)-1,2,4-triazole (METRIAP) [9]; and 2-carboxymethanothio-5-(3′,4′-dihydroxyphenylazo-1′)-1,3,4-thiadiazole (TIDAP-SO) [10]. These reagents are easily synthesized as previously proposed with 5-(1′,3′,4′-thiadiazole-2′-azo)-2,4-dihydroxybenzoic acid (TIDAREZ-β) and 5-(5′mercapto-1′,2′,4′-triazole-3′-azo)2,4-dihydroxybenzoic acid (METRIAREZ-β) [11–13].

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2. Experimental

2.1. Pharmaceutical preparations

Iron content was analysed in Biovita® tablets made by Rhöne-Poulenc Rorer (No. 32037301) (France); Multi-tabs tablets produced by Ferrasan, (No. 27775) (Denmark) and Unicap M tablets produced by Upjohn Ltd (No. RH 102 LZ, UK) (Crawley, UK).

2.2. Reagents

Stock solutions of each dye (2×10^{-4} M) were obtained by dissolving 0.00624 g of BIAP, 0.00547 g of METRIAP or 0.00625 g of TIDAP-SO in 1 ml of dimethylformamide and diluting to 100 ml with methanol. A stock $FeSO_4$ solution (2×10^{-4} M) containing 0.5 g of ascorbic acid in 100 ml (to protect Fe^{2+} ions from oxidation) was prepared. Borate buffer solution (Michaelis) (pH 10.30) was used.

2.3. Apparatus

A spectrophotometer type CE 6600 ("Cecil", UK) with 10 mm quartz cells, a pH meter type N 517 (Meratronic, Wrocław, Poland) with a glass-calomel electrode, and an atomic absorption spectrophotometer type SP 192 (Pye-Unicam, UK) were employed.

2.4. Procedure

Into each 50 ml volumetric flask, 24 ml of methanolic dye solution (BIAP, METRIAP or TIDAP-SO 2×10^{-4} M) and 0.50–13.0 ml of $FeSO_4$ solution (2×10^{-4} M) were pipetted and diluted to 50 ml with buffer solution (pH 10.30). Absorbance measurements were made after 30 min at analytical wavelengths of 490 nm for METRIAP, 560 nm for BIAP, and 600 nm for TIDAP-SO, using a reference solution (24 ml of the stock solution of the appropriate dye dilute to 50.0 ml with buffer).

Straight-line calibration curves (consistent with the Beer-Lambert law) were obtained at concentration ranges of 0.050–2.011 $\mu g\ ml^{-1}$ (METRIAP), 0.050–0.890 $\mu g\ ml^{-1}$ (BIAP), and 0.050–1.000 $\mu g\ ml^{-1}$ (TIDAP-SO). The correlation coefficients (r) were: 0.9982 for METRIAP; 0.9985 for BIAP; and 0.9960 for TIDAP-SO. The regression equations were $y = 0.2940x - 0.0206$ (METRIAP); $y = 0.1212x + 0.0267$ (BIAP): and $y = 0.1310x + 0.0616$ (TIDAP-SO).

2.5. Preparation of solutions of Biovital®, Multi-tabs and Unicap M for the determination of Fe(II)

Powdered samples, corresponding to the average tablet mass of Biovital®, Multi-tabs or Unicap M, were treated with 30 ml of 0.1 M HCl, shaken for 30 min and filtered into 100 ml volumetric flasks. The residues were washed four times with 5 ml portions of 0.1 M HCl and then twice with 10 ml portions of water, filtered, and the combined filtrates were diluted with water to 100 ml. 5.0 ml of the final solution of Multi-tabs or Unicap M or 3.0 ml of Biovital solution was pipetted into 100 ml volumetric flasks, about 0.5 g of ascorbic acid was added, and the solution was diluted with water to 100 ml.

2.6. Determination of the Fe(II) content in the preparations

Into each 50 ml volumetric flask, 24 ml of methanolic solution of BIAP, METRIAP or TIDAP-SO (2×10^{-4} M) was transferred and the following volumes of sample solution were pipetted: 4.0 ml of Biovital® or Multi-tabs solution and 8.0 ml of Unicap M solution in the case of determination by METRIAP; 2.0 ml of Biovital® solution, 3.0 ml of Multi-tabs solution or 6.0 ml of Unicap M solution in the case of determination by BIAP or TIDAP-SO. The mixture was diluted to 50 ml with borate buffer (pH 10.30). Absorbance measurements were carried out as described for construction of the calibration curves.

3. Results and discussion

The results for 10 measurements of each series of preparations, statistically evaluated and com-

Table 1
Results of Fe^{2+} determinations with statistical evaluation at the 95% probability level ($n = 10$ for each series)

Preparation	Fe(II) as declared in each tablet(mg)	Fe(II) determined in each tablet by AAS(mg)	Fe determined by BIAP (B), METRIAP (M) and TIDAP-SO (Ts)							
			$\bar{x}$ (mg)	S	$S_r = s/\bar{x}$	$\bar{S} = s/\sqrt{n}$	RSD (%)	$\pm t\bar{s}$	Difference %	
									from AAS determination	from nominal content
Biovital®	32.5000	32.3330	32.6844(B)	0.3099	0.0095	0.0980	0.95	0.3548	+1.09	+0.57
			32.5648(M)	0.3334	0.0103	0.1054	1.03	0.3818	+0.72	+0.20
			32.3206(Ts)	0.2843	0.0088	0.0899	0.88	0.3270	−0.04	−0.55
										−0.51
Multi-tabs	18.0000	17.8000	18.0371(B)	0.7630	0.0042	0.0241	0.42	0.0554	+1.33	+0.21
			18.0445(M)	0.1299	0.0072	0.0411	0.72	0.0910	+1.37	+0.25
			17.9885(Ts)	0.0478	0.0027	0.0151	0.27	0.0602	+1.06	−0.06
										−1.10
Unicap M	10.0000	9.9000	10.0320(B)	0.0297	0.0030	0.0094	0.30	0.0235	+1.33	+0.32
			10.0810(M)	0.0006	0.0001	0.0002	0.45	0.0012	+1.83	+0.81
			9.9824(Ts)	0.0750	0.0075	0.0237	0.75	0.0559	+0.83	−0.18
										−1.10

pared with those of Fe determination by the AAS method ($\lambda = 248.3$ nm; acetylene–air), are presented in Table 1.

The precision, expressed as the confidence limits at the 95% probability level, was high. The SD for Biovital was −0.473–+0.404 (METRIAP), −0.127–+0.080 (BIAP), and −0.102–+0.073 (TIDAP-SO); for Unicap M the SD was −0.166–+0.112 (METRIAP), −0.049–+0.045 (BIAP) and −0.085–+0.155 (TIDAP-SO). The determination error in relation to the declared amount was about +0.42% (METRIAP), +0.01% (BIAP) and −0.27% (TIDAP-SO); against the determination by the AAS method, the error was about −0.87% in relation to the declared amount compared to +1.31% (METRIAP), +1.25% (BIAP) and +0.62% TIDAP-SO by the AAS method.

Fe^{2+} ions in aqueous–methanolic solution at pH 10.30 react with BIAP, METRIAP and TIDAP-SO to form complexes of molar ratio ($L:Fe^{2+}$) of 3:1 and with $\log K$ values of 16.460 for METRIAP–Fe(II), 16.292 for BIAP–Fe(II) and 15.100 for TIDAP–SO–Fe(II). The formation of complexes proceeded quickly and the formed chelates are stable for at least 24 h. These properties were taken advantage of in the spectrophotometric analysis of small amounts of Fe^{2+} ions in the presence of vitamins and trace (K, Ca, Mg, Co, Mn, Zn, Cu, Cr, Mo, F, I, P and Se) in pharmaceutical preparations. In an acidic medium (0.1 M HCl), Fe^{2+} ions quantitatively pass into aqueous solution and after dilution Fe^{2+} ions are stabilized with ascorbic acid. In an alkaline medium (pH 10.30) the studied azo dyes form chelates with Fe^{2+} ions of a given composition and stability. Other components of the studied pharmaceutical preparations,

particularly vitamins, do not interfere with the determination of Fe^{2+} ions using the examined azo compounds. Most trace elements, such as K, Ca, Mg, Mn, Cr, Mo and Zn, do not form chelates with the studied ligands at pH 10.30, so that Fe^{2+} ions can be determined without sequestering or eliminating other ions from the reaction medium.

4. Conclusions

The advantage of the developed method is the high precision and reporducibility of results of Fe(II) determination in Biovital, Multi-tabs and Unicap M. The results of Fe(II) determination obtained with BIAP, METRIAP and TIDAP-SO have a smaller error than those obtained by the AAS method. From statistical analysis of the results of determinations it can be concluded that BIAP, METRIAP and TIDAP-SO can be used as reagents for the determination of Fe(II) in the presence of other trace elements and vitamins. This may be the method of choice expecially where iron is required to be determined in a compound preparation and where more sophisticated apparatus is not available.

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