

Simultaneous determination of zinc(II), manganese(II) and iron(II) in pharmaceutical preparations

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Abstract

The applicability of derivative spectrophotometry for simultaneous determination of zinc(II), manganese(II) and iron(II) in the form of PAR complexes was presented and discussed. Beer's law was obeyed in range 0.025–13 ppm for zinc 1–20, for manganese and 0.025–0.2 for iron ion. The elaborated method was applied successfully for determination of mentioned ions in pharmaceutical preparation without previous separation. The error of the determination did not exceed $\pm 3\%$. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Derivative spectrophotometry; Zinc; Manganese(II) and iron(II) ions; Pharmaceuticals; Simultaneous determination

1. Introduction

The aim of this paper is to develop a fast and sensitive spectrophotometric method for the simultaneous determination of zinc(II), manganese(II) and iron(II) ions in pharmaceutical preparations. Analysis of metal ions and minerals in multi-vitamin preparations is challenging due to a complex composition and a wide range of concentration, which may vary from ppb to percent levels. The US Pharmacopoeia [1] recommends for the determination of mentioned ions AAS-technique or an extractive-spectrophotometric method. The method presented in this work is based on the well-known reaction of Zn(II), Mn(II) and Fe(II) with 4-(pyridyl-2-azo)-resor-

cinol (PAR) [2,3]. PAR is commonly used in chemical analysis as a chromogenic reagent [2,3]. It produces with metal ions intense coloured ($\epsilon > 10^4$) and soluble in water chelates but its applicability in quantitative analysis is limited by its poor selectivity. Most of the PAR–metal ion complexes absorb in the range 410–530 nm [2,3], so the determination of metal ions in a complicated mixture demands the use of masking [2] or isolation [4] techniques. The interference of accompanied matrix can be removed by a use of a mathematical processing of mixture spectra. For this purpose the PLS [5] or derivative spectrophotometry [6,7] techniques were used. The derivative spectrophotometry is that one which allows the fast and precise resolution of a multicomponent mixture. This approach permits the separation of overlapped spectra [8] and their use for the quantification of compounds. Derivative spectrophoto-

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metry [9] is based on a mathematical transformation of the spectral zero-order curve into the derivative spectra. The negative feature of derivative method is the generation higher number of extremum [8], so called virtual extrema or satellites. As a consequence, the derivative spectrum of mixture becomes more complicated than its parent zero-order spectrum. For this reason the derivative method is rather rare used for resolution of complex mixtures with a number of components more than two. Nevertheless, some authors discussed the abilities of the derivative-spectrophotometry for resolution of binary [10], ternary and quaternary mixtures [6,7] of PAR complexes with divalent d-electron metal ions. The applicability of derivative spectrophotometry for resolution of ternary and quaternary mixtures of PAR–divalent metal ions is discussed in work [6]. The second of the mentioned methods [7] was applied for the determination of metal ions in brass and metal alloys.

Various derivative spectrophotometric methods were proposed for determination of iron, manganese and zinc in individual and multicomponent solutions. This technique was used for assay of Fe(II) [11] as a 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complexes in alloys and biological samples. First derivative spectra of complex Fe(III)-morin were used for simultaneous quantification of Fe(III) and molybdenum(VI) in cobalt–chromium and nickel–chromium alloys [12]. Toral et al. [13,14] proposed the extractive second derivative methods for simultaneous determination of iron and ruthenium [13] and iron in mixture with copper [14]. Sedaira [15] described the method for simultaneous assays of manganese and zinc using 5,8-dihydroxy-1,4-naphthoquinone (NAZA) combined with the first and second derivative spectrophotometry technique. The fourth derivative spectra were used for quantification the trace of zinc [16] in standard alloys, environmental and pharmaceutical samples.

The presented paper describes the use of PAR-method for the simultaneous determination of Zn(II), Mn(II) and Fe(II) in pharmaceutical preparation. The generation of consecutive derivative spectra improved the selectivity and allows the quantification of selected ions without previous separation.

2. Materials and methods

2.1. Reagents

1. PAR, monosodium salt, stock solution: the aqueous (0.025%) stock solution of PAR was prepared from the pure product (SIGMA) by dissolving an appropriate weight in 1 l of double distilled deionised water.
2. Standard Fe(II) solution (1000 ppm) was prepared by dissolving an appropriate weight of $(\text{FeSO}_4 \cdot 6\text{H}_2\text{O})$ in 0.5 M H_2SO_4 . The working solutions were prepared just before use by an appropriate dissolving in redistilled deionised water.
3. Standard Zn(II) solution (1000 ppm) was prepared by dissolving an appropriate weight of ZnCl_2 in redistilled deionised water. The working solutions were prepared just before use by an appropriate dissolving in redistilled deionised water.
4. The Mn(II) stock solution (1000 ppm) was prepared by dissolving an appropriate weight of MnSO_4 in 0.5 M H_2SO_4 . The working solutions were prepared just before use by an appropriate dissolving in redistilled deionised water.
5. Borate buffer pH 9.11 prepared by mixing 90 ml of 0.05 M solution of $\text{Na}_2\text{B}_4\text{O}_7$ and 10 ml 0.5 M H_3BO_4 solution.

2.2. Apparatus

A Hewlett-Packard HP-8452A diode array spectrophotometer coupled to the User Data version software was used for the acquisition and storage of spectra. The 1.0-cm quartz cuvette was used for the spectral analysis. The used diode array spectrophotometer is supplied with series of 328 individual photodiodes. The measurement parameters of the spectrophotometer were: integration time 1 s, spectral bandwidth 2 nm, and spectrum scan 0.1 s. Computer Pentium P120, 16 MB RAM equipped with Grams/32 vs. 5.2 used for mathematical treatment of the spectra.

Table 1

The chemical and mathematical conditions of simultaneous determination of Zn^{2+} , Mn^{2+} and Fe^{2+}

	Zn^{2+}	Mn^{2+}	Fe^{2+}
Volume and concentration of PAR (in 10 ml of total volume)	2 ml of 0.025%		
Volume and kind of buffer (in 10 ml of total volume)	2 ml of boric buffer pH 9.11		
Derivative order	I	III	II
Polynomial degree	2	3	3
$\Delta\lambda$ -derivatisation window (nm)	14	18	18
λ_{max} (nm)	499	539.5	537

2.3. Procedure

2.3.1. Calibration curves

Into a 10-ml calibration flask was placed an appropriate aliquot of studied ion equivalent to 2.5×10^{-2} –13 ppm for zinc, 1–20 ppm for manganese and 2.5×10^{-2} –0.2 ppm for iron, then were added 2 ml of boric buffer (pH 9.11) and 2 ml of 0.025% PAR solution. Next such prepared mixture was fulfilled to the mark with redistilled deionised water. The absorption spectra were recorded in the wavelength range 300–600 nm against mixture of reagents without metal ions as a blank. Using the mathematical parameters assembled in Table 1, the appropriate derivative spectra were generated. The amplitudes, from the base-line to the peak, at 499, 539.5 and 535 nm were related to the actual contents of the zinc, manganese and iron, respectively.

2.3.2. Sample preparation

2.3.2.1. Multi-vitamin preparation Multi tabs. Not less than 10 tablets, each included 10 mg of zinc, 1 mg of manganese, 15 mg of iron and additionally copper (1 mg), selenium (20 μ g), chromium (20 μ g), iodide (70 μ g) and organic matrix: vitamins A, B, C, D, E, starch, microcrystalline cellulose, some auxiliary appetising substances, were weighed and finely powdered. An accurately weighed portion, equivalent to about 10 mg of zinc, 1 mg of manganese and 15 mg of iron, was transferred into a 1000-ml calibrated flask and diluted to volume with deionised water. Using a mechanical shaker, the powder was completely disintegrated and solution was filtered. The working solution was prepared by an appropriate dilu-

tion. One millilitre of 40 ppm solution of potassium citrate was added to the working solution and next it was proceed according to procedure described in Section 2.3.1. The zero-order spectra of the chelates mixture were recorded in the range 300–600 nm against a reagent blank under the same experimental conditions, using 1.0 cm cuvette.

3. Results and discussion

3.1. Chemical variables

PAR is commonly used as the chromogenic reagent. It reacts in alkaline medium with many metal ions with production of intense red-coloured complexes [1,2]. The spectra of investigated complexes were shown on Fig. 1. The

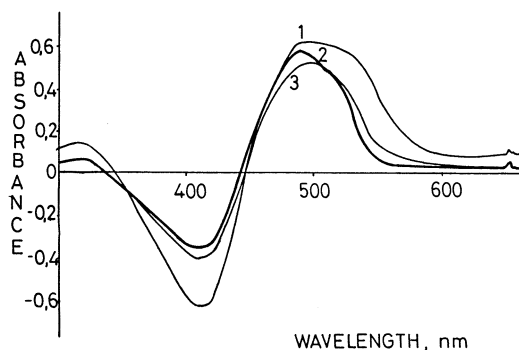


Fig. 1. The zero-order spectra of Me-PAR complexes (recorded against reagent blank): Curve 1: Fe(II)-PAR; Curve 2: Zn(II)-PAR; Curve 3: Mn(II)-PAR. Chemical conditions: concentration of metal ions—1 μ g/ml, 0.005% PAR, boric buffer pH 9.11.

maxima of complexes are situated close to each other: at 494, 498 nm for Zn– and Mn–PAR, respectively. The spectrum of complex Fe–PAR exhibits maximum at 498 with shoulder at 534 nm. The run of reaction PAR-investigated ion depends on the reagent concentration, pH of reaction medium and kind of used buffer. In order to find the optimum conditions, the influences of the reaction variables on the absorbance of complex solution were studied. Because PAR creates in the alkaline solution the diverse negative ion, initially the influence of the medium was checked. For this purposes the ammonia, boric and phosphoric buffers were used. All PAR complexes were found to be stable in boric buffer at pH 9.11. The experiments were carried out using recommended procedure. The chosen optimal volume of used buffer was 2 ml added to 10 ml total volume. The effect of PAR concentration also was investigated. In order to establish optimal concentration of PAR, the series of experiments were provided keeping constant concentration of studied ion (1 ppm), pH of reaction medium equal 9.11 and variable concentration of complexation reagent. The analytical signal increases with increase in reagent concentration in the case of Mn(II) and Fe(III) complexes. The absorbance of Zn–PAR complex increases up to concentration of PAR equals 0.005%, above, that it slightly decreases. The selected concentration of PAR was 0.005% for all investigated ions. The selected optimal chemical conditions of determination are presented in Table 1.

3.2. Derivatisation parameters

As it is shown in the Fig. 1 the spectra of complexes are strongly overlapped, so the direct reading of the absorbance value does not allow the quantitative determination of the individual ion in the presence of two others. For the separation of the spectra the Savitzky–Golay' algorithm [9] was used. The application of this procedure requires to optimise such mathematical parameters like the width of derivatisation 'windows' ($\Delta\lambda$), order of derivative and polynomial degree. For this purpose, the colour reaction using optimal chemical conditions was developed and next

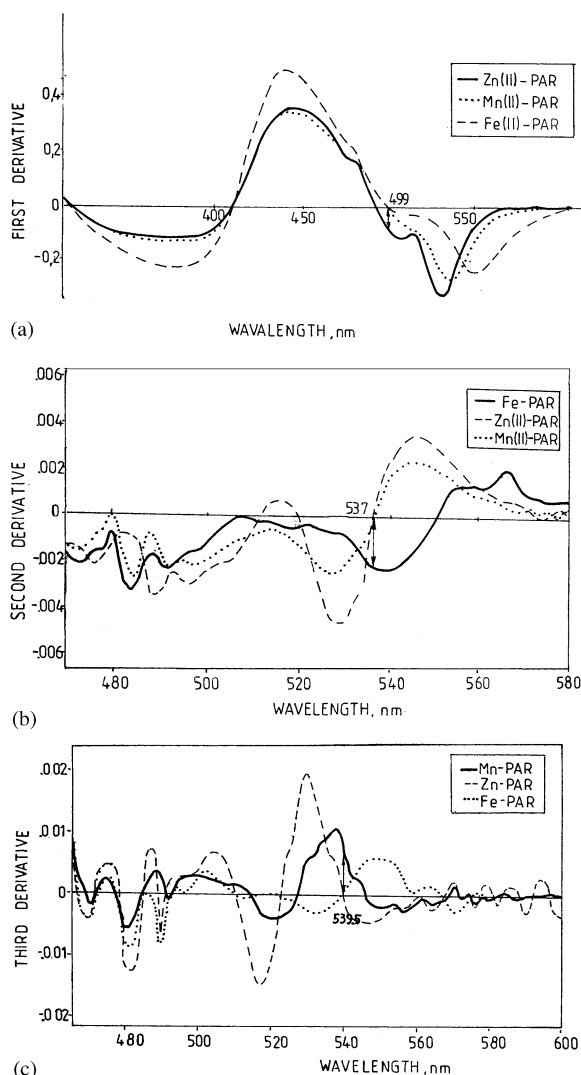


Fig. 2. First (a), second (b) and third (c) order spectra of Zn(II)–, Mn(II)– and Fe(II)–PAR complexes. Conditions of derivatisation, see Table 1. Chemical conditions: concentration of metal ions—1 $\mu\text{g/ml}$, 0.005% PAR, boric buffer pH 9.11.

spectra of solutions consisted of 1 $\mu\text{g/ml}$ of individual ion in the form of PAR complexes and their ternary mixtures were recorded. The selection of the analytical wavelengths was carried out by analysis of derivative spectra of all analytes and their mixture separately. Fig. 2(a) shows that the first derivative spectra of Fe(II)– and Mn(II)–PAR complexes cross X-axis at 499 nm. This zero-crossing point allows determining of zinc by

reading $dA/d\lambda$ values without interferences from others two ions. In order to select the optimal $\Delta\lambda$ and polynomial degree for differentiation, the series of derivative spectra of Zn(II)–PAR solutions were generated using different derivatisation windows and polynomial degrees. The choice of these parameters was done taking into account provided accuracy, precision and sensitivity of the determination of the compound. The increase of a value of polynomial degree caused the increase of noise in derivative spectrum—the second degree appeared to be optimal. The broadening of derivatisation ‘window’ ($\Delta\lambda$) caused the decrease in sensitivity and increase of the noise level. The value of $\Delta\lambda$ equal 14 nm was selected as compromise between sensitivity and acceptable noises in the derivative spectrum.

The optimisation of derivatisation parameters for iron and manganese ions were done in the same way. By using second derivative spectra generated with third polynomial degree and derivatisation window $\Delta\lambda = 18$ nm (Fig. 2(b), the determination of Fe(II) ion in multicomponent mixture is possible at 537 nm. In the third-order spectra traced with $\Delta\lambda = 18$ nm and the third polynomial degree (Fig. 2(c) exists at 595.5 nm zero-crossing point of spectra of Zn(II)– and Fe(II)–PAR solutions which gives opportunity for determination of Mn(II) ion. The mathematical parameters of derivatisation are assembled in Table 1.

Under the experimental conditions described above, the calibration graphs for all investigated compounds were constructed. Table 2 gives the equations obtained and other figures of merit,

such as limit of detection (S_L) [17], correlation coefficients and range of linearity. In order to estimate the precision (RSD) of the method, replicate samples ($n = 5$) containing 1 $\mu\text{g/ml}$ of each compound were measured individually.

3.3. Study of interference

The effect of foreign ions and compounds, which can be found in multi-vitamin formulation, in derivative method, was studied. Synthetic solutions containing 1 ppm of investigated ion and various amounts of foreign substances were measured. The tolerance limit was taken as the concentration of the foreign ion causing an error below 5% in the results of determination of studied ion concentration. The obtained results showed that the most common ions usually presented in multi-vitamin formulations do not influence on the results of simultaneous determination of zinc, manganese and iron. The most serious interference is caused by ions which create stable complexes with PAR as e.g. Cu^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} (in the case of Zn-determination) or possess strong complexing property like PO_4^{3-} , Cl^- , I^- ions. In order to reduce the interference of Cu^{2+} 1 ml of 40 ppm solution of sodium citrate was added to reaction solution. The derivatisation of spectra allows to determination of a selected ion in the presence of others main compounds. The other trace components of pharmaceutical like selenium of chromium ions do not interfere. The organic compounds of investigated pharmaceuticals also do not influence the determination.

Table 2
Analytical characteristic of elaborated methods

	Zn ²⁺	Mn ²⁺	Fe ²⁺
Quantification range (ppm)	0.025–13	1–20	0.025–0.2
Equation of calibration curve (<i>D</i> -derivative, <i>C</i> -concentration in ppm)	${}^1D = 0.0007 C + 5 \times 10^{-4}$	${}^3D = 7 \times 10^{-5} C + 2 \times 10^{-5}$	${}^2D = 0.0003 C - 7 \times 10^{-7}$
Correlation coefficient	0.9997	0.9997	0.9996
RSD (%) ($n = 5$, at 1 ppm level)	3.2	2.8	2.9
Limit of detection $\mu\text{g/ml}$ (taken as $S_L = a + 3S_{y/x}$) [17]	0.02	0.62	0.008

Table 3

The results of Zn(II), Mn(II) and Fe(II) determination in multi-vitamin preparation

	Zn	Mn	Fe
Found by described method ($n = 5$ replications) (mg)	10.02	14.89	0.97
Declared value (mg)	10	15	1
Relative error (vs. declared value)	+0.24	-0.7	-3.0
RSD (%)	3.2	2.2	3.7
Confidence interval $\pm t_{0,95} s$	± 0.05	± 0.08	± 0.10

3.4. Application

In order to establish the validity of the proposed method, the described procedure was applied for determination of investigated ions in multi-vitamin preparation. For this purpose was chosen out from large group of these kind of medical formulas the tablets Multi tabs. The obtained results are assembled in Table 3.

The results presented in Table 3 are in very good agreement with the labelled values. The error of determination does not exceed $\pm 3\%$.

4. Conclusion

In this work the simple, sensitive and selective method was developed for the simultaneous determination of zinc, iron and manganese in pharmaceuticals by derivative spectrophotometry. The calculation of derivative spectra allows the eliminating the interferences from others presented in multi-vitamin preparation compounds and ions. Only copper interferes seriously but it is easy to masking by citric ions. The method can be applied to the determination of investigated ions directly in solutions obtained after dissolving the multi-vitamin preparation in deionised water without previous separation.

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