ELSEVIER

Il Farmaco 53 (1998) 563–569

IL FARMACO

Three approaches to the analysis of zinc(II) in pharmaceutical formulations by means of different spectrometric methods

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Received 10 February 1998; accepted 20 October 1998

Abstract

The present paper constitutes three different spectral methods A, B and C developed with the purpose of determining precisely the concentrations of zinc(II), either in various ophthalmic solutions (collyria) or in insulin injections. Method A is a spectrophotometric one, based on the formation of the chelate complex of Zn(II) to be analysed with 4-(2-pyridylazo)-resorcinol(PAR) at pH 8.07 ± 0.01 and in a micellar medium produced by the non-ionic surfactant Triton X-100. This chelate complex shows a λ_{max} at 493 nm, an apparent molar absorptivity $\epsilon = 77728$ l/mol per cm and a corresponding Sandell's sensitivity of $S_s = 0.84$ ng cm^{-2}. The concentration of Zn(II) examined is calculated from the regression line equation: $A = 1.143C + 0.029$ ($r = 0.9998$, $n = 25$) with an optimum concentration range of 0.18–2.0 μg/ml. Method B exploits the fluorescence intensity of the 8-hydroxyquinolate chelate complex of Zn(II) to be analysed, measured at $\lambda_{emission} = 510$ nm ($\lambda_{excitation} = 420$ nm). The concentration of Zn(II) examined is calculated from the regression line equation: $A = 0.25C + 0.17$ ($r = 0.9996$, $n = 18$), with an optimum concentration range of 0.26–1.05 μg of Zn(II)/ml. The third method C is an AAS method, based on the following analytical parameters: $\lambda = 213.9$ nm; hollow cathode lamp (HCL): L1788-30NB; HCL current 18 mV; flame temperature 2700°K. The concentration of Zn(II) analysed is calculated from the regression line equation: $A = 1.499C - 7.409 \times 10^{-4}$ ($r = 0.9996$, $n = 25$), with an optimum concentration range of 0.2–1.2 μg/ml. The accuracy and the precision of the proposed methods A, B and C was experimentally investigated and proved to be very satisfactory. On the other hand, the methods described were successfully applied for the determination of the Zn(II) included in four eye drop solutions and in one insulin injection both provided by the pharmaceutical market. The analytical results of the afore-cited formulations were summarised in a comparative table and were considered to be very satisfactory.

Keywords: Zinc(II); Pharmaceutical formulations; Spectrometric methods

1. Introduction

A long while ago, Zn(II) compounds ($ZnCO_3$, ZnO) were used topically in a variety of skin diseases mainly for their astrigent properties [1].

Nowadays, the element 'zinc' and its products becomes from one day to the next, more and more interesting, either as an essential trace element of the human body, necessary for the formation of various metal-dependent enzymes, such as carboxypeptidase, collagenase, dipeptidase, alkaline phosphatase, carbonic anhydrase, phospholipase C, neutral protease [2] or as the principle ingredient and the pharmacological active part of a number of pharmaceutical formulations of various pharmacotechnical forms such as ointments and creams for external application to the body, ophthalmic drops (collyria) and ointments, and is an integral part of the injectable form of insulin.

Zinc salts – mainly $ZnSO_4 \cdot 7H_2O$ – are used as supplements to correct zinc deficiency; they have been tried in the treatment of a large number of conditions because of an associate reduced concentration of zinc in the human body [1,3].

In recent years, a significant number of analytical procedures of various analytical techniques, suitable for the determination of Zn(II) such as spectrophotometry [4–8], atomic absorption spectroscopy [9–12], atomic emission spectroscopy (ICP-AES) [13–16], titrimetry [17–19], polarography [20], neutron activation analysis [21] and concerning the field of pharmaceutical analysis has been published.

In the present paper, a comparison of three different spectral techniques, with the purpose of determination of Zn(II) contained in various ophthalmic solutions as well as in insulin injections, is described.

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0014-827X/98/$ - see front matter
PII S0014-827X(98)00066-4

Table 1
Evaluation of the accuracy and the precision of the proposed methods A, B and C

Method	Zinc(II) (μg/ml)		%RSD	SAE (SD/$\sqrt{n}$)[a]	Confidence limits ($P = 0.05$; $(n-1) = 2$)
	Added	Found ± SD[b]			
	0.50	0.500 ± 0.0026	0.53	0.0012	0.500 ± 0.0051
A	0.75	0.752 ± 0.0089	1.18	0.0040	0.752 ± 0.0171
	1 00	1.023 ± 0.0378	3.70	0 0169	1.023 ± 0.0728
	0.50	0.502 ± 0.0015	0.30	0.0007	0.502 ± 0.0029
B	0.75	0.757 ± 0.0058	0.76	0.0026	0.757 ± 0.0111
	1 00	1.053 ± 0 0058	0.55	0.0026	1.053 ± 0.0111
	0.50	0.502 ± 0.0017	0.34	0.0008	0.502 ± 0.0032
C	0.75	0.765 ± 0.0030	0.39	0.0013	0.765 ± 0.0058
	1.00	1.000 ± 0.0500	5.00	0.0224	1.000 ± 0.0962

[a] SAE, standard analytical error.
[b] Average of three determinations.

The above mentioned analytical techniques are:

- a spectrophotometric method based on the measurements of the Zn(II)-4-(2-pyridylazo)-resorcinol (PAR) complex formed in a suitable micellar medium resulting from the use of a non-ionic surfactant, Triton X-100 (method A);
- a spectrofluorimetric method which exploits the fluorescence of the 8-hydroxyquinolate (HOx) chelate complex formed from Zn(II) to be analysed (method B); and
- a method of atomic absorption spectrometry(AAS), especially developed for the determination of Zn(II) (method C).

Moreover, it is important to underline the purposes of the simultaneous description and publication of three spectrometric methods of clearly different techniques, instead of one as is the usual practice.

First, in this way, and as far as possible complete consideration of an important part of the spectral analysis of Zn(II), contained in various medicinal preparations, widely circulated in the pharmaceutical market, is offered to the analysts charged with the quality control of these goods. Second, in this manner, a full choice of the more suitable – in every case – method, in connection with the form of the proprietary medicine and its own concentration in Zn(II), as well with the existent and available analytical instrumentation of the laboratory at every time is presented.

Finally, a comparison of the accuracy and the precision of the described three methods was easily carried out from the effects of an experimental study the results of which appear in Table 1.

2. Experimental

2.1. Apparatus

All the spectrophotometric measurements were performed using a Milton Roy Spectronic, model 401, digital spectrophotometer matched with a set of 10-mm glass cells, while the spectrofluorimetric measurements were carried out by the use of a Perkin–Elmer LS-3 spectrofluorimeter, equipped with a RCA 931A photomultiplier and the proper prismatic silica cells.

A Perkin–Elmer, model 3300, atomic absorption spectrometer accompanied by a Perkin–Elmer 3300/5100 PC was used for all AAS measurements.

A WTW. model 537, microprocessor-controlled digital pH-meter, equipped with a precision combined glass-calomel electrode E56 and accompanied by a sensor, model TFK 150, suitable for the automatic temperature compensation, was used for the precise accomplishment of all pH-measurements. A thermostated constant temp-erature water-bath, accurate to ± 0.5°C was used throughout.

2.2. Reagents and solutions (method A)

Standard solution of Zn(II): 100 mg of metallic (pulver) Zn (Merck) was transferred to a 100-ml calibrated flask, where a mixture of 1.0 ml of water and 2.0 ml of conc. HCl (Malliinckrodt) was carefully added. The solution was diluted to volume with water. Concentration of the standard solution: 1000 μg/ml Zn.

Working solution of Zn(II): 3.0 ml of the standard solution was diluted with water to 100 ml. The latter solution (10.0 ml) was diluted again with water to 100 ml. The final concentration of the working solution was equal to: 3.0 μg/ml Zn.

Working solution of PAR: 50.0 mg of 4-(2-pyridylazo)-resorcinol, PAR (Fluka) was dissolved and then diluted to 100 ml with ethanol p.a.

Borate buffer solution (pH 8.07 ± 0.01): 1.55 g of H_3BO_3 and 1.865 g of KCl was dissolved and then diluted to 250 ml with water. The above formed solution (125.0 ml) and 14.75 ml of NaOH (0.1 mol/l) was diluted to 250 ml.

Triton X-100 (TX100) working solution: a 1.20 mmol/l Triton X-100 working solution was prepared by dilution with water of the appropriate quantity of TX100 (Aldrich).

Water: deionized and then double-distilled water was used throughout, while all other chemicals employed during this investigation (methods A, B and C) were of analytical grade.

2.3. Reagents and solutions (method B)

Working solution of Zn(II): 1.635 g of metallic (pulver) zinc (Merck) was transferred to a 250-ml calibrated flask, where a mixture of 1:2 (v/v) H_2O/conc. HCl (Mallinckrodt) was carefully added until the pulver of Zn was completely dissolved. The solution was diluted to volume with water. This solution (5.0 ml) was diluted to 500 ml with water. The concentration of the prepared working solution was 10^{-3} mol/l of Zn(II).

Working solution of 8-hydroxyquinoline: 12.5 g of 8-hydroxyquinoline (Merck) was transferred to a 250-ml calibrated flask, was dissolved and diluted with a 2.0 mol/l acetic acid solution.

Acetone: acetone (Merck) was used throughout.

Water: the water used was the same as in method A.

2.4. Reagents and solutions (method C)

Standard solution of Zn(II): the content of an ampoule of Zinc(II) standard solution (Merck), equivalent to 1.000 ± 0.002 g of Zn(II) as $ZnCl_2$ dissolved in 0.06% HCl (m/m) was quantitatively transferred into a 1000-ml calibration flask and diluted with water to volume (final concentration of the standard solution: 1000 μg/ml of Zn(II)).

Working solution of Zn(II): after the suitable dilutions with water of a fixed volume of the standard solution, a series of zinc working solutions of 0.25, 0.50, 0.75, 1.00 and 1.50 μg/ml of Zn(II) was prepared.

2.5. Preparation of calibration graph and recommended procedure (method A)

The following were pipetted into a 50-ml calibrated flask: 10 ml of the borate buffer solution (pH 8.07 ± 0.01), the appropriate volume of x ml of working Zn(II) solution ($x = 3.0, 5.0, 10.0, 15.0$ and 20.0 ml), 4.0 ml of the working PAR solution and 10.0 ml of Triton X-100 working solution.

The mixture was diluted to volume with water, homogenised by shaking and then its absorbance was measured at $\lambda = 493$ nm against a Zn(II) blank solution prepared and treated similarly.

Afterwards, a calibration graph of absorbance versus concentration of the reference Zn(II) was traced according to the regression line equation: $A = 1.143C + 0.029$ ($r = 0.9998$, $n = 25$ (five points/five replicates)). Note: sometimes the formation of the measured Zn(II)–PAR complex, was accelerated by heating of the solution at 40°C in a waterbath for about 15 min.

2.6. Application of method A in various dosage forms

2.6.1. Eye drops

2.6.1.1. Preparation of sample solution

An accurately measured volume of the mixed contents of at least five dropping-vials of eye drops examined, equivalent to about 1700 μg Zn(II) (labelled amount) was quantitatively transferred into a 100-ml volumetric flask diluted to volume with water and vortex-mixed.

2.6.1.2. Analytical assay

Two millilitres of the above prepared solution – corresponding to about 34.0 μg of Zn(II) – was pipetted into a 50-ml volumetric flask where the preceding recommended procedure (Section 2.5) was applied.

2.6.2. Insulin injection

2.6.2.1. Preparation of sample solution

The contents of at least five ampoules of the insulin injection examined were blended and an accurately measured volume from this mixture equivalent to about 650 μg of Zn(II) (labelled amount) was quantitatively transferred into a 100-ml volumetric flask, diluted with water to volume and vortex-mixed.

2.6.2.2. Analytical assay

Five millilitres of the above prepared solution – corresponding to about 32.5 μg of Zn(II) – was pipetted into a 50-ml volumetric flask, where the assay of the Zn(II) was performed as described in Section 2.5.

2.7. Preparation of calibration graph and recommended procedure (method B)

2.7.1. Preparation of the calibration graph

Into a set of six 10-ml calibrated flasks were pipetted in order: 0, 40, 80, 100, 120 and 160 μl aliquots of working solution of 10^{-3} mol/l of Zn(II) (FW_{Zn}: 65.4), 2.0 ml of 8-hydroxyquinoline (8-HOx) 5.0% (m/v) solution in CH_3COOH (2.0 mol/l) and then the calibration flasks were filled to volume with acetone and mixed well by vortexing.

The final concentration of the Zn(II) in each one of the formed solutions in the six calibrated flasks (nos. 0–5), was estimated to be 0.0, 0.26, 0.52, 0.65, 0.78 and 1.05 μg/ml of Zn(II), i.e. equivalent to 0.0, 0.4, 0.8, 1.0, 1.2 and 1.6 mmol/l of Zn(II), respectively.

Afterwards, the fluorescence intensity of the content of the six calibrated flasks was measured in three replicates at $\lambda_{em} = 510$ nm ($\lambda_{ex} = 420$ nm) and the calibration graph was traced according to the measured analytical characteristics summarised in Table 2.

2.7.2. Recommended procedure for eye drops and insulin injections

Considering that the labelled concentration of Zn(II) (in pure ionic form) included in each one of the pharmaceutical formulations analysed has the following value: 'Oculogut' 569.0 μg/ml; 'Oculosan' 45.5 μg/ml; 'Zabysept' 45.5 μg/ml; 'Zincfrin' 569.0 μg/ml and insulin in 'Monotard HM'

Table 2
Analytical characteristics of calibration curve

Characteristics	Numerical data
Regression line equation	$A = mC \pm z$
m (slope) ± SD[a]	0.252 ± 0.0018
z (intercept) ± SD[a]	0.167 ± 0.0068
Correlation coefficient (r) ($n = 18$)[b]	0.9996
Relative standard deviation (%)	0.71
Optimum concentration range (μg/ml of Zn(II))	0.26–1.05

[a] Average of 18 determinations.
[b] Six points × three replicates = 18.

130 μg/ml, it is very easy to adapt the concentrations of Zn(II) of the above formulations to the needs of the procedure described in Section 2.7.1.

2.8. Preparation of the calibration, graph and recommended procedure (method C)

2.8.1. Preparation of the calibration graph

The preparation of the calibration graph in order to determine the concentration of Zn(II) in various pharmaceutical preparations (i.e. eye drops, insulin injections) by atomic absorption spectrometry (AAS), was accomplished using the five working solutions of Zn(II), as they were previously described in Section 2.4 and under the following analytical parameters: determined element zinc; sensitivity (mg/l) 0.018; slit 0.7 nm; wavelength 213.9 nm; HCL current/HCL energy 18 mV/46; hollow cathode lamp (HCL) L1788-30NE; flame temperature about 2700°K; mixture C_2H_2/air 1.25/5.25 l/min.

The linearity of the graph traced was checked by a linear least-squares treatment using a PC, while the slope (1.499×10^{-1}) and the intercept (-7.409×10^{-4}) were obtained with a correlation coefficient of $r = 0.99964$.

3. Results and discussion

A wide range of Zn(II) salts (especially Zn(II) sulphate) contained in pharmaceutical formulations are suitable to inhibit and/or to correct the deficiency of this metal cation, as well as for the treatment either of a variety of a number of internal diseases (e.g. rheumatoid arthritis, peptic ulcer, hepatic encephalopathy, Wilson's disease, etc.) or of various external skin diseases, such as acne, alopecia, herpes simplex, etc.

Moreover, Zn(II) sulphate, is extensively used as an essential curative component for numerous mono-ingredient eye drop preparations or in combination with another medicinal agent, such as naphazoline HCl, or naphazoline HNO_3, a widely known sympathomimetic agent with marked α-adrenergic activity, which acts as a conjuctival decongestant or phenylephrine HCl usually employed as a typical mydriatic and conjuctival decongestant as well.

Finally, Zn^{2+} is an unreplaced chemical factor for the complete and faultless crystallisation of insulin and in this way the latter achieves a superior degree of purification.

In the present paper, three different spectral methods, suitable for the determination of zinc(II) contained as an ingredient in the above mentioned eye-drops preparations, as well as in those of insulin injections were described.

The methods applied are: (a) spectrophotometric (method A), (b) spectrofluorimetric (method B), (c) AAS (method C).

The spectral characteristics, the experimental conditions as well as the analytical parameters are represented in detail below.

3.1. Spectrophotometric (method A)

3.1.1. Absorption spectrum

The absorption spectrum of the binary chelate complex Zn(II)–PAR was measured against a reagent blank in the range 420–570 nm, and shows a λ_{max} at 493 nm as it appears in the Fig. 1 [5,22].

The spectral characteristics and the other analytical parameters (optimum pH, apparent molar absorptivity, Sandell's sensitivity (both referred to the Zn(II) analysed), the regression line equation, the correlation coefficient, etc. are summarised in the column 2 (i.e. without w/o surfactant) of Table 3.

3.1.2. Effect of surfactant of the absorption spectrum

In order to confirm the effect of a surfactant on the absorbance of the measured solution, a number of dispersing agents, such as: cetyltrimethylammonium bromide (CTAB, cationic); cetylpyridinium chloride (CPCL, cat-

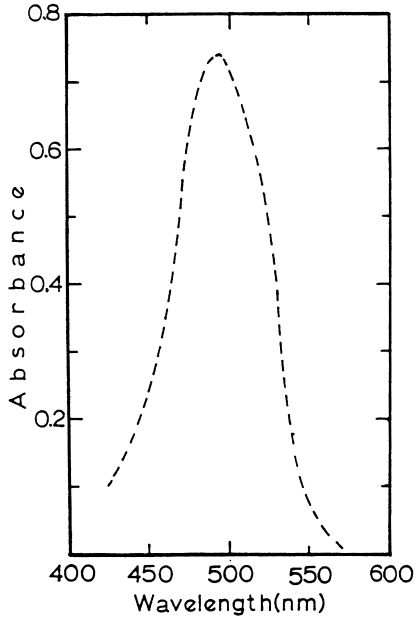

Fig. 1. Absorption spectrum of the Zn(II)–PAR chelate complex. Concentration of Zn(II): 0.633 μg/ml; pH 8.07 ± 0.01 (borate buffer).

Table 3
Spectral characteristics and other analytical parameters in connection with the surfactant used

Characteristics	Surfactant used				
	None	CTAB	CPCL	TX100	SDS
λ_{max} (nm)	493	503	503	493	493
Optimum pH			8.07 ± 0.01		
ϵ (l/mol per cm)[a]	74797	76083	75240	77728	77604
Sandell' s sensitivity (ng cm^{-2})[a]	0.87	0.86	0.87	0.84	0.84
Regression line equation			$a = mC \pm z$		
Slope (m) (μg/ml)[a]	1.127	1.159	1.144	1.143	1.149
Intercept (z)[a]	0.011	0.003	0.004	0.029	0.024
Correlation coefficient (r)[a]	0.9996	1.0000	0.9994	0.9998	1.000
Number of points/replicates: five points: 0.18, 0.30, 0.60, 0.90, 1.20 (μg/ml)/five replicates					

[a] Average of 25 measurements. The calculated values refer to the Zn(II) examined.

ionic); Triton X-100 (TX100, non-ionic); sodium dodecyl sulphate (SDS, anionic) were investigated. The absorption spectra of Fig. 2, as well as the diagram percent variation of ϵ versus surfactant used, also in Fig. 2, in combination with the numerical data of Table 3, show that amongst the surfactants examined, the non-ionic one, Triton X-100, was the most effective with respect to apparent molar absorptivity and consequently to the calculated Sandell's sensitivity.

The latter reaches its optimum and simultaneously a constant value using a 1.20 mmol/l TX100 solution in accordance with the above method A.

Obviously, the different nature of the micellar medium formed by the use of the cationic surfactants CTBR and CPCL, is the primary reason of the observed shift of the λ_{max} from 493 nm (pure aqueous solution of Zn(II)–PAR or in the presence of a non-ionic or an anionic surfactant) to 503 nm and for the fluctuation of the absorbance value of the ternary chelate complex Zn(II)–PAR-(surf) formed in any case.

3.2. Spectrofluorimetric (method B)

3.2.1. Fluorescence spectra

Fig. 3 shows the fluorescence excitation and emission spectra of 8-hydroxyquinoline (HOx) 1.0 m/v in CH_3COOH (4.0×10^{-1} mol/l) and acetone; (a,a′): HOx only; (b,b′): HOx + 0.8 mmo/l of Zn(II).

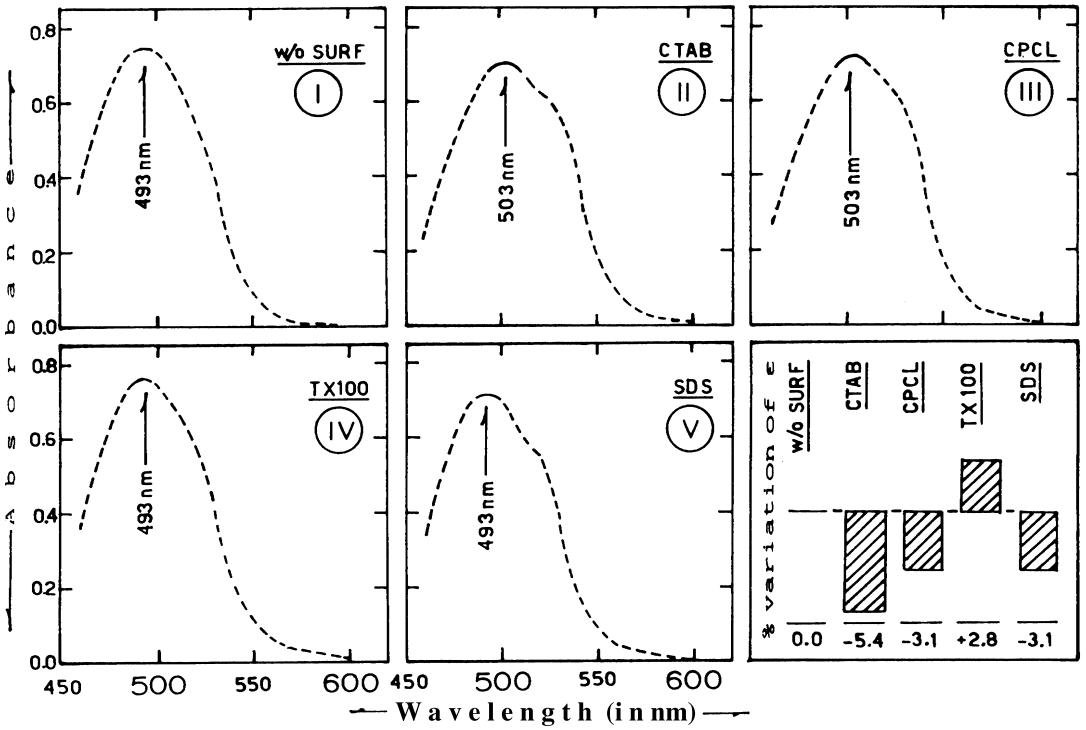

Fig. 2. Effect of the various surfactants on the molar absorptivitity of the Zn(II)–PAR chelate complex, on its absorption spectrum and on λ_{max}; pH 8.07–0.01.

Table 4
Analytical results of the determination of zinc included in various pharmaceutical formulations

Samp1e	Manufacturer/lot no.	Analytical technique (Method)	Zn(II) (μg/ml)		%RSD	SAE (SD/$\sqrt{n}$)[a]	Confidence limits ($P = 0.05$; $(n-1) = 2$)
			Labelled	Found ± SD[b]			
'Oculogut' (eye drops)	Dr. Thilo&Co./240391 (D)	A	0.6828	0.761 ± 0.003	0.40	1.8×10^{-3}	0.761 ± 0.0076
		B	0.6828	0.704 ± 0.001	0.22	8.8×10^{-4}	0.704 ± 0.0038
		C	0.6828	0.726 ± 0.005	0.73	3.0×10^{-3}	0.726 ± 0.0131
'Oculosan' (eye drops)	Dispersa S.A./914940 (CH)	A	0.6825	0.727 ± 0.005	0.76	3.2×10^{-3}	0.727 ± 0.0138
		B	0.6825	0.696 ± 0.006	0.87	3.5×10^{-3}	0.696 ± 0.0150
		C	0.6825	0.711 ± 0.004	0.51	2.1×10^{-3}	0.711 ± 0.0089
'Zabysept' (eye drops)	Rafarm S.A./12065 (GR)	A	0.6825	0.707 ± 0.001	0.21	8.8×10^{-4}	0.707 ± 0.0038
		B	0.6825	0.691 ± 0.004	0.58	2.3×10^{-3}	0.691 ± 0.0100
		C	0.6825	0.698 ± 0.008	1.09	4.4×10^{-3}	0.698 ± 0.0189
'Zincfrin' (eye drops)	Alcon-Couvreur/89J18 (B)	A	0.6828	0.750 ± 0.021	2.85	1.2×10^{-2}	0.750 ± 0.0531
		B	0.6828	0.724 ± 0.004	0.56	2.3×10^{-3}	0.724 ± 0.0100
		C	0.6828	0.736 ± 0.006	0.82	3.5×10^{-3}	0.736 ± 0.0150
'Monotard HM' (insulin Inj.)	Novo Nordisk/B141642 (DK)	A	0.6500	0.688 ± 0.021	3.02	1.2×10^{-2}	0.688 ± 0.0515
		B	0.6500	0.676 ± 0.006	0.97	3.8×10^{-3}	0.676 ± 0.0088
		C	0.6500	0.686 ± 0.004	0.52	2.1×10^{-3}	0.686 ± 0.0089

[a] SAE, standard analytical error.
[b] Average of three determinations.

The observed difference of the fluorescence intensity between the excitation spectra (a) and (b), as well as between those of the emission spectra (a′) and (b′) of Fig. 3, is obviously the expected effect of the presence of the Zn(II) analysed on the concentration of free HOx and therefore on its fluorescence [23].

3.2.2. Accuracy and precision of the proposed new methods A, B and C

With the purpose of ascertaining the accuracy and the precision of the proposed new methods A, B and C, solutions containing three different concentrations of Zn(II) (i.e. 0.2, 1.2 and 2.0 mmol/l Zn(II) for methods A and B and 0.0076, 0.0115 and 0.0153 mmol/l Zn(II) for method C, were prepared and analysed in three replicates by each one of the above mentioned methods. The analytical results obtained from this investigation are summarised in Table 1.

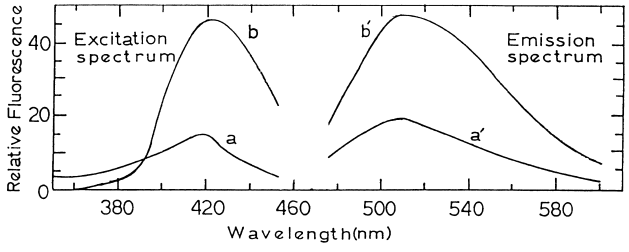

Fig. 3. Fluorescence spectra of 8-HOx (1.0% m/v in 4×10^{-1} mol/l CH_3COOH and acetone). (a,b) Excitation spectrum (emission at 510 nm); (a′,b′) emission spectrum (excitation at 420 nm); (a,a′) HOx only; (b,b′) HOx + 0.8 mmol/l Zn(II).

The mean %RSD, the standard analytical error (SAE), and the calculated confidence limits could be considered to be very satisfactory, at least for the level of the examined concentrations of Zn(II).

3.2.3. Application of the proposed new methods A, B and C to pharmaceutical analysis

The suggested methods A, B and C were successfully applied to the determination of Zn(II) contained on the one hand as an active ingredient in four well-known eye-drops solutions, such as 'Oculogut', 'Oculosan', 'Zabysept' and 'Zincfrin' and on the other hand in the insulin injectable solution 'Monotard HM' 100 iu/ml. (The manufacturers and lot no. of each one of the above medicines examined is summarised in Table 4, second column).

Table 4, gives the results obtained by application of the afore-mentioned analytical methods A, B and C. These results compared are found to be in good agreement.

Table 5
Average Zn(II) concentrations found by means of the applied methods A, B and C

Pharmaceutical preparation	Zn(II) (μg/ml ± SD)	%RSD	%Deviation[a]
Eye drops 'Oculogut'	0.730 ± 0.029	3.93	+ 6.90
Eye drops 'Oculosan'	0.711 ± 0.015	2.18	+ 4.17
Eye drops 'Zabysept'	0.699 ± 0.008	1.15	+ 2.42
Eye drops 'Zincfrin'	0.737 ± 0.013	1.77	+ 7.93
Insulin Inj. 'Monotard HM'	0.683 ± 0.006	0.94	+ 5.08

[a] Percentage deviation from the labelled content of Zn(II) in each drug examined.

Then, the average Zn(II) concentration in the medicinal samples analysed by means of the three proposed methods A, B and C are presented in Table 5.

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