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Beatriz Cañabate Díaz · Silvia Casado Terrones
Antonio Segura Carretero · José Manuel Costa Fernández
Alberto Fernández Gutiérrez

Comparison of three different phosphorescent methodologies in solution for the analysis of naphazoline in pharmaceutical preparations

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Abstract We present results from a comparative study of three proposed phosphorimetric methods for determination of naphazoline (NPZ) in solution. The first method is based on use of micelles to stabilize phosphorescence signals in solutions at room temperature (MS-RTP). The second is based on the use of a heavy atom salt and sodium sulfite as an oxygen scavenger to obtain room-temperature phosphorescence (HAI-RTP) in solution. The last method employs an optical sensor for NPZ based on the phosphorescent properties of the analyte on a solid sensor phase. The aim of this work was to compare time consumption, simplicity, sensitivity, selectivity, detection, and quantification limits for use of these three phosphorimetric methods to determine naphazoline in pharmaceutical preparations. The most simple, sensitive, and reproducible of the three methods for naphazoline analysis is the HAI-RTP method. Detection limits are 4.9, 1.7, and 9.4 ng mL^{-1}, respectively, for the MS-RTP, HAI-RTP, and optosensor methods.

Keywords Phosphorimetry · Pharmaceutical analysis · Naphazoline

Introduction

The observation of phosphorescence in the past was limited to rigid systems, especially those at liquid nitrogen temperatures (LTP) [1] or to analytes adsorbed on solid substrates (SS-RTP) [2]. A common aspect of phosphorimetry in fluid solution is the need for some form of molecular immobilization, protection, or both, to minimize non-radiative decay of luminophores, collisions with solvent, or the possibility of photochemical reaction.

Kalyanasundaram et al. [3] reported that room-temperature phosphorescence (RTP) in fluid solutions could be observed in the presence of micelles, heavy atoms, and nitrogen to effect deoxygenation. On the basis of that report, Cline Love [4] established micelle-stabilized room temperature phosphorescence (MS-RTP) as an analytical method. Following the successful application of MS-RTP, cyclodextrin-induced RTP (CD-RTP) [5], vesicle-stabilized RTP (VS-RTP) [6], and microemulsion-stabilized RTP (ME-RTP) [7], were developed. As can be seen from the evolution of RTP, there is a need to provide a protective, ordered medium to minimize self-quenching and to organize reactants on a molecular level, and to increase the proximity of heavy atoms and analytes [8, 9].

Later studies [10] have demonstrated that RTP emission of some compounds can be directly induced in aqueous solutions assisted only by addition of relatively high concentrations of a heavy atom perturber and sodium sulfite as chemical deoxygenator [11, 12, 13, 14, 15, 16, 17, 18, 19]. This type of RTP emission has been called heavy atom induced -room temperature phosphorescence (HAI-RTP) [10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21].

During the last ten years the advantages of using optical sensors for such analyses have been demonstrated [22]. The combination of flow-injection techniques with detection on optically active surfaces comprising an immobilised indicator packed in a flow-through cell has been called an “optosensor” [23] and has proved to have important advantages because of its high sensitivity and selectivity, precision, simplicity, speed, and low cost [24]. Further developments of these optosensing techniques have shortened analysis time considerably and reduced the cost of environmental monitoring.

Many non-prescription topical decongestants for ophthalmic or nasal use contain 2-imidazolidine-derived drugs. Naphazoline, 2-(1-naphthylmethyl)-2-imidazoline, is a potent *alpha*-adrenergic agonist, with vasoconstrictive and

B. C. Díaz · S. C. Terrones · A. S. Carretero (✉) · A. F. Gutiérrez
Department of Analytical Chemistry, Faculty of Sciences,
University of Granada, Campus Fuentenueva s/n,
18071 Granada, Spain
e-mail: ansegura@ugr.es

J. M. C. Fernández
Department of Physical and Analytical Chemistry,
Faculty of Chemistry, University of Oviedo,
C/ Julian Clavería 8, 33006 Oviedo, Spain

decongestive properties. Several analytical methods are used to determine naphazoline (NPZ); most are photometric [25] and chromatographic methods. Different types of chromatographic method have been reported in the literature, including thin-layer chromatography [26, 27], gas chromatography [28], and high-performance liquid chromatography [29, 30, 31, 32, 33, 34]. The literature also contains a report of the use of a fluorimetric method [35] to determine naphazoline in ophthalmic solution at $\lambda_{ex/em}$=280/327 nm, with a linear range between 0.1 and 0.5 mg mL^{-1}. NPZ has native phosphorescence and four phosphorimetric methods have been described. Among these, one was developed on filter paper ($\lambda_{ex/em}$=290/485, 520 nm) [36] and the others were proposed by our research group [11, 37].

In this work we compare different experimental conditions and analytical characteristics of three phosphorimetric methods. The first is based on the micelle-stabilized media, another on HAI-RTP methodology, and the last is a flow-through optosensor. All these methods were proposed for analysis of naphazoline in pharmaceutical preparations.

Material and methods

Reagents

The surfactant sodium dodecyl sulfate (SDS), analytical reagent grade thallium(I) nitrate, potassium iodide, sodium chloride, sulfuric acid, acetone, and anhydrous sodium sulfite (all from Sigma) were used as received. Aqueous solutions were made with doubly distilled water. The sodium sulfite solutions were prepared daily and kept in tightly stoppered containers. The non-ionic resin Amberlite XAD 7 (Sigma) was sieved and the 80–120 μm grain size was used. Naphazoline (Sigma) was used without further purification. Working solutions were prepared by suitable dilution of the stock solution (30 mg L^{-1}) with deionized water.

Instrumentation and flow set-up

A Varian Cary-Eclipse fluorescence spectrophotometer (Varian Iberica, Madrid, Spain) was used to obtain the phosphorescence spectra and the relative phosphorescence intensity measurements. The spectroluminometer is equipped with a xenon discharge lamp (75 kW), Czerny-Turner monochromators, R-928 photomultiplier tube which is red sensitive (900 nm) with manual or automatic voltage control, using the Cary Eclipse software for Windows 95/98/NT. The photomultiplier detector voltage was 850 V and the instrument excitation and emission slits were both set at 20 nm. The delay time used was 0.12 ms and the gate time was 5 ms.

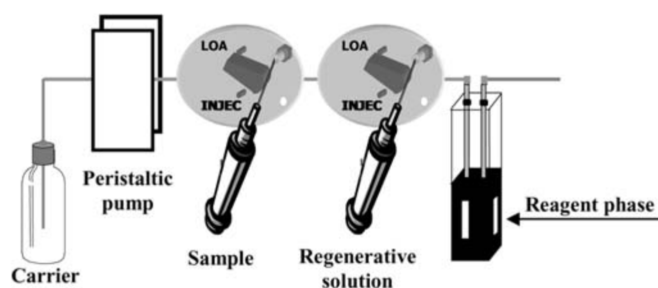

Fig. 1 The flow manifold employed

Figure 1 illustrates the optosensing FIA manifold used. A Hellma Model 176.052-QS flow-through cell of 25 μL volume was packed with the corresponding resin and placed in the conventional sample compartment of the detector. Two rotary valves (Supelco 5020) were used for sample introduction and for elution of the retained NPZ. PTFE tubing (1.1 mm i.d.) and fittings were used for connecting the flow-through cell, the rotary valves, and the carrier solution reservoirs. A Gilson Miniplus-3 peristaltic pump was used to generate the flow stream.

pH was measured with a MicropH 2002 meter (Crison, Barcelona, Spain).

General procedure

MS-RTP method

An aliquot of the NPZ stock standard solution, 0.45 mL 0.5 mol L^{-1} SDS, 0.84 mL 0.25 mol L^{-1} thallium nitrate, 0.64 mL 0.1 mol L^{-1} sodium sulfite, and 0.67 mL 0.02 mol L^{-1} sulfuric acid were introduced into a 10-mL calibrated flask and diluted to volume with water. The inclusion of the heavy atom salt in the solution can cause slight precipitation, which disappears on warming the flask in a water-bath, before the other reagents are added. After thorough mixing the flask was placed in a water bath at 25.0±0.1 °C for 1 min. At 25 °C the critical micellar concentration for SDS is 8.1 mmol L^{-1}, so the formation of micelles is spontaneous at the SDS concentration chosen. Standard 10-mm fused-silica cells were filled with this solution. Relative phosphorescence intensities (RPI) were measured at 524 nm with excitation at 290 nm. Reagents blanks lacking NPZ were prepared and measured by following the same procedure.

HAI-RTP method

An aliquot of the NPZ stock solution, 5 mL 2 mol L^{-1} potassium iodide and 1 mL 0.1 mol L^{-1} sodium sulfite were introduced into a 10 mL calibrated flask and diluted to volume with water. Standard 10-mm fused-silica cells are filled with this analyte solution. Reagents blanks lacking naphazoline were prepared and measured following the same procedure. The intensities of the samples and the corresponding blanks were measured at the phosphorescence wavelength maxima $\lambda_{ex}/\lambda_{em}$ 288/524 nm.

Flow-through optosensor

Naphazoline sample (2 mL, with 1.6 mol L^{-1} KI and 15 mmol L^{-1} Na_2SO_4) were injected via the first valve into a channel of 1.6 mol L^{-1} KI and 15 mmol L^{-1} Na_2SO_3 carrier. At 2 mL min^{-1} flow-rate the naphazoline is retained in the flow cell on Amberlite XAD7 and the phosphorescence was measured at $\lambda_{ex/em}$=290/520 nm. Regenerative solution (1 mL 2 mol L^{-1} NaCl with 15% acetone) was injected through second valve to elute the analyte retained on the sensing zone, before proceeding with the next sample injection.

Procedure for pharmaceutical preparations

Two different commercial products were analysed using these three methods – Euboral Oftálmico (Bama-Geve SA) with a nominal content of 10 mg g^{-1} naphazoline and also containing sodium tetraborate, 970 mg g^{-1}, and methyl *p*-hydroxybenzoate 20 mg g^{-1}, and Colirio Alfa (Rivofarma SA) with a nominal content of 3 mg mL^{-1} and also containing copper sulfate, trisodium citrate, potassium chrome alum, boric acid, camphor, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, sodium hydroxide, and sodium chloride, without indication of their concentration.

Portions of two products were dissolved in doubly distilled water. Aliquots of these solutions were treated as indicated under General procedure for each method.

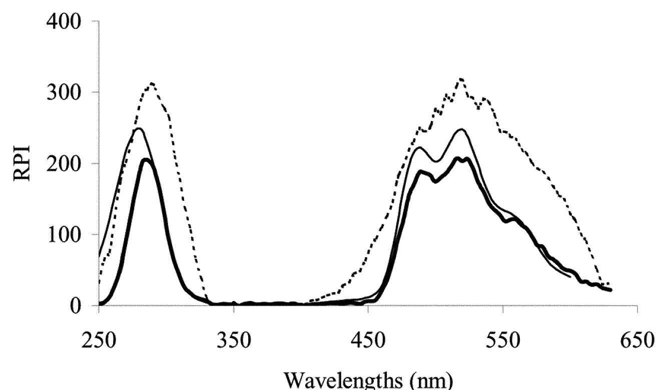

Fig. 2 Phosphorescence spectra obtained from 400 ng mL^{-1} NPZ: MS-RTP (*thin continuous line*), HAI-RTP (*thick continuous line*), and optosensor (*broken line*)

Results and discussion

Phosphorescence spectral characteristics

The different instrumental conditions which could affect the phosphorescence response, such as wavelength maxima, decay time, gate time, and detector sensitivity were conveniently selected.

The phosphorescence wavelengths for the MS-RTP method are 290 and 524 nm for excitation and emission, respectively. In HAI-RTP phosphorescence wavelengths of 288 nm and 524 nm were obtained as optimal values. On the Amberlite XAD7 (optosensor) naphazoline emits phosphorescence with a maximum excitation intensity at 290 nm and maximum emission intensity at 520 nm. These wavelengths are very similar those in solution. The phosphorescence spectra obtained for NPZ by use of the three different methods are showed in Fig. 2.

As is apparent from the figure, the shape of MS-RTP and HAI-RTP spectra were very similar but for the optosensor spectrum the influence of the solid support changes the shape of the emission spectrum.

Table 1 Instrumental conditions for the three methods

Method	$\lambda_{ex/em}$ (nm)	t_d (ms)	t_g (ms)	Detector voltage (V)	Slits$_{ex/em}$ (nm)
MS-RTP	290/524	0.12	5	600	20/20
HAI-RTP	288/524	0.12	5	1000	20/20
Optosensor	290/520	0.12	5	850	20/20

The delay time used was typically 0.12 ms and the gate time was 5 ms. The photomultiplier detector voltage was 600 V for MS-RTP, 1000 V for HAI-RTP, and 850 V for the optosensor. Detector voltage is the uniquely different instrumental condition for the three methods. The instrument excitation and emission slits were both set at 20 nm. All these instrumental variables were kept constant for the rest of the experimental work (Table 1).

Experimental variables

Table 2 shows the optimum experimental variables necessary to develop the three different methods proposed for NPZ. The careful selection of these experimental conditions has been described elsewhere [11, 37].

It is apparent from this table that in the three methods it is necessary to use a heavy atom (KI or $TlNO_3$) and deoxygentator to develop the phosphorescence of the analyte.

On other hand, only for MS-RTP and the optosensor, respectively, are an organized medium (SDS) or a solid support (Amberlite XAD7) necessary to obtain a phosphorescence signal for naphazoline.

It is also necessary to indicate the use of an acid medium (H_2SO_4) in MS-RTP to minimise the stabilization time or time necessary for deoxygenation of the samples. Deoxygenation was necessary for use of the optosensor because the solid support is in contact with the solution.

The most simple method is HAI-RTP because it uses only a heavy atom and a deoxygenator as experimental variables.

Analytical characteristics

Analytical performance characteristics of the three methods under these experimental conditions were evaluated. Standard calibration graphs, prepared according to recommended procedure, were linear, passing through the origin for all the methods studied. All the features of the methods are summarized in Table 3.

The wide linear ranges and standard errors and correlation coefficients indicate very good calibration linearity. The detection and quantification limits and sensitivity [38] were calculated. The precision, expressed as relative standard deviation, was determined by measuring RTP intensities of ten replicates, containing 400 ng mL^{-1} naphazoline, for each method.

Table 2 Reagents and optimum concentrations for each method

Method	Solid support	Organized medium [SDS] (mmol L^{-1})	Heavy atom		Deoxygenator [Na_2SO_3] (mmol L^{-1})	Acid medium [H_2SO_4] (mmol L^{-1})
			[KI] (mol L^{-1})	[$TlNO_3$] (mmol L^{-1})		
MS-RTP	–	22.0	–	21.0	6.4	1.3
HAI-RTP	–	–	1.0	–	10.0	–
Optosensor	Amb. XAD7	–	1.6	–	15.0	–

Table 3 Analytical properties of the proposed methods

Property	MS-RTP	HAI-RTP	Opto-sensor
Slope	18.30	13.20	0.34
Intercept	0.53	0.69	3.07
Correlation coefficient	0.998	0.999	0.994
Sensitivity (ng mL^{-1})	16.66	8.22	11.6
Detection limit (ng mL^{-1})	4.9	1.7	9.4
Quantification limit (ng mL^{-1})	16.3	5.6	31.2
Linear range (ng mL^{-1})	16.3–1000	5.6–1000	31.2–1000
RSD (%) (at 400 ng mL^{-1} level)	2.05	1.05	2.32

Table 4 Study of recoveries from samples of both pharmaceuticals

Method	Colirio Alfa		Euboral Oftálmico	
	Recovery (%)	*RSD* (%)[a]	Recovery (%)	*RSD* (%)[a]
MS-RTP	97.9	1.60	96.2	2.20
HAI-RTP	93.2	2.02	90.0	4.75
Optosensor	104.9	9.38	101.4	8.92

[a]For seven replicates

Table 5 Comparison of analytical performance of the three RTP methods under scrutiny

	MS–RTP	HAI–RTP	Optosensor
Simplicity	Average	Excellent	Good
Sensitivity	Bad	Good	Average
Time consumption	Bad	Average	Good
Reproducibility	Average	Good	Bad

Applications

The three phosphorimetric methods were satisfactorily applied to the determination of naphazoline in two eye drops, Colirio Alfa and Euboral Oftálmico.

Results obtained from recovery experiments are shown in Table 4.

For both products tested, statistical analysis of the assay results showed the precision of the three proposed phosphorimetric methods was satisfactory with no significant differences between indicated and experimental results except for the optosensor method, for which repeatability was worst.

The shape of spectra from naphazoline standard and from pharmaceutical samples were very similar and no interferences were observed.

Conclusions

These phosphorimetric techniques provide good sensitivity and selectivity for determination of small amounts of chemicals in real samples.

These methods can be recommended for the routine determination of naphazoline because of their sensitivity, precision, speed, and simplicity, which are superior to those of liquid chromatographic methods described previously.

A summary of conclusions reached from comparison of the three phosphorimetric methods studied is given in Table 5.

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