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Online naphazoline quality control by micellar-enhanced spectrofluorimetry

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ABSTRACT: The aim of this study was to develop a method for online spectrofluorimetric quality control of naphazoline (NPZ) in pharmaceuticals and raw drugs. A combination of a flow-injection analysis (FIA) system with micellar-enhanced fluorescence detection is presented as a powerful alternative for the rapid and sensitive analysis of naphazoline. Since NPZ shows low native fluorescence, the use of an anionic surfactant, such as sodium dodecyl sulphate (SDS), provides a considerable enhancement of fluorescence intensity and the nature of the technique allows a possible and easy adaptation to a FIA system. Using $\lambda_{exc} = 280$ nm and $\lambda_{em} = 326$ nm, a good linear relationship (LOL) was obtained in the range 0.003–10 μg mL^{-1} with a detection limit (LOD) of 3×10^{-4} μg mL^{-1} (s/n = 3). Parameters related to the nature of the analytical signal and to the FIA manifold were optimized. Satisfactory recoveries were obtained in the analysis of commercial pharmaceutical formulations. The proposed method is simple, accurate and allows for high-speed sampling and considerably shorter analysis times. In addition, it requires inexpensive equipment and reagents and has easy operational conditions and no side effects, thus avoiding environmental pollution through toxic waste. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: naphazoline; micellar enhancement; flow injection; spectrofluorimetry; pharmaceuticals

Introduction

Naphazoline (NPZ) is the common name for 2-(1-naphthylmethyl)-2-imidazoline hydrochloride and its molecular formula is $C_{14}H_{14}N_2$(p$K_a^{25} = 10.81$) (1). NPZ is a sympathomimetic agent with marked α-adrenergic activity and is a relatively long-lasting vasoconstrictor with a rapid action in reducing swelling when applied to the mucous membrane. It acts on α-receptors in the arterioles of the conjunctiva to produce constriction, resulting in decreased congestion. In addition, NPZ is an active ingredient in some commercial nasal and eye drops, and is widely used to relieve redness due to minor eye irritation caused by cold, dust, wind, smog, pollen, swimming or wearing contact lenses (2).

There are several analytical methods to determine NPZ (Table 1), the most widely used ones being photometry (3–5), gas chromatography (6) and high-performance liquid chromatography (7–10). Capillary electrophoresis (11–13), atomic absorption and emission (14) and electrochemical (15) methods have also been reported. Different luminescence methods have also been developed for NPZ determination, based on its intrinsic fluorescence and phosphorescence emission (16,17). However, most of the reported methods for NPZ determination present some practical complications for routine laboratory use: photometric methods are not selective and sensitive enough, separative methods are relatively expensive and time consuming and luminescence methods have been developed in a batch modality. Additionally, some of these methods produce a lot of waste material, causing significant environmental pollution. In this sense, and considering that environmental concerns in analytical chemistry is a recent consideration in order to assure the sustainable development of this discipline, direct monitoring methods involving the use of non-toxic reagents and suitable to be employed without sample preparation are desirable.

The aim of this study was to develop a method for online spectrofluorimetric quality control of NPZ in pharmaceuticals and raw drugs. The loss in sensitivity produced in flow-injection systems by dispersing the analyte into the carrier led us to design a system to enhance the detection signal.

Experimental

Instrumental

A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp and 1 cm quartz cells, was used for the fluorescence measurements. For flow measurements, a LC flow cell unit (12 μL cell) was used. A home-made valve was used for FIA configuration. Solutions were propelled by Gilson Minipuls 3 peristaltic pumps with pumping tubes. All tubes connecting the different components of the flow system were PVC, 0.8 mm i.d. A pH meter (Model EA 940, Orion Expandable Ion Analyser, Orion Research, Cambridge, MA, USA) with combined glass electrode was used for monitoring pH adjustment.

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Table 1. Methods for naphazoline determination

Instrumental methodology	Experimental detail	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	LOL (ng mL^{-1})	Samples	Ref.
DSph	295.5 nm	20×10^9	60×10^9	$60–100\times10^9$	Pharmaceuticals	(4)
SIC	Mobile phase: methanol:water (40:65, v/v), pH 5.2 (triethylamine/ acetic acid), flow rate 0.9 ml min^{-1}, UV detection	20	60	65–1250	Nasal drops	(10)
MEKC-PLS	Fused-silica capillary (57 cm 675 lm ID), 258 C 30 kV, 5 mmol/L phosphate-5 mmol/L borate, pH = 8.2, 40 mmol/L sodium dodecylsulphate background electrolyte, run time 6.6 min	30	130	200–39900	Pharmaceuticals	(11)
CZE	Silica capillary with 75 mm i.d., 70 cm length, 17.7 kV, phosphate buffer, pH 3.72, 0.063 mol L^{-1}	4700	15 800	15 800–60 000	Nasal solutions	(12)
MEKC	Fused-silica capillary (57 cm, 375 mm i.d.) at 25 °C 30 kV, 5 mmol/L phosphate–5 mmol/L borate buffer, pH 8.2, 50 mmol/L SDS and 10% methanol/water (v/v) as background electrolyte	980	2940	100–32 300	Pharmaceuticals	(13)
AES	Precipitation of NPZ ion-association complexes with $[Co(NO_2)_6]^{3-}$ and $[Fe(CN)_6]^{3-}$. Indirect determination Fe(III)			2940–14 760	Pharmaceuticals	(14)
Potentiometric: ISE	pH range 3.0–8.0	525	WD	$1050–1050\times10^4$	Pharmaceuticals Pure drug	(15)
FOS	Nonionic-exchanger solid support (Amberlite XAD-7) in a flow cell $\lambda_{exc/em}$ = 294/306 nm.	2.6	8.7	2.6–225	Eye drops	(16)
This work	$\lambda_{ex/em}$ = 280/326 nm; pH 9.0; ionic strength, 0.1 mol.L^{-1}; SDS, 8.10^{-3} mol L^{-1}; flow rate, 20 rpm; sampling rate, 30 samples h^{-1}	0.3	3	3–10 000	Pharmaceuticals	–

LOD, limit of detection, LOQ, limit of quantification, LOL, limit of lineality, DSph, derivative spectrophotometry, CZE, capillary zone electrophoresis, MEKC, micellar electrokinetic chromatography, AES, atomic emission spectroscopy, FOS, fluorescence optosensor, SIC, sequential injection chromatography, ISE, ion-selective membrane electrode, WD, without datum.

Reagents

Naphazoline was kindly provided by Andrómaco S. A. (Buenos Aires, Argentina). The following chemicals of analytical grade were purchased from Tokyo Kasei Industries, (Chuo-Ku, Tokyo, Japan): sodium dodecyl sulphate (SDS), hexadecyltrimethylammonium bromide (HTAB), polyethylene glycol mono-4-nonylphenyl ether (PONPE 7.5). High-purity water was obtained from a Millipore (Milford, MA, USA) Milli-Q Plus system.

The pH values in the optimization stage were adjusted by the addition of solutions of NaOH (0.01 mol L^{-1}) NaOH (2 N) HCl (.01 mol $^{-1}$) or Cl (12 N) until the target pH value was reached.

Standard solutions. Naphazoline standard solution containing 0.5 mg mL^{-1} was prepared by dissolving the reagent in water. Under these conditions, naphazoline solution was found to be stable for several weeks when kept in dark. A standard working solution of 4 µg mL^{-1} was prepared daily by dilution of stock standard solution with ultra-pure water and stored in a dark bottle.

SDS solution (4.0×10^{-2} mol L^{-1} SDS), electrolyte solution (1 mol L^{-1} NaCl) and basic solution (0.02 mol L^{-1} borax) were prepared in ultra-pure water.

Sample solutions. An accurately measured volume of NPZ hydrochloride ophthalmic solution, equivalent to about 0.1 mg NPZ hydrochloride, was transferred to a 25 ml volumetric flask, diluted with ultra-pure water to volume and mixed. The same procedure was applied to NPZ hydrochloride nasal solutions. In all cases, the final concentration was 4 µg mL^{-1}.

FIA configuration

The manifold used was built using a four-channel Gilson Minipuls-3 peristaltic pump fitted with rate selectors, a home-made injection valve, acting as selecting valve, and PVC tubing of 0.8 mm i.d.

A stream of SDS/borax/NaCl was introduced into the detector, producing the baseline. After changing the valve position, NPZ was mixed in the reaction coil (R) with a stream of SDS/borax/NaCl and then impelled through the spectrofluorimeter for measurement.

General procedure

A stream of sample or standard solutions containing naphazoline at pH 9 was combined with the carrier stream. The carrier stream consisted of a solution prepared with SDS (1.62×10^{-2} mol L^{-1}), buffer (pH 9) and NaCl (0.2 mol L^{-1}) to obtain the optimal conditions

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for naphazoline fluorescent emission. The drug contained in the sample or standards and the carrier stream interacted in the reactor (R) and flowed to the fluorescence detector. The valve was switched in such a manner that in one position it allowed the flow of the carrier stream, and in the second position the flow of the sample or standards and the carrier solution. In this way the fiagram (diagram of flow injection analysis) always had the same background produced by the same concentration of carrier solution.

Validation procedure

In order to demonstrate the validity of this method, 100 µL commercial NPZ hydrochloride nasal drops (Dazolin®) containing 0.1 mg NPZ were transferred to 10 100 mL volumetric flasks. Six of these portions were taken to volume with ultra-pure water and analysed by the proposed method, and the average quantity of NPZ determined was taken as a base value. Then increasing volumes of NPZ solution were added to other four aliquots of sample and total NPZ was determined by applying the standard addition method. A second validation was performed by determining NPZ in pharmaceuticals using the official United States Pharmacopea (USP) chromatographic method (18).

Results

Fluorescence characteristics of naphazoline in aqueous media

Figure 1 shows the three-dimensional (3D) excitation–emission plot for the excitation and emission spectra for 4 µg mL^{-1} aqueous solution of NPZ, SDS 8.1×10^{-3} mol L^{-1} and NaCl 0.1 mol L^{-1}, pH 9, in batch. The drug showed a maximum emission at 326 nm when excited at 280 nm. These wavelengths were selected to measure the fluorescence intensity for the subsequent assays.

Figure 2 shows the emission spectra of NPZ aqueous solution (4 µg mL^{-1}) ($\lambda_{exc} = 280$ nm) and NPZ (4 µg mL^{-1}) in the optimal working conditions (pH 9; NaCl 0.1 mol L^{-1}; SDS 8.1×10^{-3} mol L^{-1}). As can be seen, the native fluorescent response achieved in the latter conditions was 2.5-fold that obtained in the aqueous medium.

Diphenhydramine is a drug commonly present in pharmaceuticals containing NPZ. The spectrum of diphenhydramine hydrochloride performed in the same conditions presented a maximal emission at 310 nm.

Figure 3 depicts the effect of pH value in the fluorescence signal. Analysis of spectrofluorimetric data for NPZ in water solutions showed a maximal intensity in the pH range 8–9 and began to decrease at pH > 9.8. The working pH selected was 9, using borax as the buffer solution.

Nature and concentration of surfactant agent

In order to improve the native luminescent emission, the fluorescent properties of NPZ were studied in different surfactant media: anionic (SDS, $0–9 \times 10^{-3}$ mol L^{-1}); cationic (HTAB, 9×10^{-3} mol L^{-1}); and non-ionic (PONPE 7.5, 8.5×10^{-5} mol L^{-1}). Experimental data showed that the enhancement factor for the NPZ–SDS system (2.2-fold with respect to NPZ fluorescence in water medium) was higher than for NPZ–HTAB, while for NPZ-PONPE 7.5 an important spectral interference was observed; thus, the anionic surfactant SDS was chosen for further work.

The fluorescence spectrum of NPZ in ultra-pure water and SDS solution at pH 9 reveals that, above the critical micellar

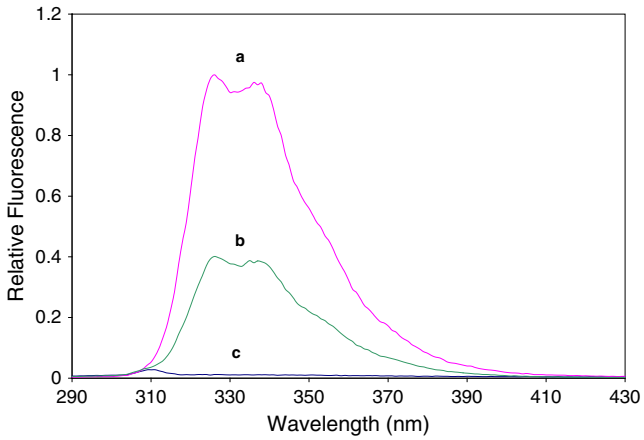

Figure 2. Emission spectra of: (a) naphazoline (4 µg mL^{-1}) in the optimal working conditions (pH 9, NaCl 0.1 mol L^{-1}, SDS 8.1×10^{-3} mol L^{-1}); (b) naphazoline aqueous solution, 4 µg mL^{-1}; (c) diphenhydramine (0.08 µg mL^{-1}) in the optimal working conditions ($\lambda_{exc} = 280$ nm).

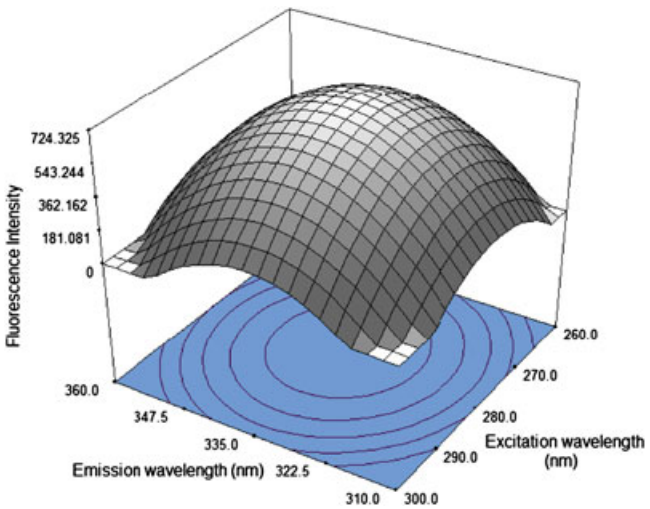

Figure 1. Three-dimensional excitation–emission plot for the excitation and emission spectrum for 4 µg mL^{-1} aqueous solution of naphazoline, SDS 8.1×10^{-3} mol L^{-1}, NaCl 0.1 mol L^{-1} at pH 9 in batch.

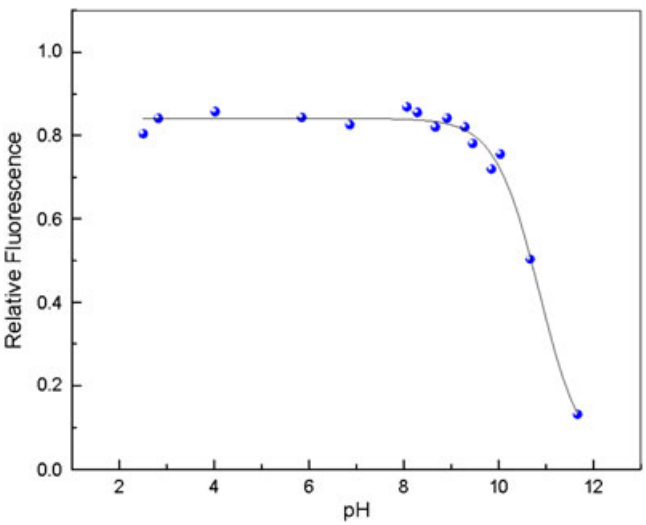

Figure 3. Effect of pH on naphazoline fluorescence intensity. Naphazoline 4 µg mL^{-1}; NaCl 0.1 mol L^{-1}; SDS 8.1×10^{-3} mol L^{-1}.

concentration (CMC), the fluorescent intensity increased with SDS concentration, and this increase was more evident at 8.1×10^{-3} mol L^{-1} (Fig. 4).

Effects of electrolytes on the fluorescence intensity of NPZ

The presence of 0.1 mol L^{-1} NaCl in NPZ in surfactant medium at pH 9 showed that the fluorescence increases almost 2.5 times with respect to that of NPZ in aqueous medium. The effect produced by the addition of inert salts to the system is shown in Fig. 5.

FIA system: effect of flow rate

Several experiments were made in order to study the effect of the flow rate of the FIA system on the fluorescent signal. The results showed that the fluorescent signal increases with the increase of rate. For rates >5 mL min^{-1} great turbulence was observed, due to the introduction of bubbles into the flow system, with consequent fluctuation in the fluorescent values. A flow rate of 5 mL min^{-1} was selected as optimal. Under these optimal conditions the sampling rate was 30 samples h^{-1}.

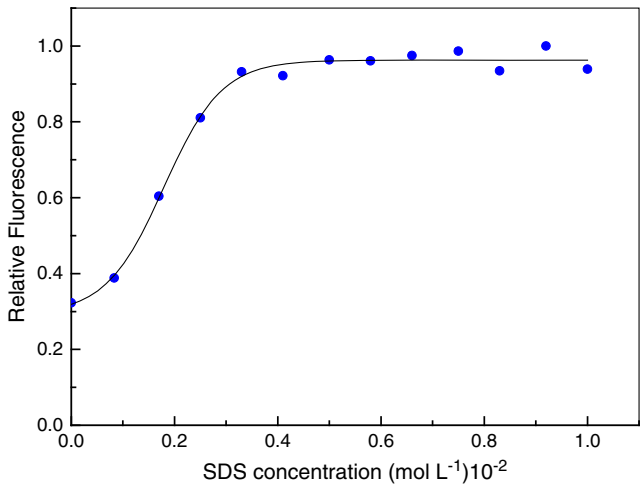

Figure 4. Effect of surfactant concentration on naphazoline fluorescence intensity. Naphazoline, 4 µg mL^{-1}; NaCl, 0.1 mol L^{-1}, pH 9.

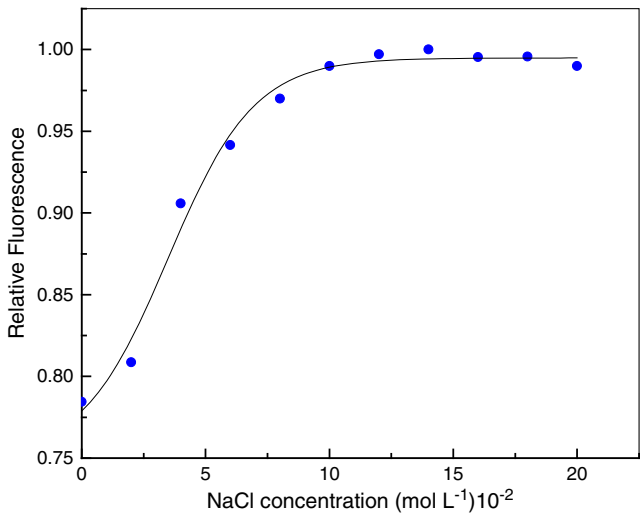

Figure 5. Effect of ionic strength on naphazoline fluorescence intensity. Naphazoline, 4 µg mL^{-1}; SDS, 8.1×10^{-3} mol L^{-1}, pH 9.

Method validation

Linearity. The equation for the calibration graph was obtained by least-squares regression analysis, employing the areas of the analyte standard fluorescent signals:

$$F = 435285\ C + 847.13$$

where F is the relative fluorescence intensity and C the concentration of NPZ. The method was linear in the range 0.003–10 µg mL^{-1} NPZ. The correlation coefficient was 0.9998.

Limit of detection and limit of quantification. The limit of detection (LOD) was defined as the compound concentration that produced a signal:noise (s:n) ratio >3, while the limit of quantification (LOQ) of the assay was evaluated as the concentration equal to 10 times the value of the s:n ratio. LOD and LOQ values for this method, based upon these criteria, are 3×10^{-4} and 3×10^{-3} µg mL^{-1}, respectively.

Sensitivity. LOD and LOQ values confirmed the high sensitivity of the proposed method.

Reproducibility. The fiagram was registered for different standards of NPZ: (a) 2 µg mL^{-1}; (b) 3 µg mL^{-1}; (c) 4 µg mL^{-1}; (d) 6 µg mL^{-1}; (e) 8 µg mL^{-1}; (f) 10 µg mL^{-1} (Fig. 6). The triplicate signals demonstrated good reproducibility for the developed methodology.

Specificity. The presence of other active drugs with which pharmaceuticals containing NPZ can be formulated (such as pheniramine or diphenhydramine), or of the common excipients of the analysed formulations, were observed not to cause spectral interferences. This is an important advantage of the method, since it allows for the analysis of NPZ without a prior separation step.

Accuracy and precision. The intraday and interday precision of the method, based on repeatability, was determined by replicating injections ($n = 6$) of five standard solutions prepared by the standard addition method at different concentration levels. A relative standard deviation (RSD) <6.1% was obtained in all cases. The intraday and interday recoveries were in the range 91–106.4%. The method was also validated against the USP HPLC method (18); validation results are shown in Table 2. The developed method was applied to the determination of

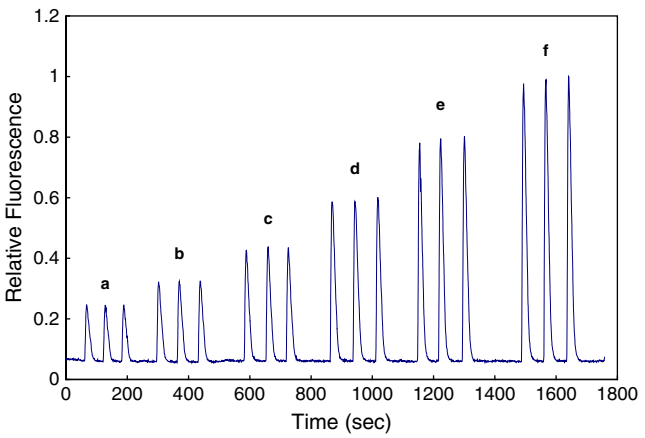

Figure 6. Naphazoline fiagram. Standard solution of NPZ. (a) 2 µg mL^{-1}; (b) 3 µg mL^{-1}; (c) 4 µg mL^{-1}; (d) 6 µg mL^{-1}; (e) 8 µg mL^{-1}; (f) 10 µg mL^{-1}.

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Table 2. Validation of the method for the determination of NPZ in commercial pharmaceutical formulations

Sample ($n = 6$)	Base value (μg mL^{-1})	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery* (%)	RSD (%)
Intraday	1.00	–	0.973	97.3	5.3
	1.00	0.5	1.455	91.0	4.5
	1.00	1	1.980	98.0	3.8
	1.00	2	2.980	99.0	2.6
	1.00	3	3.920	97.3	2.7
Interday	1.00	–	0.981	98.1	6.1
	1.00	0.5	1.532	106.4	5.8
	1.00	1	1.975	97.5	5.6
	1.00	2	3.098	104.9	4.3
	1.00	3	3.971	99.0	3.7

n, number of replicates for each sample.
[a]×100 [(found-base)/added].

Table 3. Analysis of NPZ in pharmaceutical samples by the proposed spectrofluorimetric method and HPLC official methods

Samples	NPZ nominal quantity (mg mL^{-1})	NPZ found (mg mL^{-1})[a]	E (%)	NPZ found (mg mL^{-1})[b]	E (%)
1	0.12	0.123	2.5	0.1235	2.9
2	1.00	0.973	2.7	1.035	3.5
3	0.25	0.238	4.8	–	–
4	0.50	0.525	5.0	–	–

[a]Spectrofluorimetric method developed in this work.
[b]USP official method using HPLC.
1, Mirus-S Ophthalmic® (Alcon, Bs.As., Argentina), NPZ content, 0.12 mg mL^{-1}.
2, Dazolin Nasal® (Roux-Ocefa, Bs.As., Argentina), NPZ content, 1 mg mL^{-1}.
3, Mirus Ophthalmic® (Alcon, Bs.As., Argentina), NPZ content, 0.25 mg mL^{-1}, and 3 mg mL^{-1} of pheniramine maleate.
4, Panoptic Ophthalmic® (Bausch & Lomb, Bs.As., Argentina), NPZ content 0.5 mg mL^{-1}, and 0.01 mg mL^{-1} of diphenhydramine chlorhydrate.

NPZ in four commercial pharmaceutical samples, as described in Table 3. The results were in good agreement with the labelled content of NPZ.

Robustness. This was examined by evaluating the influence of small variations of method variables, including concentration of SDS, NaCl concentration, pH and flow rate, on the method's suitability and sensitivity. It was found that none of these variables significantly affect the analytical parameters of method. This provides an indication of the reliability of the proposed method during normal usage, and so it can be considered robust.

Discussion

NPZ presents low native fluorescence with a maximal emission at 326 nm. This emission can be improved by working at the proper pH. In this case, the working pH selected was 9, due to the highest intensity obtained and the possibility of using a buffer solution of borax that did not interfere with the NPZ fluorescence measurements.

The fluorescence increase in micellar media may be attributed to stabilization/protection of the excited singlet state, which hinders decay by quenching, and other non-radioactive deactivation processes (19,20). The higher enhancement in fluorescence intensity produced by the presence of SDS in NPZ solutions (2.2-fold) and the important spectral interference observed for the PONPE 7.5–NPZ system led to the choice of SDS.

The fluorescent emission in surfactant solution can be affected by the addition of an inert electrolyte. The salting-out electrolyte decreases the solubility of organic substances in water (21). In aqueous surfactant media, the electrolyte may contribute to enhancing the fluorescence because it helps to push the organic substance into the micellar interior.

The beneficial effect produced on the native fluorescence of NPZ by the presence of both the electrolyte and the surfactant agent represents an essential factor to develop a FI system. The analyte in such a system is dispersed in the carrier, with the consequent loss of sensitivity (lower intensity). In addition, the low fluorescence intensity of NPZ solution and the allowed maximal concentration to avoid the inner filter effect made it necessary to improve the signal in order to obtain good sensitivity and reproducibility.

With the new working conditions in the presence of SDS, inert electrolyte and borate buffer, pH 9, an enhancement of 2.5 in

NPZ native fluorescence is achieved. These conditions allow working in a wider range of concentrations because the inner filter effect begins to appear at 10 μg mL^{-1}. These facts allow the detection of NPZ in a flow system.

By comparing the spectra of NPZ and diphenhydramine hydrochloride (Fig. 2) obtained in the same concentration proportion as they are in pharmaceuticals, it can be seen that diphenhydramine hydrochloride has a maximum emission at 310 nm, with low fluorescence intensity with respect to NPZ. This difference in intensity increased on adding SDS and an electrolyte to the solutions, allowing for the determination of NPZ without the interference of diphenhydramine hydrochloride.

In sample analysis a common step is sample dissolution; sample conditioning and the required concentrations require the original formulations to be diluted prior to analysis. On this point is important to remark that the NPZ concentration found in Table 3 has been calculated by applying the corresponding dilution factor. In the chromatographic (HPLC) USP official method, the sample is diluted to a solution containing 250 μg mL^{-1} NPZ, and in the absorptiometric method the sample is diluted to a solution of 20 μg mL^{-1} NPZ. Although the concentration of NPZ in the pharmaceutical preparations is high, it is not enough to be detected with precision in a FIA system with a high speed of sampling (in this study, 30 samples h^{-1}).

In comparison with the method proposed here, HPLC (the USP method) probably involves more difficult parameters to be optimized than in other separative instrumental methods. In addition, column efficiency constitutes a prime requirement for an HPLC system, and the obtained sensitivity and selectivity depend on the detection system used. It can be concluded that the method developed in this work is simple, sensitive and offers a higher rate for sample processing than the official method.

An important point of novelty of this work arises from the small volumes of sample and reagents employed and their non-toxic characteristics. Comparing this method with the official chromatographic and absorptiometric methods of USP, we observed that the mobile phase used in chromatography is sodium 1-heptane sulphonate, acetonitrile (25%), glacial acetic acid (10%) and water; and in the absorptiometric method, methanol is used to dilute the samples and standards. In both cases, highly pollutant reagents are used.

Conclusions

Spectrofluorimetric analysis is a very attractive detection technique, mostly due to the instrument simplicity, low detection limits and wide calibration ranges. In the present study the combination of this instrumental technique with a flow-injection method, such as FIA, stimulates more extensive use of spectrofluorimetric methods in drug analysis.

The addition of SDS/NaCl gives an approximately 2.5-fold increase in sensitivity and improves the limit of detection without further sample manipulation. Fluorescent detection allows for greater selectivity, free from the interferences produced by the common excipients found in commercial NPZ pharmaceutical forms. The method avoids the interference of other active drugs that coexist with NPZ in the formulation, without prior separation. The enhancement effect produced on the native fluorescence of NPZ by the presence of the electrolyte and the surfactant agent is essential to combine the developed spectrofluorimetric method with a FI system, since the analyte in such systems is dispersed into the carrier, with the consequent loss of sensitivity. The advantages of the developed methodology make it appropriate for pharmaceutical quality control of NPZ as routine analysis.

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