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Short communication

Determination of poloxamer 188 and poloxamer 407 using high-performance thin-layer chromatography in pharmaceutical formulations

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Abstract

Poloxamers (PXMs) are amphiphilic non-ionic block polymers commonly used in the cosmetic and pharmaceutical industries. In spite of the wide use of PXMs, few studies have dealt with the analysis of these polymers in pharmaceutical preparations. In this work, high-performance thin-layer chromatography (HPTLC) has been used to quantify both PXM-188 and PXM-407 in pharmaceutical preparations. The separation of these compounds was carried out using reverse phase HPTLC plates with a chloroform–methanol mixture as the mobile phase. Detection was performed densitometrically using the Dragendorff's reagent for the visualization of PXMs. Quality parameters were established, and the detection limits ranged from 24 to 47 ng/spot. A good precision (day to day and run to run), with relative standard deviations <11.18%, was obtained. The proposed method was satisfactorily applied to the analysis of laboratory-made and commercial pharmaceutical products.

Keywords: Poloxamer; Pluronic®; Triblock polymers; Reverse-phase High-performance thin-layer chromatography; Dragendorff's reagent

1. Introduction

Poloxamers (PXMs) are amphiphilic non-ionic block polymers of hydrophobic propylene oxide and hydrophilic ethylene oxide (Fig. 1) comprising a central poly(oxypropylene) (PPO) molecule, which is flanked on both sides by two chains of poly(oxyethylene) (PEO) [1]. PXMs have similar chemical structures, but they have a variable number of PEO and PPO units, and therefore, they differ in their molecular weight [2].

Poloxamer 188 (PXM-188) and Poloxamer 407 (PXM-407) are among the most commonly used PXMs. PXM-188 is an ABA block type copolymer which contains around 80% POE units and 20% PPO units. It is used as an emulsifier, solubilizer, dispersing and wetting agent in the preparation of solid dispersions, and for the enhancement of the bioavailability of low-solubility drugs in oral solid dosage forms [3,4]. Given its low toxicity, PXM-188 is one of the most common surfactants in parenteral applications [3,5]. PXM-407 is also an ABA block type copolymer, containing approximately 70% PEO and 30% PPO. PXM-407 exhibits reverse thermal gelation in concentrations above 20%, and has a non-toxic nature [6,7]. Due to these favorable properties, PXM-407 has a potential use in the development of controlled drug delivery systems [7–9].

In spite of the wide use of PXMs, few studies have dealt with the analysis of these polymers in pharmaceutical prepa-

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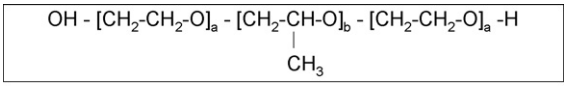

a = chains of poly(oxyethylene) (PEO)
b = central poly(oxypropylene) (PPO)

Fig. 1. Chemical structure of PXMs, *a*, chains of poly(oxyethylene) (PEO), *b*, central poly(oxypropylene) (PPO).

rations. Most analytical methods make use of the ability of PXMs to complexate with metal ions [4,10], and reagents such as tetraiodobismuthate or cobalt (II) thiocyanate have been used [2,5,10–13]. Quantification of PXMs was achieved either spectrophotometrically or by titration of the complex. Although, some efforts have been made in order to improve selectivity, these methods have been used mainly for biological samples, and in most cases, the analytical performance of the methods has not been satisfactorily evaluated. Mao et al. [2] have reported the quantification of PXMs in pharmaceutical products using a colorimetric method based on the formation of a colored complex between PXMs and cobalt (II) thiocyanate, with a good performance only for certain products.

Chromatographic methods have also been developed for PXMs, although they have been focused mainly on determining the molecular weight distribution of these polymers. Size exclusion chromatography with two coupled columns has been successfully used in the quantification of PXMs in pharmaceutical products [2,14]. Quintanar et al. [15] have recently proposed the use of Dragendorff's reagent for detection in the high-performance thin-layer chromatographic (HPTLC) analysis of PXM-188. Here, we describe the development of an HPTLC procedure for the determination of both PXM-188 and PXM-407 in pharmaceutical formulations. The proposed method was fully validated and applied to the analysis of some laboratory-made and commercial products.

2. Experimental

2.1. Reagents and chemicals

The PXM-188 and PXM-407 were provided by Nutrer, Mexico (BASF, Ludwigshafen, Germany). Bismuth nitrate, barium chloride, potassium iodide and glacial acetic acid were obtained from JT Baker (Mexico) and were all of analytical grade. Organic solvents were HPLC grade and were purchased from Fermont (Monterrey, Mexico). Water was purified using a Milli-Q system (Millipore, MA, USA). Stock solutions of PXMs (5 mg mL^{-1}) were prepared in water. Working solutions were obtained by dilution of stock solutions.

The modified Dragendorff's reagent was prepared as follows: Solution A was an 80:20 v/v mixture of bismuth nitrate in glacial acetic acid (85 mg mL^{-1}) and aqueous potassium iodide (0.4 g mL^{-1}). Solution B consisted of barium chloride (0.2 g mL^{-1}) in Milli-Q water. The final reagent solution was obtained by mixing 10 mL of solution A and 5 mL of solution B. The Dragendorff's reagent was stored in a brown bottle and discarded after 48 h.

2.2. HPTLC analysis

Analyses were performed on 20 cm × 5 cm Alugram® SIL G/UV254 plates (Machery–Nagel, Düren, Germany; 0.20 mm Silica Gel 60) and 20 cm × 5 cm Alugram® RP-18W/UV254 plates (Machery–Nagel, 0.15 mm Silica Gel C_{18}), both impregnated with a fluorescent indicator. The plates were cleaned by predevelopment with the mobile phase to the top of the plate and dried in a fume hood.

Sample and raw material zones were applied as bands by means of an Automated TLC Sampler III (ATS3, CAMAG, Muttenz, Switzerland) which was operated with the following settings: delivery speed, 200 nL s^{-1}; band length, 3 mm; distance between bands, 12 mm; distance from the plate side edge, 10 mm; and distance from the bottom of the plate, 15 mm.

The chromatographic chamber was a 20 cm × 10 cm flat bottom chamber from CAMAG (Muttenz, Switzerland). The mobile phase consisted of a chloroform–methanol (90:10 v/v for PXM-188 and 88:12 v/v for PXM-407) mixture. The chamber was equilibrated with 10 mL of mobile phase for 20 min prior to inserting the plate. The plates were developed in the mobile phase during 30 min. The separation was performed in the ascending mode up to 4 cm. After each analysis, the mobile phase was removed and substituted by a fresh one.

Bands were developed by spraying the plate with Dragendorff's reagent through a glass atomizer reagent sprayer and were air-dried. The visualized bands of PXMs on HPTLC plates were quantitated with a densitometer CAMAG TLC-Scanner 3 (Muttenz, Switzerland) in the absorbance-reflection mode, measuring the absorbance of the bands at 512 nm, where the complex has the maximum absorption. The monochromator bandwidth was 5 nm and a tungsten source was used. The slit dimensions were set at 2.0 mm in length and 0.2 mm in width, with a scanning rate of 0.5 mm s^{-1}. All the plates were scanned and measured within 10 min following detection. The automatic scanning was controlled by the CATS 4 software (version 4.06).

2.3. Sample preparation

Poloxamers were determined in the following products: a commercial buccal antiseptic solution containing PXM-407 as excipient, PXM-188 laboratory-made nanoparticles (NPs) and a PXM-407 gel prepared in our laboratory.

NPs were prepared by the emulsification–diffusion technique using PXM-188 as stabilizer. Briefly, a solution was prepared by dissolving 400 mg of poly(lactic acid) (PLA) in 20 mL of propylene carbonate saturated with water. Forty milliliters of aqueous phase saturated with propylene carbonate containing 5% w/v of the stabilizing agent (PXM-188) were added to the polymer solution under mechanical stirring. An emulsion was formed by using a standard laboratory mixer at 8000 rpm during 10 min. Then, 160 mL of pure water were added to allow diffusion of the organic solvent into the water, leading to the precipitation of PLA and the formation of nanospheres. The batches were adjusted to 200 mL before purification. The suspension was subjected to tangential filtration using polyvinylidene fluoride membranes to discard soluble impurities as well as any excess of solvent

or stabilizing agent. NPs were purified by cross-flow filtration using 6 L of water as filtration medium [15].

The PXM-407 gel was prepared as follows: 27 g of PXM-407, 24.8 g of Transcutol® and 3 g of sodium naproxen were weighted and added slowly to 100 mL of cold water (4 °C) under soft mechanical stirring for 3 min. This solution was stored at 4 °C overnight to allow complete hydration. Adjustment to 100 g was performed with water and then, the mixture was stored at 30 °C overnight to allow a complete dissolution of the mixture components [16].

2.4. Method validation

The analytical methods were validated according to the guidelines of the International Conference on Harmonization (ICH) [17] and the IUPAC [18]. Precision, linearity and the limits of detection (LODs) and quantitation (LOQs) were determined using solutions of each PXM prepared in water. It should be noted that an important advantage of using an automated sample applicator is that variable volumes of a single raw material solution and the sample can be applied, obtaining equalized initial zones that lead to an accurate and precise densitometric determination. In this study, the 5 mg mL^{-1} stock raw material solution was used directly.

Calibration curves were constructed using five analyte concentrations (100–900 nL of the stock raw material solution), representing 500–4500 ng/spot of PXM-188 and 1750–3500 ng/spot of PXM-407. The system's precision was assessed by consecutively analyzing six replicates of raw material solutions containing various amounts of PXMs (PXM-188: 1500, 3000 and 4500 ng/spot; PXM-407: 1750, 2500 and 3250 ng/spot) on a single day and on two different days. The LODs were estimated based on the standard deviation (S.D.) of the response and the slope (S) of the calibration curve at low calibration levels (100–400 ng/spot), according to the following formula: LOD = 3.3 (S.D./S). The S.D. of the response was determined based on the standard deviation of the y-intercepts of regression lines. Likewise, the LOQs were calculated using the calibration curves with the formula: LOQ = 10 (S.D./S).

3. Results and discussion

3.1. Method development

In a preliminary study, an attempt to establish a colorimetric method for the determination of PXMs was made. The original idea was to isolate PXMs by forming an insoluble colored complex with Dragendorff's reagent. After separation, the orange precipitate would be dissolved and the amount of PXMs in the sample would be measured spectrophotometrically. For the dissolution of the precipitate, a wide variety of solvents, such as methanol, ethanol, 2-propanol, propylene glycol, diethyl ether, ethyl acetate, chloroform, tetrahydrofuran, acetone and mixtures of these were evaluated. However, no successful solubilization of the precipitate was attained. Two main problems were encountered: the complex showed only a partial solubility and a very low chemical stability. Since this first approach did not work, we decided to evaluate HPTLC using the Dragendorff's reaction for the visualization of PXMs.

In order to obtain the best sensitivity for the detection of PXMs, the maximum absorption wavelength was determined by the measurement of the *in-situ* UV absorption spectrum of a zone of each PXM treated with the visualization reagent. The plate was scanned in the absorbance-reflection mode and the absorption spectra were recorded between 400 and 800 nm; maximum responses were obtained at 513 nm for PXM-188 and at 512 nm for PXM-407. So, these wavelengths were selected for the quantitative analysis. All plates were scanned and measured within 10 min following detection, as the bands slowly lost the orange color.

Both reverse- and normal-phase HPTLC plates were evaluated in order to establish the optimum separation conditions for PXMs. Since commercial products do not use mixtures of these non-ionic surfactants, all this work was carried out using individual raw material solutions. Although several mobile phases were evaluated, no retention was achieved when silica HPTLC-plates were used, and in all cases, both PXM-188 and PXM-407 migrated along with the solvent front.

With RP-18 plates, many solvent systems were also tested. For PXM-188, the best results were obtained using tetrahydrofurane–water (70:30 v/v) and chloroform–methanol (90:10 v/v), although an important band tailing was observed for both systems. In order to improve the band shapes, a washing step was introduced, the plate was developed with mobile phase before sample application, and less tailing bands were obtained due to the deactivation of the plate.

The saturation of the chamber atmosphere with eluent vapor is recommended for crucial mobile phases to obtain reproducible migration distances. In order to establish the optimum conditions for the analysis of PXMs, the saturation time was studied, and a strong influence of this parameter on the band shapes was observed. An increase in the saturation time produced an improvement in the band shapes due to the decreased layer activity. However, no further improvements were observed when using saturation times longer than 20 min. In order to maintain a constant saturation degree in the chamber, the mobile phase was replaced after washing and developing one plate. Since a low reproducibility was found with the tetrahydrofurane–water system, the chloroform–methanol mixture was chosen as the mobile phase.

Thus, the R_f value obtained for PXM-188 was 0.55. When the analysis of PXM-407 was performed using these conditions, low R_f values were observed. In order to increase the migration distance of this compound, slight modifications to the mobile phase were made, and the use of a 88:12 v/v chloroform–methanol mixture as the mobile phase resulted in an R_f value of 0.5 for PXM -407.

3.2. Quality parameters

The performance of the proposed HPTLC method was evaluated by establishing the quality parameters. These parameters were determined using raw material solutions, and the results obtained are shown in Table 1. Linear ranges were estab-

Table 1
Quality parameters

	PXM-188	PXM-407
Linear range (ng/spot) *y*-intercept	500–4500	1750–3250
Coefficient	405.76	−1472.59
Standard error	177.68	151.14
p-Value	0.0312	7.03E-08
Slope		
Coefficient	4.24	2.22
Standard error	0.063	0.58
p-Value	9.05E-30	1.96E-16
Determination coefficient (r^2)	0.994	0.990
Factor response RSD (%)	6.46	8.87
Repeatibility (RSD%, $n=6$)		
1500 (ng/spot)	1.93	1.78
3000 (ng/spot)	1.08	1.56
4500 (ng/spot)	0.98	1.03
Intermediate precision (RSD%, $n=2$)		
1500 (ng/spot)	3.26	6.15
3000 (ng/spot)	3.64	5.84
4500 (ng/spot)	4.24	11.18
LOD (ng/spot)	24.40	51.60
LOQ (ng/spot)	73.93	156.38

lished for each compound, from the curves constructed using an unweighted linear least squares regression of the peak areas as a function of the analyte's nominal mass. As can be observed from the data shown in Table 1, although PXM-407 exhibited a narrower linear range than PXM-188, both PXMs showed a linear relationship over the selected concentration range, with determination coefficients (r^2) better than 0.99 and relative standard deviations (RSDs) for the response factor below 4.5%. The lack-of-fit test was used to confirm the linear relationship of the method and no significant deviation from linearity was found. For each calibration point, the concentrations were back-calculated from the equations of the linear regression curves. The linear regression of the back-calculated concentrations versus the nominal concentrations provided a unit slope and an intercept equal to 0 (Student *t*-test).

The run-to-run precision was evaluated by performing six replicate determinations, while day-to-day precision was evaluated by performing six replicate determinations on two different days. The results, which are summarized in Table 1, demonstrated a good method precision, with relative standard deviations (RSDs) below 2% for run-to-run precision, and below 11.18% for day-to-day precision.

In both cases, the variation observed for PXM-407 was higher than that obtained for PXM-188. The LODs and LOQs were estimated from the regression lines, and for PXM-188, the LOD was 24 ng/spot, while for PXM-407, a slightly higher value of 47 ng/spot was obtained. Likewise, the LOQs obtained were 73.9 ng/spot for PXM-188 and 141.6 ng/spot for PXM-407.

3.3. Sample analysis

As mentioned above, the validated method was used for the determination of PXMs in a commercial buccal solution (0.5% w/w according to the label), as well as in two laboratory-made formulations. For the analysis of the lab-made gel (PXM-407) and the commercial buccal antiseptic (PXM-407), samples were only diluted and analyzed in triplicate, with application volumes of 700 and 900 nL, respectively. The HPTLC determination of PXM-188 in NPs was determined by digesting approximately 2000 mg of the lyophilized cake in 5 mL 0.1 N NaOH during 24 h; the solution was then neutralized with 0.1 N HCl, and the final volume was adjusted to 10 mL with pure water. This solution was analyzed in triplicate, using an application volume of 2000 nL.

Table 2
Results obtained for the analysis of pharmaceutical products

Sample	Analyte	Expected value (%, w/w)	Value obtained (%, w/w)[a]	Error (%)
Gel	PXM-407	27.00	26.80	0.74
Buccal antiseptic solution	PXM-407	0.50	0.50	0.54
NPs[b]	PXM-188	–	1.12	–

[a] $n=3$.
[b] NPs, nanoparticles.

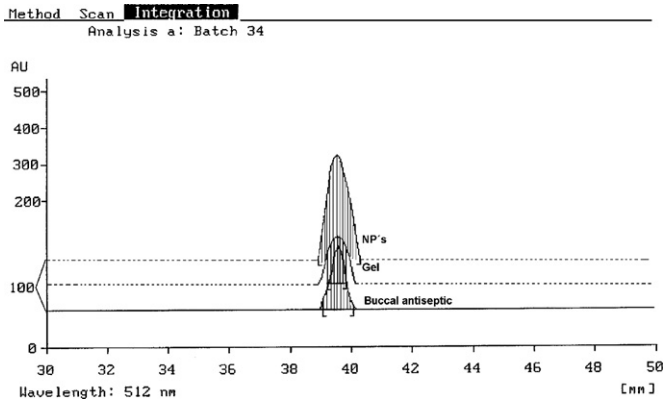

Fig. 2. Chromatograms of the evaluated samples. NPs, nanoparticles.

The values obtained for the analysis of PXMs samples are presented in Table 2.

As can be observed, a good agreement of the results with the expected values was obtained for the gel and buccal antiseptic samples. Although in the case of NPs no reference value was available and no comparisons can be made, the PXM-188 content is similar to that previously reported for this kind of formulations [15,19]. Fig. 2 shows the chromatograms obtained for the analyses of the samples.

4. Conclusions

High-performance thin-layer chromatography with RP-18 plates and densitometric detection has been shown to constitute a good approach for the analysis of PXM-188 and PXM-407 in pharmaceutical formulations. Quality parameters have shown that a good precision and low detection limits can be obtained, providing an easy and rapid procedure for the determination of these compounds compared to other methodologies in which the

formation of a complex and a separation step are involved. The developed method represents a good alternative to save money and time because the quantity of solvents used is lower than HPLC, the steps to obtain the results are fewer than methods that involve complex formation in which is required a specific instrument to concentrate the complex and at least 1 L of sample to be analyzed. The method was applied to laboratory-made products and commercial products, and it is able to quantify the PXMs content in pharmaceutical formulations.

References

[1] S.M. Moghimi, A.C. Hunter, Trend. Biotechnol. 18 (2000) 412–420.
[2] Y. Mao, M.J. Thompson, Q.X. Wang, E.W. Tsai, J. Pharm. Biomed. Anal. 35 (2004) 1127–1142.
[3] BASF, Lutrol® F68: Poloxamer 188 for the pharmaceutical industry, Ludwigshafen, Germany, 1997.
[4] BASF, Pluronic® Block Copolymer NF Grade, Ludwigshafen, Germany, 1995.
[5] C. Li, W.K. Palmer, T.P. Johnston, J. Pharm. Biomed. Anal. 14 (1996) 659–665.
[6] BASF, Lutrol® F127: Poloxamer 407 for the Pharmaceutical Industry, Ludwigshafen, Germany, 1997.
[7] S. Miyazaki, T. Tobiyama, M. Takada, D. Attwood, J. Pharm. Pharmacol. 47 (1995) 455–457.
[8] S.D. Desai, J. Blanchard, J. Pharm. Sci. 87 (1998) 1190–1195.
[9] O. Felt, S. Einmahl, R. Gurny, P. Furrer, V. Baeyens, in: D. Severian (Ed.), Polym. Biomater., second ed., Marcel Dekker Inc, New York, 2001, pp. 377–421.
[10] T.M. Schmitt, Analysis of Surfactants, CRC Press, New York, 2001.
[11] H. Ghebeh, A. Handa-Corrigan, M. Butler, Anal. Biochem. 262 (1998) 39–44.
[12] R.A. Greff, E.A. Setzkorn, W.D. Leslie, J. Am. Oil Chem. Soc. 42 (1965) 180–185.
[13] A.M. Tercyak, T.E. Felker, Anal. Biochem. 187 (1990) 54–55.
[14] Z. Takats, K. Vekey, L. Hegedus, Rapid Commun. Mass Spectrom. 15 (2001) 805–810.
[15] D. Quintanar-Guerrero, A. Ganem-Quintanar, E. Allemann, H. Fessi, E. Doelker, J. Microencapsul. 15 (1998) 107–119.
[16] J.J. Escobar-Chávez, D. Quintanar-Guerrero, A. Ganem-Quintanar, Drug Dev. Ind. Pharm. 31 (2005) 447–454.
[17] ICH, ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2 (R1), Step 4 version, November 2005.
[18] IUPAC, Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), Pure Appl. Chem. 74 (2002) 835–855.
[19] S. Galindo-Rodríguez, E. Allémann, H. Fessi, E. Doelker, Pharm. Res. 21 (2004) 1428–1439.