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Quantitation of poloxamers in pharmaceutical formulations using size exclusion chromatography and colorimetric methods

Yun Mao*, Mark J. Thompson, Qingxi Wang, Eric W. Tsai

Pharmaceutical Research & Development, Merck Research Laboratories, Merck & Co., Inc., West Point, PA 19486, USA

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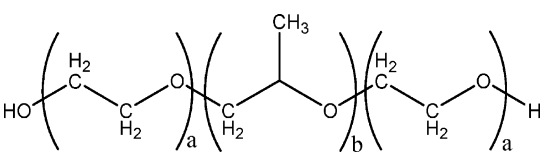
Abstract

Poloxamers have been used as functional excipients in pharmaceutical products. They function as surfactants, emulsifying agents, solubilizing agents, dispersing agents, and in vivo absorbance enhancer. Despite their wide range of applications, limited analytical techniques have been reported in literature for characterizing poloxamers and few are targeted to quantify poloxamer contents in formulations with desired sensitivity and accuracy. In this paper, two distinct analytical methods for quantifying low levels of poloxamers in pharmaceutical formulations have been developed and optimized: a colorimetric method and a size exclusion chromatography method. The colorimetric method is based on the formation of a colored complex between poloxamers and cobalt(II) thiocyanate in aqueous medium, which has a maximum UV absorbance at 624 nm. The feasibility of this method is product specific. In this report, adequate specificity and sensitivity was demonstrated for only one of the several products tested. The size exclusion chromatography (SEC) method utilizes size exclusion columns with THF as mobile phase and refractive index detection. The SEC method provides a limit of quantitation (LOQ) of 0.005 mg/mL (0.0005%, w/w) and at least three orders of magnitudes of linear range. We applied the SEC method to pharmaceutical products containing 0.3–10% poloxamer 188 or poloxamer 407, such as Avapro, Neurontin, Sudafed and other developmental formulations. The results obtained with the SEC method agreed very well with literature and theoretical values with 97–102% recovery. The SEC method was proven to be widely applicable, accurate, precise and simple to use.

Keywords: Poloxamer; Size exclusion chromatography; Colorimetric method; Pharmaceutical formulation

1. Introduction

Poloxamers are non-ionic poly (ethylene oxide) (PEO)–poly (propylene oxide) (PPO) copolymers. They are used in pharmaceutical formulations as surfactants, emulsifying agents, solubilizing agent, dispersing agents, and in vivo absorbance enhancer [1–5]. Poloxamers are often considered as "functional excipients" because they are essential components, and play an important role in the formulation [6]. Poloxamers are synthetic triblock copolymers with the following formula:



* Corresponding author. Tel.: +1-215-6525383; fax: +1-215-9931266.
E-mail address: yun_mao@merck.com (Y. Mao).

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All poloxamers have similar chemical structures but with different molecular weights and composition of the hydrophilic PEO block (a) and hydrophobic PPO block (b). Two of the most commonly used poloxamers are poloxamer 188 ($a = 80$, $b = 27$) with molecular weight ranging from 7680 to 9510 Da, and poloxamer 407 ($a = 101$, $b = 56$) with molecular weight ranging from 9840 to 14600 Da [5].

Despite their wide range of applications, there are limited analytical techniques in literature for characterizing poloxamers. A colorimetric method for the analysis of the non-ionic surfactant was first developed by Greff and coworkers. They found that the polyethylene oxide segment and cobalt(II) thiocyanate can form a colored complex, which has an UV absorbance maximum at 320 nm and 620 nm [7]. Tercyak and coworkers further modified this method with an improved solvent system to separate and dissolve the complex. They monitored the complex at 624 nm and measured poloxamer concentration (2.54%) in organic extract of rat livers perfusates [8]. Li and coworkers used this colorimetric method to detect poloxamer 407 in plasma, urine and the supernatants of kidney and hepatic tissues within a narrow concentration range [9]. Recently, Ghebeh and coworkers altered the washing procedure, and measured the absorbance of the complex at 328 nm to further improve sensitivity and reproducibility of the method. They found that the method was linear from 0.04 to 0.16% (w/v) of poloxamers in the serum samples [10]. These reported colorimetric methods are all targeted towards quantitation of poloxamer in biological samples. It is interesting to explore whether the colorimetric method can be modified for poloxamer quantitation in pharmaceutical solid dosage forms. In addition, the developed method needs to have desired accuracy, sensitivity and precision to suit routine quantitative analysis.

Various chromatography techniques were reported in literature for the analysis of non-ionic surfactants. Miszkiewicz and Szymanowski reviewed the analysis of non-ionic surfactants with polyethylene oxide segments by normal phase, reversed phase, size exclusion, and ion exchange chromatography [11]. Given the multiple ingredients in pharmaceutical formulations, size exclusion chromatography (SEC) will probably provide the best specificity towards poloxamer analysis compared to other modes of chromatography. Size exclusion chromatography has been used to determine the number and weight average molecular weights (Mn and Mw) and the molecular weight distribution of poloxamers [12–14]. However, none of them are developed for quantitation purposes, and the analyte consists only of poloxamers.

In addition, Takats and coworkers used matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) to determine molecular weights of poloxamers. The peak molecular weights were measured to be 9000 Da for poloxamer 188, and 13800 Da for poloxamer 407, respectively. They also determined poloxamer concentrations by monitoring fragment ions with a standard addition method. The detection limit for this method is 0.02% [15].

Despite the literature reports on poloxamer characterization, the quantitation of poloxamers in formulations to support drug development has never been reported to the best knowledge of the authors. The goal of the present work is to develop a method for quantitation of low level of poloxamers in pharmaceutical formulations. The developed methods should be accurate, sensitive, reproducible and simple for routine analysis. First, the colorimetric method was explored for pharmaceutical samples. We modified the literature method in terms of reagent volume, ratio and sample preparation procedure. Then, a SEC method with refractive index detection was developed. The quantitation linear range and limit of quantitation were determined for both methods. The method accuracy and precision were evaluated by applying the optimized method to several commercial products as well as Merck developmental formulations containing poloxamers. Since poloxamers are the key ingredients in the formulation, the developed method can be used to monitor poloxamer content uniformity and distributions in the sieve cut of the formulation to support formulation and process development for drug products.

2. Experimental

2.1. Reagents

All chemicals used in this study were reagent grade or better. HPLC-grade tetrahydrofuran, acetone

and ammonium thiocyanate were purchased from Fisher Scientific (Pittsburgh, PA). Ethyl acetate and Cobalt (II) nitrate hexahydrate were obtained from Aldrich (Milwaukee, WI). Poloxamer 188 (lutrol F68NF) and poloxamer 407 (lutrol F127 NF) were gifts from BASF (Mount Olive, NJ). The 300 mg potency Avapro tablets (Lot #: MKM35) were manufactured by Sanofi–Syntheiabo (New York, NY). The 800 mg potency Neurontin tablets (Lot #: 21891V) were manufactured by formerly Parke Davis Pharmaceuticals (Merris Plains, NJ). 30 mg potency Non-Drowsy Sudafed Nasal decongestant tablets (Lot #: 10231V) were manufactured by formerly Warner–Lambert consumer healthcare (Morris Plains, NJ).

2.2. Colorimetric assay

Poloxamer contents in the formulation are determined by forming a water-insoluble complex with cobalt(II) thiocyanate. The poloxamer complex can dissolve in acetone, and its UV absorbance at 624 nm is proportional to the poloxamer–cobalt concentration.

2.2.1. Colorimetric assay procedure

A cobalt thiocyanate solution was prepared by dissolving 3 g of cobalt(II) nitrate and 20 g of ammonium thiocyanate in 100 mL of water. Two milliliter of ethyl acetate, 1 mL of the cobalt thiocyanate solution, and 2 mL of poloxamer solution were transferred into a 10 mL centrifuge tube with screw-on cap. After thorough mixing, the mixture in the tube was centrifuged immediately in the IEC HN-SII centrifuge (International Equipment Group, Needham heights, MA) at 3000 rpm for 1 min. The upper ethyl acetate and aqueous solution layers were discarded. Two milliliter increments of ethyl acetate were added in the centrifuge tube to rinse the sediment till there is no color in the ethyl acetate solution (usually 2–3 times). Ten milliliter of acetone was added to the tube, and immediately cap the tube. Shake vigorously till all the sediments were dissolved. All colorimetric experiments were performed on a Hewlett-Packard Model 8453 UV–vis Spectrophotometer with a ChemStation software. A sipper was utilized for UV measurement, and enough flush volume was used to eliminate carryover.

2.2.2. Standard and sample preparation

Poloxamer 188 and poloxamer 407 stock standards were prepared by dissolving 200 mg of poloxamer in 100 mL of water to make 2.0 mg/mL standard solution. Poloxamer 188 and 407 standards with concentrations of 1.0, 0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 mg/mL were prepared by diluting the stock solution with water.

One Neurontin tablet was put into 50 mL of water and the solution was stirred for 2 h. A portion of the sample was centrifuged, and the supernatant was used for the poloxamer colorimetric assay.

2.3. Size exclusion chromatography (SEC)

2.3.1. Chromatographic conditions

All chromatographic work were performed on a Hewlett-Packard 1100 chromatography system, equipped with a quaternary pump, an autosampler, a thermostatted-column compartment, a G1362 refractive index detector and a computer-based Chemstation (Hewlett Packard S.A., Wilmington, DE). The Polysep-GFC-P3000 column (300 mm × 7.8 mm) was purchased from Phenomenex (Torrance, CA). The Plgel 3 μm Mixed E columns (300 mm × 7.5 mm each) were purchased from Polymer Laboratories Inc. (Amherst, MA). Two columns with the same dimension were connected in series to increase the separation efficiency. Chromatography was performed at a flow rate of 1 mL/min with refractive index detection. The injection volume was 100 μl. The column temperature was controlled at 35 °C. HPLC grade water was prepared by filtering through Milli-Q deionized water system from Millipore (Billerica, MA). The poloxamer standards and samples were prepared in the mobile phase. Analyte concentration ranged from 5 to 0.005 mg/mL.

2.3.2. Standard and sample preparation

A stock standard of poloxamer 188 and 407 was prepared by dissolving 200 mg of poloxamer 188 and 407 in 100 mL of THF to make 2.0 mg/mL standard solution. Poloxamer standards with concentrations of 1.0, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.005 mg/mL were prepared by diluting the stock solution with THF.

One Neurontin tablet was placed into a 100 mL volumetric flask, diluted to volume with THF and stirred for 2 h. A portion of the solution was cen-

trifuged and the clear supernatant was used for the chromatography analysis. Two Avapro tablets were put into 50 mL volumetric flask, diluted to volume with THF and the solution was stirred for 2 h. A portion of the sample was centrifuged, and the clear supernatant was used for the chromatography analysis. Twelve Sudafed tablets were put into a 100 mL volumetric flask, diluted to volume with THF, and the solution was stirred for 2 h. A portion of the sample was centrifuged, and the supernatant was filtered through a 0.22 μm filter for chromatography analysis. Four hundred milligram formulation A of Compound 1 was dissolved and extracted with 10 mL of THF, the centrifuged clear supernatant was used for analysis. 100 mg formulation B of Compound 1 was dissolved in 25 mL of THF, after stirring for two hours, a portion of the solution was centrifuged for analysis.

3. Results and discussion

3.1. Quantifying poloxamers using the colorimetric method

3.1.1. Colorimetric method development

There are several reports in the literature on the characterization and quantitation of poloxamers in plasma or urine samples using colorimetric methods [7–10]. The assay depends on the formation of a colored complex between poloxamer and cobalt(II) thiocyanate. The complex forms a precipitation that sediments upon centrifugation. The precipitate can be dissolved in acetone, and the intensity of the color is proportional to the amount of the poloxamers in the sample. The structure of the complex was not fully understood, but it was hypothesized that the oxygen atoms in the ether group of the poloxamer interact with the inorganic ion (cobalt ion) and form a complex with a helix configuration. The blue color is due to poloxamer–cobalt thiocyanate complex [16].

To develop a colorimetric method for quantifying poloxamers in the pharmaceutical formulations, we initially adopted the literature conditions [10]. However, the percent R.S.D. for triplicate measurements of poloxamer standards were 2–70% depending on the poloxamer concentration, which is due to the small sample volume used (micro liters). Since there is no sample size limitations for formulation analysis, we modified the method with more than a 10-fold increase in reagent volumes for the sample preparation procedure, as described in detail in the experimental session. The modified method dramatically improved the method precision for replicate standard analysis. Fig. 1 shows the typical UV profiles for the complex with poloxamer 188 and 407. Next, the linear range and the limit of quantitation were determined by preparing a series of poloxamer solutions with concentration ranges from 0.01 to 2 mg/mL. We could not measure a statistically significant absorbance value at 624 nm for poloxamer samples with concentrations less than 0.1 mg/mL. Thus, the limit of quantitation (LOQ) was determined as 0.1 mg/mL for this method. Fig. 2 plots the average absorbance versus poloxamer concentration from 0.1 to 2 mg/mL. Excellent linearity was observed for both poloxamer 188 ($r = 0.9990$) and poloxamer 407 ($r = 0.9993$).

3.1.2. Determination of the poloxamer content in pharmaceutical formulation using colorimetric methods

After developing the method, we tried to utilize the method to quantify the poloxamer 188 and 407 content in two developmental pharmaceutical formulations. Compound 1 is a developmental drug candidate at Merck. Two formulations were developed containing 1% poloxamer 188 (formulation A) and 10% poloxamer 407 (formulation B), in which poloxamer played an important role as a dispersant. We are interested in measuring and controlling the poloxamer content in the formulation. Formulations A and B for Compound 1 were extracted with water, and the centrifuged supernatant was then complexed with cobalt(II) thiocyanate. However, after dissolving the complex in acetone, the solution has a greenish-blue color instead of the blue color observed with the poloxamer standard. As a result, the calculated poloxamer contents in developmental formulation were much higher than the theoretical values. Investigation showed that Compound 1 can also form a colored complex with cobalt thiocyanate, which has a green color and also absorbs at 624 nm. Therefore, the colorimetric method cannot be used for poloxamer quantitation in the developmental formulations of Compound 1.

Next, we applied the colorimetric method to a commercial pharmaceutical product containing poloxamer 188 or poloxamer 407. Neurontin (gabapentin) is

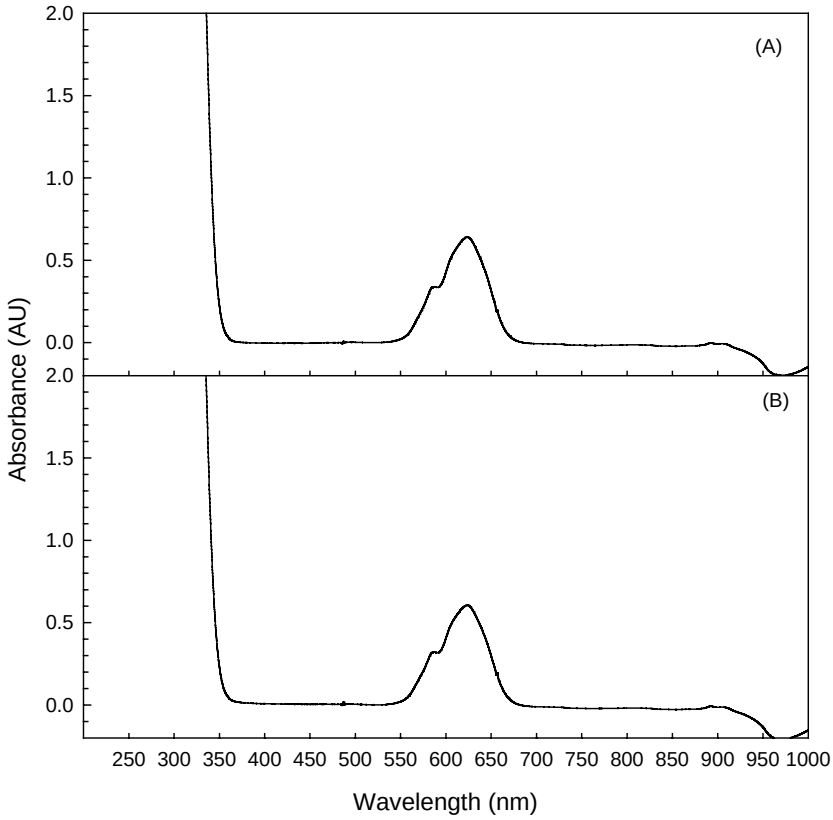

Fig. 1. UV spectrum of the (A) poloxamer 188 complex, and (B) poloxamer 407 complex at 1.0 mg/mL concentration.

for the treatment of nerve pain and partial seizures. The poloxamer 407 content in Neurontin tablet was determined by colorimetric method. Five replicate samples were prepared, and the results are shown in Table 1. The reported poloxamer 407 in Neurontin tablet found in FDA web-site is 106.7 mg per tablet [17]. The average poloxamer content in the tablet was calculated to be 102.28 mg, which corresponds to a 96% theoretical value. The difference might be contributed to the possible lose of complex during sample preparation. In addition, 4.8% R.S.D. was observed for the analysis, which is quite large for quantitation purposes.

Avapro (irbesartan) is an angiotensin II receptor antagonist containing poloxamer 188, and Sudafed is an over the counter drug containing poloxamer 407. We also used colorimetric method for Avapro and Sudafed tablet, but no sediments were formed. We speculated that this is due to either the low poloxamer contents present in these tablets or the matrix effect from other excipients, which prevents the complex formulation.

Table 1
Determination of Poloxamer 407 contents in Neurontin tablets using colorimetric method

Concentration	UV absorbance at 624 nm	Calculated Poloxamer 407 concentration (mg/mL)	Poloxamer 407 per tablet (mg)
Sample 1	1.172	1.92	96.0
Sample 2	1.338	2.19	109.6
Sample 3	1.246	2.04	102.1
Sample 4	1.227	2.01	100.6
Sample 5	1.259	2.06	103.1
Average	1.248	2.044	102.28
R.S.D. (%)			4.80

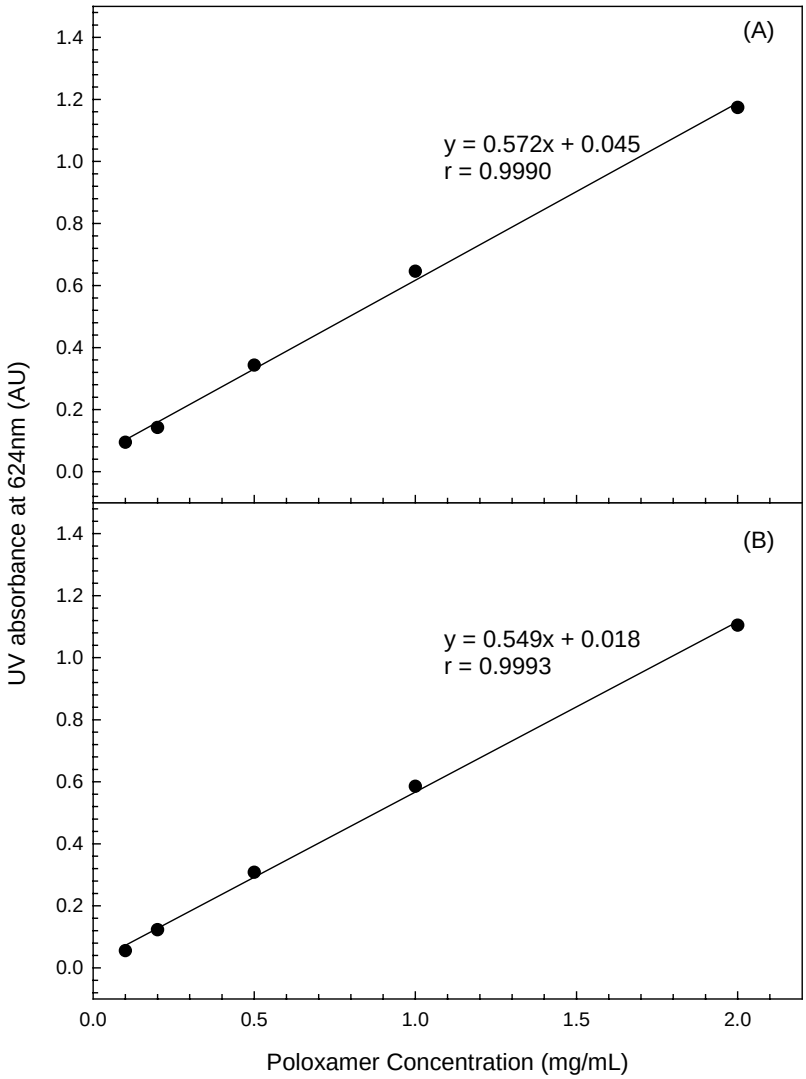

Fig. 2. Calibration curves of (A) poloxamer 188; and (B) poloxamer 407 with the colorimetric method.

To summarize, the colorimetric method did not provide the needed specificity, and sensitivity for the most pharmaceutical formulations analyzed. In addition, the method is relatively time consuming and labor intensive. Although it provided acceptable results (96% theoretical value) for poloxamer 407 determination in Neurontin tablet, high variability was observed. In conclusion, the colorimetric method seems not adequate for routine quantitation of poloxamer in solid formulation dosage form. Other methods with better accuracy, precision and applicability are desired.

3.2. Quantifying poloxamers using size exclusion chromatography (SEC)

3.2.1. Size exclusion chromatography method development

Size exclusion chromatography has become a extensively used technique for separating and characterizing polymers [11,18–20]. SEC columns are packed with small particles composed of polymeric or silica-based gels with controlled pore size to separate samples of different molecular sizes in the mobile

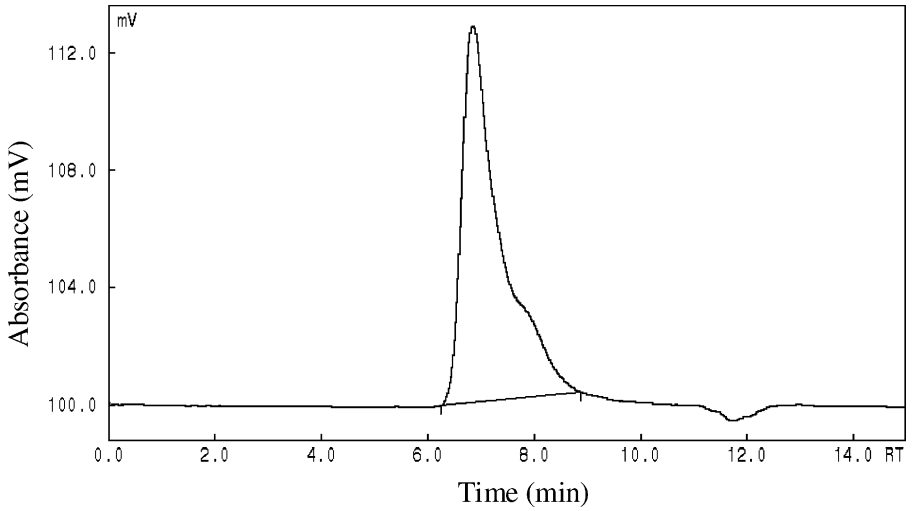

Fig. 3. Size exclusion chromatogram of poloxamer 188. Experimental conditions: stationary phase, phenomenex Polysep-GFC-P3000 column (300 mm × 7.8 mm); mobile phase, water; flow rate, 1 mL/min; detection, RI; temperature setting for column and detector, 35 °C; solute concentration, 1.0 mg/mL.

phase. The commonly used mobile phase in SEC are organic solvents such as tetrahydrofuran, chloroform, toluene or aqueous solvents such as water or buffered solutions. Poloxamers are freely soluble in water and most tablets/capsules are easily disintegrated in an aqueous solvent. Therefore, a SEC method with an aqueous mobile phase was first evaluated. Phenomenex Polysep-GFC-P3000 column was first tested. The column has a highly hydrophilic synthetic polymer phase, and is suitable for water-soluble polymers. The mobile phase was pure water with a flow rate at 1 mL/min. Fig. 3 shows the chromatogram of a standard solution of poloxamer 188. The poloxamer peak was very broad with a shoulder and tailing at the end of the peak. Due to the broad peak shape and low peak height, we cannot detect poloxamer 188 standard at a concentration of 0.05 mg/mL. The broad peak can be contributed to the low efficiency of the SEC column, which usually has a significantly less plate count than a typical reversed phase HPLC column [20]. In addition, secondary interactions between the poloxamers and the mobile phase and the stationary phase may contribute to the broad peak shape as reported in the literature [21,22].

An alternative approach is to change the column type and use an organic mobile phase. The Polymer Laboratory column Plgel mixed-E column has ultra high efficiency. This column is packed with 3 μm particles and suitable for low molecular weight polymers (<30,000 Da). In theory, it would be applicable to separate and analyze poloxamers whose molecular weight is well below 30,000 Da. To further improve the separation efficiency, two identical columns were coupled in series. Tetrahydrofuran (THF), a good solvent for poloxamers, was chosen as the diluent and mobile phase. With the serially connected columns, the back-pressure was about 110 bar at 1 mL/min flow rate, which was reasonable. Under otherwise similar experimental conditions, we observed the chromatogram of poloxamer 188 as shown in Fig. 4A. The peak shape was dramatically improved with much less tailing. The small peak eluting after the main peak was separated from the main peak, instead of a shoulder peak observed using an aqueous mobile phase. Due to the improved column efficiency, the poloxamer peak becomes narrower and the peak height was enhanced by at least three-fold, which will significantly improve the method sensitivity. As a result, the SEC method with an organic mobile phase was chosen. Fig. 4B shows the chromatogram of poloxamer 407 using this method. Similar to poloxamer 188, good peak shape was also observed. The retention time of poloxamer 407 (10.67 min) is slightly shorter than that of the poloxamer 188 (11.15 min), which is expected since

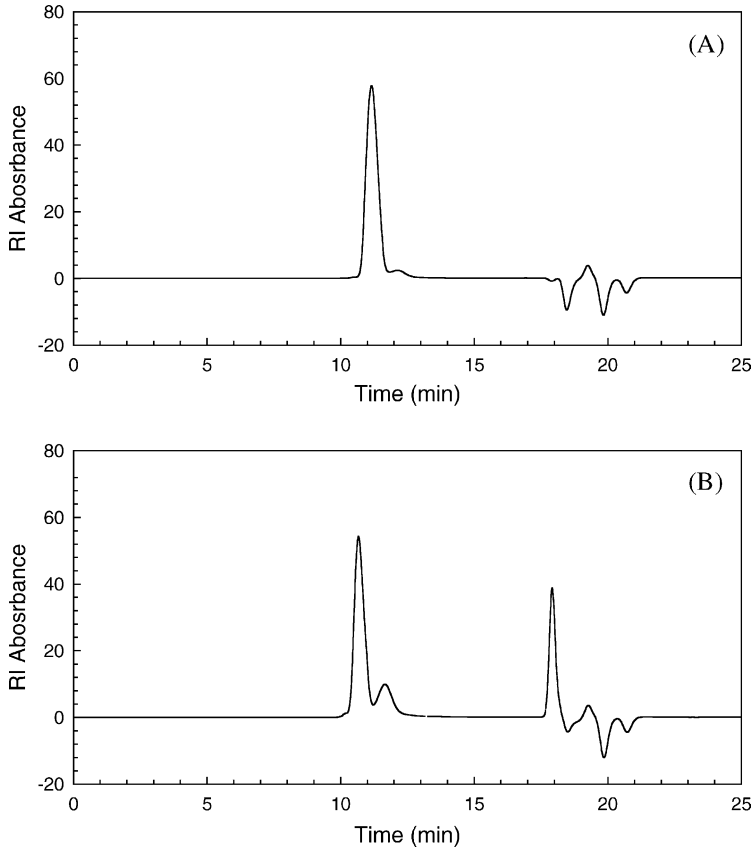

Fig. 4. Size exclusion chromatograms of (A) poloxamer 188 and (B) poloxamer 407. Experimental conditions: stationary phase, two serially connected Plgel 3 μm mixed E columns (300 mm × 7.5 mm each); mobile phase, tetrahydrofuran; flow rate, 1 mL/min; detection, RI; temperature setting for column and detector, 35 °C; solute concentration, 2.0 mg/mL.

poloxamer 407 has a higher molecular weight than poloxamer 188. A small peak eluting after the large peak was also observed in the poloxamer 407 chromatogram as a result of the bimodal distribution of poloxamers.

The bimodal distributions of poloxamer observed in the chromatograms have been reported in the literature [12,13,23,24]. Poloxamer is synthesized via sequential anionic polymerization. First, propylene oxide (PO) is added to a propylene glycol initiator to form a polyoxypropylene glycol (PPO). Then ethylene oxide is added in a controlled manner to form two polyoxyethylene blocks (PEO). The product is then neutralized with acid. However, during the synthesis of the PPO block, significant quantities of PO rearrange to become allyl alcohols, which leads to the formation of allyl ethers of PPO. Therefore, when ethylene oxide (EO) is added, only one chain end of the PPO block is available to react with EO. As a result, some diblock copolymers (PEO-PPO) are formed instead of the triblock copolymer (PEO-PPO-PEO). The smaller peak observed in the chromatogram, which has a longer retention (i.e. lower molecular weight), corresponds to the diblock copolymer formed during synthesis. Poloxamer 407 sample has significant amount of diblock co-polymers impurities (25% area), and its quantitation can be difficult if the poloxamer has different amount of impurities in the standard and the sample.

3.2.2. Evaluation of the limit of quantitation (LOQ) and linear range for the SEC method

Several poloxamer samples with a concentration range from 5.0 to 0.005 mg/mL were prepared, and

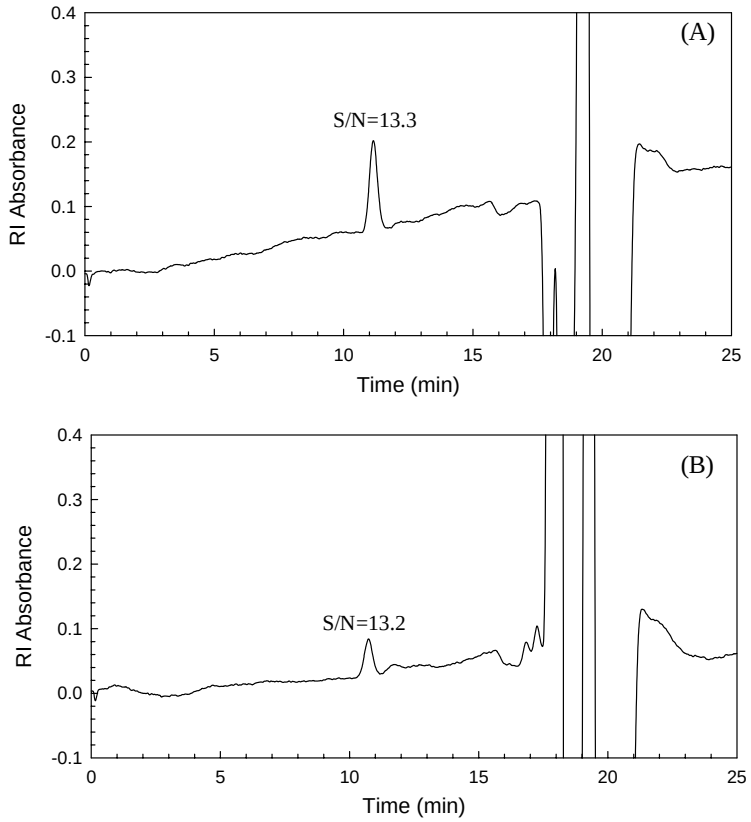

Fig. 5. Size exclusion chromatograms of (A) poloxamer 188 and (B) poloxamer 407 at the limit of quantitation. Experimental conditions are the same as in Fig. 4; solute concentration, 0.005 mg/mL.

the samples were injected to the column. The chromatograms at the lowest concentration, 0.005 mg/mL, were shown in Fig. 5. The signal to noise ratio (S/N) was calculated based on the peak height of the poloxamer and the average peak height of the baseline noise. The limit of quantitation was determined to be 0.005 mg/mL (0.0005%, w/w) for both poloxamer 188 (S/N of 13.3) and poloxamer 407 (S/N of 13.2). In comparison, the SEC method using aqueous mobile phase has a LOQ of 0.1 mg/mL (data not shown here). Thus, the chosen method has improved sensitivity by 20-fold.

To assess the linearity of the method, the peak area of poloxamer 188, eluting at 11.15 min, versus its concentration was plotted as shown in Fig. 6. Good linearity was observed over three-orders of magnitude concentration range. The correlation coefficient (R) is 1.00000. For poloxamer 407, the diblock impurity peak consists of 25% area compared to the poloxamer 407 peak. We noticed that the two peaks are not completely baseline resolved. The peak area was integrated by drawing a vertical line at the valley point between the two peaks, and the linearity of the poloxamer 407 peak may vary at different concentrations. Therefore, calibration curves were plotted for the peak area of poloxamer peak, diblock impurity peak and the sum of the two peaks, as shown in Fig. 7. Line (a) represents the small impurity peak eluting at 11.68 min, with a correlation coefficient of 0.9996. In Fig. 7B, the lower left portion of the calibration curve was zoomed in, and it showed that the impurity peak was not detected at 0.005 mg/mL concentration. Line (b) and (c) were the calibration curves for poloxamer 407 peak eluting at 10.71 min and the sum of the peak areas, respectively. Excellent correlation coefficients ($r = 0.99999$) were observed. Therefore, all three calibrations should be suitable for quantitation of poloxamer 407 if the sample has the same diblock content as the standard.

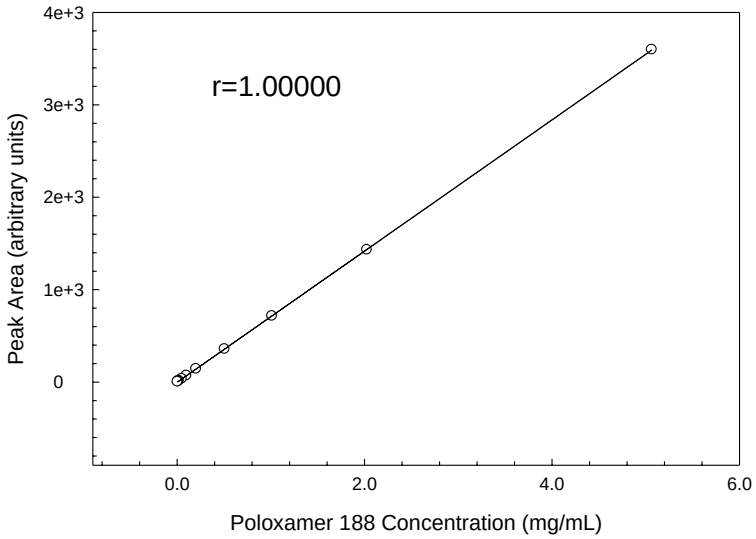

Fig. 6. Calibration curve for poloxamer 188 with the concentration from 0.005 to 5 mg/mL.

3.2.3. Determination of the poloxamer 188 content in the pharmaceutical formulations

After developing the method, we utilized the method to quantify the poloxamer 188 content in commercial pharmaceutical products as well as developmental formulations. Avapro tablets have many excipients including lactose, microcrystalline cellulose, starch, croscarmellose sodium, poloxamer 188, silicon dioxide and magnesium stearate. A typical chromatogram of the Avapro sample is shown in

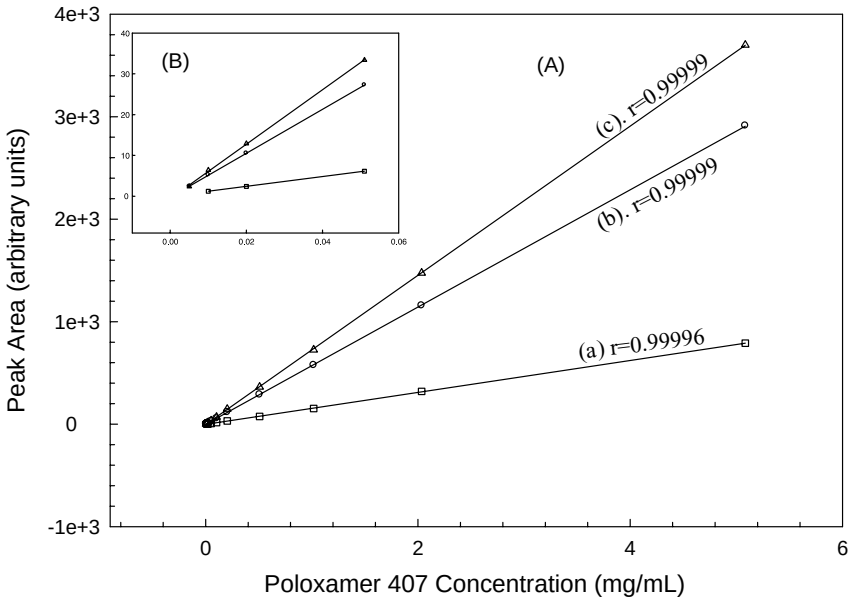

Fig. 7. (Plot A) Calibration curve for poloxamer 407 with the concentration from 0.005 to 5 mg/mL. Symbol (□) and line (a): peak area for 11.68 min peak (diblock impurity peak) only; symbol (○) and line (b): peak area for the 10.71 min peak (poloxamer 407 peak) only; symbol (△) and line (c): sum of the peak areas at 10.71 min and 11.68 min. (Plot B) Zoom in plot at the lower concentrations.

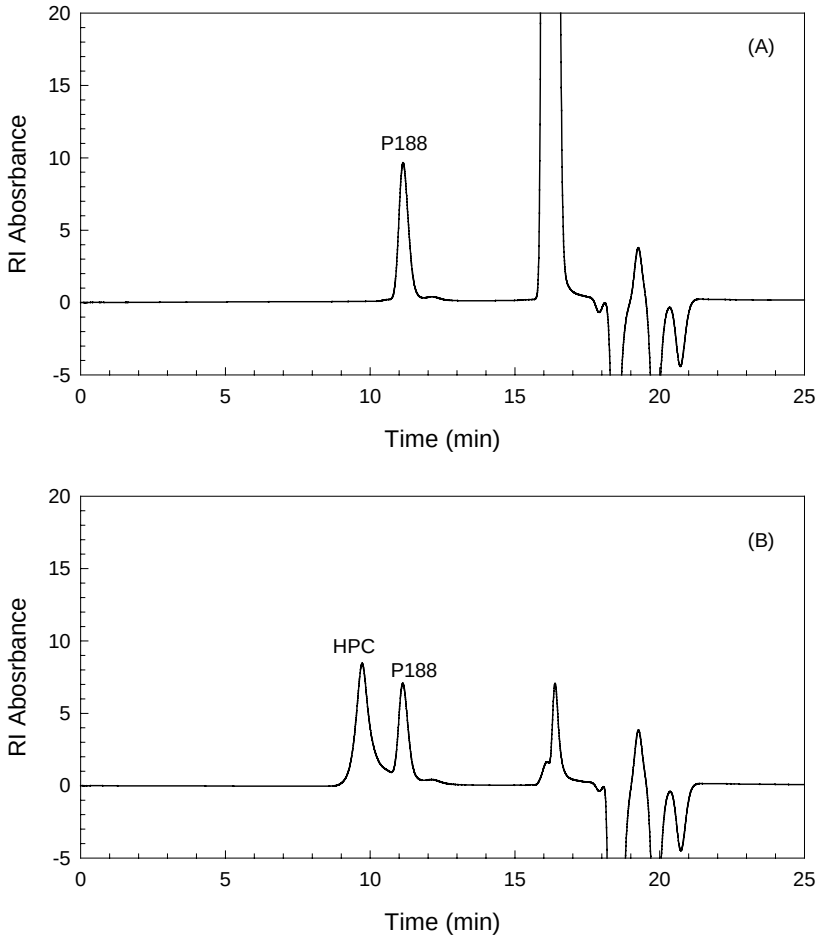

Fig. 8. Size exclusion chromatograms of (A) Avapro tablet and (B) formulation A of Compound 1. Experimental conditions are the same as Fig. 4.

Fig. 8A. There was no observable interference from the active or other excipients. Using the calibration curve established in Fig. 6, poloxamer 188 contents in Avapro tablets were calculated and summarized in Table 2. The average content of poloxamer 188 per tablet was determined as 16.22 mg, which corresponded to 2.68% (w/w) in each tablet. The SEC method is precise with relative standard deviation of 0.67% for three replicates. The reported poloxamer 188 in Avapro tablet found in FDA web-site is 18 mg per tablet [17]. The difference in the measurement is believed to come from the difference in the poloxamer 188 used as the standard and in the product, as the molecular weight distribution of poloxamer 188 varies with different batches and supplies.

Compound 1 is a developmental drug candidate at Merck. During the formulation development, 1% poloxamer 188 was added as an absorbance enhancer in formulation A. We are interested in measuring and controlling the poloxamer 188 content in the formulation. A typical chromatogram of formulation A sample is shown in Fig. 8B. A large peak eluting shortly before the poloxamer 188 peak was also observed in the chromatogram, which corresponded to molecules with higher molecular weight. After analyzing other excipients present in the formulation with the same method, the peak was identified to be hydroxypropyl cellulose

Table 2
Determination of Poloxamer 188 contents in Avapro tablets[a]

Concentration	Tablets weight (mg)	Peak area (AU)	Calculated Poloxamer 188 concentration (mg/mL)	Poloxamer 188 per tablet (mg)	Percent Poloxamer 188 per tablet
Sample 1	1214.15	513700	0.65	16.3	2.68
Sample 2	1205.14	508263	0.64	16.1	2.66
Sample 3	1224.24	514572	0.65	16.3	2.67
Average				16.22	2.67
R.S.D. (%)				0.67	0.30

[a] Experimental condition is the same as described in Fig. 4.

(HPC). In formulation A, about 2.4% HPC was used as binder, which is freely soluble in THF and has a broad molecular weight distribution depending on the types and grade of HPC. The low molecular weight HPC can have a molecular weight distribution overlapping with the molecular weight range of poloxamer 188. Since size exclusion chromatography separates analytes based on their hydrodynamic size in the mobile phase (directly related to molecular weight), overlapped peaks are expected if there is an overlap of molecular weight between the analytes. If the poloxamer 188 peak was integrated by drawing a perpendicular line in the valley of the two peaks, we calculate that there was 1.28% poloxamer in the tablet. This obviously overestimated the poloxamer 188 content in the formulation due to the interference HPC. However, a more accurate result was obtained when tangent skimming was used to integrate the peak. Tangent skimming deconvolute the overlapped peaks based on their peak shape. We calculated 0.97% of poloxamer 188 in the formulation, which corresponding to a 97% recovery based on 1.0% theoretical value. Thus, the SEC method provides adequate accuracy for analysis of poloxamer in this formulation with a proper integration technique.

3.2.4. Determination of the poloxamer 407 content in pharmaceutical formulations

Poloxamer 407 is another functional excipient used in solution and solid formulations, and there are seven marketed prescription medicines as well as many OTC drugs listing poloxamer 407 as an excipient [17]. During the further formulation development of Compound 1, it was found that poloxamer 407 was a more effective absorbance enhancer than poloxamer 188. Therefore, formulation B was developed which incorporated 10% poloxamer 407. Because poloxamer plays an important role in the formulation, it was desirable to measure and control the poloxamer 407 content in the formulation and know the content uniformity of the poloxamer in the dosage form, within the granule sieve cuts, and the poloxamer content in the formulation after storage. The SEC method developed here can allow us to quantify the poloxamer 407 contents in this developmental formulation B.

A typical chromatogram is shown in Fig. 9A. Comparing to formulation A, the surfactant was changed from 1% poloxamer 188 to 10% poloxamer 407, and the HPC level was decreased from 2.4% in formulation A to 2.0% in formulation B. Therefore, minimal interference was observed in the chromatogram. Three composite samples were analyzed and the poloxamer contents were calculated based on calibration curve established in Fig. 7 with line b (see Table 3). The average content of poloxamer 407 in the formulation was calculated as 10.02%. This agrees very well with the theoretical value of 10%, demonstrating the satisfactory accuracy of this method.

To further understand the poloxamer distribution in the formulation, a sieve fraction analysis of poloxamer 407 was also performed. 50 mg of granules from different sieve cuts were dissolved and extracted in 25 mL of THF. The percent claim of poloxamer is calculated assuming 10% poloxamer 407 in the formulation. Similarly, an active drug assay was performed on the corresponding sieve cut, and the active percent claim was calculated based on its theoretical content. In Fig. 10, we plotted the percent claim of the Compound 1 and poloxamer 407 for the same sieve fractions. Fairly good distribution of poloxamer 407 was observed for the entire sieve cut except the smaller granule fractions. Interestingly, the same trend was observed for the Compound 1 assay across

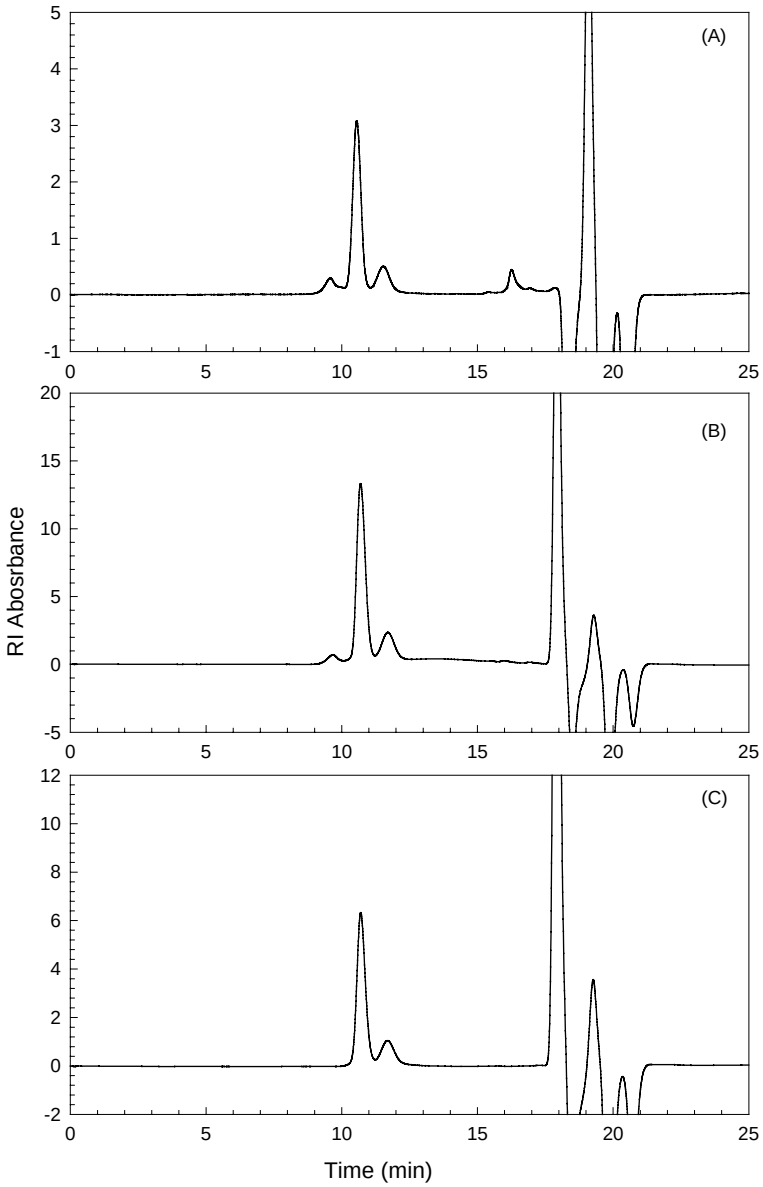

Fig. 9. Size exclusion chromatograms of (A) formulation B of Compound 1; (B) Neurontin tablets; and (C) Sudafed tablets. Experimental conditions are the same as Fig. 4.

the sieve cut. It shows that poloxamer 407 could be associated with the active drug in the formulation.

We also investigated some marketed pharmaceutical products, such as Neurontin and Sudafed tablets. Using the developed SEC method, we obtained chromatograms for Neurontin tablets and Sudafed tablets as shown in Fig. 9B and C. Minimal interference was observed with the poloxamer 407 peak, and the bi-model distribution was observed in the chromatogram as seen with the Poloxamer 407 standard. Quantitation was performed using the calibration curve b as shown in Fig. 7 and the results were also

Table 3
Determination of Poloxamer 407 contents in pharmaceutical formulations[a]

Sample	Weight (mg)	Calculated P407 concentration (mg/mL)	Amount of Poloxamer 407 per tablet (mg)	Percent of Poloxamer 407 per tablet
Comp.1 formulation B 1	103.79	0.41	10.25	9.97
Comp.1 formulation B 2	104.31	0.43	10.75	10.22
Comp.1 formulation B 3	104.47	0.41	10.25	9.86
Average		0.418	10.45	10.02
R.S.D. (%)			1.85	1.82
Neurontin tablet 1	1105.15	1.061	106.1	9.60
Neurontin tablet 2	1111.15	1.065	106.5	9.59
Neurontin tablet 3	1103.82	1.065	106.5	9.64
Average		1.06	106.35	9.61
R.S.D. (%)			0.23	0.31
Sudafed sample 1	1752.29	0.651	5.42	0.309
Sudafed sample 2	1764.31	0.685	5.71	0.324
Sudafed sample 3	1750.38	0.658	5.49	0.313
Average		0.67	5.57	0.317
R.S.D. (%)			2.72	2.32

[a] Experimental condition is the same as described in Fig. 4.

summarized in Table 3. The poloxamer 407 content in Neurontin tablet was calculated as 106.35 mg per tablet, which has excellent agreement with the literature value of 106.7 mg per tablet [17]. This once again demonstrated the accuracy of the method. In addition, excellent precision with 0.31% R.S.D. was observed for Neurontin tablet, which has about 9.61% poloxamer in the formulation. Relatively higher R.S.D. (2.32%) was observed for Sudafed tablet, which has only 0.317% poloxamer in the formulation. It is not surprising that the measurement precision is better for formulations with higher content of poloxamers.

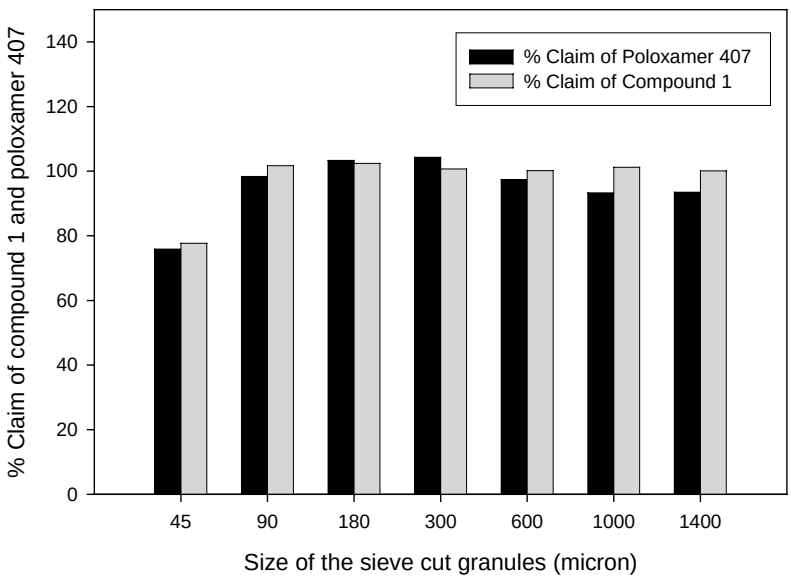

Fig. 10. Correlation of Compound 1 (active drug) and the poloxamer 407 distribution in the formulation sieve cuts assay.

Table 4
Comparison of the size exclusion chromatography method with the colorimetric method for Poloxamer quantitation

	Size exclusion chromatography	Colorimetric method
Limit of quantitation (LOQ)	0.005 mg/mL	0.1 mg/mL
Linear range	0.005–5 mg/mL	0.1–2 mg/mL
Correlation coefficient in calibration curve	$r > 0.99999$	$r > 0.999$
Poloxamer contents determined	0.3–10% (w/w)	10% (w/w)
Measurement precision	0.3–2.3%	4.8%
Sample treatment time	5 min	30 min

To summarize, we have developed a SEC method using the coupled-columns with THF as mobile phase, and a RI detector. This method is proven to be accurate, sensitive, reproducible and simple for the quantitation of poloxamer 188 and poloxamer 407. The method has been applied to pharmaceutical products and the results obtained have good agreement with the literature or theoretical values. Interference could happen if there are other excipients with similar molecular weights as poloxamer. But for most of the products that we evaluated, the SEC method was proven to be a useful approach to accurately and precisely quantify the poloxamer content as low as 0.3% w/w in the formulation.

3.3. Comparison of the SEC and colorimetric methods for quantifying poloxamer in pharmaceutical formulations

The two methods were compared according to linear range, limit of quantitation, analysis time, and measurement precision as summarized in Table 4. The SEC method has several advantages over the colorimetric method. It has at least three-orders of magnitude of linear range, which is much wider than the colorimetric method. In terms of sensitivity, the LOQ of the SEC method is 20 times lower than that of the colorimetric method. Both methods have good linearity within the calibration range, with correlation coefficients of 0.99999 and 0.999 for SEC and colorimetric method, respectively. The SEC method also has better measurement precisions. For sample preparation, the SEC method is relatively easy, while the colorimetric method can be labor intensive with multiple steps of sample treatment. Therefore, the SEC method developed will be more suitable and applicable for poloxamer quantitation in pharmaceutical solid dosage forms, and in-process samples to support formulation and process development.

4. Conclusions

Two analytical methods have been developed and compared for quantifying poloxamer contents in pharmaceutical solid formulations. The major conclusions for this work can be summarized as follows:

1. A size exclusion chromatography method utilizing a coupled column with THF as mobile phase was developed. The method demonstrated three orders of magnitude of linear range with LOQ of 0.005 mg/mL. Several pharmaceutical compounds containing 0.3–10% poloxamer 188 or 407 were analyzed and the measured poloxamer contents agreed very well with the literature and theoretical values.
2. A colorimetric method based on the formation of a colored complex between poloxamer and cobalt thiocyanate was also developed. The method has a LOQ of 0.1 mg/mL. For certain formulations with high poloxamer contents, the colorimetric method provide acceptable results in terms of precision and accuracy, but it failed for other formulations which have low levels of poloxamers or have interference from other ingredients in the formulation. In addition, the sample preparation step could be labor intensive.
3. By comparison, the SEC method is a better method suited for poloxamer quantitation. It has better sensitivity, accuracy, and precision and is simple to use.

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