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Development and validation of HPLC methods for the determination of potential extractables from elastomeric stoppers in the presence of a complex surfactant vehicle used in the preparation of parenteral drug products

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Abstract

HPLC methods were developed and validated for potential extractables [zinc dithiocarbamate, 2,6-di-*tert*-butyl-*para*-cresol (BHT), octylated diphenylamine antioxidant, sulfur, pentylphenol, and tetrakis(methylene(3,5-di-*tert*-butyl-4-hydroxyhydro cinnamate))methane] from commercial elastomeric stoppers in a complex surfactant matrix. These stoppers were proposed to be part of the container-closure system for experimental formulations containing the surfactant, polyoxyethylated Castor oil (USP/NF) (POE Castor oil) and ethanol. The presence of POE Castor oil in the formulation posed unique challenges to the development and validation of the HPLC methods. POE Castor oil, also known as Cremophor, is a viscous and complex solubilizing agent with a number of uncharacterized fractions. Hence the goal was to identify HPLC conditions that would be suitable for the separation, detection, and quantitation of the potential stopper extractables in the presence of such a complex drug product matrix. A number of experiments were performed to evaluate the effects of different columns, mobile phase composition, injection volume, and gradient profile on the separation and detection of the potential stopper extractables. The quantitation limits of these stopper extractables are between 1 and 10 ppm. The methods demonstrate good linearity, acceptable accuracy, and precision.

Keywords: HPLC; Potential extractables; Container closures; Polyoxyethylated Castor oil; Cremophor; Parenteral drug product; Elastomeric stopper; Rubber stopper; Surfactant; Method development; Method validation; Ultraviolet detection; Zinc dithiocarbamate; 2,6-Di-*tert*-butyl-*para*-cresol (BHT); Octylated diphenylamine antioxidant; Sulfur; Pentylphenol; Tetrakis(methylene(3,5-di-*tert*-butyl-4-hydroxyhydro cinnamate)) (Organox 1010)

1. Introduction

Elastomeric or rubber stoppers have long been key components of container/closure systems for parenteral drug products. The reason for their ubiquitous use is that they possess such desirable physicochemical properties as penetrability, elasticity, resiliency, chemical inertness, and ability to exclude vapor and gas [1–3]. Manufacturers of elastomeric stoppers use curing agents, activators, accelerators, antioxidants, plasticizers, fillers, and pigments to make the base material of their products acquire these ideal characteristics. While these ingredients impart desirable properties to the elastomer base, they can also pose potential health hazards [4,5], cause adulteration, or interfere with analytical methods [6] if residual amounts left in the stoppers from manufacturing leach or are extracted into the parenteral product. In a recent report [7], leaching plasticizers from rubber stoppers used in single-use syringes was found to have interacted with an excipient in a parenteral product to form an immunogenic adjuvant which led to pure red-cell aplasia (PRCA), a severe form of anemia, in patients receiving the product. The United States Code of Federal Regulations (CFR) stipulates that "drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, quality, or purity of the drug beyond the official or established requirements" [8]. Regulatory bodies in Europe and Japan have similar requirements for container/closure systems. Various reports in the literature have,

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however, shown measurable amounts of these stopper ingredients in parenteral solutions [9–13].

The potential deleterious effects of extractables in pharmaceutical products and the need to preserve product quality throughout its shelf life have led the three major pharmacopoeias [14–16] and FDA [17] to require extractable and toxicity testing of stoppers and evidence of compatibility with the drug product prior to their use. The potential extractable tests are, generally, non-specific wet-chemistry methods employing water, isopropanol, and product vehicle as the extractants. Manufacturers of elastomeric stoppers carry out the required safety tests for their products and the results are included in a Drug Master File (DMF) to be referenced by the drug industry intending to use that particular stopper in a product container/closure system. Drug manufacturers are required to show that potential extractables from the selected stopper or stoppers will not interfere with the physicochemical integrity of their product throughout its shelf life. Testing for extractables is recommended even if the target stoppers have passed compendial suitability tests [18]. A number of analytical methods have been reported in the literature to quantitate and characterize stopper extractables found in parenteral drug products. These include LC [12,19–21]; LC–MS [20,22,23]; TLC [24]; GC–MS [19]; and atomic absorption spectroscopy [21]. The majority of these methods were applied to stopper ingredients extracted into aqueous product media and, therefore, not subject to matrix challenges posed by more complex formulations such as limitations to sensitivity and reproducibility. The literature is, however, scant with chromatographic methods for the analysis of stopper extractables in surfactant or oil-based products. While this may be related to the majority of parenteral products being aqueous-based, the scarcity of methods for non-aqueous (oily) parenterals can also be attributed to the analytical challenges posed by such samples. Not only do such methods have to deal with more difficult sample preparation procedures, but components of the surfactant excipient may also chromatograph making it difficult to resolve and quantify the extractables of interest. This problem is compounded by the typically low levels of the potential extractables in the presence of a large excess of the surfactant excipient. Additionally, non-specific absorption of the components of the surfactant excipient may also limit the routine application of the method to real samples. In this paper, we report on the development of HPLC methods for the determination of six potential extractables typically used in the manufacture of commercial elastomeric stoppers. The commercial stoppers were proposed to be used in the primary container/closure system for an experimental parenteral product formulated in a purified polyoxyethylated Castor oil (USP/NF)(POE Castor oil):ethanol mixture. POE Castor oil, also known as Cremophor, is a non-ionic surfactant used as an emulsifying or solubilizing agent. For proprietary reasons, neither the method for the purification of the POE Castor oil nor the commercial sources of the elastomeric stoppers will be discussed in this report. The potential stopper extractables studied are zinc dithiocarbamate (Z), 2,6-di-*tert*-butyl-*para*-cresol (BHT) (B), octylated diphenylamine antioxidant (ODA), sulfur (S), pentylphenol (P), and tetrakis(methylene(3,5-di-*tert*-butyl-4-hydroxyhydro cinnamate)) methane (Organox 1010) (I).

2. Experimental

2.1. Chemicals and reagents

Acetonitrile was purchased from EM Science (Gibbstown, NJ), trifluoroacetic acid (TFA) was obtained from Burdick & Jackson (Muskegon, MI), and ethanol, Absolute-200 proof was purchased from AAPER Alcohol and Chemical Company (Shelbyville, KY). Deionized water was purified using Milli-Q Ultra-Pure Water System (Millipore Corp., Bedford, MA).

Authentic samples of the following standards were purchased and used without further purification: Z, B, S, and I were obtained from Aldrich (Milwaukee, WI). ODA was purchased from ICN Biomedicals Inc. (Aurora, OH). P was purchased from Fluka (St. Louis, MO). POE Castor oil, NF was obtained from BASF (Florham, NJ) and purified by a proprietary process. Purified POE Castor oil was mixed in equal volumes with ethanol and vialed in the identical container/closure system to be used for the drug product. This POE Castor oil:ethanol (1:1) mixture is referred to as the placebo hereafter. Vialed and stoppered placebo samples were stored inverted and in upright positions at elevated temperatures and under recommended storage conditions for 3 months and analyzed for potential extractables.

2.2. Liquid chromatography

The HPLC system consisted of a Waters Alliance 2690 Separation Module, and Waters 2487 Dual λ Absorbance Detector/Waters 996 Photodiode Array Detector (Waters Corp., MA). Waters Millennium 32 software was used for the data acquisition, processing, and reporting.

Three HPLC methods were developed and validated using the above-named commercially available standards. Method I was used for the analysis of Z, P, and B; and it employed a YMC-Pack Cyano column (YMC-Pack Cyano, S-5 μm, 150 mm × 4.6 mm, YMC Co., Kyoto, Japan). The column was maintained at room temperature. Mobile phases A and B consisted of water:acetonitrile:TFA (90:10:0.05, v/v/v) and acetonitrile:TFA (100:0.05, v/v), respectively. The analysis began with a linear gradient from 0% to 50% B in 30 min, then 30–32 min linear gradient to 100% B, 32–35 min held at 100% B, 35–35.1 min, 100% A and finally, 35.1–40 min held at 100% A. The flow rate was 1.0 ml/min and the UV detector wavelength was set at 215 nm.

Method II was used for the analysis of S and I. Mobile phases A and B were acetonitrile:water (25:75, v/v) and acetonitrile (100), respectively. Analysis was performed on a 50 mm × 4.6 mm, 5 μm Waters XTerra™ MS C 18 column, eluted using the following gradient profile: 0–15 min, 50% B, 15–25 min, 55% B, 25–35 min, 65% B, 35–75 min, 100% B, 75–78 min, held at 100% B, and 78.1–85 min, 100% A. Flow rate was 1.0 ml/min and the UV detector wavelength was set at 210 nm.

Method III uses the same conditions as Method II except the gradient profile was changed to facilitate the analysis of ODA. Method III used the following gradient profile: 0–15 min, 50%

B, 15–25 min, 55% B, 25–28 min, 58% B, 28–30 min, 100% B, 30–35 min, held at 100% B, and 36–45 min, 100% A.

2.3. Preparation of the standard solutions

2.3.1. Diluent

Standards were dissolved in a diluent prepared by mixing equal volumes of the placebo with ethanol such that the ratio of POE Castor oil to ethanol in the final solution was 1:3 (v/v).

2.3.2. Standard solution of Z, P, and B

Appropriate amounts of Z, P, and B standards were weighed into a 200-ml volumetric flask, dissolved, and diluted to volume with the diluent such that the final stock concentration of each standard was 100 ppm. Serial dilutions were prepared from this stock solution in the diluent and used for subsequent experiments.

2.3.3. Standard solution of S and I

Stock standard solution for S and I was prepared in the same way as described above for Z, P, and B except a 500-ml volumetric flask was used and the stock concentration was 50 ppm. Serial dilutions of the stock solution were also prepared and used as described above.

2.3.4. Standard solution of ODA

ODA stock standard concentration was 200 ppm and serially diluted using the above-described described procedure.

2.3.5. Working standard solutions

The working concentration for all the standards except ODA was 20 ppm. ODA working concentration was 50 ppm.

2.4. Preparation of sample solutions

Test samples were directly mixed in equal volumes with ethanol to obtain a final POE Castor oil:ethanol ratio of 1:3 (v/v) prior to analysis.

3. Results and discussion

3.1. Method development and optimization

Different columns were evaluated to identify chromatographic conditions that would be suitable for the separation and quantitation of the six potential stopper extractables in the presence of the complex drug product matrix consisting of the surfactant, POE Castor oil, and ethanol. Due to the high background from the POE Castor oil vehicle and the presence of several matrix components, the chromatographic conditions had to have the rugged capability to elute each potential extractable peak in a relatively "clean" area in the chromatogram with little or no interfering background peaks. This considerably increased the difficulty of the method development process. No single chromatographic condition was found to be adequate for the resolution of all six potential extractables in the drug product matrix so three methods, involving the use of Cyano and C-18 columns, were developed. Adding to the challenge of the method development was the observation that peak shape for the potential extractables differed in the presence (POE Castor oil:ethanol mixture) and absence (ethanol alone) of POE Castor oil, respectively hence necessitating the need to match the sample and standard matrix in the analysis. An example of this matrix effect is shown in the overlay chromatograms (Fig. 1) for Z, P, and B analysis (Method I). It can be seen that the peak for each potential extractable was much sharper in the absence of POE Castor oil (top chromatogram) than in the placebo at identical concentrations (middle chromatogram). Similar matrix effect was observed for the potential extractables measured in Methods II and III, respectively. This peak broadening due to the sample matrix was detrimental to achieving low detection limits and therefore needed to be minimized. To eliminate the matrix difference, standards were prepared in the diluent and test sample solutions were diluted 1:1 with ethanol to achieve the same 1:3 POE Castor oil:ethanol ratio as in the diluent. The 1:1 dilution of the test samples with ethanol also had the added advantage of decreasing the viscosity of the final solution thus facilitating its quantitative preparation and handling.

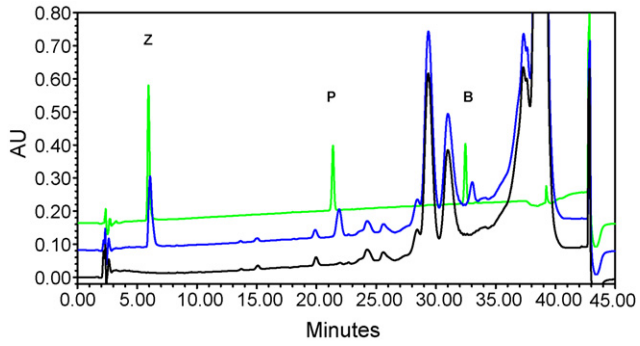

Fig. 1. Chromatogram of ethanolic solution of Z, P, B (top), placebo spiked with Z, P, B (middle) and unspiked placebo (bottom) in acidic mobile phase (see text for conditions). Placebo (1:1, v/v, POE Castor oil:ethanol) samples were injected without further dilution.

The UV spectra of the six potential extractables were obtained using a Waters 996 Photodiode Array Detector. Based on the results obtained (Fig. 2a–c) and the inherent UV absorption of the surfactant matrix, 215 nm was used for Method I and 210 nm was selected for Methods II and III. These analytical wavelengths gave the best signal/noise ratio for the analysis and were a pragmatic compromise between the lambda maximum for each potential extractable and the need for reducing background interferences from POE Castor oil.

Studies were also carried out to evaluate the impact of injection volume on peak shape and quantitation. These studies were performed in anticipation of the potential band broadening that might occur, as evidenced by the middle chromatogram of Fig. 1, when the more viscous sample and standard solutions are injected into the relatively less viscous mobile phase. Ten-microliter injection was found to give adequate peak shape and was used in all three methods.

The influence of mobile phase pH on the retention time of all the potential extractables was investigated. P, B, S, I, and ODA

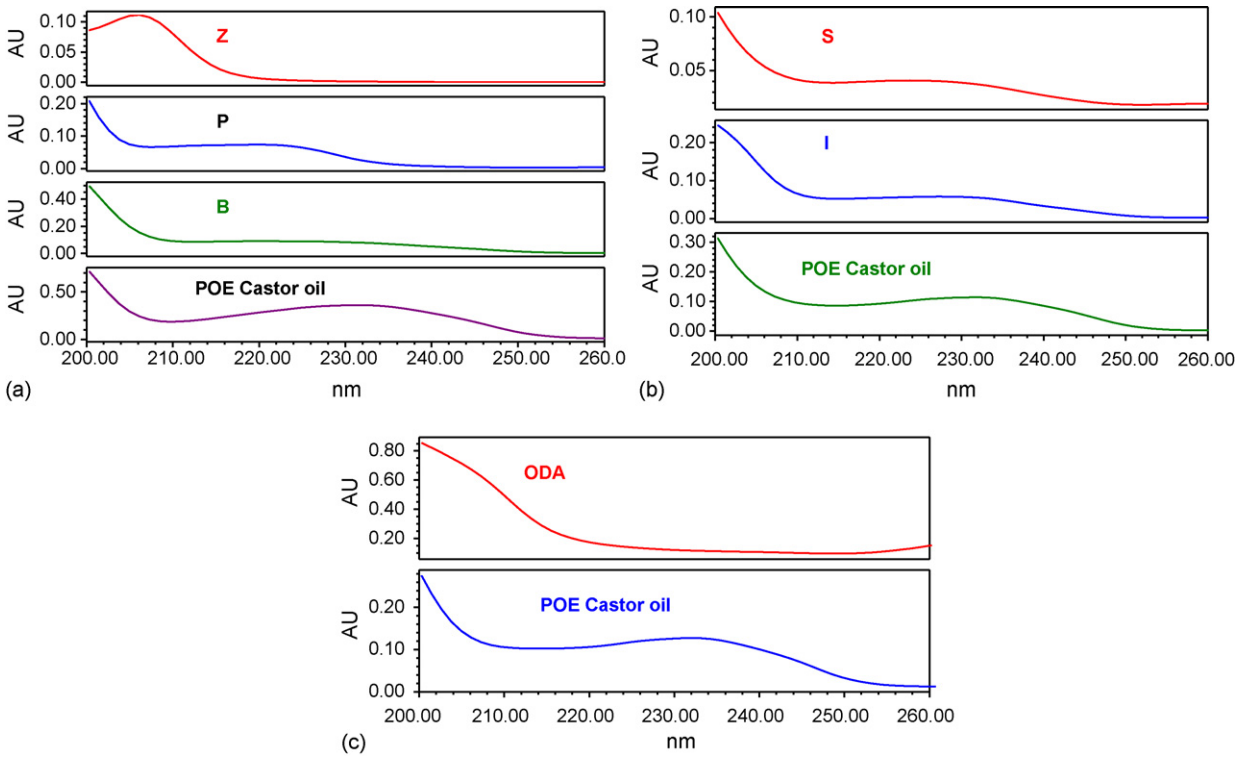

Fig. 2. (a) Ultraviolet absorption spectra of injected working standard concentrations of Z, P, B in diluent, and of the diluent (1:3, v/v, POE Castor oil:ethanol) obtained from the photodiode array detector under the conditions of Method I. Different expansions on the *y*-axis are used to highlight what minor spectral differences there were between the potential extractables and POE Castor oil. (b) Ultraviolet absorption spectra of injected working standard concentrations of S and I in diluent, and of the diluent obtained as described in (a) but under the conditions of Method II. (c) Ultraviolet absorption spectra of injected working standard concentration of ODA, and of the diluent obtained as described in (a) but under the conditions of Method III.

were not markedly affected by pH changes in the mobile phase. The effect of pH was, however, dramatic on the retention time of Z. As shown in Fig. 1, Z elutes in about 6 min in the mobile phase containing 0.05% TFA, the final Method I assay conditions used in its analysis. In the absence of TFA, Z eluted in 29 min (Fig. 3) with a peak that tailed into one of the components of POE Castor oil and made its quantitation more difficult. The goal of the acidification of Method I mobile phase was to elute Z in a region in the chromatogram to facilitate its determination but it also had the added benefit of providing a stable environment for the analyte since dithiocarbamates are generally more stable under acidic conditions. Comparison of Figs. 1 and 3 also shows that the non-specific absorption of the POE Castor oil components did not change markedly with changes in mobile phase pH. The stability of the chromatographic profile of this major component of the drug product was very important from the standpoint of the methods' accuracy, repeatability, and transferability. No pH adjustment to Methods II and III mobile phases was necessary due to the lack of effect on the retention times of the extractables assayed in these methods. Because S and ODA elute about 1 minute apart under similar gradient conditions, both Methods II and III can be used for their analysis (Fig. 4). However, for practical reasons, Method III was exclusively used for the analysis of ODA since it enabled a significant amount of time be saved for the analysis of test samples expected to contain only this potential extractable. P (~10 min in Fig. 4) also separates under these conditions but it was analyzed using Method I due to observed drifts in its retention time towards the adjacent

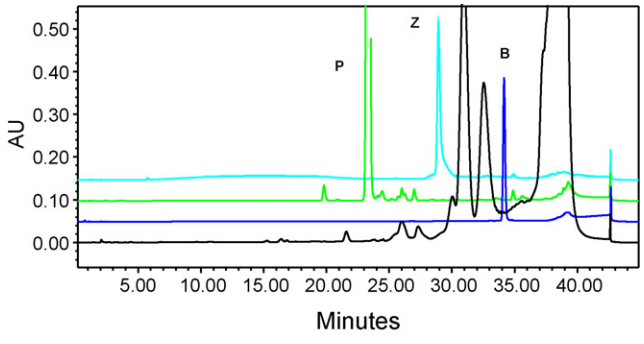

Fig. 3. Chromatogram of ethanolic solutions of Z, P, B (top) and unspiked placebo (bottom) in un-acidified mobile phase (see text for conditions).

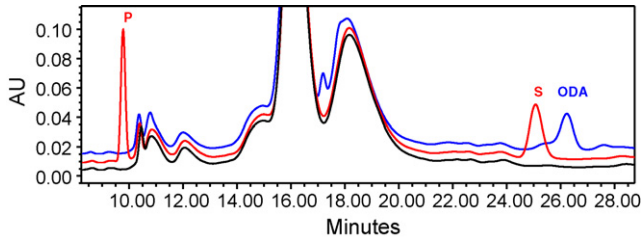

Fig. 4. Expanded chromatograms of S and ODA showing their resolution under gradient conditions common to Methods II and III. P elutes under similar conditions but neither method is used for its assay (see text for details).

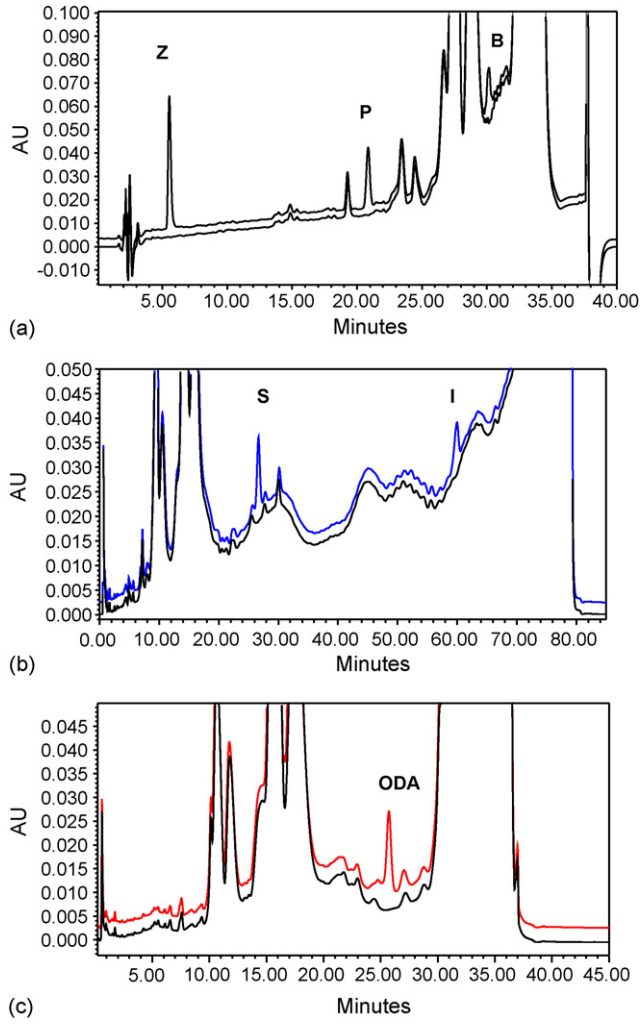

Fig. 5. (a) Typical chromatograms of the working standard concentrations of Z, P, and B (Method I) compared with the diluent. (b) Typical chromatogram of the working standard concentrations of S and I (Method II) compared with the diluent. (c) Typical chromatogram of the working standard concentration of ODA (Method III) compared with the diluent.

POE Castor oil components. Typical chromatograms from the final and optimized assay conditions for Methods I, II, and III are shown in Fig. 5(a–c) for the working concentrations of the potential extractables. Comparison of the middle and bottom placebo chromatograms in Fig. 1 to the chromatograms in Fig. 5a of diluted samples shows the success of the 1:1 ethanol dilution procedure in matching the standard and sample matrices.

3.2. Method validation

The following parameters were evaluated for the validation of the methods.

3.2.1. Linearity

The linearity of detector response to various concentrations of the potential extractables was studied by preparing serially diluted solutions in the diluent and analyzing them by their respective methods. A minimum of six solutions, including that corresponding to the quantitation limit, were studied for each potential extractable. Each solution was injected in duplicate. Linearity curves were obtained for Z (1–100 ppm), P (5–100 ppm), and B (10–100 ppm) standard solutions using Method I. Method II was used for S and I at concentrations ranging from 5 to 50 ppm. ODA was studied in the 5–100 ppm concentration range using Method III. The average area counts were subjected to regression analysis and the results are presented in Table 1. All the plots showed acceptable linearity over the ranges studied.

Table 1
Linearity results

Potential extractable	Range (ppm)	Regression equation	r^2
Z	1–100	$Y = 44700x + 17975$	0.999
P	5–100	$Y = 22569x + 64954$	1.000
B	10–100	$Y = 20373x - 15425$	1.000
S	5–50	$Y = 26668x - 55198$	0.994
I	5–50	$Y = 23042x - 59307$	0.997
ODA	5–100	$Y = 8745x + 36424$	0.990

3.2.2. Accuracy/precision

Accuracy (percent recoveries) was determined by spiking the placebo with known amounts of the potential extractables and determining the percent recovered using the respective methods. The spiked placebos were diluted 1:1 with ethanol and analyzed against external standards in matched matrices. Recoveries were determined at multiple levels within the method range for each potential extractable. Each level was analyzed in triplicate. Percent recovery at each concentration level was calculated by multiplying the ratio of the amount recovered to the spiked amount by 100. The results are presented in Table 2.

Table 2
Accuracy results

Potential extractable	Concentration level (ppm)	%Average recovery ($n = 3$)	%R.S.D.
Z	5	102.0	1.4
	10	105.2	1.8
	15	97.2	3.8
	20	97.9	1.7
P	10	121.2	3.4
	15	105.8	2.4
	20	99.7	0.9
B	10	93.0	5.5
	15	94.1	7.2
	20	97.8	3.3
S	5	93.0	12.6
	10	99.7	1.6
	20	91.9	0.7
I	5	91.0	3.0
	10	91.9	0.8
	20	94.2	2.1
ODA	15	116.1	4.9
	25	107.9	4.0
	50	102.1	0.7

$n = 3$: average of three determinations; R.S.D.: relative standard deviation.

Table 3
Precision results

Potential extractable	Working concentration (ppm)	Average area counts ($n=6$)	%R.S.D.
Z	20	910698	2.6
P	20	522098	1.8
B	20	398257	1.6
S	20	545091	1.8
I	20	385998	4.2
ODA	50	537357	4.0

$n=6$: average of six determinations.

The percent recoveries ranged from 91.0 to 121.2 for the lowest concentrations of I (5 ppm) and P (10 ppm), respectively. The corresponding percent relative standard deviation (%R.S.D.) values were 3.0 and 3.4, respectively.

The precision of the methods was determined from the percent R.S.D. of six replicate injections of each of the potential extractables at their respective working standard concentrations. The percent R.S.D. ranged from 1.6 to 4.2 (Table 3). The assay precision for each potential extractable was determined from its recovery data. The variations in the percent recoveries obtained for the various studied levels were expressed in terms of %R.S.D. and used as the measure of the precision of the method for that potential extractable. The results are presented in Table 4. The assay precision ranged from 2.4% for I to 9.1% for P. Intermediate precision, or within-laboratory variation, was determined by analyzing three fresh preparations of the working standard concentrations of each potential extractable on 3 different days using different instrumentation. To account for differences in detector response and slight differences in the standard concentrations, the area counts were divided by the solution concentration to obtain the response factor. The variations in the response factors for the preparations were determined for each potential extractable and expressed as %R.S.D. The %R.S.D. values for the intra-day results were well below 10 indicating good within-day precision (Table 5). Acceptable inter-day/inter-instrument precision was also obtained for each extractable (Table 6).

The overall results obtained for accuracy and precision, in the presence of the surfactant matrix, were all within acceptable limits for trace level impurities determination, as is the case for potential extractables, thus indicating the adequacy of the methods for their intended purpose.

Table 4
Assay precision

Potential extractable	Average of percent recoveries ($n=9$)	%R.S.D.
Z	100.6[a]	3.9
P	108.9	9.1
B	95.0	5.3
S	94.9	7.3
I	92.4	2.4
ODA	108.7	6.5

$n=9$: average of nine determinations.
[a] $n=12$ for this determination.

Table 5
Intra-day precision

Potential extractable	Day	Average response factor ($n=3$)	%R.S.D.
Z	1	43021	1.3
	2	39441	0.7
	3	42697	3.7
P	1	23479	1.5
	2	21549	2.3
	3	19180	4.4
B	1	14674	0.2
	2	11615	1.1
	3	13539	3.2
S	1	27750	0.4
	2	21949	4.0
	3	27443	2.1
I	1	16421	0.5
	2	16075	3.1
	3	17378	1.3
ODA	1	8154	0.9
	2	8864	6.8
	3	10480	1.6

$n=3$: average of three determinations.

3.2.3. *Specificity and selectivity*

The interference, or lack thereof, from POE Castor oil components in the analysis of each potential extractable was determined by comparing the chromatogram of the placebo alone with those obtained for the placebo and ethanol separately spiked with the potential extractables. As shown in Fig. 5a for Method I, none of the POE Castor oil-related peaks interfered with the potential extractable peaks. Similar lack of background interference is shown in Fig. 5b and c for Methods II and III, respectively. Additionally, the purity of each potential extractable peak was confirmed using a diode array detector. This was achieved by comparing the spectral characteristics of the potential extractable peaks in the chromatogram with spectral libraries obtained for each standard at its working concentration. Spectral characteristics of each potential extractable peak and components of POE Castor oil eluting next to it were also compared. The potential extractables and components of POE Castor oil do not possess very characteristic ultra-violet spectral features but they were sufficiently different to enable confirmation of the purity and identity of each of the analyte peaks. As expected, ethanol did not show any chromatographic peaks.

Table 6
Inter-day precision

Potential extractable	Average of response factors ($n=9$)	%R.S.D.
Z	41730	4.6
P	21403	9.1
B	13276	10.2
S	25714	11.2
I	16625	3.9
ODA	9166	11.8

$n=9$: average of nine determinations.

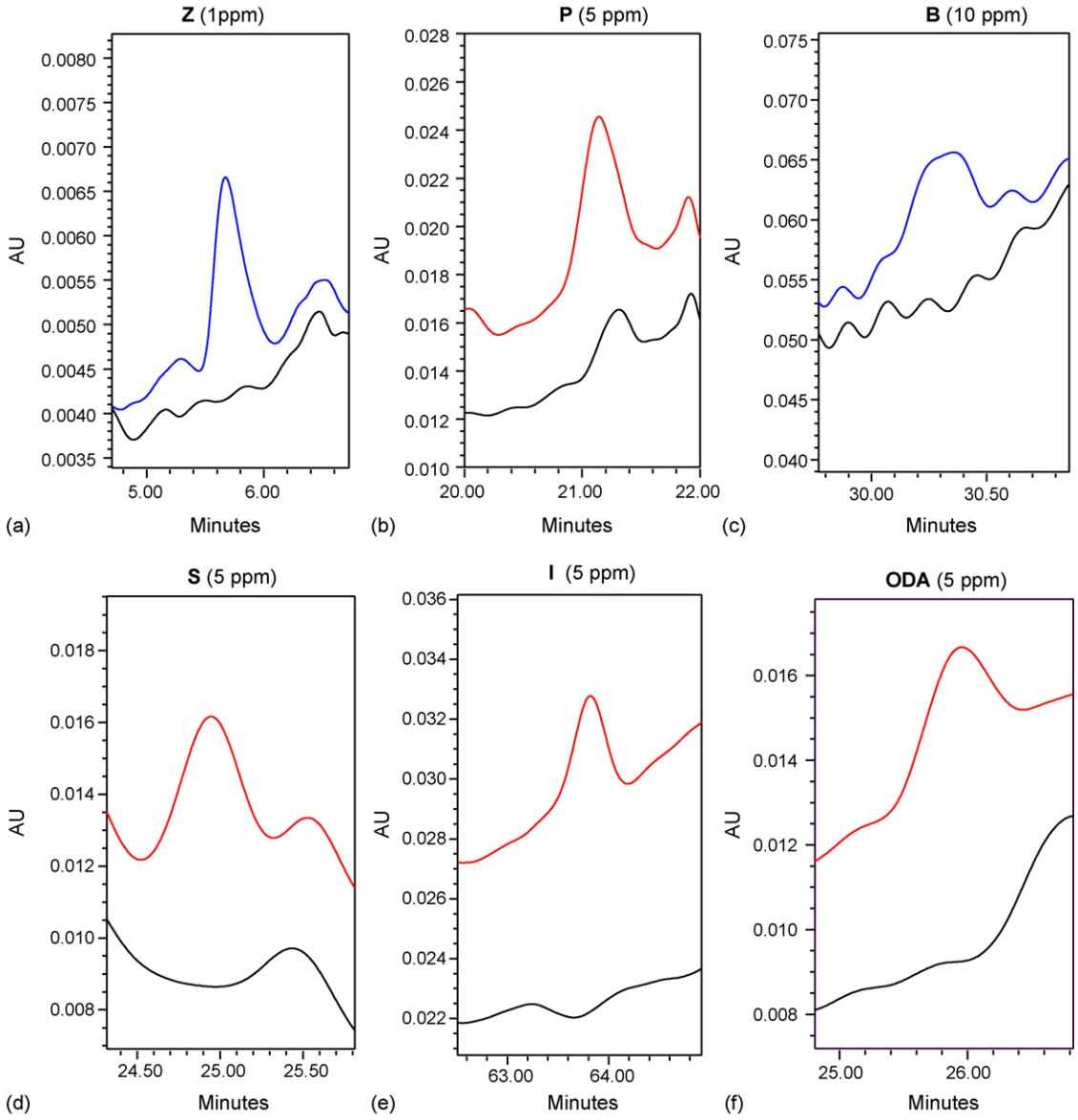

Fig. 6. Comparative expanded chromatograms of the quantitation limit concentrations for the potential extractables. Diluent chromatogram is at the bottom in each plot, and quantitation limits are in parenthesis. The expansions used preclude the showing of the full chromatograms so only regions close to the retention times of the potential extractables are shown.

3.2.4. Detection (DL) and quantitation limits (QL)

The DL and QL for each potential extractable were determined by injecting serially diluted solutions and comparing the chromatograms with that of the placebo. The lowest concentration of each potential extractable that could reliably be differentiated from placebo background was selected as the DL for that compound. The QL was the next higher level concentration that could be chromatographed repeatedly with acceptable precision. Fig. 6(a–f) shows expanded chromatograms obtained for the potential extractables at their respective quantitation limits.

3.2.5. Stability

The stability of working standard solution of each potential extractable in diluent was determined at both room temperature and at 5 °C by analyzing the solutions over a period of 2 days. The solutions were considered stable if the variability in the assay results was less than 10% of initial. The goal here was to ensure that the preparation of samples for analysis did not initiate and/or contribute to the degradation of the analytes of interest and that the samples will remain stable during the course of the analysis. The results indicated neither the sample preparation nor the length of the analysis times contributed to sample degradation.

4. Conclusion

Three gradient liquid chromatographic methods were developed, optimized, and validated for the determination of six potential extractables from two commercially-available elastomeric stoppers used in the container/closure system for parenteral drug products. The methods enable the detection and

quantification of these potential extractables at low parts per million levels in the presence of a complex drug product vehicle made up of a 1:1 mixture of the surfactant, POE Castor oil, and ethanol. Resolution of all six potential extractable peaks from the components of the surfactant drug product vehicle was achieved using the three methods. Good linearity was achieved for each potential extractable in the presence of the drug product vehicle. Acceptable precision and accuracy were also obtained for each potential extractable. The different physicochemical properties of the six potential extractables coupled with the analytical challenges posed by the viscous and complex surfactant matrix of the test article made it difficult to develop a single HPLC method that was capable of analyzing all the analytes of interest. These problems were overcome through careful screening of columns, different mobile phases with or without modifiers, experimentation with an array of gradient profiles, and employing a "divide-and-conquer" approach of bundling the potential extractables in one of the three final methods based on the results of the screening experiments. The matching of the standard and sample matrices was key to the success of these methods, and low part per million detection levels were achieved through a number of pragmatic choices including the selection of appropriate analytical wavelengths to maximize signal-to-noise ratio. The utility of the methods was successfully demonstrated through their use in the evaluation of the compatibility of container-closure systems for an experimental parenteral drug product.

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