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Solid-Phase Extraction and HPLC Analysis of Methylparaben and Propylparaben in a Concentrated Antibiotic Suspension

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ABSTRACT An accurate and precise solid-phase extraction coupled with high performance liquid chromatography (SPE/HPLC) method developed for the quantification of antimicrobial preservatives (methylparaben and propylparaben) in oxytetracycline injectable suspension is described in this article. The SPE technique was necessary to quantify the preservatives since the high concentration of the drug and excipients was masking low levels of preservatives, making quantification difficult. This developed HPLC method was stability-indicating and found to be linear between 1.3 to 2.4 mg/mL for methylparaben and 0.15 to 0.27 mg/mL for propylparaben in this concentrated antibiotic suspension formulation. The extraction recoveries were 98.8–101.6%. System precision and sample extraction precision (RSD) were less than 1%.

KEYWORDS Methylparaben, Propylparaben, Preservatives, Solid Phase Extraction, High-Performance Liquid Chromatography, antibiotic, suspension, oxytetracycline

INTRODUCTION

Antimicrobial preservatives are often added to pharmaceutical formulations to manage microbial contamination. Preservatives such as methylparaben and propylparaben are used in a wide variety of drug products such as creams, ointments, and suspensions (Majlat & Barthos, 1984). Parabens are preferred due to their broad antimicrobial spectrum and effectiveness at low concentrations (Dhaliwal & Theobald, 1995). In the current regulatory environment, the validation of a specific, stability-indicating test method is required for preservatives. However, the quantification of preservatives can be complicated by their low concentration (less than 1%) with respect to the active ingredient. The complexity of suspensions can require extensive sample clean up before chromatographic analysis. Specific gas chromatographic methods have been reported; however, prior derivatization is often required (Dhaliwal & Theobald, 1995). Other reported methods for the quantification of preservatives in complex matrices use time-consuming liquid/liquid extractions or solid-phase extraction (SPE) that requires a solvent exchange step (Weisenberg et al., 1977;

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Radus & Gyr, 1983; Tarbin & Shearer, 1992). Microbiological assays can also be used to analyze parabens; however, the sensitivity and accuracy are often much lower than HPLC assays (Abuirjeie et al., 1990). Simultaneous determination of parabens has been reported in oral suspension formulations without the use of sample cleanup; however, the concentration differences between the formulation components (25 mg/mL active, 1 mg/mL parabens) are less than what is described in this article (Ali et al., 1990).

The antibiotic (oxytetracycline) containing injectable suspension is under product development. It is an intramuscular sustained release formulation and primarily indicated for the treatment of bovine respiratory disease (or commonly known as shipping fever) in cattle. Single treatment of this suspension provides therapeutic antibiotic blood level over a 12-day period. Homogenization reduces the raw material particle size from approximately 15 μm to the volume-weighed mean of approximately 5 μm in the suspension product. This suspension formulation was filled in 500-mL amber glass containers and then terminally sterilized by gamma irradiation (Wong et al., 2004).

During the product development process, several potential suspension formulations were evaluated. One of these formulations contains a combination of methylparaben and propylparaben (see Fig. 1) as preservatives. The oxytetracycline, USP (25% w/v), suspension formulation described in this study contains phospholipids as dispersing agents (20% w/v), mannitol, USP (2.25% w/v), methylparaben, NF (0.18% w/v), and propylparaben, NF (0.02% w/v).

This article describes sample extraction of methylparaben and propylparaben using SPE. This quick and accurate method involves a single extraction of preservatives using strong cation exchange (SCX) cleanup column and HPLC compatible solvents. A cation exchange SPE cartridge was chosen because the antibiotic (oxytetracycline, see Fig. 1) is cationic in nature and was retained in the cartridge while the preservatives were collected.

The preservative solutions were analyzed by a gradient HPLC method. The resulting method is specific and stability-indicating.

EXPERIMENTAL

Material

Cation exchange cartridges (SCX Maxi Clean 600 mg) were purchased from Alltech Associates, Inc. (Deerfield, IL, USA) for sample cleanup (see Instrument

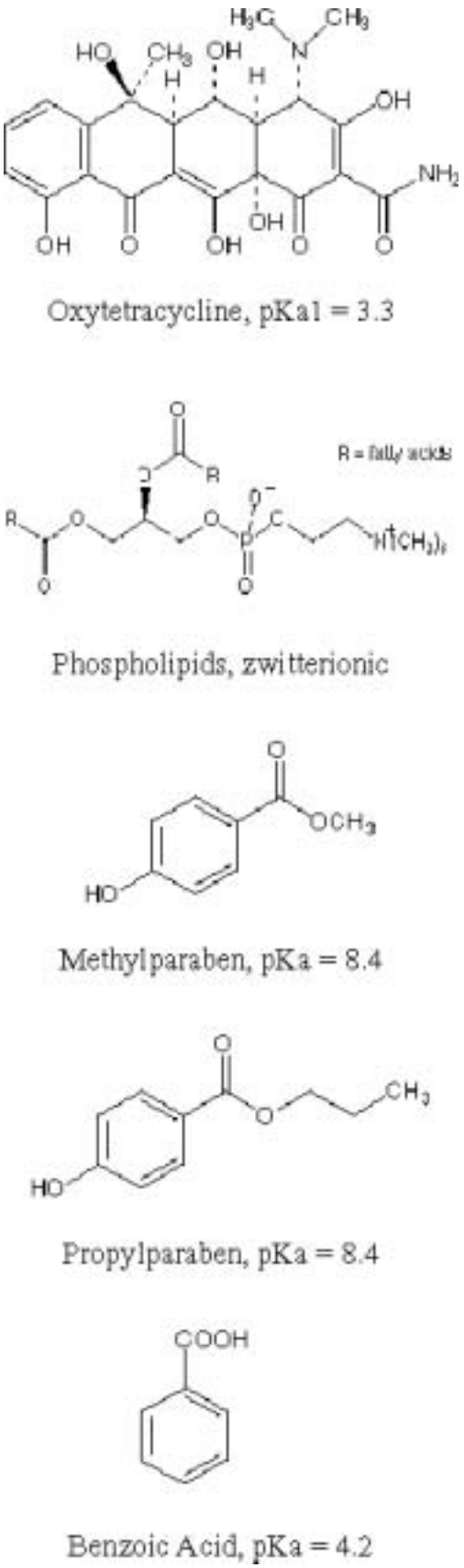

FIGURE 1 **Representative Chemical Structures of the Active and Excipients.**

section). Methanol and acetonitrile solvents were HPLC grade purchased from Burdick and Jackson (Muskegon, MI, USA). ACS grade potassium phosphate, monobasic, potassium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Oxytetracycline was USP grade purchased from Long March Pharmaceuticals, 98.8% pure (Sichuan, China). Except for phospholipids, all other excipients were either USP or NF grade. Phospholipids syrup was obtained from Pfangstiehl Laboratories, Inc. (Waukegan, IL, USA). The preparation of the oxytetracycline suspension evaluated in this study and the sterilization by gamma irradiation have been published (Wong et al., 2004).

Instrument

The instrumental components of the Hewlett Packard 1100 series liquid chromatography system (Hewlett Packard, Palo Alto, CA, USA) used in this study included a binary pump, autosampler, and detector. An Alltech Associates, Inc. (Deerfield, IL, USA) phenyl column (250 × 4.6 mm i.d., 5 μm particles) was used at ambient conditions. The mobile phase for methylparaben and propylparaben quantification was composed of the following components:

1. Mobile Phase A: 80% phosphate buffer (0.025 M of potassium phosphate, monobasic at pH 6.80) and 20% acetonitrile.
2. Mobile Phase B: 20% phosphate buffer and 80% acetonitrile.

The gradient program is outlined in Table 1. The flow rate was kept at 1.0 mL/min and the injection volume was 50 μL. The detection wavelength was 254 nm. Quantification was performed by peak area against USP methylparaben and propylparaben reference standards or high purity benzoic acid (USP grade). A 1 mg/mL methylparaben (or benzoic acid) and 0.2 mg/mL propylparaben stock standard solutions were prepared in methanol. The stock solutions were serially diluted in 50:50 methanol/water to form a single solution containing 0.004 mg/mL methylparaben (or benzoic acid) and 0.0004 mg/mL propylparaben.

TABLE 1 **Gradient Program: Linear Between the Times Specified Below**

Step	Time (min)	% Mobile Phase A	% Mobile Phase B
1	0	100	0
2	20	100	0
3	20.01	90	10
4	44	50	50
5	44.01	100	0

Sample Extraction

To prepare samples for the analysis it was necessary to dissolve the suspension aliquot in 0.1 N HCl. However, interferences from matrix-related peaks at the retention time of methylparaben were observed. In addition, a large injection volume (50 μL) was required for adequate detector response, which resulted in column overload and high column backpressure.

Subsequently, a sample cleanup procedure was developed. Oxytetracycline is cationic at low pH (less than pH 2) while the preservatives are neutral. The SCX column was chosen to retain the dissolved oxytetracycline and allow the elution of the preservatives. The resulting preservatives dissolved a mixture of 0.1 N HCl and methanol could be analyzed directly by a gradient reversed-phase method. The sample preparation procedure is outlined as follows:

1. 300 mg of the suspension was weighed into a 50-mL volumetric flask.
2. 25 mL of 0.1 N HCl was added to dissolve the oxytetracycline solid drug particles in the suspension.
3. The solution was diluted to volume with methanol for the extraction of the preservatives.
4. 10 mL of the above solution passed through the SCX cartridge and the preservative solution was eluted into a 25-mL volumetric flask.
5. The cartridge was rinsed with 2–4 mL portions of methanol.
6. The resulting sample was diluted to volume with water.

Recovery Samples

Test solutions were prepared at 73%, 100%, and 134% of target methylparaben (or propylparaben) concentration in the suspension formulation (see Table 2). Recovery was computed against the control articles prepared from the same raw material used in the test articles. The paraben concentrations in test and control articles were determined against bracketing USP reference standards.

TABLE 2 Method Accuracy

	%Recovery					
	Methylparaben			Propylparaben		
Test Article	Experimental Conc.	Analyst 1	Analyst 2	Experimental Conc.	Analyst 1	Analyst 2
1	73% (1.3 mg/ml)	99.6	99.5	73% (0.15 mg)	99.8	99.3
2		99.6	99.6		100.4	100.0
3		99.7	100.4		99.5	99.0
Mean		**99.7**	**99.8**		**99.9**	**99.4**
95% CI		**0.3**	**1.2**		**1.1**	**1.2**
%RSD		**0.1**	**0.5**		**0.5**	**0.5**
1	100% (1.8 mg/ml)	99.2	98.8	99% (0.20 mg/ml)	99.4	99.5
2		100.2	99.4		100.7	101.1
3		99.1	99.1		99.2	99.4
Mean		**99.5**	**99.1**		**99.8**	**100.0**
95% CI		**1.5**	**0.7**		**2.0**	**2.3**
%RSD		**0.6**	**0.3**		**0.8**	**0.9**
1	134% (2.4 mg/ml)	99.8	99.6	132% (0.27 mg/ml)	100.1	101.0
2		100.0	99.5		100.6	101.6
3		100.7	100.7		101.1	101.1
Mean		**100.2**	**99.9**		**100.6**	**101.2**
95% CI		**1.3**	**1.6**		**1.2**	**0.8**
%RSD		**0.5**	**0.6**		**0.5**	**0.3**

Specificity Samples

A suspension matrix blank containing oxytetracycline drug particles, mannitol, and phospholipids was prepared at 100% of the target formulation concentration at pH 5 (see Table 1 for the gradient program). This suspension was dissolved per the procedure listed in the Sample Extraction section. In addition, a container blank containing water was sterilized by gamma irradiation in a 500-mL. Type III amber glass bottle with radiation compatible stopper (Wong et al., 2004) and prepared per the Sample Extraction section. A mixture of 50:50 methanol and 0.1 N HCl was filtered through a SCX cartridge and diluted in the same manner as the sample was prepared to account for any interferences resulting from the cartridge. In addition, oxytetracycline suspension samples were stressed for approximately 9 months at 30°C to evaluate specificity with respect to formulation related degradation products.

RESULTS AND DISCUSSION

Specificity

Specificity was evaluated using the gamma radiation sterilized oxytetracycline suspension (Wong et al., 2004) that had been stored for 9 months at 30°C (stressed test article) and USP reference standards. Peak purity was evaluated by using the UV absorbance indexing technique described by Poile and Conlon (1981). The UV spectrum from 200 to 400 nm was taken for each paraben peak as it eluted from the HPLC column under nominal operating conditions. Absorbance values were evaluated at the leading edge, apex, and trailing edge of each peak. Discriminator values, which are an indication of peak purity, were computed for each paraben. Discriminator values of 1.5 or less indicate that a peak is pure. Discriminator values were computed from absorbance values in the wavelength ranges from 208 to 222 nm and 248 to 270 nm for methylparaben, to obtain adequate absorbance. Discriminator values were computed from absorbance values in the wavelength ranges from 208 to 224 nm and 248 to 270 nm for propylparaben. Discriminator values ranging from 1.0 to 1.5 were obtained for the methylparaben and propylparaben in the stressed test article indicating that pure peaks were obtained.

In addition to the peak purity experiment, the matrix suspension blank, water blank, and SCX cartridge blank were tested to ensure that no peaks originating from these sources could elute at the same times as the paraben peaks. The chromatographic profiles of the blanks and stressed test article injections were compared to confirm the absence of peaks at the retention times of

both methylparaben and propylparaben. Figs. 2, 3, and 4 demonstrate the sample cleanup of the SCX method. Fig. 4 shows an injection of the stressed test article after sample cleanup. There were no peaks detected at the retention times of methylparaben or propylparaben in injections of the matrix blank or cartridge blank. In addition, there were no distortions visually observed for the methylparaben and propylparaben peaks in the injections of the stressed test article. In addition, benzoic acid was spiked into the formulation at a concentration of 0.2% (w/v). The method was specific with respect to benzoic acid (retention time of 10 min), which is a potential degradation product of methylparaben and propylparaben. However, benzoic acid was not observed in the heat-stressed test article.

Accuracy

The accuracy (recovery) and sample linearity of the method was evaluated by testing triplicate laboratory

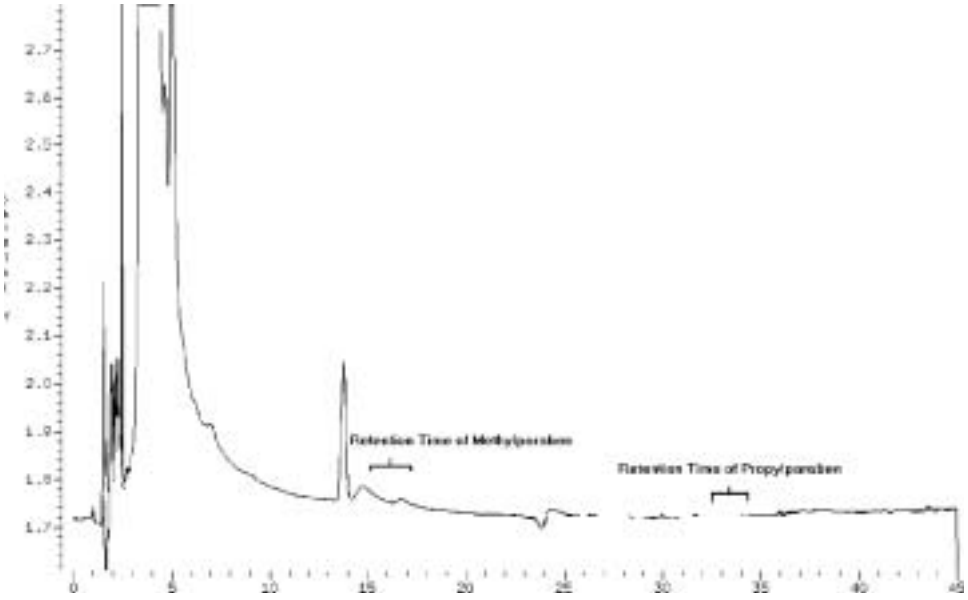

FIGURE 2 **Chromatogram of the Antibiotic Suspension Formulated Without the Preservatives. The SCX Cartridge Was Not Used to Remove the Matrix Components.**

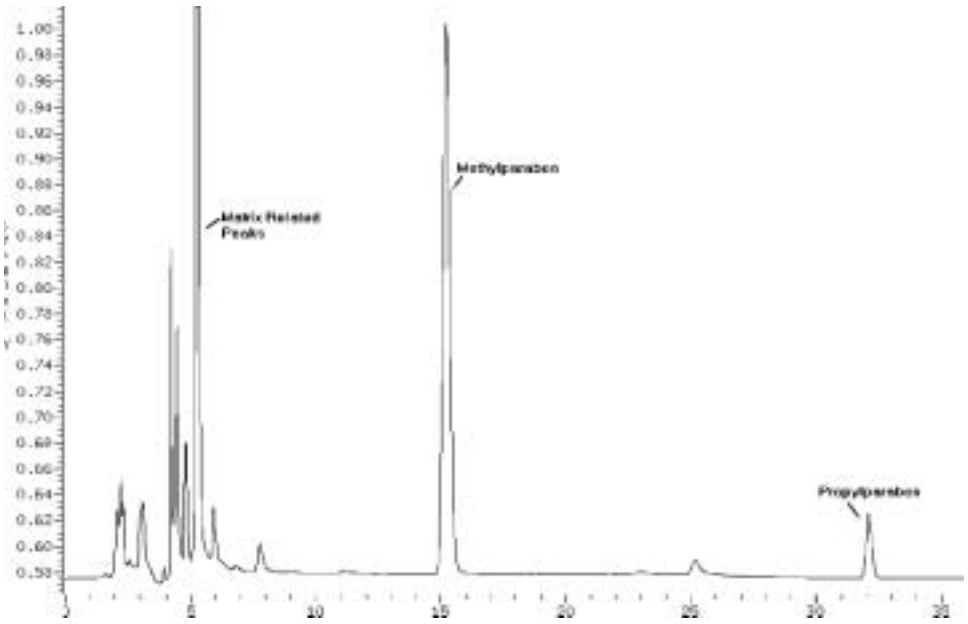

FIGURE 3 **Chromatogram After Using the SCX Sample Extraction Method.**

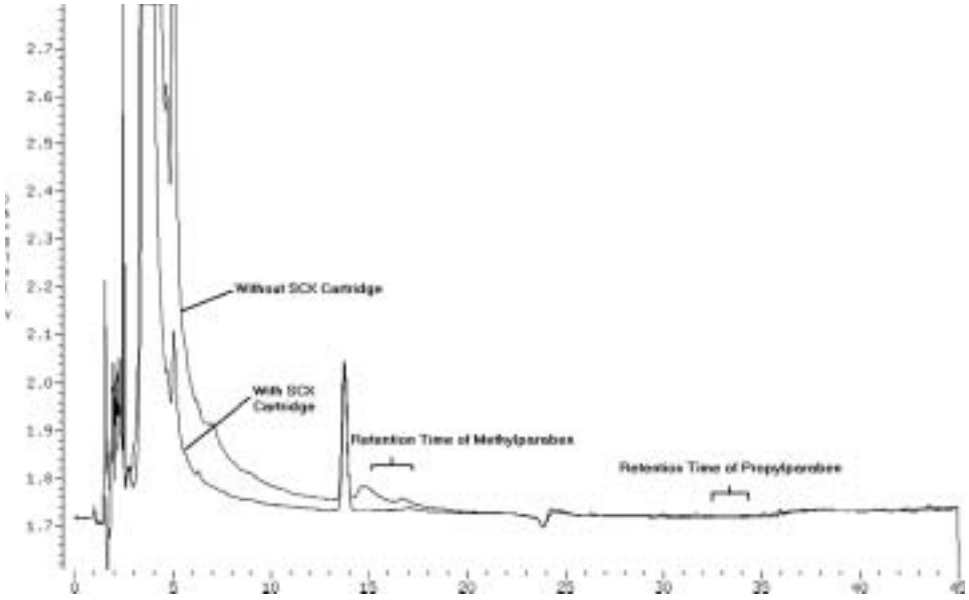

FIGURE 4 **Chromatogram Demonstrating the Effectiveness of the SCX Cleanup Cartridge.**

preparations of the suspension formulation containing approximately 73% to 134% of the target formulation concentrations of methylparaben and propylparaben. Recovery was computed against the control articles prepared from the same raw material used in the stressed test articles. The paraben concentrations in stressed test and control articles were determined against bracketing USP reference standards. The extraction recoveries were between 99% and 101%. The recovery data are presented in Table 2.

Standard/Sample Linearity

The linearity of response for the standards was evaluated in the range from approximately 50% to 150% of the working standard concentrations. The method was linear for test articles over the specified range of methylparaben and propylparaben. Regression analysis using three replicate dilutions at five concentration levels produced a straight line for methylparaben that could be represented by the equation

$$y = 21162054x + 274715$$

and a correlation coefficient (r^2) of 1.000. The equation for propylparaben was

$$y = 18431191x - 3216.3$$

and the correlation coefficient was 0.9999. The % y-intercept (y-intercept/mean peak area of middle standard multiplied by 100) was less than 0.5% for both preservatives. The response factor percent relative standard deviations (%RSD) for both analysts were less than 0.3% for methylparaben and propylparaben.

The linearity of response was also evaluated using the stressed test article described in the accuracy section. The linearity was evaluated in the range from 73% to 134% of the nominal methylparaben concentration and propylparaben concentrations (see Table 2). The method was linear for test articles over the specified range of methylparaben and propylparaben. Regression analysis using three replicate dilutions at three concentration levels produced a straight line for methylparaben that could be represented by the equation

$$y = 21321771x - 7987095$$

and a correlation coefficient (r^2) of 0.9996. The equation for propylparaben was

$$y = 18573553x - 113772$$

and the correlation coefficient was 0.9995. The % y-intercept was less than 3% for both preservatives. The response factor %RSDs for methylparaben and propylparaben were less than 1% for both analysts.

Precision and Ruggedness

In terms of ruggedness, two separate analysts evaluated system suitability, accuracy (see Table 2) and precision on two separate HPLC systems and chromatographic columns. Each analyst prepared separate standards and mobile phase and tested the same suspension test articles. Acceptable accuracy and precision were obtained by both analysts, demonstrating method ruggedness (Tables 2 to 3).

The system precision (see Table 3) was confirmed by a %RSD of less than 0.5% for methylparaben and propylparaben ($n = 5$). Method precision (see Table 3) was determined by extracting five replicate sample preparations of a stressed test article. The stressed test article represents the finished product with respect to processing, sterilization, and storage under accelerated temperatures. As a result, this sample was chosen for method precision. As can be seen in Table 3, the %RSDs of the replicate sample preparations were less than 0.8% for two analysts. In addition, the percent difference of the methylparaben and propylparaben concentrations for analysts 1 and 2 was less than 2.0%.

CONCLUSIONS

Sample cleanup was needed to remove interfering peaks resulting from the formulation matrix. The preservatives (methylparaben and propylparaben) constitute less than 1% of the oxytetracycline and phospholipids ingredients. The phospholipids dispersing agent complicated analyte extraction by solubilizing a portion of the preservatives. Complete solubilization of all of the components in the suspension was necessary to quantify the preservatives accurately and precisely. As a result, concentration differences between the oxytetracycline suspension and the preservatives caused column overloading (column overpressure) when a solution containing 0.004 mg/mL methylparaben and 0.0004 mg/mL propylparaben was injected. The preservatives and oxytetracycline contain similar chromophores and the corresponding UV maximas are close to the same wavelength. In addition, matrix related peaks at the retention times of methylparaben and propylparaben interfered with quantification. Low level paraben quantification by HPLC was therefore feasible only after implementation of a sample cleanup procedure. Per the results presented in this article, the HPLC assay method developed for the determination of methylparaben and propylparaben is therefore validated–the HPLC method can accurately and precisely determine the concentration of each preservative in this oxytetracycline suspension formulation. The method has been shown to be accurate and linear in the concentration ranges tested. Ruggedness was also demonstrated for multiple columns, HPLC systems, and analysts. Specificity

TABLE 3 System/Method Precision and Ruggedness

	Methylparaben		Propylparaben	
Sample	Analyst 1	Analyst 2	Analyst 1	Analyst 2
System Precision: Standard (peak area)	85324252	62467045	7395859	5441873
	85271193	62382670	7357471	5435663
	85207102	62465174	7380358	5417066
	85107566	62693181	7375706	5471929
	85397771	62816620	7404183	5466078
Mean	85261577	62564938	7382715	5446521
%RSD	0.1	0.3	0.2	0.4
Method Precision: Stressed	1.786	1.809	0.200	0.201
Formulation Sample (mg/mL)	1.779	1.805	0.199	0.201
	1.781	1.810	0.200	0.202
	1.756	1.807	0.197	0.202
	1.796	1.800	0.201	0.201
Mean	1.78	1.81	0.20	0.20
%RSD	0.8	0.2	0.8	0.3
% Difference Between Concentration Obtained by Two Analysts	1.5		1.0	

was confirmed by performing UV spectral analyses of the paraben peaks, and by the absence of peaks from injections of appropriate blanks at the retention times of methylparaben and propylparaben.

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