Development and Validation of a Single RP-HPLC Assay Method for Analysis of Bulk Raw Material Batches of Four Parabens that are Widely Used as Preservatives in Pharmaceutical and Cosmetic Products

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Abstract

A stability-indicating, robust, fast, and user friendly reversed-phase high-performance liquid chromatographic (RP-HPLC) assay method has been developed and validated for the analysis of commercial raw material batches of methylparaben, ethylparaben, propylparaben, and butylparaben. These four parabens are widely used as preservatives in pharmaceutical and cosmetic products. Accurate assay value of each of the parabens in their respective commercial lots is critical to determine the correct weight of the paraben that is needed to obtain the target concentration of the paraben in a specific lot of pharmaceutical or cosmetic products. Currently, there are no single HPLC assay methods (validated as per ICH requirements) available in the literature that can be used to analyze the commercial lots of each of the four parabens. The analytical method reported herein analyzes all four parabens in less than 10 min. The method presented in this report was successfully validated as per ICH guidelines. Therefore, this method can be implemented in QC laboratories to analyze and assay the commercial bulk lots of the four parabens.

Introduction

Parabens (p-hydroxy benzoates) are commonly used as preservatives in pharmaceutical, food and cosmetic products because of their anti-fungal and anti-bacterial properties (1). Nonspecific assay method (titration) with a long sample preparation time is reported in the United States Pharmacopeia (USP) (2). Separation and quantitation of parabens in pharmaceutical and cosmetic products using capillary electrophoresis (CE) has been reported in the literature (3,4). RP-HPLC methods have been reported for the assay of various parabens in cosmetic and pharmaceutical products (5,6,7,8). Manojlovic et al. reported an RP-HPLC method for analysis of methylparaben in multivitamin syrup (9). Except for the method reported by Manojlovic et al. (9), none of the other HPLC methods reported in the literature demonstrated method specificity as prescribed in the ICH guidelines (10). In addition, most of the methods reported in the literature have a relatively long chromatographic run-time. The HPLC methods reported in the literature are for the assay of parabens (mainly methyl and propyl parabens) in finished pharmaceutical products, processed food, and cosmetic products.

Pharmaceutical or cosmetic products companies purchase parabens from commercial sources in bulk quantity. Before the usage of a commercial bulk raw material batch of any of the four parabens in the manufacturing of drug and or cosmetic products, in-house testing (for assay, purity etc.) is conducted to obtain accurate assay value of the paraben (in a given lot) and also to ensure that each batch meets the formal specifications for all other testing. Testing and release (against formal specifications) of all bulk raw material batches of each of the four parabens for the manufacturing of drug products is required to comply with the cGMP requirements. Currently, individual method is used for analysis and assay of the bulk raw material batches of each of the four parabens (i.e., four different methods for the four parabens).

The main objective of our work was to eliminate the need of four individual methods by developing a single analytical method that can be used to analyze and assay the bulk raw material batches of all four parabens. A single method capable of resolving all four parabens was also desired because other parabens can be present as an impurity in the bulk raw material lot of a given paraben (3). The primary focus during RP-HPLC method development activities was to develop a method that is simple (including LC–MS compatible mobile phase), specific, robust, and has a short run time. Successful achievement of all the method development goals would generate a friendly and desired quality control laboratory method for rout ine analysis of bulk raw material lots of the four parabens. The LC–MS compatible mobile phase of the new method would be useful in the identification of any future potential unknown and or new chromatographic peaks that may be present in the new bulk raw material lots and or in the stability samples of the parabens.

Accurate potency and purity values are also required to certify/qualify materials from bulk lots that are intended for use as a reference standard. Therefore, the new method presented in this paper can also be used to characterize and certify in-house primary or secondary reference standard lots for each of the four

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parabens. In pharmaceutical industry, large quantities of reference standards are required to conduct all cGMP work including instrument calibration and qualification (11).

In this paper, we report the development and validation of a single RP-HPLC method for the analysis of bulk raw material lots of each of the four parabens namely methylparaben, ethylparaben, propylparaben and butylparaben (Figure 1). The Phenomenex Gemini C18 column (5 cm × 4.6 mm i.d., 5 µm particle size) (Torrance, CA) was identified and established as a true alternate column. The alternate column can be used as a back-up column in case the primary column prescribed in this method is no longer commercially available. The new analytical method has been successfully validated as per ICH guidelines (10) and has demonstrated to be accurate, linear, precise, reproducible, specific, and robust. This new method can be adopted and implemented in a quality control (QC) laboratory for the analysis of bulk raw materials of each of the four parabens namely methylparaben, ethylparaben, propylparaben, and butylparaben. This method can also be used for the certification and characterization of in-house reference standards of each of the four parabens.

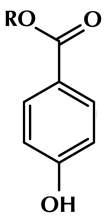
Experimental

Chemicals and reagents

Parabens were either obtained from Schering-Plough (Union, NJ) or purchased from vendors such as Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Waltham, MA), or MP Biomedicals. The reagents/solvents (HPLC grade) used to prepare the mobile phase or diluent for the HPLC analyses were purchased from Fisher Scientific. Water purified by the Milli-Q system (Millipore, Milford, MA) was used for mobile phase and sample diluent preparation.

Instrumentation

Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with Chromsword method development (12) software (Merck KGaA, Darmstadt, Germany) and LC Spiderling line of HPLC Heated/Cooled Column Selector (Chiralizer Services, L.L.C., Newtown, PA) and Waters 2695 Alliance HPLC system were used for method development and/or method validation. All HPLC systems were equipped with a column compartment with temperature control, an on-line degasser, and a diode array detector or a dual wavelength UV detector. Data acquisition, analysis, and reporting were performed (except Chromsword simulation) by ChemStation (Agilent) or Millennium 4.0 or Empower 2 (Waters) chromatography software. The HPLC columns (50 × 4.6 mm ID) were purchased from vendors as needed - ACE 3 (3 µmM) (MAC-MOD Analytical, Inc. Chadds Ford, PA), YMC Hydrosphere (3 µm) (YMC, Kyoto, Japan), TSK Gel Super ODS (2 µm) (Tosoh Bioscience, Tokyo, Japan), Gemini (5 µm) (Phenomenex, Torrance, CA), and Atlantis (3 µm) (Waters). Detection wavelength of 254 nm and sample injection of 20 µL using an auto sampler was used in the method. Linear regression data analysis was performed using SAS System JMP 4.0.4 software. A photostability chamber Caron Model 6540-2 (Caron, Marietta, OH) or Q-Sun Xenon Test Chamber (Q-Lab Corporation Westlake, OH) was used for stressing the sample [~100 mg of sample in a round 2 cm (i.d.) × 3 cm quartz cell with lid] with light consistent with the ICH guidelines (13).



R	Name	Approximate t_R (min)
CH_3	Methylparaben	1.3
C_2H_5	Ethylparaben	1.9
C_3H_7	Propylparaben	3.7
n-C_4H_9	Butylparaben	6.1

Figure 1. Scheme of the four parabens, namely methylparaben, ethylparaben, propylparaben, and butylparaben.

Sample preparation

For method validation experiments, solutions (linearity and accuracy) of each paraben were prepared by diluting the stock solution (0.25 mg/mL in acetonitrile, in triplicate) with mobile phase (acetonitrile– the water (34:66, v/v), to achieve the desired concentration level (150%, 125%, 100%, 75%, and 50%) relative to the analytical concentration (0.02 mg/mL, also referred as 100% level in this article). All samples and the mobile phase were stored at room-temperature.

Duplicate solutions of each of the four parabens at a concentration of approximately 0.02 mg/mL were also prepared to evaluate their solution stability at the ambient laboratory temperatures (RT) and under refrigeration (2°C to 8°C). Solid samples of all four parabens were stressed using heat (7 days, 80°C) and light (2 × ICH light conditions). Also all four parabens' solutions at analytical concentration level (0.02 mg/mL) were also stressed using 3% H_2O_2 (30 min) and analyzed. For acid and base stress studies, 10 mL of a paraben stock solution (0.2 mg/mL in acetonitrile) was mixed with 10 mL of 0.1 N HCl or 0.1 N NaOH and kept at room temperature for 24 h. After which a portion of the solution was neutralized using 0.1 N (or further diluted) HCl or NaOH solutions and diluted to analytical concentration before analysis.

Results and Discussion

General strategies and highlights of method development

The alkyl part of the ester functional group in parabens (Figure 1) plays the principal role in the separation of homologous parabens on reversed-phase columns. The retention times of parabens increase with increased hydrophobicity of the parabens [i.e., methylparaben (least hydrophobic) to butylparaben (most hydrophobic)]. Due to the difference in hydrophobicity, C18 HPLC columns are ideal for the chromatographic

separation of parabens. Based on this rationale, only C18 HPLC columns were selected for evaluation. A detection wavelength of 254 nm was selected since parabens are known to have significant absorption around 254 nm (7).

Parabens do not contain any easily ionizable functionality (acid or base) and therefore should not have any significant impact on chromatographic characteristics at different buffer and/or pH conditions of the mobile phase. Therefore, the different mobile phase conditions that were evaluated during method development were based on the variation of solvent strengths using various organic modifiers. Initial experiments were conducted using a computer-assisted chromatographic method development tool [Chromsword and an automated column switching system (LC Spiderling, Chiralizer, Newtown, PA)]. Chromsword is one of the computer-assisted chromatographic method development tools commercially available. Chromsword is capable of fully automated method development. The software can be used in two modes. In one mode, the analyst puts the chromatographic retention data (such as peak area, retention time, and peak half width) and mobile phase gradient information from real chromatographic runs into the software. The details, specifics and strategies of HPLC method development using a computer assisted method development tool has been reported in the literature (12).

Various experiments were conducted using each pair of the aqueous-organic combinations of the mobile phase (methanol–water and acetonitrile–water) on ACE C18, 50 × 4.6 mm column.

The retention time (RT), peak width at 50% peak height ($W_{1/2}$) and peak areas associated with the analytes obtained from these experiments were used to create the separation model on the Chromsword. A representative simulated chromatogram from the Chromsword separation model for methylparaben and propylparaben is provided in Figure 2. By sliding the modifier composition bar along the x-axis in the separation model, optimal chromatographic conditions [short isocratic method with tailing factor for both analytes ≤1.5; 2 ≤ retention factor (k) ≤ 108, and resolution ≥ 5] were selected. Next column screening was conducted using LC Spiderling automated column selector to search for the most suitable HPLC column. The LC Spiderling system has 9-column switching capability and can automatically test multiple mobile phase conditions on up to nine columns. Due to its automation capability, the LC Spiderling system allows the analyst to screen large number of HPLC columns with minimal supervision and in much shorter time compared to traditional one column HPLC systems. In this study, the column screening conducted included YMC Hydrosphere (3 µm) (YMC, Japan), TSK Gel Super ODS (2 µm) (Tosho Bioscience, Japan), Gemini C18 (5 µm) (Phenomenex), and Atlantis dC18 (3 µm) (Waters) (see Table I) columns. Based on the column screening experiments, the Atlantis dC18, 3 µm, 4.6 mm × 50 mm column was selected as the primary column as it showed desired separation [tailing factor ≤1.5; 2 ≤ k ≤ 810, and resolution ≥ 5)]. Final optimized chromatographic conditions for the method are summarized in Table II.

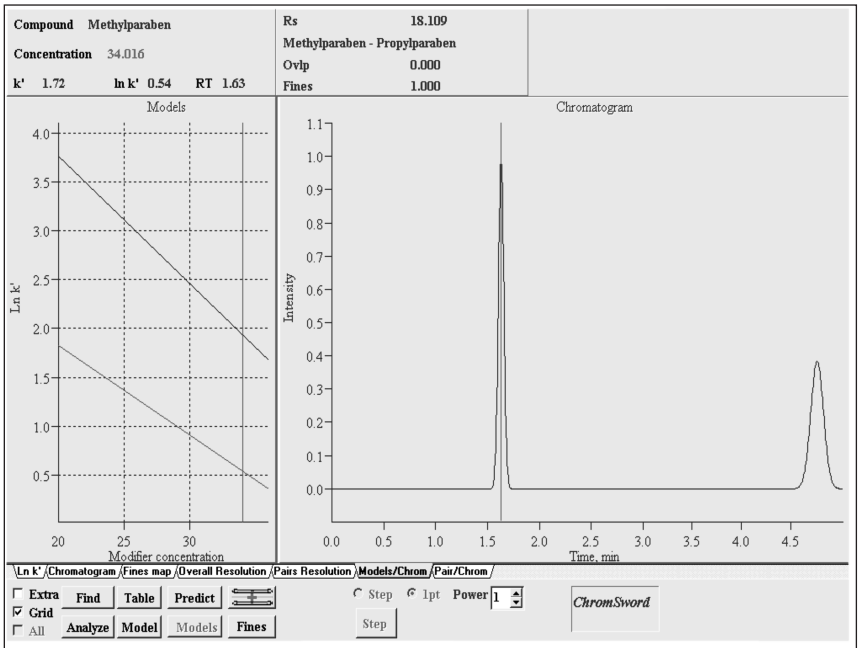


Figure 2. Simulated chromatogram of methylparaben and propylparaben on MAC-MOD Analytical Ace 3 C18 50 × 4.6 mm column at 34% acetonitrile–water mobile phase composition (flow rate = 1.0 mL/min).

Table I. HPLC Columns (50 × 4.6 mm) Screened for the Primary Column Selection

HPLC column	Particle Size, Pore Size, Carbon Loading and Surface area	Rt (min)* MP and PP	Resolution (Tailing)†	(MP) and (PP)
YMC Hydrosphere	3 µm, 120 Å, 12%, 340 m^2/g	1.1 and 2.6	9.5 (1.2)	1 and 4
Comments: Methylparaben elutes too close to solvent front (k less than 2)‡				
TSK-Gel Super ODS	2 µm, 110 Å, 8% and 96.8 m^2/g	1.9 and 5.5	12 (2.1)	3 and 10
Comments: Significant peak tailing for propylparaben				
ACE 3 C18	3 µm, 100 Å, 15.5%, 300 m^2/g	1.6 and 4.4	14 (1.1)	2 and 8
Comments: Good separation, nosignificant peak tailing				
Gemini C18	5 µm, 110 Å, 14%, 375 m^2/g	1.9 and 5.5	14 (1.2)	3 and 10
Comments: Good separation, no significant peak tailing				
Atlantis dC18	3 µm, 100 Å, 12%, 336 m^2/g	2.0 and 5.6	15 (1.3)	3 and 10
Comments: Good separation, no significant peak tailing				

* Flow rate = 1.0 mL/minute; column temperature = 35°C ± 5°C; MP = methylparaben; PP = propylparaben.
† In all cases tailing factor ≥ 1.0; highest tailing factor is reported.
‡ $k = (t_r - t_0)/t_0$, where t_R is retention time for the analyte and t_0 is column dead time.

Selection of an alternate column

During the life-cycle of an analytical method, the column of the validated HPLC method may no longer be commercially available. To mitigate the risk from such a scenario, an alternate column should be identified for the method. Two C18 HPLC columns [ACE 3 (3 µm) and

Gemini (5 µm)] that had similar carbon loading and surface area to the primary column [Atlantis (3 µm)] (Table III) were tested using the optimized isocratic conditions to identify the alternate column. As Gemini (5 µm) gave a separation profile similar to that of the primary column, it was selected as the alternate column for the new method.

Figure 3 shows representative chromatograms of all four parabens. After a few preliminary method validation experiments and PDA analysis of stressed samples, the final method (Table II) was subjected to the method validation studies.

Method validation studies

Accuracy, linearity, precision, range, specificity, and robustness characteristics of the analytical method (Table II) were validated. System suitability requirements [blank baseline: no interfering peak with signal-to-noise ratio (S/N) ≥ 10; standard check (≤ 2.0%), tailing factor (≤ 1.8), injector precision (five injections %RSD ≤ 2.0%)] were met prior to performing the method validation experiments. The samples assay values were calculated by comparing their responses to the bracketing standards injections. To ensure system suitability during the sample run, the % difference between the bracketing standard injections was calculated and evaluated against the acceptance criterion, (i.e., % difference between response factors for parabens from bracketing standard injections is not more than 2.0%).

Table II. Chromatographic Conditions of the Method

Equipment:	HPLC system with autosampler, column-temperature-controller and UV/PDA detector
Column:	Atlantis dC18, 3 µm, 4.6 mm × 50mm (Waters Corp.)
Alternate column:	Gemini C18, 5 µm, 4.6 × 50 mm (Phenomenex)
Column Temp.e:	35°C ± 5°C
Flow Rate:	1.5 mL/min
Injection Volume:	20 µL
UV Detector:	254 nm
Run Time:	~9 min
Mobile Phase:	Acetonitrile–water, 34:66 (v/v)
Analytical Method Concentration:	0.02 mg/mL
Diluent:	Mobile Phase

Table III. HPLC Columns (50 × 4.6 mm) Screened for the Alternate Column Selection

HPLC column	t_R (min)* MP and PP	Resolution (Tailing)†	k (MP) and (PP)
Atlantis dC18	1.3 and 3.7	12 (1.2)	3 and 19
Comments: Primary Column			
ACE 3 C18	1.0 and 3.0	12 (1.1)	2 and 8
Comments: Methylparaben elutes too close to solvent front			
Gemini C18	1.3 and 3.7	11 (1.1)	3 and 10
Comments: t_R for the analytes are similar to those obtained on primary column			

* Flow rate = 1.5 mL/min; column temperature = 35°C ± 5°C; MP = methylparaben; PP = propylparaben.
† In all cases tailing factor ≥ 1.0; highest tailing factor is reported.

Accuracy (recovery)

The method accuracy (recovery was demonstrated by analyzing separate solutions of each of the four parabens at 50%, 75%, 100%, 125%, and 150% (triplicate at each level) of the analytical (procedural) concentration (0.02 mg/mL). This validation characteristic was evaluated on two different days by two analysts on two different HPLC instruments. The recovery for all sample preparations was 98.1–102.8% and met the acceptance criterion (97.0–103.0%) (Table IV). The assay accuracy (recovery) was also evaluated for the alternate (equivalent) column (Phenomenex, Gemini C18, 5 cm × 4.6 mm i.d., 5 µm) using the same solution concentrations. The recovery range was 99.0–100.4% on the alternate column.

Linearity

Linearity for the assay using the primary HPLC column was demonstrated by analyzing the peak response versus the analytical concentration from the data generated during the accuracy (recovery) studies. A linear least square analysis of the data gave correlation coefficient (r) ≥ 0.9999 for the range of 50–150% for each paraben. The y-intercepts obtained were ≤ 0.5% of the 100% level indicating that there is no significant bias for quantitation of any of the parabens within the defined range (50–150%) of the analytical method. Linearity for all four parabens (50–150% relative to the analytical concentration) was also

Table IV. Accuracy and Recovery* on Primary and Alternate Column

Sample	Primary Column† Analyst 1 (Day 1)	Primary Column† Analyst 2 (Day 2)	Alternate Column‡ Analyst 1
Methylparaben	99.4–100.1%	99.4–100.9%	99.4–100.3%
Ethylparaben	99.5–102.8%	99.5–100.7%	99.3–100.2%
Propylparaben	99.6–100.2%	99.2–100.4%	99.6–100.2%
Butylparaben	100.0–101.1%	98.1–101.8%	99.0–100.4%

* Acceptance criterion: (97.0–103.0%).
† Waters Atlantis dC18, 5 cm x 4.6 mm i.d., 3 µm.
‡ Phenomenex, Gemini C18, 5 cm x 4.6 mm i.d., 5 µm.

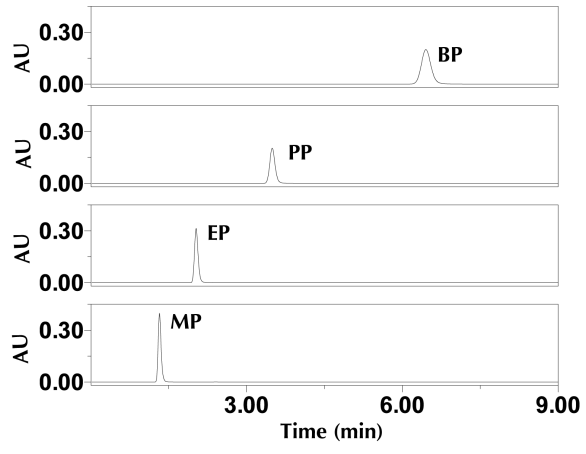


Figure 3. Representative chromatograms of methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) at 100% analytical concentration (0.02 mg/mL).

demonstrated on the alternate column. Linearity data is summarized in Table V.

Precision (Repeatability and Intermediate)

Method precision (repeatability) was demonstrated by calculating the percent relative standard deviation (%RSD) of the [low (50%), middle (100%) and high (150%)] percent recoveries (n = 9) of the samples used to determine accuracy (recovery). For all four parabens intermediateprecision was also successfully demonstrated by comparing precision-repeatability data obtained by analyst 1 and analyst 2. Results are summarized in (Table VI). It should be noted that the repeatability (%RSD) for Analyst 2 for butylparaben is greater (although within the acceptance criterion of ≤ 3.0%) than all of the other repeatability results.

Table V. Linearity* on Primary and Alternate Column

	Primary Column					
	Analyst 1 (Day 1)		Analyst 2 (Day 2)		Alternate Column	
Sample	r	y-intercept	r	y-intercept	r	y-intercept
Methylparaben	1.0000	–0.4%	0.9999	0.5%	0.9999	0.3%
Ethylparaben	0.9998	0.2%	1.0000	–0.3%	0.9999	–0.3%
Propylparaben	1.0000	–0.2%	0.9999	0.1%	1.0000	0.2%
Butylparaben	0.9999	0.3%	1.0000	0.5%	0.9999	–0.0%

* Acceptance criteria: $r \geq 0.9900$ for 50 to 150% range and y-intercept NMT (not more than) ± 3% of the 100% response.

Table VI. Precision (Repeatability and Intermediate)

	Repeatability (%RSD)*		
Sample	Analyst 1	Analyst 2	Intermediate (%RSD)†
Methylparaben	0.2	0.3	0.1
Ethylparaben	0.1	0.3	0.3
Propylparaben	0.2	0.5	0.3
Butylparaben	0.3	1.5	1.2

* Acceptance criteria: %RSD obtained for the 50, 100, and 150% sample recovery levels (n = 9) should be ≤ 3.0%.
† The percent RSD achieved by each of the two (2) analysts should not differ more than ± 2.0% (absolute difference).

Assay Range

Based on the linearity, accuracy/recovery, and precision (repeatability and intermediate) results, the assay range of 0.01 mg/mL (50%) to 0.03 mg/mL (150%) is considered validated for methylparaben, ethylparaben, propylparaben, and butylparaben.

Method Specificity

Method specificity was established by demonstrating that there is no interfering peak from the diluent or potential degradants. The specificity was demonstrated by performing peak homogeneity on the stress degraded samples of paraben peaks (Table VII) using a HPLC PDA detector. Peak homogeneity (purity) was assessed by comparing the peak purity angle with the threshold angle for methylparaben and propylparaben peaks. A peak purity angle that is less than its threshold angle indicates that peak is spectrally homogenous (14). On the other hand, a peak purity angle greater than its threshold angle indicates that the peak is spectrally inhomogenous and possibly has a co-eluting peak(s).

Solid samples of all four parabens were stressed using heat (7 days, 80°C) and light [2 × ICH light conditions; total exposure of 2.4 million lux h and integrated near UV energy of 400 watt h/square meter (13)]. Also all four parabens' solutions at analytical concentration level (0.02 mg/mL) were stressed using 3% H_2O_2 (30 min), acidic conditions (0.05 N HCl for 24 h) and basic conditions (0.05 N NaOH for 24 h). No significant degradation was observed for light and heat stressed samples. This indicates that parabens have good stability towards heat and irradiation to light. However, more degradation occurred under basic conditions compared to acidic stress condition. The principal degradation pathway for parabens is hydrolysis of the ester group in the presence of an acid or base. As base assisted hydrolysis of the ester group is much more facile than the acid catalyzed hydrolysis, higher amount of degradation was observed in basic condition. In all cases the principal degradant, identified as p-hydroxy benzoic acid (RT ~ 0.6 min), and the diluent peaks were well separated from the main analyte peak. Degradant, p-hydroxy benzoic acid, was confirmed by injecting a reference solution of p-hydroxy benzoic acid (0.02 mg/mL). Representative chromatograms of ethylparaben and butylparaben from acidic and basic stress conditions are shown in Figure 4. Figure 5 shows the

Table VII. PDA Peak-Purity Results (Method Specificity)*

		Methylparaben		Ethylparaben		Propylparaben		Butylparaben	
Stress Condition	Expose Time and condition	% deg†	Peak Homogeneity	% deg†	Peak Homogeneity	% deg†	Peak Homogeneity	% deg†	Peak Homogeneity
None	Control at RT	0.00	Pass	0.00	Pass	0.00	Pass	0.00	Pass
3% H_2O_2	30 min at RT	0.05	Pass	0.10	Pass	0.00	Pass	0.08	Pass
Heat	7 days, 80°C	0.00	Pass	0.03	Pass	0.00	Pass	0.00	Pass
Light	2 × ICH‡	0.00	Pass	0.00	Pass	0.00	Pass	0.00	Pass
~ 0.05 N HCl	24 h, RT	0.6	Pass	0.00	Pass	0.89	Pass	0.0	Pass
~ 0.05 N NaOH	24 h, RT	13.6	Pass	3.7	Pass	3.0	Pass	1.6	Pass

* Acceptance criteria: (a) separation of all degradant and diluent peaks from the peak of interest, and (b) peaks of stressed samples pass peak purity (homogeneity) test.
† %degradation = % of total peak area from degradants.
‡ Light Condition: Total exposure of about 2.4 million lux hours and an integrated near UV energy of 400 watt h/meter square.

analytes chromatograms from oxidative (3% H_2O_2) stress study. Very limited degradation (~0.1%) was seen under the strong oxidative conditions. In all stress studies, the paraben peaks were found to be spectrally homogenous and well separated from degradants and diluent peaks demonstrating method's specificity.

Robustness

HPLC parameters variation. Small but deliberate variations in HPLC parameters were made to verify the robustness of the analytical method. Variations made in the HPLC parameters are consistent with those typically studied for the methods submitted to the regulatory agencies. The HPLC parameter variations studied include, flow rate (1.5 ± 0.1 mL/min), column temperature (35 ± 5°C), wavelength (254 ± 2 nm), injection volume (20 ± 5 µL), a different lot of the column and acetonitrile content in the mobile phase [34 ± 4% (absolute)]. It should be noted that for butylparaben the lowest validated range for acetonitrile content in the mobile phase is 32% (30% for all other parabens). This is because decreasing the mobile phase strength lower than 32% acetonitrile–water causes butylparaben to retain longer than the runtime specified in the method.

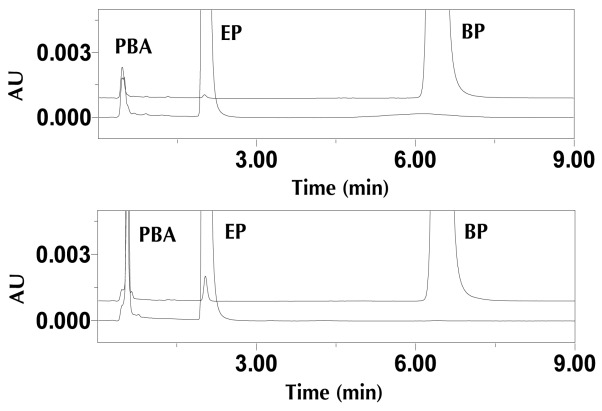


Figure 4. Representative chromatograms of analyte under various stress conditions: A. Ethylparaben and Butylparaben in acidic (0.05 N HCl, 24 h) conditions; B. Ethylparaben and Butylparaben in basic (0.05 N NaOH, 24 h) conditions. PBA: p-hydroxy benzoic acid; EP: Ethylparaben; BP: Butylparaben.

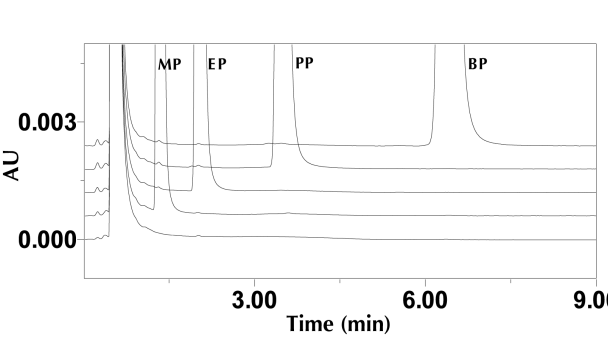


Figure 5. Chromatograms of methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BP) at 100% analytical concentration (0.02 mg/mL) and blank stressed with 3% H_2O_2 for 30 min.

For all the HPLC variations, the chromatography was comparable to the procedural conditions in terms of for the assay, tailing, and injection precision. Based on these robustness, the method is considered robust under the variation ranges studied.

Solution stability

Solution stability of each paraben (in duplicate) was investigated by storing preparations at room temperature (RT) and under refrigeration (i.e., 2°C–8°C). Solutions were stored up to seven (7) days. The HPLC analysis of the solutions stored for 1, 3, and 7 days versus freshly prepared standards showed no significant changes in the assay values. The maximum change in the assay value was 0.8% and 0.9% for paraben solutions stored at RT and under refrigeration, respectively. Based on these solution stability results, all four paraben solutions are considered stable up to seven days when stored at room temperature (RT) or under refrigeration (i.e., 2°C–8°C).

Method application

The method was employed to conduct the assay of a commercial batch of methylparaben using USP reference standard. Solutions of methylparaben sample (triplicate preparations) and the USP reference standard (duplicate preparations) were prepared at the analytical concentration (0.02 mg/mL) in acetonitrile–water (34:66, v/v). Assay values of 99.6%, 98.9%, and 99.7% (mean = 99.4%) were obtained which met the pre-established acceptance criterion of 98.0–102.0%. Assay values of the sample were also consistent with the assay values obtained using mass balance equation [i.e., assay (mass balance) = chromatographic purity X (100 – %inorganic impurities – %volatiles) / 100] (99.9%) and titration (100.1%). These results clearly demonstrate that the new method presented in this report is suitable for the analysis and assay of commercial bulk raw material lots of methylparaben.

Conclusion

A QC friendly, efficient, reproducible, and robust RP-HPLC method has been successfully developed and fully validated as per ICH guidelines for the assay of methylparaben, ethylparaben, propylparaben, and butylparaben in the commercial bulk raw material lots of each of the four parabens. This method can also be used to characterize and/or certify reference standard lots of each of the four parabens. This method is stability indicating as it can adequately separate all four parabens and other degradation products from each other as demonstrated by analysis of acid, base, light, heat, and 3% H_2O_2 stress degraded samples. This analytical method would be valuable for QC laboratories because this method can replace four methods that are currently used to test and release the commercial bulk lots of four parabens. As the new method is a simple, rugged, and reproducible method, it can be easily implemented in a quality control laboratory. To the best of our knowledge, this is the first fully ICH validated RP-HPLC method that can be used as a single method to accurately assay methylparaben, ethylparaben, propylparaben and butylparaben in commercial bulk lots of these four parabens in less than 10 min.

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