APPLICATION NOTE

Liquid Chromatography

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Rapid UHPLC Determination of Common Preservatives in Cosmetic Products

Introduction

P-hydroxybenzoic acid esters also called parabens (Figure 1 – Page 2) are widely used as antimicrobial preservatives in cosmetic products. Parabens have a potential of endocrine effect from weak estrogenic activity, and it is known that some estrogens can drive the growth of tumors. As a result their use is limited in the EU countries to a concentration up to 0.4% when used individually and 0.8% as a mixture. In the U.S., under the FDA and Cosmetic Act (FD&C Act), it is against the law to market products with ingredients that may cause injuries under label conditions. Thus, in the U.S. parabens are typically used at levels ranging from 0.01 to 0.3%. Because of these concerns and regulations, it is necessary to control the level of parabens in cosmetic products, and therefore methods to quantify paraben levels are needed. This application note will present a fast, sensitive and reliable UHPLC analysis of six common parabens.

The method was developed using a 1.9 µm LC column to achieve very high throughput at a low flow rate, to improve throughput while reducing solvent consumption. The throughput will be compared to that of a conventional HPLC analysis with a 5 µm particle column. In addition to throughput comparisons, method conditions and performance data including precision and linearity are presented. The results of the method applied to a sample of hydrating face lotion and a sample of body lotion are reported.

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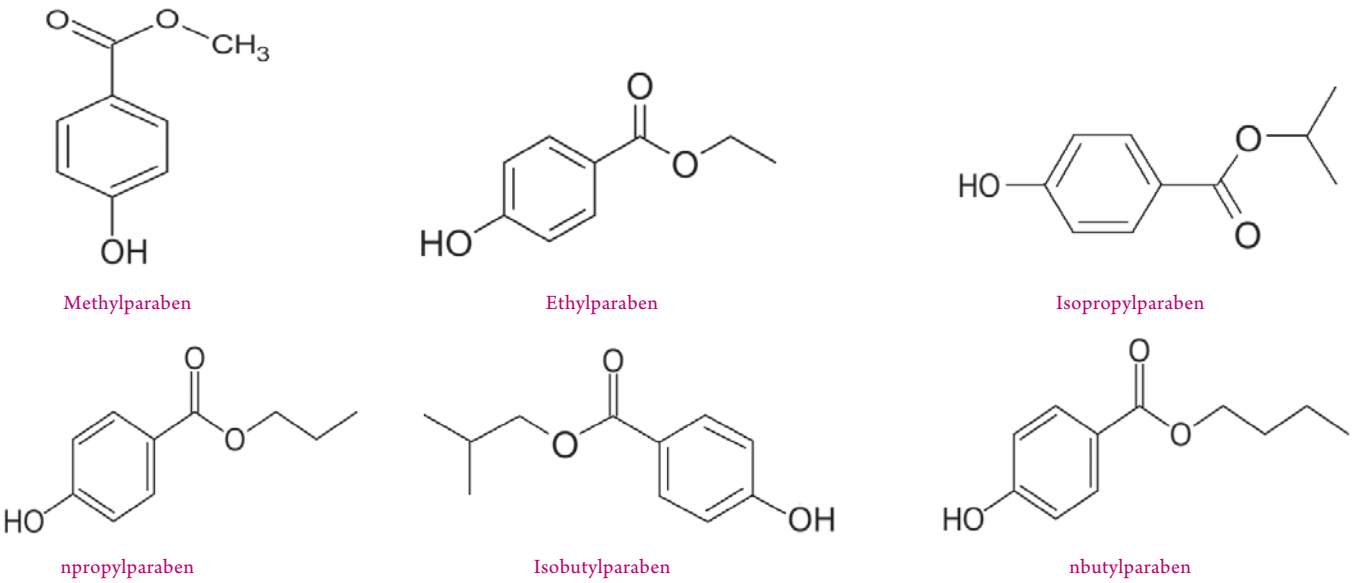

Figure 1. Names and structures of six parabens studied.

Experimental

A stock solution containing 250 µg/mL of each paraben was prepared by dilution from neat material. Working standards with 5000 ng/mL of each paraben were prepared from the stock standard using 50/50 (v/v) methanol/water as a solvent.

Repeatability was studied with six injections of the working standard. Linearity was determined across the range of 60 – 5000 ng/mL with injections at concentrations of 60, 125, 250, 500 and 5000 ng/mL.

Samples of a face hydrating lotion and a body lotion were tested. Solutions of 0.1 g/mL of each lotion were diluted with methanol, vortexed for three minutes, allowed to stand for three minutes, vortexed a second time for three minutes and centrifuged at 5000 RPM for ten minutes. The supernatant was used to prepare a 10 mg/mL sample solution filtered with a 0.2 µm nylon membrane prior to dispensing into testing vials.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was used. The separation was achieved using a PerkinElmer Brownlee™ Analytical C18, 1.9 µm 50 mm x 2.1 mm column. The run time was approximately 3.5 min with a back pressure of 7000 PSI (483 bar).

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC Setting: 50 µL loop and 15 µL needle volume, partial loop mode Injection: 2 µL for UHPLC column, 10 µL for HPLC column
Oven:	Flexar Peltier Column Oven
Detector:	Flexar FX PDA UHPLC Detector
Analytical Wavelength:	254 nm
Pump:	Flexar FX-15 UHPLC Pump
UHPLC Column:	PerkinElmer Brownlee Analytical C18 DB, 1.9 µm, 50 x 2.1 mm (Cat # N9303853)
HPLC Column:	PerkinElmer Brownlee Spheri-5 RP-18, 5 µm, 100 x 4.6 mm (Cat # 0711-0015)
Column Temperature:	Ambient, 45° C
Mobile Phase:	B: 70/30 (v/v) acetonitrile/methanol, A: 0.02% formic acid in water (HPLC grade solvent and ACS grade reagent)

Conventional C18 HPLC column

Time (min)	Flow rate (mL/min)	B %	Curve
10	1.5	55	1
9	1.5	55-65	1

UHPLC C18 column

Time (min)	Flow rate (mL/min)	B %	Curve
2	0.7	54-56	1
1.5	0.7	56-72	1

Software:	Chromera® Version 3.0

Results and discussion

Initially, the method was developed with a conventional C18 100 x 4.6 mm, 5 µm particle size HPLC column. The optimal flow rate of this method was determined to be 1.5 mL/min at ambient temperature. All the parabens peaks eluted within 19 min (Figure 2). By using a shorter column with smaller particle size (C18 50 x 2.1 mm, 1.9 µm particle size) suitable for UHPLC, the run time was dramatically reduced from 19 min to about 3.5 min (Figure 3).

In addition to the greater than five times reduction in chromatographic run time, the resolution of analyte peaks and sensitivity of the determination were improved. The optimal flow rate was 0.7 mL/min at a temperature of 45 ˚C. An improved separation with sharper peaks and better signal to noise characteristics was obtained.

Greater than 90% solvent reduction was achieved by moving to the UHPLC method. The final analysis was completed in 3.5 minutes with a total solvent usage of 2.45 mL per injection, an impressive improvement from 19 minutes run time and 28.5 mL solvent usage when the conventional HPLC column was used. This is important not only because of the relatively high cost of HPLC-grade solvents, but also because far less solvent must be disposed of as waste. This results in a much lower cost of ownership and a much greener lab operation.

Excellent method performance was achieved. The linearity of the analysis achieved an average r^2 value of 0.9999. The average precision ranged from 0.9 - 1.1% RSD. Details of the method performance are presented in Table 2.

A spectrum of each paraben was obtained from the analysis of the standard solution over a range of 190 nm to 700 nm, and the wavelength maximum was determined, enabling a choice of suitable wavelength setting for the parabens tested. A spectral library was created within Chromera using the standard preparation run, and was used to confirm the identity of peaks in lotion samples.

The spectrum of two parabens and an annotated UHPLC chromatogram of the body lotion sample are shown in Figures 4 and 5 (Page 4).

Confirming the identity of compounds in the chromatogram of a known or an unknown sample is an important aspect of quality assurance, and adds another level of confidence to the analysis. Confirmation of the presence of ethylparaben in the hydrating face lotion and methylparaben in the body lotion samples is demonstrated in Figures 6 and 7 (Page 4). In these figures, the spectra at the peak apex of the highlighted peaks are compared with the spectra of the standards to confirm peak identification.

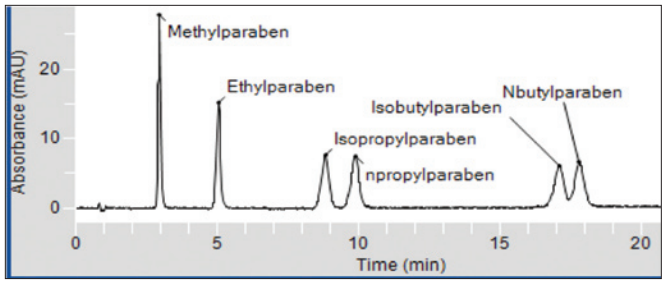

Figure 2. Example chromatogram from the analysis of a standard of common parabens in cosmetics with conventional HPLC C18 100 x 4.6 mm, 5 µm particle size column.

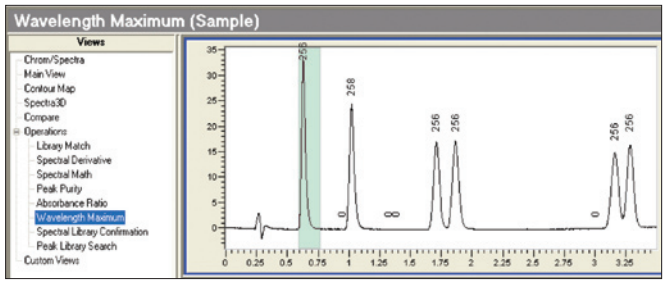

Figure 3. Example chromatogram from the analysis of a standard solution with a UHPLC 50 x 2.1 mm 1.9 µm particle size column showing the wavelength maximum of each peak.

Table 2. **Precision, Linearity and Recovery.**

	Methylparaben	Ethylparaben	Isopropylparaben	npropylparaben	Isobutylparaben	nbutylparaben
Response precision (% RSD) n = 6	1.1	1.0	1.0	1.1	1.0	0.9
Linearity (R^2)*	0.9999	0.9999	0.9999	0.9999	0.9999	1.0000
Face Hydrating Lotion	0.11%	0.03%	ND	0.01%	0.01%	0.02%
Body Lotion	0.12%	ND	ND	ND	ND	ND

** 60, 125, 250, 500 and 5000 µg/mL solution, 1 injection per level; ND – not detected.*

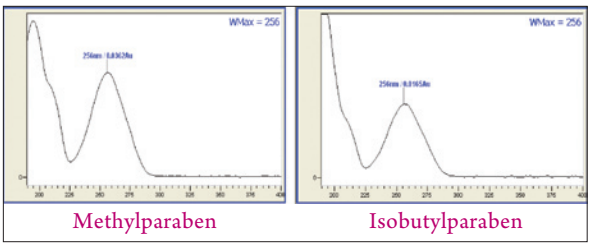

Figure 4. Stored spectra of two parabens from the analysis of a standard solution.

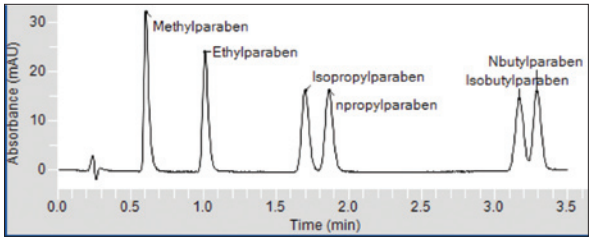

Figure 5. Example chromatogram from the analysis of a standard solution of common parabens in cosmetics with a UHPLC 50 x 2.1 mm 1.9 µm particle size column.

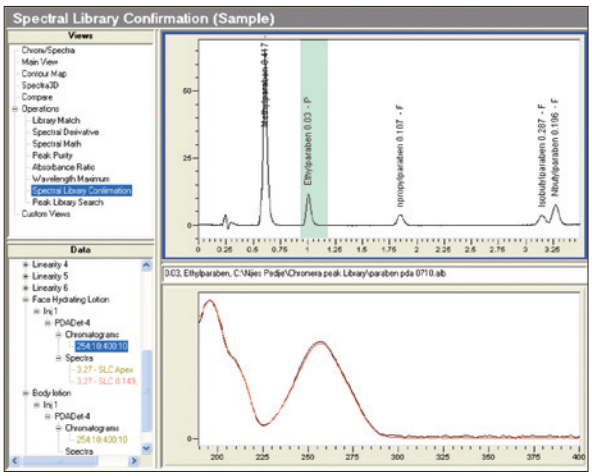

Figure 6. Chromatogram of the analysis of a hydrating face lotion and the spectra confirmation using a Chromera PDA spectral library.

Conclusion

The application of UHPLC to the analysis of common anti-microbials in cosmetics has resulted in a 15 minute, or about 80% reduction in run time, as well as a reduction of solvent usage of 26 mL, or about 90%. The PerkinElmer Flexar FX-15 UHPLC system and Brownlee Analytical C18, 1.9 µm 50 x 2.1 mm column, resolved all of the six parabens studied in about three and half minutes.

The method was shown to be linear and the peaks were well resolved. The level of preservative in lotions tested were in compliance with the EU regulation as well as with the U.S. Cosmetic Ingredient Review (CIR) recommendations. The PerkinElmer FX PDA provides a rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3D and contour maps, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectra library search function allowed the storage of standard parabens spectra, which could be later used for peak identification and confirmation in the lotion samples.

References

1. Robert Golden, Jay Gandy, Guenter Vollmer Critical Review in Toxicology, Vol. 35: 435-458, (2005).

2. European Scientific Committee on Consumer Product (SCCP), SCCP/0873/05.

3. Federal Food, Drug, and Cosmetic Act (FD&C Act) Chapter VI: Cosmetic Sec. 601 [21 USC § 361] Adulterated cosmetics.

4. Cosmetic Ingredient Review (CIR), cosmetic ingredient found safe as used. 2007 Compendium.

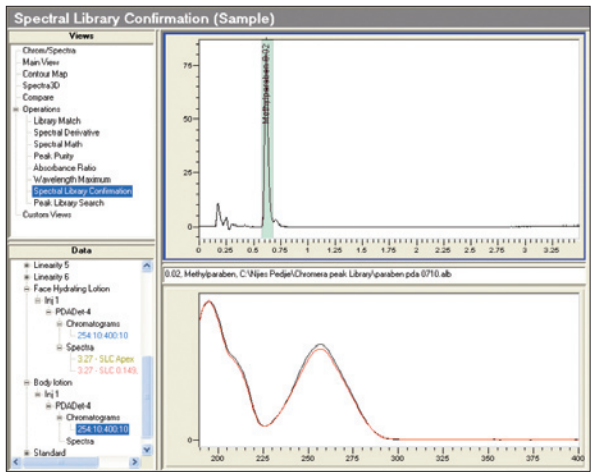

Figure 7. Chromatogram of the analysis of a body lotion and the spectra confirmation using a Chromera PDA spectral library.

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