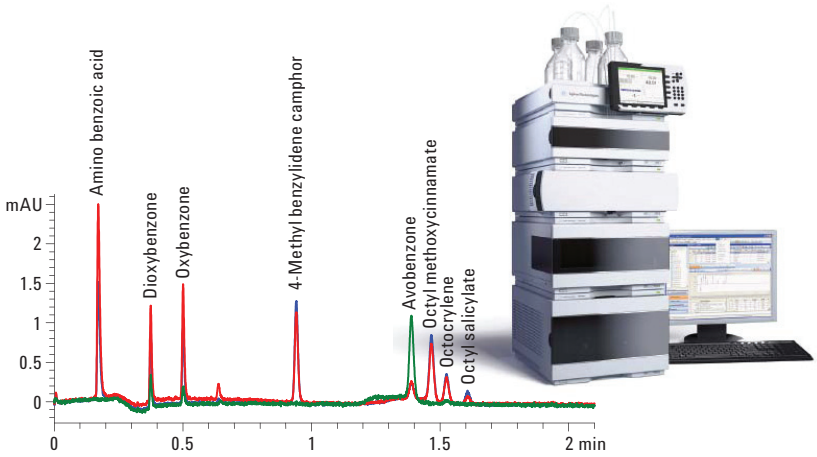
Agilent 1290 Infinity LC with Agilent Poroshell columns for simultaneous determination of eight organic UV filters in under two minutes

Application Note

Consumer Products



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Abstract

Levels of UV filters in personal care products are regulated by the FDA and European Pharmacopeia (EP). Liquid chromatographic (LC) methods are widely accepted analytical techniques for the qualitative and quantitative analysis of these UV filters. Most of these traditional LC methods require about 25–50 minutes. In this Application Note, the Agilent 1290 Infinity LC, in combination with Agilent Poroshell columns, were used for development of a short, sensitive, robust and well resolved separation of eight FDA/EP approved active UV filter ingredients in 99 seconds. Standard deviation (SD) and relative standard deviation (RSD) values of retention time for replicate injections confirmed the excellent performance of the Agilent 1290 Infinity Binary Pump. Exceptional performance of the Agilent 1290 Infinity Diode Array Detector was established by minimum area RSD values and a wide linear range with standard organic UV filters in amounts from 0.25 ng to 200 ng on-column. In addition, the method was effectively used to identify active UV filters extracted from six international personal care products.

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Introduction

The wavelength of UV rays lies between X-rays and visible light (~ 10 – 400 nm)[1]. Production of melanin, a pigment that causes the darkening of skin, is a natural defense of the human body against UV radiation. Melanin absorbs UV radiation and dissipates the energy as harmless heat, though the response to UV radiation and production of melanin pigmentation depends on skin color and other genetic factors [2, 3]. The intensity of UV radiation and length of exposure are the main parameters involved in sunburn, irrespective of skin tone and ability of the skin to produce melanin. The major classifications of UV light are presented in Table 1[4].

Sl No:	Component	Wavelength (nm)	Effects of over exposure on skin
1	UV-A I	340-400	Can cause tanning but has minimal erythemal effect. Can cause long term damage. Penetrates deeply. it can contribute to skin cancer via indirect DNA damage.
2	UV-A II	320-340	Slightly erythemal contribution
3	UV-B	290-320	Causes sunburn and is a major contributor to skin cancer development.
4	UV-C	100-290	Very energetic radiation. Absorbed by the ozone layer. Direct DNA damage

Table 1
Major classifications of UV radiation.

Sunscreens protecting the skin against sunburn contain one or more of the following types of active ingredients:

- Organic chemicals that absorb UV light.
- Inorganic particulates that reflect, scatter, and absorb UV light.
- Organic particulates that have all the above features.

Organic UV filters are usually aromatic compounds conjugated with carbonyl groups. The FDA has approved seven UV-A filter compounds and nine UV-B filter compounds for sunscreen formulations in the United States, while the European Commission has approved the use of ten additional UV filters in European countries[5].

Though several approved UV filters are available in the market, extensive use of these UV filters may have several major concerns. Some sunscreen ingredients have been shown to have carcinogenic properties. Additionally, older and more widespread sunscreen chemicals cannot dissipate the energy of the excited state as efficiently as melanin, therefore the penetration of these sunscreen ingredients into the lower layers of the skin may increase the amount of free radicals and reactive oxygen species[6]. Therefore, extensive testing of sunscreens is advisable to reveal the efficacy of the ingredients. This Application Note discusses a short LC method to separate eight widely used UV filters within 99 seconds. Cosmetic manufacturers can adopt this method to simplify the analysis of sunscreen raw materials and personal care products in product development, regulatory compliance, and quality control to increase the efficiency of analysis.

Experimental

Instrument configuration

An Agilent 1290 Infinity LC, controlled by ChemStation (Version B.04.02) and equipped with a binary pump with integrated vacuum degasser, autosampler, thermostatted column compartment and a diode array detector, was used for data collection. The injection volume was set to 1 µL and the needle wash was enabled using acetonitrile for three seconds. The sample thermostat was set at 5 °C, while the columns were operated at 55 °C. The binary pump was operated at a flow rate of 1 mL/min. The detector was programmed for three different wavelengths (288, 304 and 358 nm) and operated at a sampling acquisition rate of 80 Hz (response time 0.062 seconds, >0.003 min). An Agilent Poroshell 120 EC-C18 column (75 mm × 2.1 mm, 2.7 µm) was used for the chromatographic separation.

Chemicals and standards

All eight UV filter standards and acetic acid (mobile phase modifier) were purchased from Aldrich (India). Super gradient grade acetonitrile (ACN) was purchased from Lab-Scan (Bangkok, Thailand). HPLC grade water was freshly taken from a Milli-Q water purification system. Six different brands of international sunscreen formulations were purchased locally. The details of organic UV filter standards used in this study are tabulated in Table 2.

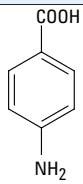
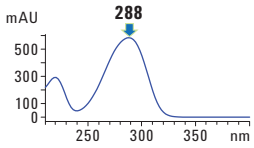
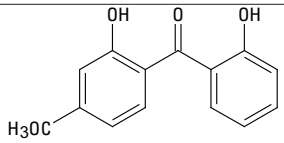
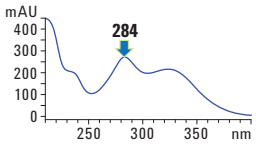
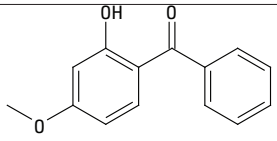
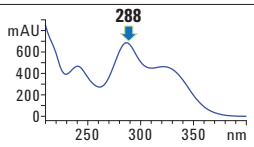
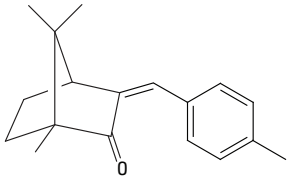
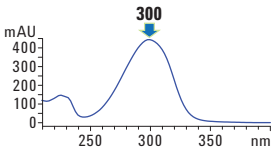
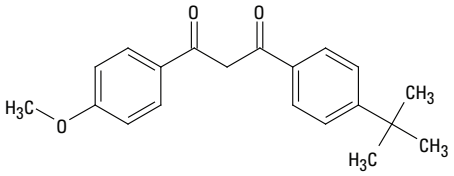
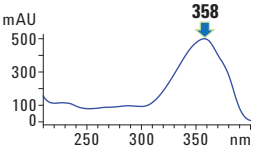
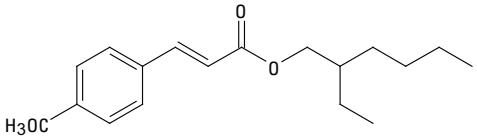
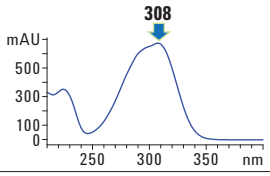
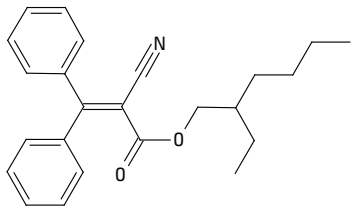
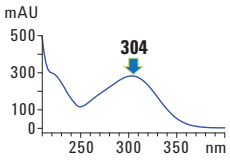
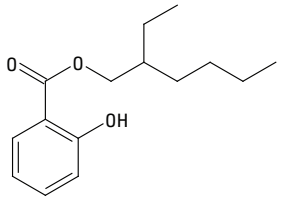
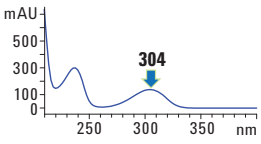
Compound number	Compound	Structure	UV Spectra
1	Name: Aminobenzoic acid CAS No: 150-13-0 Mol Formula: $C_7H_7NO_2$ Mol Wt: 137.14 Detection: 288 nm		
2	Name: Dioxybenzone CAS No: 131-53-3 Mol Formula: $C_{14}H_{12}O_4$ Mol Wt: 244.24 Detection: 288 nm		
3	Name: Oxybenzone CAS No: 131-57-7 Mol Formula: $C_{14}H_{12}O_3$ Mol Wt: 228.24 Detection: 288 nm		
4	Name: 4-Methyl benzylidene camphor CAS No: 36861-47-9 Mol Formula: $C_{18}H_{22}O$ Mol Wt: 254.37 Detection: 304 nm		
5	Name: Avobenzone CAS No: 70356-09-1 Mol Formula: $C_{20}H_{22}O_3$ Mol Wt: 310.39 Detection: 358 nm		
6	Name: Octylmethoxycinnamate CAS No: 5466-77-3 Mol Formula: $C_{18}H_{26}O_3$ Mol Wt: 290.397 Detection: 304 nm		
7	Name: Octocrylene CAS No: 6197-30-4 Mol Formula: $C_{24}H_{27}NO_2$ Mol Wt: 361.48 Detection: 304 nm		
8	Name: Octyl salicylate CAS No: 118-60-5 Mol Formula: $C_{15}H_{22}O_3$ Mol Wt: 250.33 Detection: 304 nm		

Table 2
Detailed list of organic UV filter standards used in this study.

LC parameters

Premixed solutions of 0.1% acetic acid in water and acetonitrile in the ratio 90:10 (A) and 10:90 (B) were used as mobile phase. The gradient used for the study is presented in Table 3. A post run time of 1 minute was set for column re-equilibration.

Time (min)	B (%)
0	50
0.1	70
2	85

Table 3
Gradient used for experiment.

Standard mix

A premixed solution of acetonitrile and 0.1% acetic acid in the ratio 50:50 was used as the diluent. A stock solution of each standard was prepared individually at a concentration of 1000 ppm (1000 ng/µL). A standard mixture of p-aminobenzoic acid, dioxybenzone, oxybenzone, 4-methyl benzylidene camphor, avobenzone, octyl methoxycinnamate, octocrylene and octyl salicylate, all 100 ppm (ng/µL) each, was prepared by diluting individual standard stock solutions using diluent. For detector linearity analysis, seven more mixed standard solutions with analyte concentrations of 50, 25, 10, 5, 1, 0.5 and 0.25 ng/µL were prepared by subsequent dilution of the higher concentrated standard mix.

Extraction of UV filters from formulation samples

UV filters from six different locally available international brands were extracted by a simple extraction procedure using acetonitrile. Two hundred fifty milligrams of each formulation were extracted with 5 mL of acetonitrile, sonicated and centrifuged. The supernatant liquid was filtered using 0.2 µ Agilent syringe filters (p/n 5061-3361). The resulting filtrate was diluted five times with diluent to get a stock solution of extracted sample. A diluted extracted sample for injection was prepared by further diluting the stock solution with equal volumes of diluent and injecting 1µL. Extracted samples spiked with standards were prepared by mixing equal volumes of 100 ppm standard mix solution and extracted sample stock solution. This spiked sample was used for the confirmation of peak identity in extracted samples by means of retention time and UV spectra.

Procedure

A blank injection was performed in all trials to check the chromatographic interference in the resolution. Standard mix, linearity levels, diluted extracted samples and spiked diluted extracted samples were also injected. The retention time of each standard was confirmed by individual standard injections.

Results and Discussion

LC chromatogram of standard mixture

The results showed excellent baseline separation of all eight active sunscreen ingredients, without chromatographic blank interference. The last peak of the standard mix (octyl salicylate) eluted at 1.62 minutes. A chromatographic representation of the standard mix is as shown in Figure 1. An unknown peak was observed at approximately 0.65 minute, which is an impurity present in the avobenzone standard. A peak purity check by spectral scanning in the range of 200 to 400 nm revealed that all eight compounds eluted without co-elution of any detectable impurities. Three different wavelengths were selected for detection as the maximum absorbance values vary for individual components. The peak width (half height), peak symmetry, USP tailing factor, and resolution values confirm the baseline separation of all the standard analytes in 99 seconds using the Agilent Poroshell 120 EC-C18 column (Table 4).

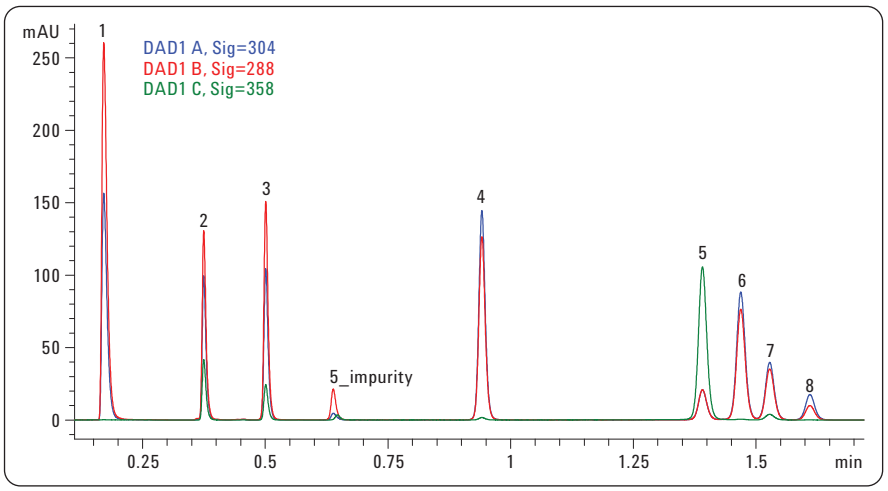


Figure 1
Chromatographic elution profile of eight organic UV filters in 99 seconds using an Agilent 1290 Infinity LC and an Agilent Poroshell 120 EC-C18 column (three different wavelengths are overlaid).

Comp No:	Name of the compound	Half peak width	Symmetry	USP tail	Resolution
1	Aminobenzoic acid	0.011	0.556	1.438	NA
2	Dioxybenzone	0.008	0.648	1.449	12.662
3	Oxybenzone	0.009	0.720	1.306	8.794
4	4-Methyl benzylidene camphor	0.014	0.862	1.112	22.988
5	Avobenzone	0.018	0.896	1.090	16.456
6	Octyl methoxycinnamate	0.019	0.920	1.070	2.453
7	Octocrylene	0.019	0.912	1.061	1.805
8	Octyl salicylate	0.020	0.910	1.061	2.447

Table 4
Peak width (half height), peak symmetry, USP tailing factor, and resolution values of the eight sunscreen ingredient standards in an injection of a standard mix (50 ng) with detection at 304 nm. Injection volume was 1 µL.

The chromatographic overlay of six replicates at 10 ng/µL confirms the excellent reproducibility of the data (Figure 2). Despite similarities in chemical structures, the components are well resolved within 99 seconds. At this level, the observed standard deviation (SD) value for retention time (RT) was < 0.0005, relative standard deviation (RSD) was < 0.32% and area RSD value was < 1.43%.

Signal-to-noise ratio

Figure 3 shows the chromatogram of the standard mix where all the analytes were at 0.25 ng on-column concentration. The observed signal-to-noise (S/N) values for each standard is calculated by taking the signal from a readily detectable peak height for each component and noise as absolute noise from the baseline in a compound-free area. The S/N values along with SD, and RSD values are tabulated in Table 5. At this concentration, S/N values for the first six compounds are > 20 and a least S/N value of 5 was observed for octyl salicylate (compound 8).

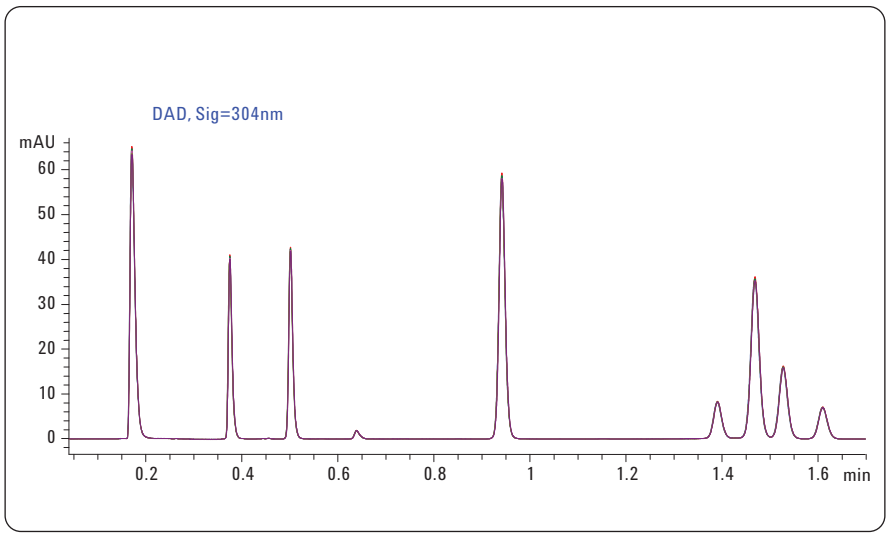


Figure 2
Chromatographic overlay of six replicates of standard mix injections at 10 ng/µL level.

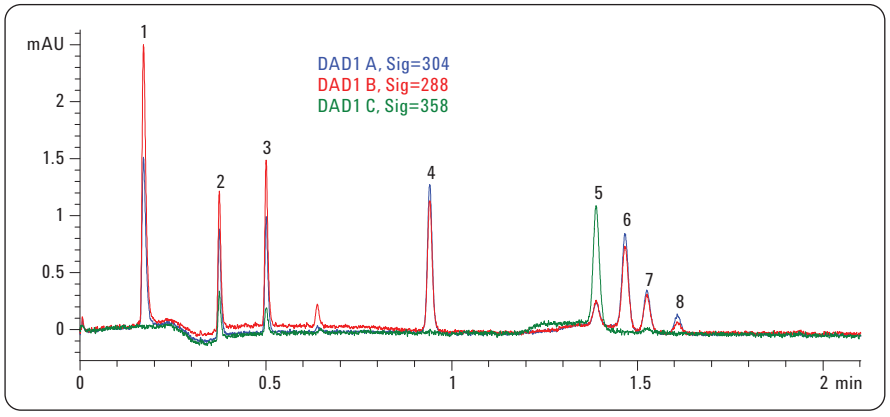


Figure 3
Chromatogram of standard mix where all the analytes were at 0.25 ng on-column concentration (three different wavelengths are overlaid).

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 8
Injections	**288 nm**			**304 nm**	**358 nm**	**304 nm**		
3	51.5	26.5	31	36.1	21.1	22.9	10.4	4.6
5	50.3	25.5	28.8	34.7	20.2	22	9.4	4.4
6	56.5	27.3	31.8	34.1	20.8	22	10	4.6
7	50.2	25.6	30	35.1	19.8	21.9	9.5	4.5
8	52.8	26.1	31.4	35.7	19.3	22.2	9.7	4.4
9	50.2	25.3	29.8	35.3	18.6	21.8	9.8	4.5
Average	51.9	26.1	30.5	35.2	20.0	22.1	9.8	4.5
SD	2.47	0.75	1.13	0.71	0.94	0.40	0.36	0.09
RSD (%)	4.75	2.89	3.71	2.02	4.68	1.80	3.71	1.99

Table 5
Signal-to-noise values for each standard at 0.25 ng on-column concentration.

Linearity

A linearity study was performed in the concentration range of 0.25 ng to 200 ng (nine levels and five replicates) on-column concentration. The levels were 200 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 1 ng, 0.5 ng, 0.25 ng. The precision of area and retention time was demonstrated by calculating the SD and RSD values of five replicate injections for each level. The graphical representation of RSD for RT is shown in Figure 4. The observed RSD values are well within the acceptance limit of 1.0% confirming the excellent precision in retention time.

The results show an excellent assurance of area reproducibility above 0.5 ng on-column for all components except octyl salicylate (compound 8). For compound 8, from 1 ng and above, the area RSD values are well within the allowed limit of 2.0%. A smaller peak area showed poor UV response of octyl salicylate compared to other standards. This was the reason for a higher RSD value. The observed area RSD values throughout the linearity levels are tabulated in Table 6.

A calibration graph was constructed by plotting the peak area of each standard against nominal concentrations (0.25 ng, 0.5 ng, 1 ng, 5 ng, 10 ng, 25 ng, 50 ng, 100 ng, 200 ng). The linearity of the relationship between peak area and concentration is established by the correlation coefficients (R^2) > 0.9997. The overlaid linearity curves for all standards are shown in Figure 5. Observed R^2 values for individual components are tabulated in Table 7.

Extracted sample analysis

A spectral library was generated for all the standards to confirm peak identities and to provide data for spectral peak purity or the absence of coelution. UV filters from six different international brands were extracted and analyzed. Observed elution patterns for all the samples are overlaid and shown in Figure 6. An unknown peak was

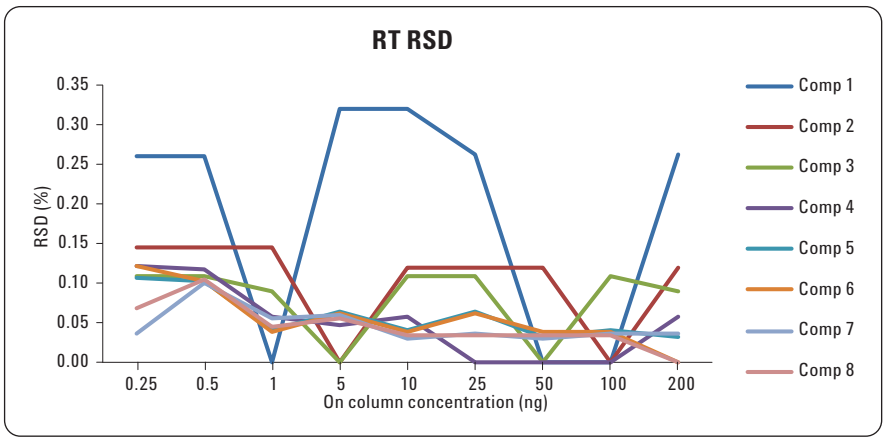


Figure 4
Retention time RSD.

On-column Concentration (ng)	Area RSD values (%)							
	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 8
0.25	1.70	4.40	3.57	1.53	1.95	2.80	5.93	9.67
0.5	1.48	1.46	1.48	1.45	0.98	1.46	1.80	4.24
1	0.47	0.55	0.73	0.87	1.00	1.34	1.20	1.94
5	0.22	1.37	0.06	0.09	0.31	0.21	0.20	0.50
10	0.95	1.02	0.96	1.10	1.16	1.29	1.43	1.21
25	0.27	0.45	0.50	0.46	0.55	0.54	0.61	0.53
50	1.11	1.16	1.02	0.98	0.80	0.80	0.70	0.79
100	0.11	0.16	0.20	0.26	0.25	0.29	0.27	0.33
200	0.16	0.24	0.19	0.16	0.19	0.18	0.22	0.11

Table 6
Area RSD values for all compounds at all linearity levels (n=5). Values > 2.0 are marked in red.

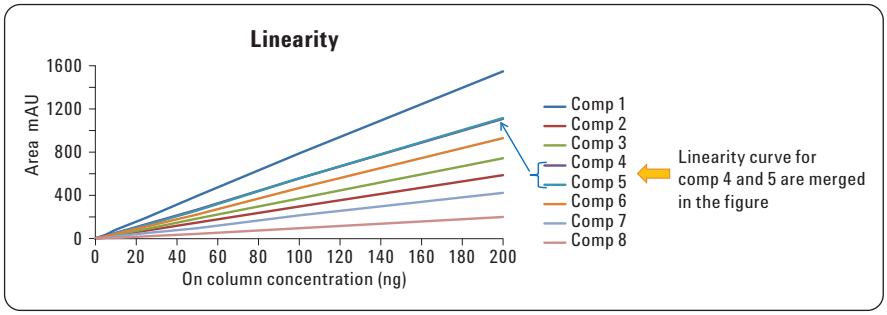


Figure 5
Overlaid linearity curves for all standards.

Compound	Name	R^2
1	Aminobenzoic acid	0.9999
2	Dioxybenzone	1.0000
3	Oxybenzone	0.9999
4	4-Methyl benzylidene camphor	0.9999
5	Avobenzone	0.9997
6	Octyl methoxycinnamate	0.9998
7	Octocrylene	0.9997
8	Octyl salicylate	0.9998

Table 7
Observed R^2 values for individual components.

observed in sample 5 at 1.71 min. Results show that avobenzone and octocrylene are the most widely used UV filter components in the sunscreen personal care products tested. From the extracted sample chromatogram, it is clear that samples b and d provide a broad range of protection against UVA, UVB and UVC rays.

Sunscreen products with higher SPF values may contain higher amounts of sunscreen components and consequently chances are high that these products contain significant amounts of impurities. The Agilent 1290 Infinity LC provides an overall picture of impurity profiles in personal care products in the shortest amount of time, as demonstrated for sample e in Figure 7.

Conclusions

This Application Note demonstrates the baseline separation of eight FDA/EP approved sunscreen compounds in 99 seconds using the Agilent 1290 Infinity LC and the Agilent Poroshell 120 EC-C18 column. The minimum observed resolution value in the standard mix chromatogram was > 1.8. S/N values for each component at 0.25 ng level (on-column concentration) were demonstrated. Linearity was demonstrated from 0.25 ng to 200 ng on-column for all compounds. The poorest R^2 value is 0.9997 (nine levels and five replicates). Across the linearity levels, the highest observed RT standard deviation value was 0.0018 and the highest observed RT RSD value was 0.32% (n=5). This method can be effectively used to chromatograph UV filters and impurities present in sunscreen and personal care cosmetic products.

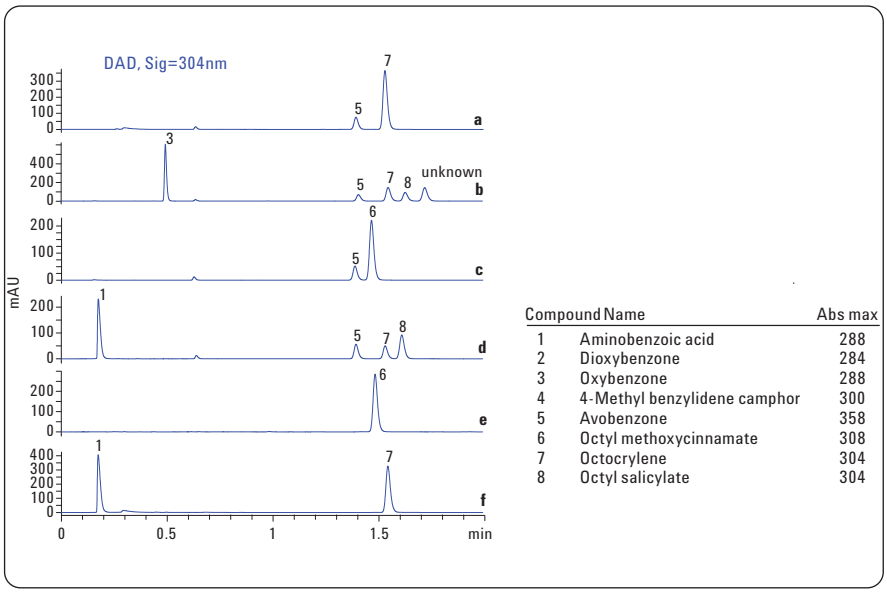


Figure 6
Overlay of chromatogram for all six extracted samples.

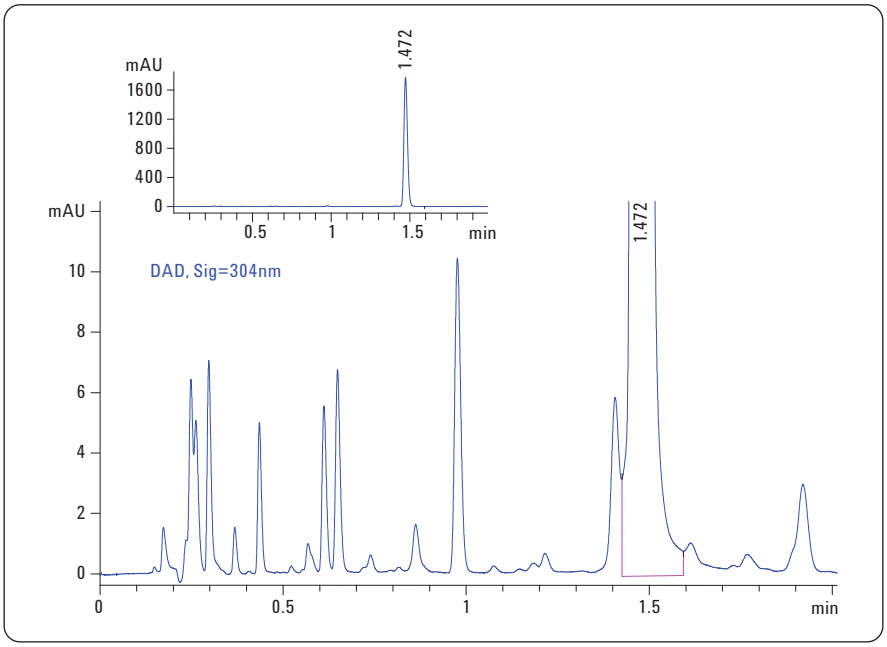


Figure 7
Full scale and zoomed chromatogram of sample "e", which demonstrates well resolved impurity peaks. These minor unidentified peaks may be parabens or other listed ingredients of the formulations and related impurities.

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