

Determination of UV Absorbers from Sunscreens by UHPLC with Photodiode Array Detection

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Goal:

Measure UV-absorbing compounds in consumer products and characterize the UV absorption spectra of the individual components by employing high-speed liquid chromatography with photodiode array detection.

Introduction

Skin cancer accounts for almost half of all cancers in the U.S. In 2007 over 1 million new cases were diagnosed and over 10,000 people died from skin cancers. A majority of skin cancers are associated with exposure to harmful radiation from the sun. The most damaging radiation occurs in two wavelength regions, designated UVB and UVA. Radiation from 290 – 320 nm (UVB, as in “burn”) is absorbed mostly near the skin surface, causing the immediate redness and pain of sunburn. Radiation from 320 - 400 nm (UVA) penetrates deeply into the skin and causes DNA damage to cells in the under layer of skin, increasing the risk of malignant melanoma¹. UVA radiation also causes much of the skin damage associated with aging.

Sunscreens are topical lotions that disperse UV-absorbing compounds in an oily base that may also contain emollients, skin moisturizers, and light reflecting particles of zinc oxide. Common UV-absorbers added to sunscreens include oxybenzone (benzophenone), avobenzone, octinoxate (octyl methoxycinnamate), octisalate (octylsalicylate), homosalate, and octocrylene.

Useful physicochemical properties of these UV-absorbers are listed in Table 1.

The same additives that protect skin from sun damage may have a dark side as endocrine disruptors, causing abnormal development of fish, frogs and other aquatic organisms. Research performed in Switzerland in 2006 demonstrated that the common sunscreen additives 4-methylbenzylidene camphor (4-MBC) and octocrylene were present in surface water and bioaccumulate in fish², and a study of six common sunscreen chemicals found that octyl-methoxycinnamate (OMC) and homosalate (HMS) exerted significant estrogenic activity, as measured by the increase in proliferation rates of human breast cancer cells (MCF-7 cells) grown in vitro³.

The oily matrix of sunscreen lotions coupled with the high UV absorbance of the analytes makes HPLC the method of choice for analysis of sunscreens, and by using photodiode array detection, the complete absorption spectrum of each compound is obtained as it elutes. The sensitivity of HPLC/PDA is also sufficient to measure these compounds in environmental water samples, which facilitates research on exposure and environmental fate. In this application, an HPLC method used by a commercial analytical laboratory is improved by using ultra high-performance liquid chromatography (UHPLC) on a Hypersil GOLD 1.9 μm column. The resolution of adjacent peaks is improved even as method run time is reduced from 45 to 7 min. Applications include

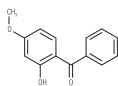
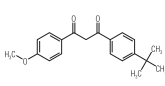
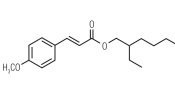
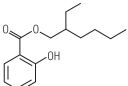
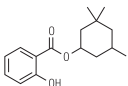
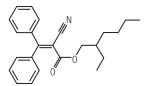
CAS#	Oxybenzone 131-57-7	Avobenzone 70356-09-1	Octinoxate 5466-77-3	Octisalate 118-60-5	Homosalate 118-56-9	Octocrylene 6197-30-4
						
UV band	UVA	UVA	UVB	UVB	UVB	UVA
max, nm ¹	241, 288, 386	360	238, 303, 310	238, 305	238, 305	<220, 305
Formula	C ₁₄ H ₁₂ O ₃	C ₂₀ H ₂₂ O ₃	C ₁₈ H ₂₆ O ₃	C ₁₅ H ₂₂ O ₃	C ₁₆ H ₂₂ O ₃	C ₂₄ H ₂₇ NO ₂
MW (g/mol)	228.24	310.39	290.40	250.33	262.36	361.48
Log P _{o-w} ²	3.79	5.3,4.51	5.8	5.97	6.16	6.88
Water solubility, mg/L at 25 C ³	69 (est)	2.22 (est)	0.16 (est)	0.72 (est)	0.42 (est)	0.004 (est)

Table 1. Useful properties of sunscreen agents determined by UHPLC/DAD

¹UV Max from this application work

²ChemID Plus, United States National Library of Medicine <http://ntp.niehs.nih.gov>

³Data From SRC PhysProp Database: <http://esc.syrres.com/interkow/webprop>

Key Words

- Accela UHPLC
- Hypersil GOLD
- Sunscreens
- UV-Absorbers
- Water Contamination
- Estrogenic Compounds

determination of UV absorbers in three consumer products and in water from a public swimming pool. Also documented is chromatographic method performance including resolution, calibration range, method detection limits, and precision of retention time and peak area.

Experimental

Instrumentation

Thermo Scientific Accela High-Speed Liquid Chromatography system with PDA Detector

Thermo Scientific ChromQuest 5.0 Chromatography Data System (CDS)

Chromatographic conditions

Columns:	Thermo Scientific Hypersil GOLD, 1.9 μm , 100 x 2.1 mm (Thermo Scientific 25002-102130)
Mobile phase:	A: Water B: Acetonitrile
Isocratic:	40:60
Flow rate:	1000 $\mu\text{L}/\text{min}$
Run time:	7 min
Detector:	PDA, D ₂ and W lamps, 10-mm flow cell, 20Hz, 0s rise time Scans: 200-500 nm, 1Hz, 1nm bandwidth, Step1 Discrete: 313 nm, 20Hz, 11nm bandwidth
Column temp.:	45 °C
Injection:	1 μL "no waste" injection from 25 μL sample loop, 4 $\mu\text{L}/\text{sec}$ 1 mL Flush and 1 mL Wash, using 90:10 acetonitrile: water from Flush Reservoir

Chemicals

Water, HPLC-grade	Fisher Scientific W5
Acetonitrile, HPLC-grade	Fisher Scientific A998-1
Avobenzene	USP 1045337
Homosalate	USP 1311408
Oxybenzone	USP 1485001
Octocrylene	USP 1477411
Octinoxate	USP 1477900
Octylsalicylate	USP 1477943

Consumables

Syringe filters, 0.45 μm Nylon	Thermo Scientific A5307-010
Autosampler vials, 1.8 mL glass	Thermo Scientific A4954-010
HyperSep C18, 100 mg, 1 mL	Thermo Scientific 60108-302

Mobile Phase

Proportioned mobile phase: Filled Solvent Reservoir Bottle A of the Accela pump with fresh HPLC-grade water and purged the solvent line with at least 30 mL of the water. Connected a fresh bottle of HPLC-grade acetonitrile to Reservoir B and purged as above.

Calibration Standards

Stock Standard, 200 mg/L: Accurately weighed 10 mg of each neat compound into a 50-mL volumetric flask. Added 25 mL acetonitrile, vortex, sonicate 5 min, brought to volume with acetonitrile and mix.

Calibration standards: Used a calibrated pipette to dilute the intermediate standard with mobile phase in volumetric glassware to 100, 50, 20, 5, 1, 0.2, 0.05 and 0.03 mg/L.

Analyte	k' ^a	R ^a	Linear range, mg/L	r ²	MDL ^b mg/L	Precision, Retention Time %RSD ^c	Precision, Peak Area Partial Loop %RSD ^c	Precision, Peak Area Full Loop %RSD ^d
oxybenzone	1.9	0	1-200	0.9998	0.19	0.33	0.55	0.12
avobenzene	12.6	27.1	1-100	0.9988	0.35	0.31	0.60	0.13
octinoxate	14.1	2.2	1-200	0.9998	0.09	0.29	1.39	0.30
octisalate	15.8	2.3	1-200	0.9997	0.82	0.25	0.76	0.14
homosalate	16.9	1.5	1-200	0.9996	0.85	0.24	0.66	0.14
octocrylene	18.2	1.5	1-200	0.9998	0.47	0.29	0.61	0.14

Table 2: Performance of UHPLC method for sunscreens performed on Hypersil GOLD 1.9 μm , 1 x 100 mm column

^aCapacity factor (k') and Resolution (R) calculated according to Reference 4

^bDetection limit (MDL) calculated by multiplying the standard deviation of the concentration determined for a low level standard by Students' t for n = 7 replicates

^cfor 1 μL injection from 25 μL sample loop, n = 30 replicates

^dfor 5 μL full loop injection, n = 30 replicates

Samples

Samples of three commercially available sunscreens were purchased from local retail stores; Coppertone Water Babies SPF 50 Sunscreen Lotion, Coppertone Sunscreen Lotion SPF 45 (Schering-Plough HealthCare Products), and Banana Boat Kids Sunblock Lotion SPF 30 (Sun Pharmaceuticals Corp.). To prepare the sample for UHPLC analysis, transfer 0.2 g of the lotion to a 100 mL volumetric flask and add about 5 mL of HPLC-grade water. Stopper and vigorously shake the vol flask to mix, then add about 50 mL acetonitrile and sonicate with occasional shaking for 10 min to dissolve or evenly disperse the lotion. Bring to volume with acetonitrile and mix. Filter through a 0.45 μm nylon syringe filter into a glass autosampler vial before analysis.

A sample of swimming pool water was collected from a local public pool and prepared by using solid phase extraction. A HyperSep SPE cartridge (bed weight of 100 mg and bed volume of 1 mL) was placed in a vacuum manifold and conditioned by washing with 2 mL methanol followed by 2 mL LC/MS-grade water. During conditioning, the vacuum was adjusted to produce a flow rate through the SPE cartridge of about 1-2 mL per minute. 20 mL of sample was passed through the filter at a rate of 1-2 mL/min. Analytes were eluted with 5 mL of acetonitrile into a clean 5-mL glass test tube. The extract was evaporated to dryness in a Caliper LifeSciences TurboVap®LV Concentration workstation at 35 °C under a stream of nitrogen at 17 psi. The sample was

reconstituted in 200 μL of mobile phase, filtered into an autosampler vial, and injected.

System Preparation

To ensure good performance of this application, prepare the system as directed in Appendix A.

Results

The UHPLC separation of sunscreen agents provided by this application (Fig 1A) provides better resolution, peak shape and run time compared to the original method used by the commercial laboratory (Fig 1B). Particularly noteworthy is the 6-fold improvement in run time and sample throughput.

We measured the performance of this application including peak resolution, linear calibration range, dynamic range, limits of detection, and precision of retention time and peak area, as summarized in Table 2. For most of the analytes, the peak area precision for partial loop injections was better than 1% RSD, and for full loop injections was better than 0.15% RSD. For the best precision and accuracy, use a full loop injection from a calibrated sample loop. Minimum detection limits obtained with a 1 μL partial loop injection were on the order of 0.1 – 0.8 mg/L, more than sufficient for analysis of commercial sunscreen products. For analysis of environmental water samples, the SPE procedure provided a 100-fold concentration of the sample. If necessary, a larger injection volume could also be used to further lower the detection limits.

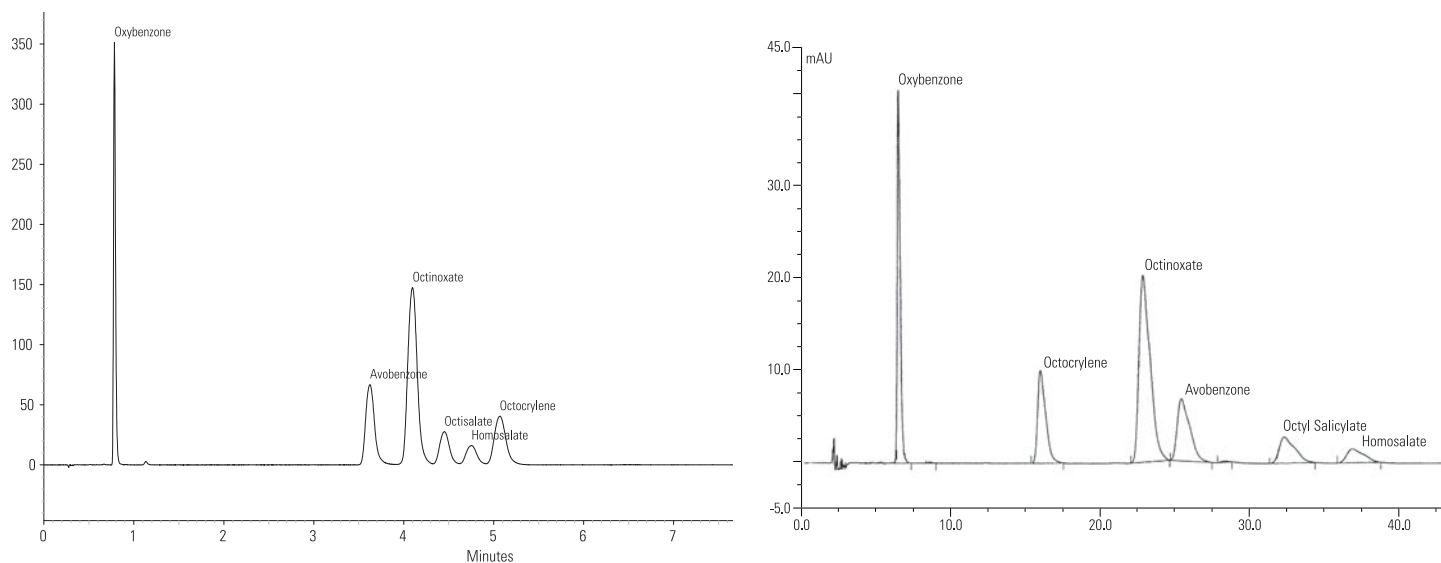


Figure 1. Separation of UV absorbing sunscreens: A) on the Accela™ High Speed LC by reversed phase chromatography with photodiode array detection at 313 nm. Peak 1, oxybenzone; peak 2, avobenzone; peak 3, octinoxate; peak 4, octisalate; peak 5, homosalate; peak 6, octocrylene, 100 mg/L each. Conditions: Isocratic separation using 40:60 water: acetonitrile on Hypersil GOLD 1.9 μm , 2.1 x 100 mm column at 45 °C and 1000 $\mu\text{L}/\text{min}$. B) Conventional separation used by a commercial laboratory, for comparison. Details are proprietary.

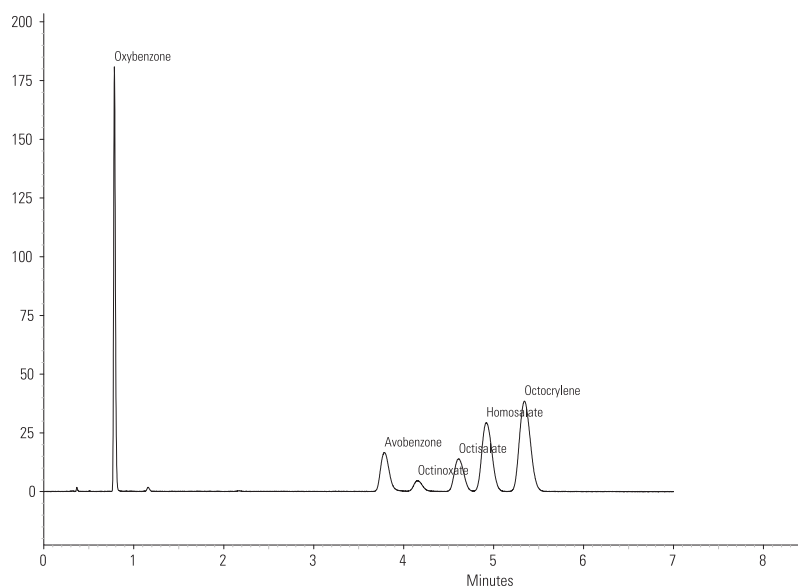


Figure 2. Determination of UV absorbing compounds in a sunscreen lotion by reversed phase chromatography with photodiode array detection at 313 nm. Sample, Sunscreen 2 from Table 2. Peak 1, oxybenzone; peak 2, avobenzone; peak 3, octinoxate; peak 4, octisalate; peak 5, homosalate; peak 6, octocrylene, 100 mg/L each. Conditions: Isocratic separation using 40:60 water: acetonitrile on Hypersil GOLD 1.9 μm , 2.1 x 100 mm column at 45 $^{\circ}\text{C}$ and 1000 $\mu\text{L}/\text{min}$.

To demonstrate the efficacy of the present method for analyzing real samples, we analyzed samples of three off-the-shelf sunscreen products and water from a public swimming pool. Figure 2 shows a chromatogram of Sunscreen 2, and Table 3 summarizes the concentration of UV absorbers determined in each sample. The results from the commercial products agreed well with the nominal values on the product label, with none deviating by more than 10%. For the pool water sample, the detection limits after solid phase extraction were 50-times lower than for direct injection. In the sample of pool water, only oxybenzone was detected, at a concentration of 10 $\mu\text{g}/\text{L}$.

The UV absorption spectra collected by the PDA detector provide three important benefits. First, the spectrum of each compound can be stored in a library and later matched with unknown samples to identify and confirm analytes present in a sample. Second, the peak purity index automatically detects poorly resolved peaks, such as degradation products. Third, because sunscreens are formulated to protect against specific regions of the solar spectrum, the spectrum of each agent clearly identifies it as a UVB, UVA or broad-spectrum absorber. Figure 3 shows the spectral data displayed by ChromQuest for Sunscreen 2. It is easy to determine from either the

Sample/ Analyte	Sunscreen 1 (weight percent)	Sunscreen 2 (weight percent)	Sunscreen 3 (weight percent)	Pool water ($\mu\text{g}/\text{L}$)
oxybenzone	5.39	3.84	2.32	10
avobenzone	n.d.	2.39	1.19	n.d.
octinoxate	7.32	0.30	0.18	n.d.
octisalate	4.15	3.86	2.91	n.d.
homosalate	n.d.	15.4	9.91	n.d.
octocrylene	n.d.	7.73	1.47	n.d.

Table 3: Concentration of UV-absorbing compounds determined in various matrices by High Speed Liquid Chromatography. Concentrations in sunscreens 1- 3 given in weight percent of original sample. "n.d." indicates not detected. N = 3 replicates.

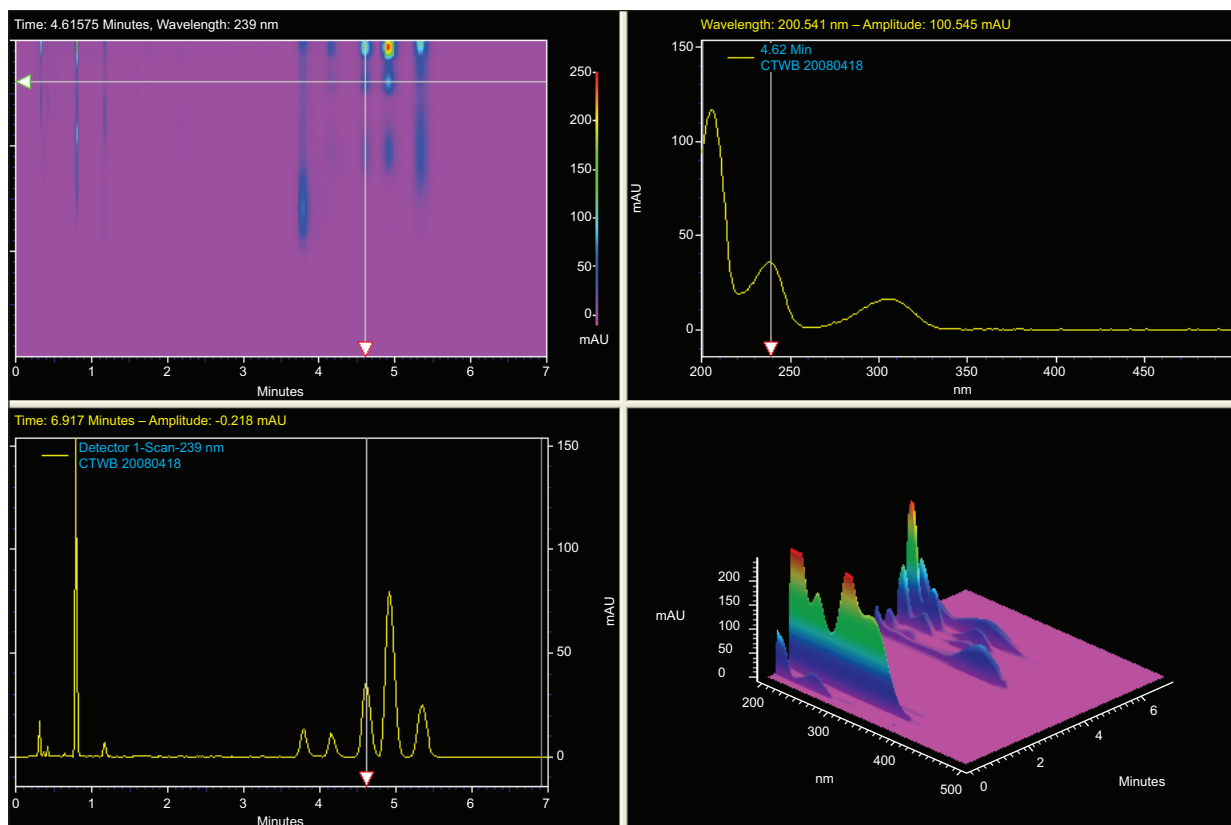


Figure 3. Spectral data of UV absorbing compounds in Sunscreen 2. Obtained on the Accela™ High Speed LC by reversed phase chromatography with photodiode array detection. Peak 1, oxybenzone; peak 2, avobenzene; peak 3, octinoxate; peak 4, octisalate; peak 5, homosalate; peak 6, octocrylene. Conditions: Isocratic separation using 40:60 water: acetonitrile on Hypersil GOLD 1.9 μm , 2.1 x 100 mm column at 45 °C and 1000 $\mu\text{L}/\text{min}$.

isoabsorbance plot or the 3-D plot which compounds protect against UVA (absorbing at less than 320 nm), which protect against UVB (absorbing at greater than 320 nm), and which protect against a broad spectrum of wavelengths.

Conclusion

A fast HPLC separation performed on the Accela high speed chromatography system equipped with a PDA detector resolves six UV-absorbing components of sunscreens in about six minutes with retention time and peak area precision better than 1% RSD. The UV-absorbance spectrum of each compound is acquired as it elutes and can be matched against a stored spectral library to aid in compound identification.

References

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2. Hans-Rudolf Buser, Marianne E. Balmer, Peter Schmid, and Martin Kohler. *Environ. Sci. Technol.*, 2006, 40, (5), pp 1427-1431.
3. Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger, W (2001). *In vitro and in vivo estrogenicity of UV screens.* Environmental Health Perspectives 109(3): 239-244.
4. United States Pharmacopeia 30-National Formulary 25, United States Pharmacopeia, Rockville, Maryland 20852-1790, USA.

Appendix A.

System Preparation

Pump: Always plumb the Accela system with precut and polished 0.005" i.d. high-pressure tubing and high pressure fittings as shown in Figure 15 of the Accela Pump Hardware Manual (Document 60157-97000 Revision B). For all tubing connections that you make, ensure that the tubing end is square-cut and burr-free. Firmly push the tubing into the injection valve port as you tighten the high-pressure fitting, in order to maximize peak efficiency. Prime the pulse dampener and purge the solvent lines as instructed in Chapter 4 of the Accela Pump manual. Verify that the pump is performing well by monitoring the pressure pulsation and by testing the pump proportioning accuracy as described in Chapter 5 of the pump manual.

AS: Open the Instrument Configuration and verify that the Accela AS Configuration entry for "Dead volume" is correct (the calibrated volume in μL written on the transfer tubing between the injection port and injection valve). Verify that the entry for "Loop size" is correct for the currently installed sample loop. Fill the Flush reservoir with 90:10 (v/v) acetonitrile:water and flush the syringe with solvent to purge any air bubbles from the syringe and tubing.

Install the HypersilGOLD, 1.9 μm 2.1 x 100 mm column, using a 10-cm length of precut and polished 0.005" i.d. high-pressure tubing and the high pressure fitting consisting of a nut, back ferrule and front ferrule. Ensure that the tubing is fully pushed into the column inlet when you tighten the high-pressure fitting. Consult the Accela Getting Connected manual (Document 60057-97001 Revision A) for details.

Detector: Use a 10 mm LightPipe flow cell. Add a short section of 0.005" PEEK backpressure tubing to the flow cell outlet to suppress bubble formation in the flow cell. Verify that the deuterium lamp has been used for less than 2000 hours.

Use Direct Control or a downloaded method to equilibrate the Accela system under the conditions shown in Table 3: 45 °C, 823 $\mu\text{L}/\text{min}$, and 0.8 μL injection. Create a method based on these operating conditions and then create a Sequence to make several injections of HPLC grade water. The system is ready to run standards and samples when the peak-to-peak baseline oscillation is between 50 – 200 $\mu\text{AU}/\text{min}$ (average of 10 1-min segments) and no significant peaks elute in the retention time window of the analytes.

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