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Experiment #7: Determination of Methylparaben in Lotion by Capillary Electrophoresis (CE).

CHEM 3170

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Sample: Vaseline intensive care aloe vera hydration lotion

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Abstract:

A sample of Vaseline intensive care aloe vera hydration lotion was analyzed by capillary electrophoresis (CE) to determine the concentration of methylparaben. Methylparaben is a type of paraben, a class of molecule commonly used as a preservative in cosmetic products. Some publications suggest that methylparaben can have adverse health effects, but there is little evidence to support this. Over two weeks, five standard solutions and a number of samples, some spiked for recovery studies, were analyzed with varying success. A solvent extraction procedure of methylparaben from the lotion using methanol was designed and tested to be successful. All prepared samples and methylparaben standards were analyzed on a SCIEX P/ACE System MDG Plus capillary electrophoresis instrument. A calibration curve was generated from standard results and an R squared value of 0.9964 was attained. The concentration of methylparaben in the lotion was determined to be 2516 ppm with a relative standard deviation of 5.21%. Recovery studies results gave an average percent recovery of 165.8%, which indicated inaccuracy. An increased number of standards would have increased the linear range of the calibration curve and improved the accuracy of the spiked samples.

Introduction:

Parabens are a class of similar compounds commonly used as preservatives in cosmetic products[2,4]. The four most common parabens are methylparaben, ethylparaben, propylparaben and butylparaben. They are effective at preventing growth of microorganisms in cosmetic products and multiple parabens are often used along with other preservatives in one product[2]. Methylparaben is the most commonly used and is listed in the ingredients of many cosmetic products. Many scientific publications have claimed that excessive use of paraben containing products can cause adverse health effects including higher risk of certain cancers, reproductive harm, weaker immune system or obesity. However, there is little experimental evidence of parabens being linked to any health effects and the United States' Food and Drug Administration does not restrict parabens in any way[5]. Nevertheless, a method for efficient extraction and quantification of parabens from a variety of matrices is useful. The four main paraben compounds have relatively similar solubilities in alcohols, so the simplest and most common paraben, methylparaben, was selected as the analyte for this experiment.

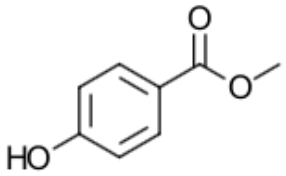

Figure 1. Structure of Methylparaben

Capillary electrophoresis is a separation technique which uses a strong electric field and a fused silica capillary to separate solutes based on their relative mobility[3]. The capillary is connected to an electrode on either end, and an ionic buffer is used to carry sample solutions through[1]. The buffer generates electroosmotic flow (EOF) through the capillary due to ionic interactions with the silica surface creating potential[1]. Analyte ions in solution are separated based on charge and size. An ultraviolet detector is used in this experiment, and it is placed near the negative cathode end of the capillary. Cations are detected first as they travel faster with the EOF toward the negative cathode[1]. The determination of methylparaben was well suited to this technique since it is deprotonated to an anion at the buffer pH of 9.51, and therefore can be separated from other parabens and similar solutes by relative size. Solvent extraction using methanol also eliminates some matrix compounds in the hand lotion.

Experimental:

Instrumental Parameters:

Table 1. Optimized instrumental parameters for capillary electrophoresis (CE) analysis of methylparaben in lotion.

Capillary:	Fused silica, 50 μm I.D. x 375 μm O.D. x 50 cm total length (40 cm to detector)
Operating Temperature:	25 °C
Run Time:	15 min
Detection:	UV, 214 nm (direct absorbance)
Rinse Pressure (0.1 M NaOH):	20 psi for 3.0 min
Rinse Pressure (water):	20 psi for 1.0 min
Rinse Pressure (rinse buffer):	20 psi for 3.0 min
Injection Pressure:	1 psi for 5.0 s
Separation Voltage:	20 kV
Polarity:	Normal
Buffer Concentration:	20 mM
Buffer pH:	9.51

Sample and reagents:

- Vaseline intensive care aloe vera hydration lotion
- 1000 ppm methylparaben stock solution
- 20 mM sodium borate buffer
- Methanol

Instrument:

- SCIEX P/ACE System MDQ Plus capillary electrophoresis system

Procedure:

Table 2. Volume of 1000 ppm methylparaben solution used to prepare standard solutions of various concentrations in ppm.

Vial #	Concentration methylparaben (ppm)	Volume 1000 ppm methylparaben (μL)	Volume Methanol (μL)	Total volume (μL)
1	1.0	1.0	999.00	1000.00
2	5.0	5.0	995.00	1000.00
3	10.00	10.00	990.00	1000.00
4	20.00	20.00	980.00	1000.00
5	25.00	25.00	975.00	1000.0

Preparation of Standards:

To prepare each standard solution, appropriate volumes of 1000 ppm methylparaben stock solution and methanol were dispensed into each CE vial using a micropipette. Each vial was briefly vortexed.

Preparation of first three samples (week 1):

To prepare each of the three lotion samples, approximately 0.6000 g of lotion was weighed into a 15 mL centrifuge tube using an analytical scale. Next, 1.6 mL of methanol was added to the tube, and it was vortexed for 3 min. The tube was then spun in a centrifuge for 10 min at 6000 rpm. Avoiding the solid/lotion layer in the tube, the solution was then transferred to a CE vial through a 0.45 μm syringe filter.

Preparation of sample for recovery studies (week 2):

To prepare the four sample vials for recovery studies, one solvent extraction was done using the same extraction method as week 1. Instead of 1.6 mL of methanol, 9.6 mL was used, but the vortex and centrifuge steps remained unchanged. The methanol solution was filtered through a 0.45 μm syringe filter and then further diluted 10 times by pipetting 1.00 mL into a 10.00 mL volumetric flask and diluting to the mark with methanol. The diluted solution was then transferred to four CE vials, and the appropriate volumes of 1000 ppm methylparaben stock (Table 5) were added to each vial as a spike.

Analysis:

An SCIEX P/ACE System MDQ Plus capillary electrophoresis system was used to determine methylparaben concentrations in lotion. All standards were run once. Week one samples were run in triplicate and week two unspiked and spiked samples were run once.

Data and Results:

Table 3. CE data for standard solutions used to generate a calibration curve, Figure 2.

Standard #	[methylparaben] (ppm)	Migration Time (min)	Peak Area
1	1.00	8.917	306
2	5.00	8.871	1128
3	10.00	8.912	2072
4	20.00	8.988	3885
5	25.00	8.921	5211

Table 4. CE data for spiked and unspiked lotion samples from CE instrument including migration times and peak areas.

Sample	Mass Lotion (g)	Migration Time (min)	Peak Area
Week 1 unk 1	0.6303	8.738	166411
Week 1 unk 2	0.6017	8.683	163050
Week 1 unk 3	0.6041	8.838	163618
Unspiked	0.6000	8.771	3037
80 % spiked	0.6000	8.775	6595
100 % spiked	0.6000	8.787	7987
120 % spiked	0.6000	8.838	7573

Table 5. Recovery study data including total methylparaben concentration, methylparaben concentration added, and percent recovery used to determine the accuracy of the method.

Sample	Vol 1000 ppm methylparaben stock added (μL)	[Methylparaben] added (ppm)	[Methylparaben] (ppm)	% recovery
Unspiked	0	0.00	14.80	-
80% spiked	10.8	10.68	32.69	167.5%
100% spiked	13.5	13.32	39.69	186.9%
120% spiked	16.2	15.94	37.61	143.1%

Table 6. Summary of results for the determination of methylparaben in lotion by CE including experimental concentration, % RSD, equation of the line, R^2, and uncertainty values.

Experimental methylparaben content in diluted sample =	14.80 ppm
Experimental methylparaben content in undiluted sample =	2516 ppm ± 5.21%
mg methylparaben per g lotion =	2.516 mg/g
% RSD =	5.2%
Equation of the line =	y = 198.86x + 94.297
R^2 =	0.9964
Uncertainty of the y-intercept (S_b) =	104.33
Uncertainty of the slope (S_m) =	6.88 ppm^{-1}
Uncertainty of the unknown (S_x) =	0.77 ppm

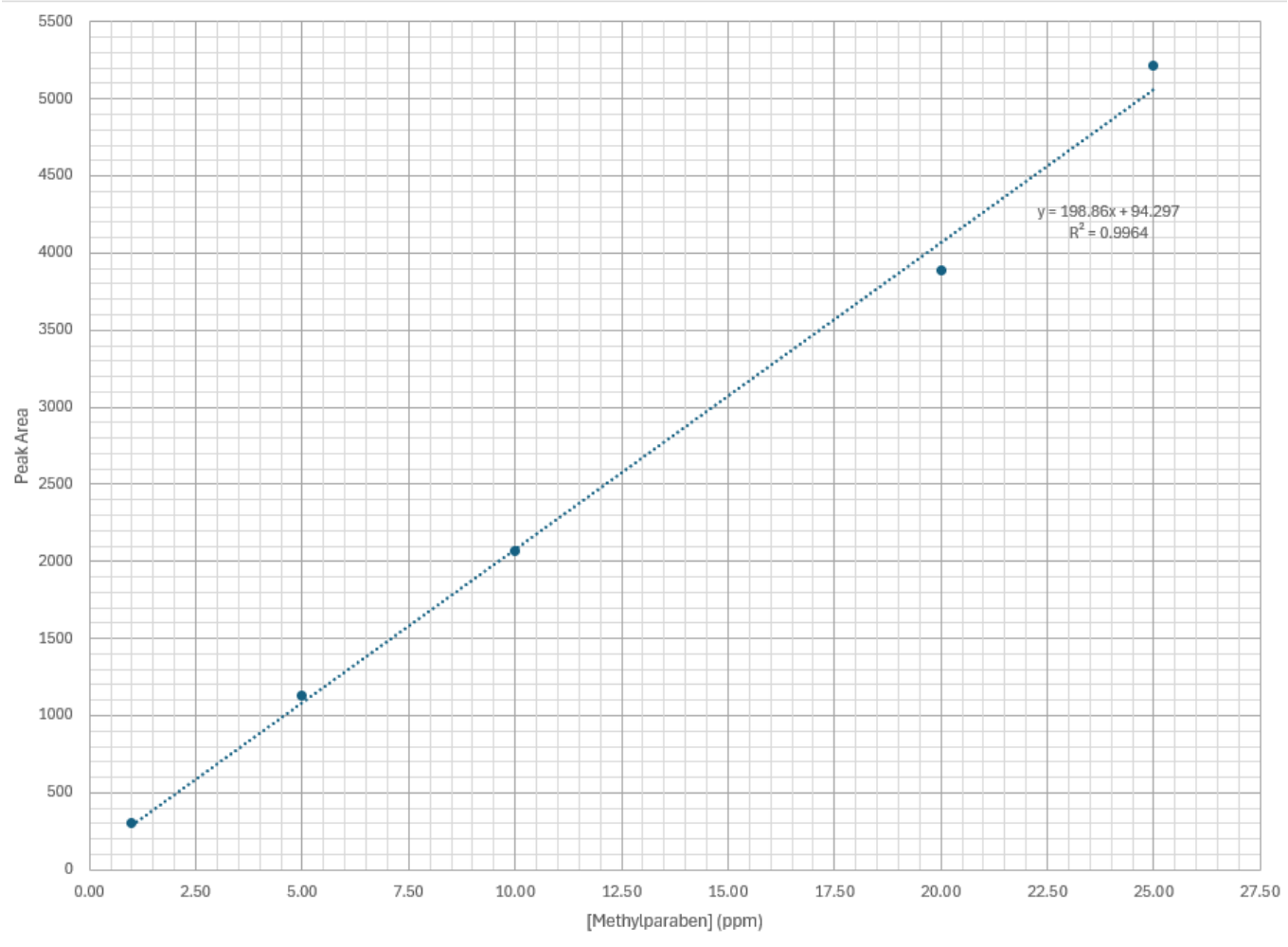

Figure 2. Calibration curve of peak areas as methylparaben concentration (ppm) in standard solutions increases

Calculations:

Concentration of methylparaben in standard solutions (Standard 2):

$$C_2 = \frac{C_1 V_1}{V_2} = \frac{1000 \text{ ppm} \times 5.0 \text{ µL}}{1000 \text{ µL}} = 5.0 \text{ ppm}$$

[methylparaben] in unspiked prepared sample from equation of the line:

Sample peak area $= 3037$

Equation of the line: $y = 198.86x + 94.297$

$$x = \frac{3037 - 94.297}{198.86} = 14.80 \text{ ppm}$$

Uncertainty Calculations for [methylparaben] in prepared sample:

$$S_y^2 = \frac{\sum(d_i^2)}{n-2} = \frac{5.7701 \text{ x } 10^4}{5-2} = 19234$$

$$S_y = \sqrt{19234} = 138.68$$

$$D = n\sum x_i^2 - (\sum x_i)^2 = 5(1151) - (61)^2 = 2034$$

$$S_m = \sqrt{\frac{S_y^2 \times n}{D}} = \sqrt{\frac{(19234)(5)}{2034}} = 6.876$$

$$S_b = \sqrt{\frac{S_y^2 \times \sum x_i^2}{D}} = \sqrt{\frac{19234 \times 1151}{2034}} = 104.3$$

$$S_x = \frac{s_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y}_i)^2}{m^2(x_i - \bar{x})^2}} = \frac{138.7}{198.9} \times \sqrt{\frac{1}{1} + \frac{1}{5} + \frac{(3037 - 2520)^2}{198.9^2\ (61 - 12.2)^2}}$$

$$S_x = 0.77 \text{ ppm}$$

$$\%\text{RSD} = \frac{0.77 \text{ ppm}}{14.80 \text{ ppm}} \times 100\% = 5.2\%$$

[methylparaben] added to 80% spiked sample:

$$C_{added} = \frac{(10.8\ \mu L \times 1000\ ppm)}{1.0108\ mL} = 10.68\ ppm$$

%Recovery calculation (80% spike):

$$\%Recovery = \frac{Conc'n\ Spiked - Conc'n\ Unspiked}{Conc'n\ added} \times 100\%$$

$$= \frac{32.69\ ppm - 14.80\ ppm}{10.68\ ppm} \times 100\% = 167.5\%$$

Methylparaben concentration in hand lotion (undiliuted sample):

$$\text{Second dilution: } C_1 = \frac{C_2V_2}{V_1} = \frac{14.80\ ppm \times 10.00\ mL}{1.00\ mL} = 148.0\ ppm$$

$$\text{First dilution (solvent extraction): } C_1 = \frac{C_2V_2}{V_1} = \frac{148.0\ ppm \times 10.2\ mL}{0.600\ mL} = 2516\ ppm$$

NOTE: Assuming lotion has density of 1.00 g/mL.

Propagation of uncertainty in dilutions:

$$\frac{S_{C_1}}{C_1} = \sqrt{\left(\left(\frac{S_{vol1}}{vol_1}\right)^2 + \left(\frac{S_{vol2}}{vol_2}\right)^2 + \left(\frac{S_x}{C_2}\right)^2\right)}$$

$$\text{Dilution 2: } S_{C_1} = 148.0ppm\sqrt{\left(\frac{1.2\mu L}{1000\mu L}\right)^2 + \left(\frac{20\mu L}{10000\mu L}\right)^2 + \left(\frac{0.77ppm}{14.8ppm}\right)^2}$$

$$S_{C_1} = 7.71\ ppm$$

$$\text{Dilution 1: } S_{C_1} = 2516ppm\sqrt{\left(\frac{0.0002mL}{0.6000mL}\right)^2 + \left(\frac{0.01224mL}{10.2mL}\right)^2 + \left(\frac{7.71ppm}{148.0ppm}\right)^2}$$

$$S_x(\text{undiluted}) = 131\ ppm$$

$$\text{Undiluted \%RSD} = \frac{131\ ppm}{2516\ ppm} \times 100\% = 5.21\%$$

Discussion:

Capillary electrophoresis (CE) with UV detection and the external standard method were used to determine the concentration of methylparaben in Vaseline lotion. The experiment was run over the course of two weeks. Week one was spent optimizing the experimental method. Methylparaben was extracted from three replicate lotion samples and six standards ranging from 1 ppm to 100 ppm were prepared. On day two a recovery study was conducted and two additional standards, 15 ppm and 20 ppm, were run. All standards and samples were run on the SCIEX P/ACE System MDQ Plus capillary electrophoresis system and electropherograms were generated for each solution. The methylparaben peak was identified based on increasing peak area of the peak around 8.9 min as concentration of methylparaben in standards increased. Another peak in sample electropherograms around 8.5 was also identified as potentially propylparaben, another paraben present in the lotion sample. Tables 3 and 4 show the peak area and migration time data for standards and samples respectively. After analysis, the results demonstrated the successful solvent extraction of methylparaben from lotion and the concentration of methylparaben was found to be 2516 ± 5.21 %.

Throughout the course of the experiment many successes and challenges were encountered. After the first week, two of the six standards run, 50 ppm and 100 ppm, were unsuccessful because no methylparaben peaks were present. Moreover, the samples run had high methylparaben concentrations that were out of the linear range of the calibration curve (see Figure 2). Therefore, although displaying little noise and well separated peaks, the electropherogram data for the samples was not analyzed further. Despite these setbacks, the solvent extraction method used to separate methylparaben from the lotion samples was proved successful. Because the samples from day one were too concentrated, on day two sample solutions were diluted to fall within the linear range of the calibration curve. A recovery study performed by preparing four sample solutions, unspiked, 80 % spiked, 100 % spiked, and 120 % spiked, resulted in an average percent recovery of 165.8 % (see Table 6). This indicated a high degree of error in the analysis. The high percentage was likely due to the spiked samples being highly concentrated and lying outside the linear range. Random errors and impurities like other parabens or neutral molecules present in the lotion could have also contributed to the high percent recovery. Only one of the two standards run on day two was successful for a total of five

successful standard solutions. The standard solutions that were successful were used to generate a calibration curve, Figure 2, with an R^2 value of 0.9964 which indicated good linearity. The %RSD was 5.21 % indicating good precision. This % RSD included standard deviation calculated using the method of least squares as well as error calculated using the propagation of uncertainty from diluting the samples. Uncertainty calculations resulted in S_b and S_m values of 104.33 and 6.88 ppm^{-1} respectively.

The Vaseline lotion sample did not provide methylparaben concentration information on the product label. Therefore, percent error was not calculated. However, the experimental concentration of methylparaben was compared to literature values. In Ye et al. a similar experiment was conducted to determine the concentrations of multiple parabens in hand creams[7]. The study did not provide brand names, but the concentrations of methylparaben in two hand creams were found to be 0.977 ± 0.156 mg/g and 0.939 ± 0.159 mg/g. A statement from European Scientific Commitees, recommended a maximum of 4 mg/g of any single paraben in cosmetic products[4]. Therefore, a literature range for the methylparaben content in lotion was established as 0.939 – 4 mg/g. The experimental value, 2.516 ± 5.21 %, fell within this literature range.

A potential source of error could have been micropipettes not being calibrated or poor micropipette technique. Impurities like other parabens or neutral compounds in the lotion could have interfered with the methylparaben signal making the peak area larger than it should have been. A good portion of the experimental error could have probably been accounted for by increasing the linear range of the calibration curve. To continue this research more calibration standards at higher methylparaben standards could be run to accommodate for the high concentrations in spiked samples. Additionally, unspiked and spiked samples could be re-run in triplicate to help minimize error by averaging results. This experiment could also be run using micellar electrokinetic capillary chromatography (MEKC) to determine concentrations of multiple parabens in cosmetic products at once. Lotion samples from different brands could also be analyzed using CE and then compared using PCA. Overall, this experimental method, especially the solvent extraction method, is very applicable to the detection of a variety of analytes from complex matrices often found in cosmetics.

Conclusion:

The concentration of methylparaben in Vaseline intensive care aloe vera hydration lotion was determined by CE and UV detection to be 2516 ± 5.21 % ppm or 2.516 mg/g indicating high precision. A recovery study resulted in an average percent recovery of 165.8 % indicating a high degree of error.

References:

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Appendix A:

Table A1. Uncertainty table for the determination of methylparaben in lotion by CE.

Number of points			**[methylparaben]**	**Peak Area**				
n =	5							
			x_i	y_i	x_iy_i	x_i^2	d_i	d_i^2
			1.00	306	306	1.0	12.842	164.91
Unknowns			5.00	1128	5640	25.0	39.398	1552.22
3037			10.00	2072	20720	100.0	-10.906	118.94
			20.00	3885	77700	400.0	-186.515	34787.75
			25.00	5211	130275	625.0	145.181	21077.50
			Σx_i	Σy_i	Σx_iy_i	Σx_i^2	Σd_i	Σd_i^2
		Sums	61.00	12602.0	234641.00	1151.00	0.000	57701.3
			D =	2034.00	**S_y =**	138.6859		
	Method of least squares							
			slope =	198.86	intercept=	94.29744		
			R^2 =	0.9964	**S_y^2 =**	19233.77		
			S_m =	6.8761	**S_b =**	104.33		
	Measured y value =		3037.0		**k =**	1		
	derived x=	14.798		**s_x =**	0.769228			

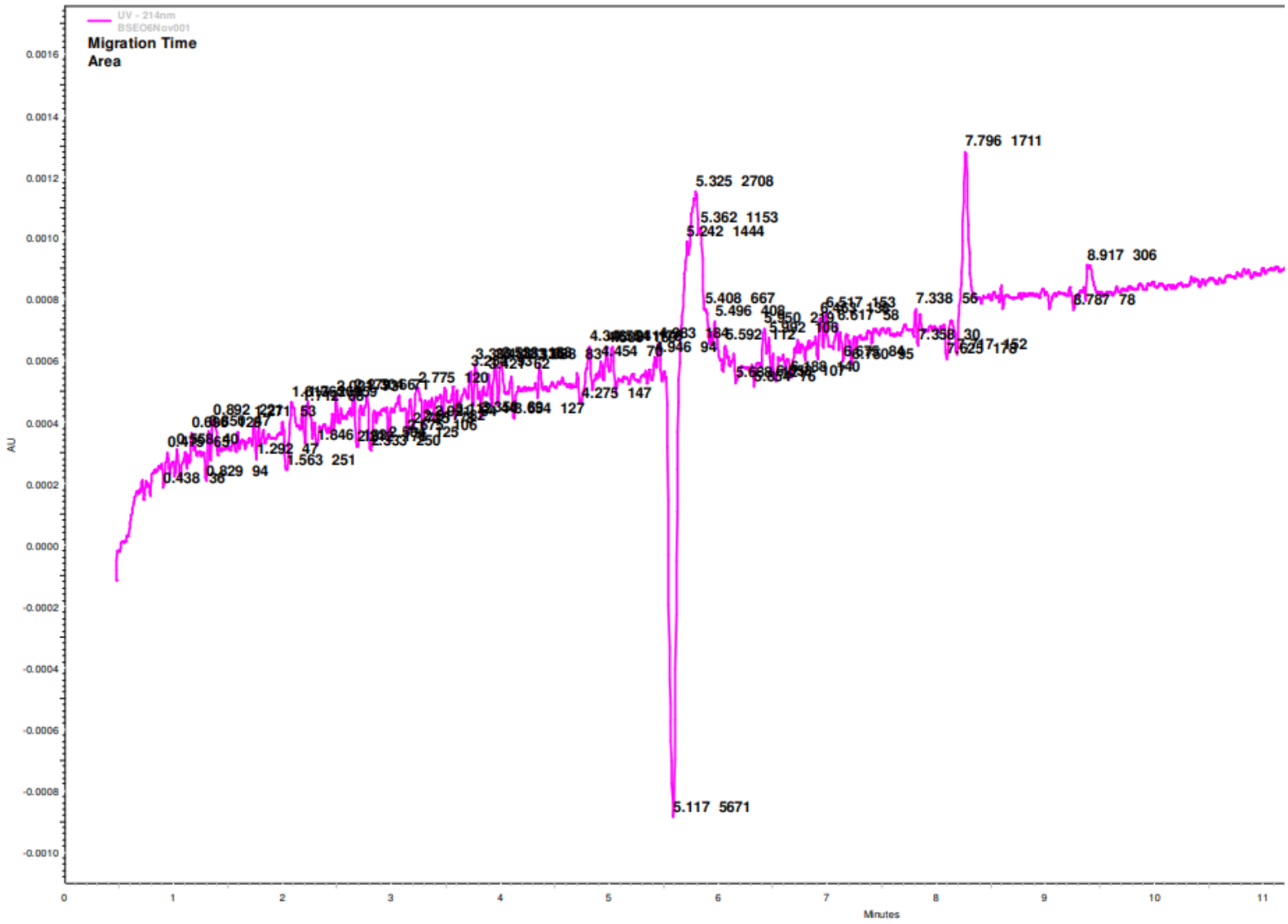

Figure A1. Electropherogram displaying analyte peak as a function of migration time for standard solution 1, 1.0 ppm methylparaben, standard ran week 1.

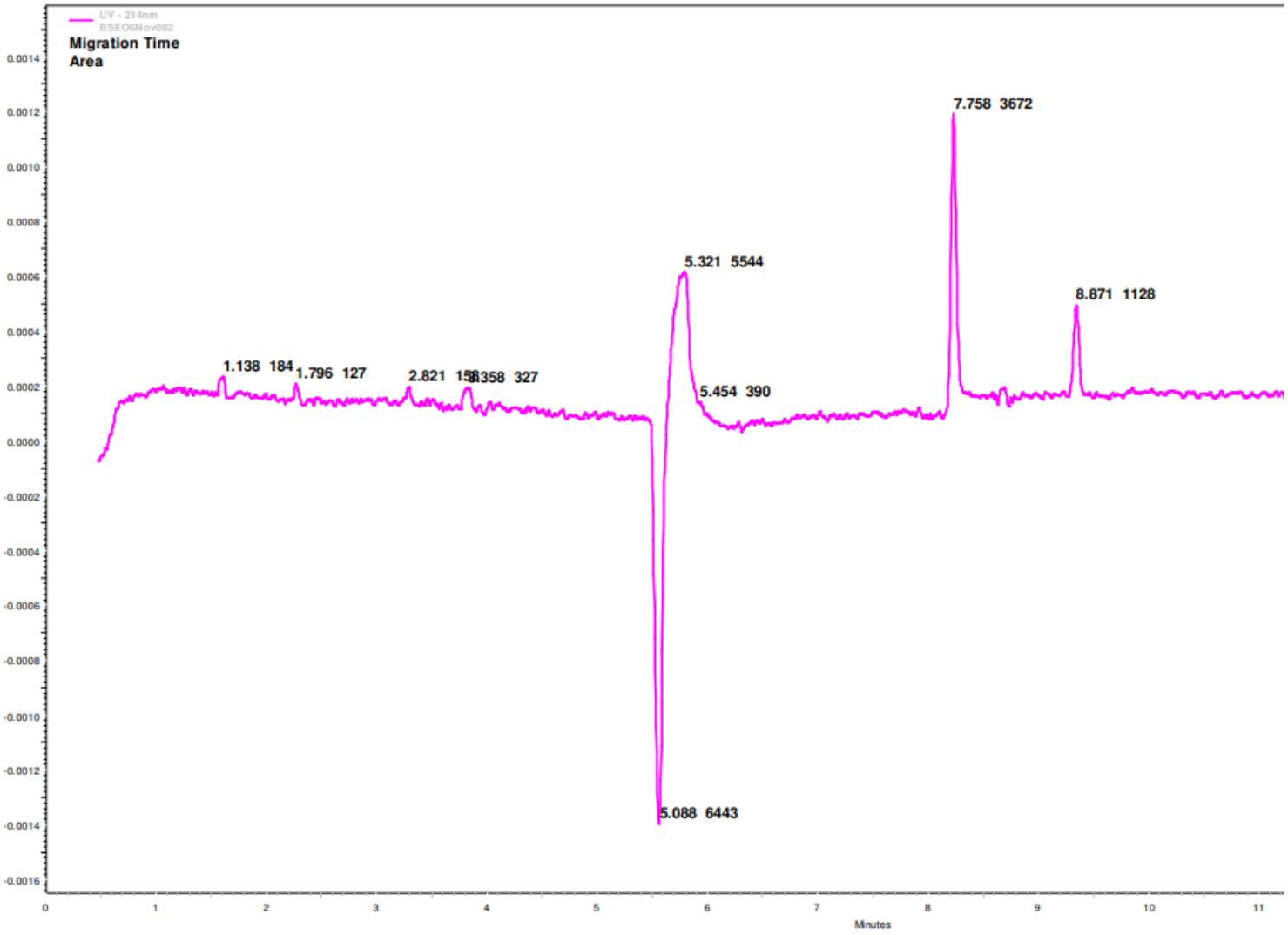

Figure A2. Electropherogram displaying analyte peak as a function of migration time for standard solution 2, 5.0 ppm methylparaben, standard ran week 1.

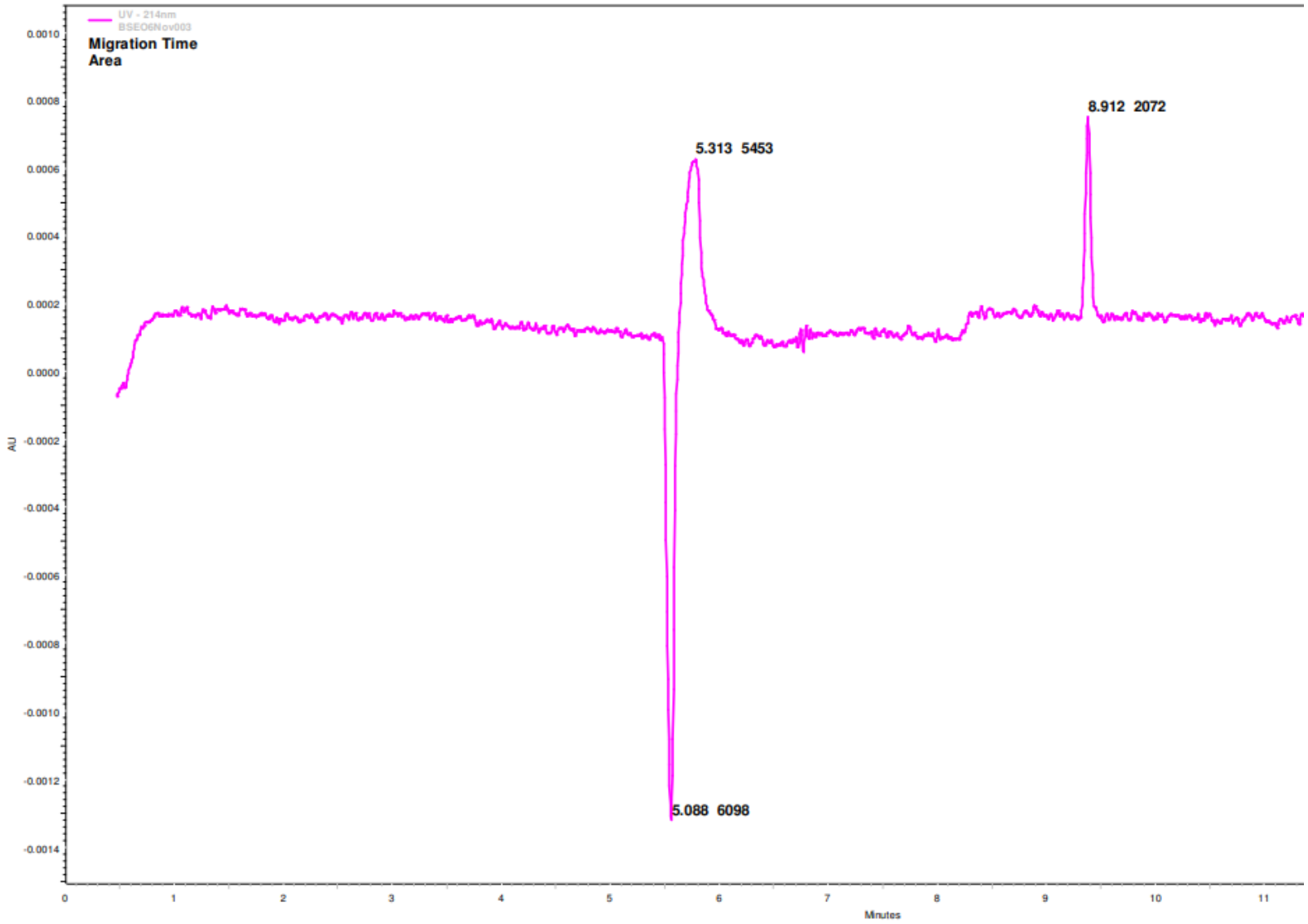

Figure A3. Electropherogram displaying analyte peak as a function of migration time for standard solution 3, 10.0 ppm methylparaben, standard ran week 1.

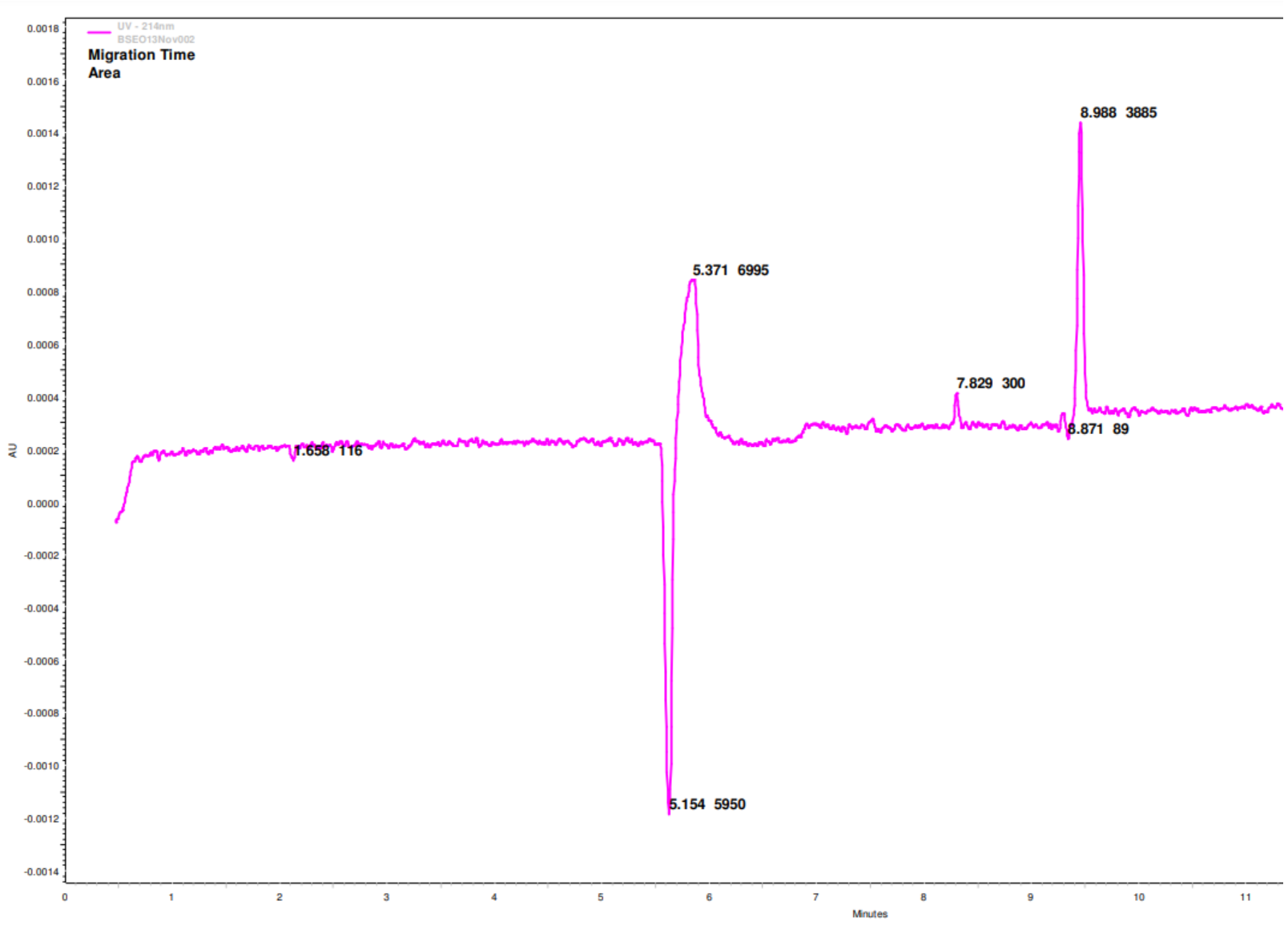

Figure A4. Electropherogram displaying analyte peak as a function of migration time for standard solution 5, 20.00 ppm methylparaben, standard ran week 2.

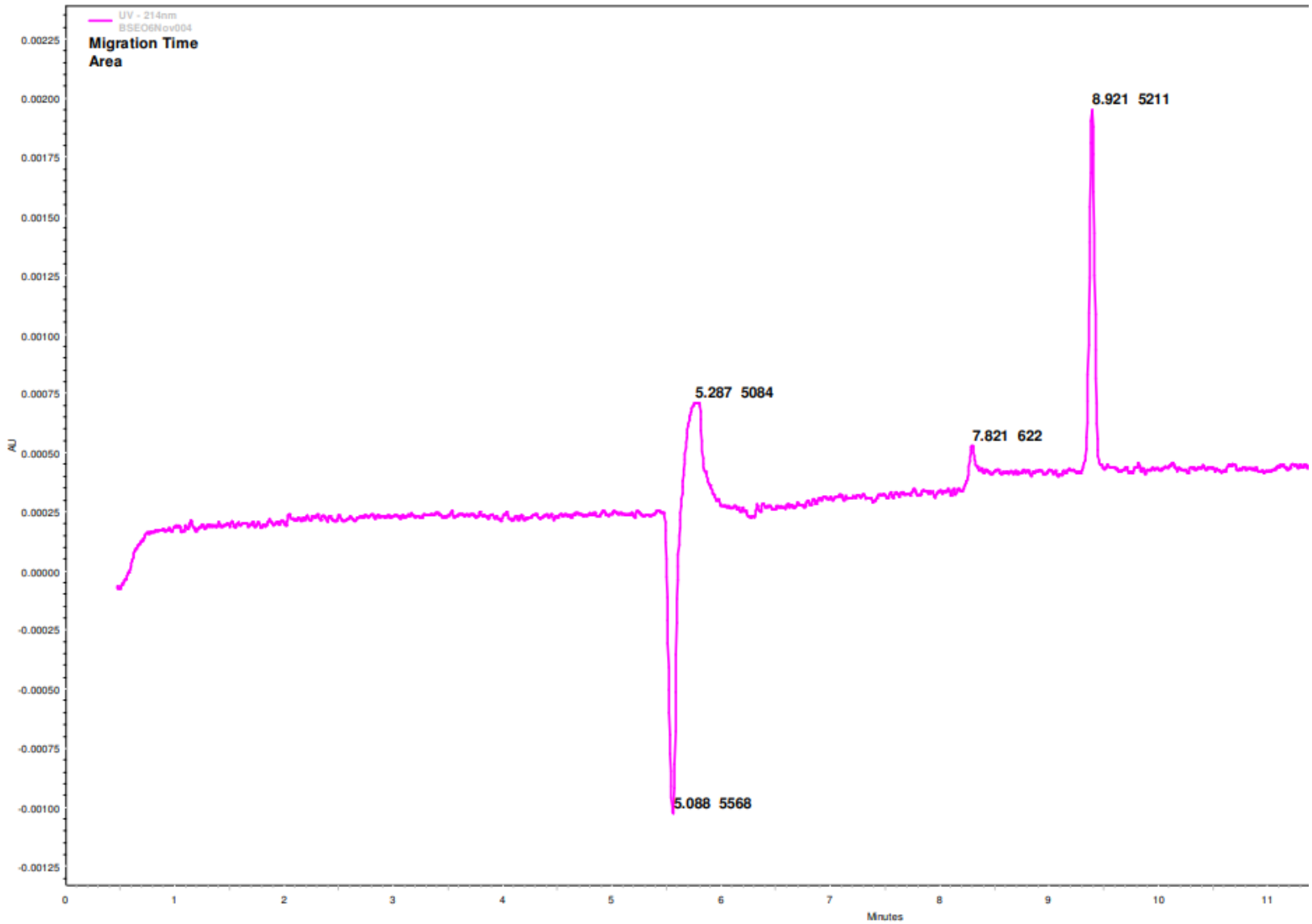

Figure A6. Electropherogram displaying analyte peak as a function of migration time for standard solution 6, 25.00 ppm methylparaben, standard ran week 1.

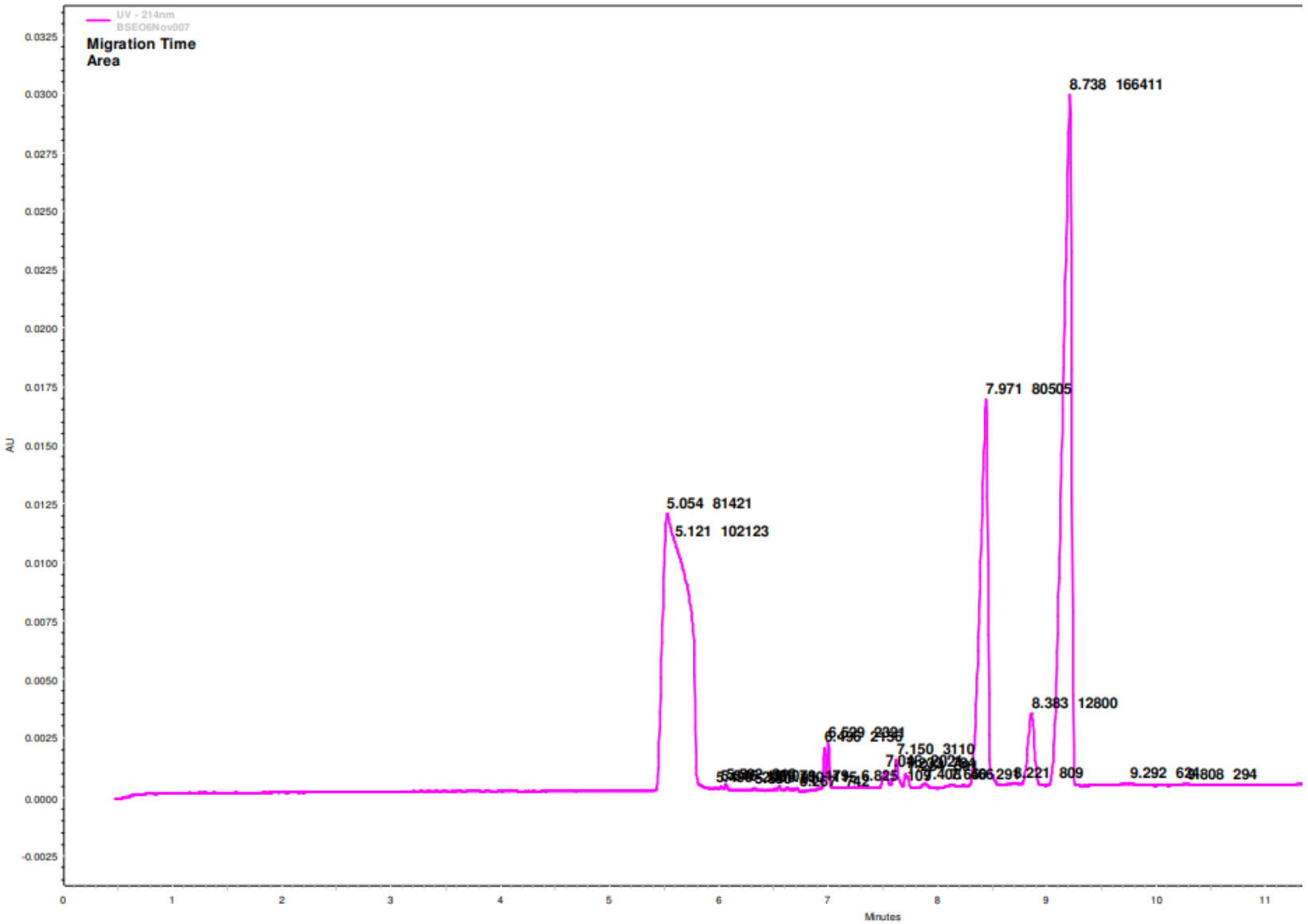

Figure A7. Electropherogram displaying analyte peak as a function of migration time for lotion sample, replicate 1, week 1.

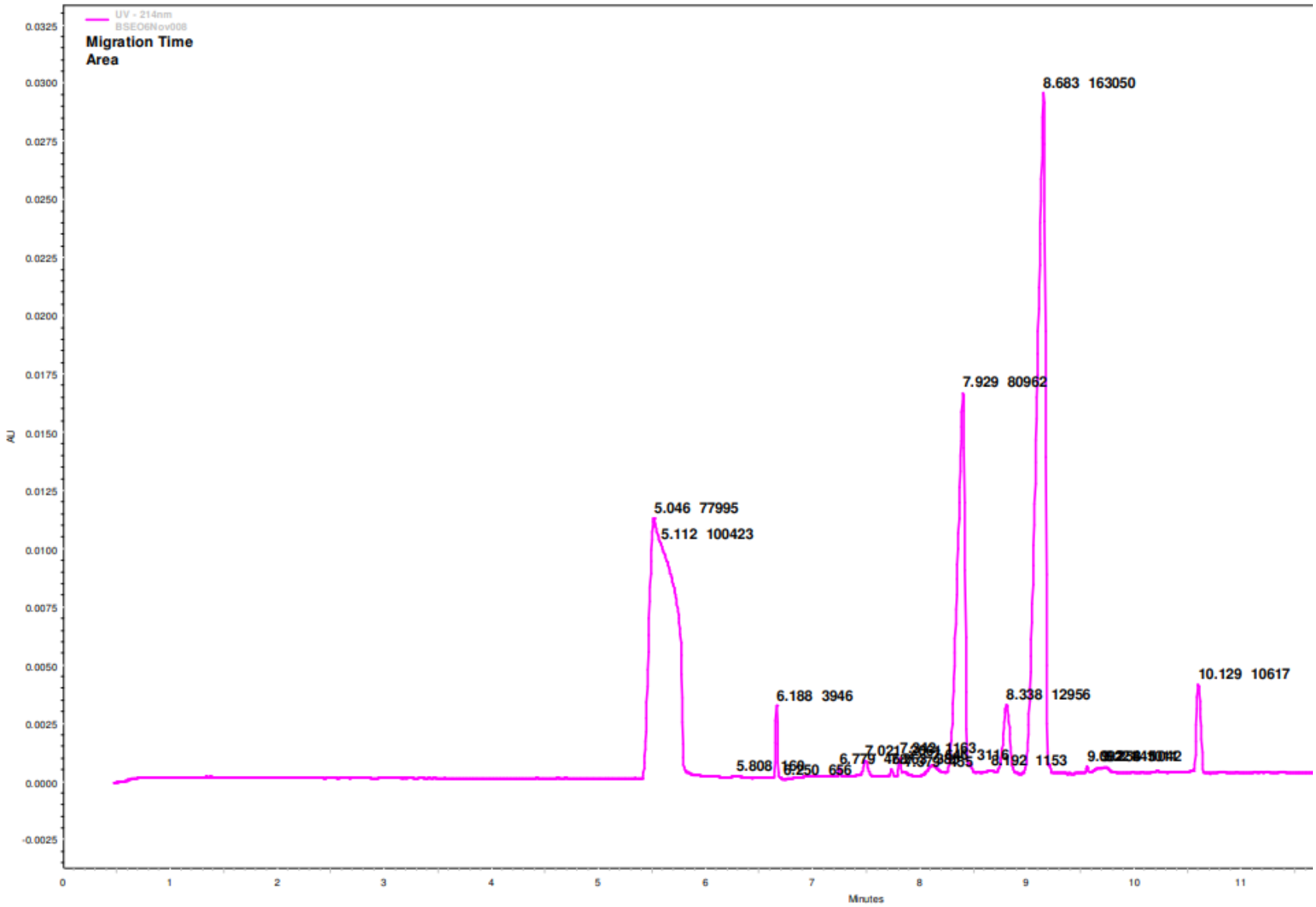

Figure A8. Electropherogram displaying analyte peak as a function of migration time for lotion sample, replicate 2, week 1.

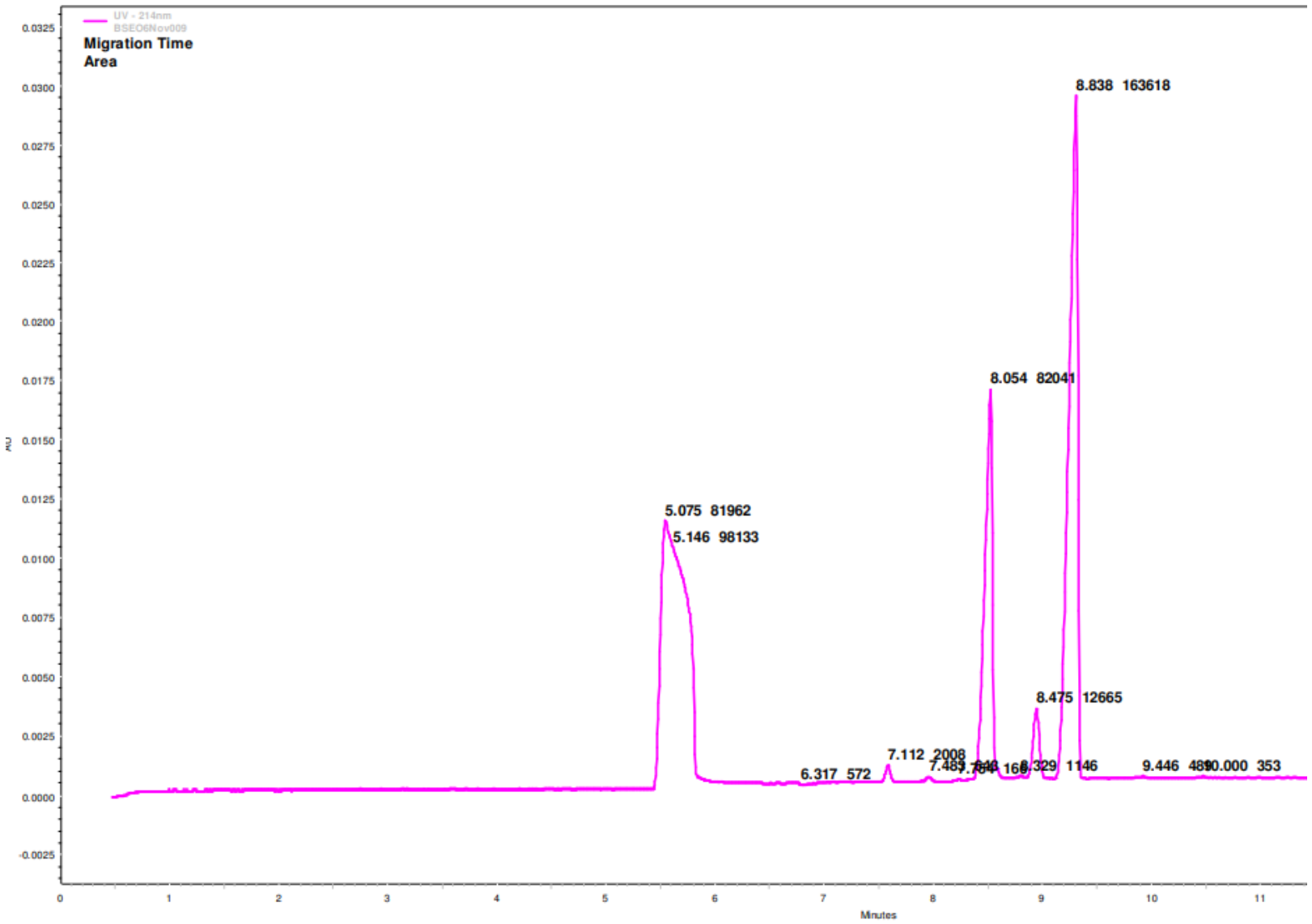

Figure A9. Electropherogram displaying analyte peak as a function of migration time for lotion sample, replicate 3, week 1.

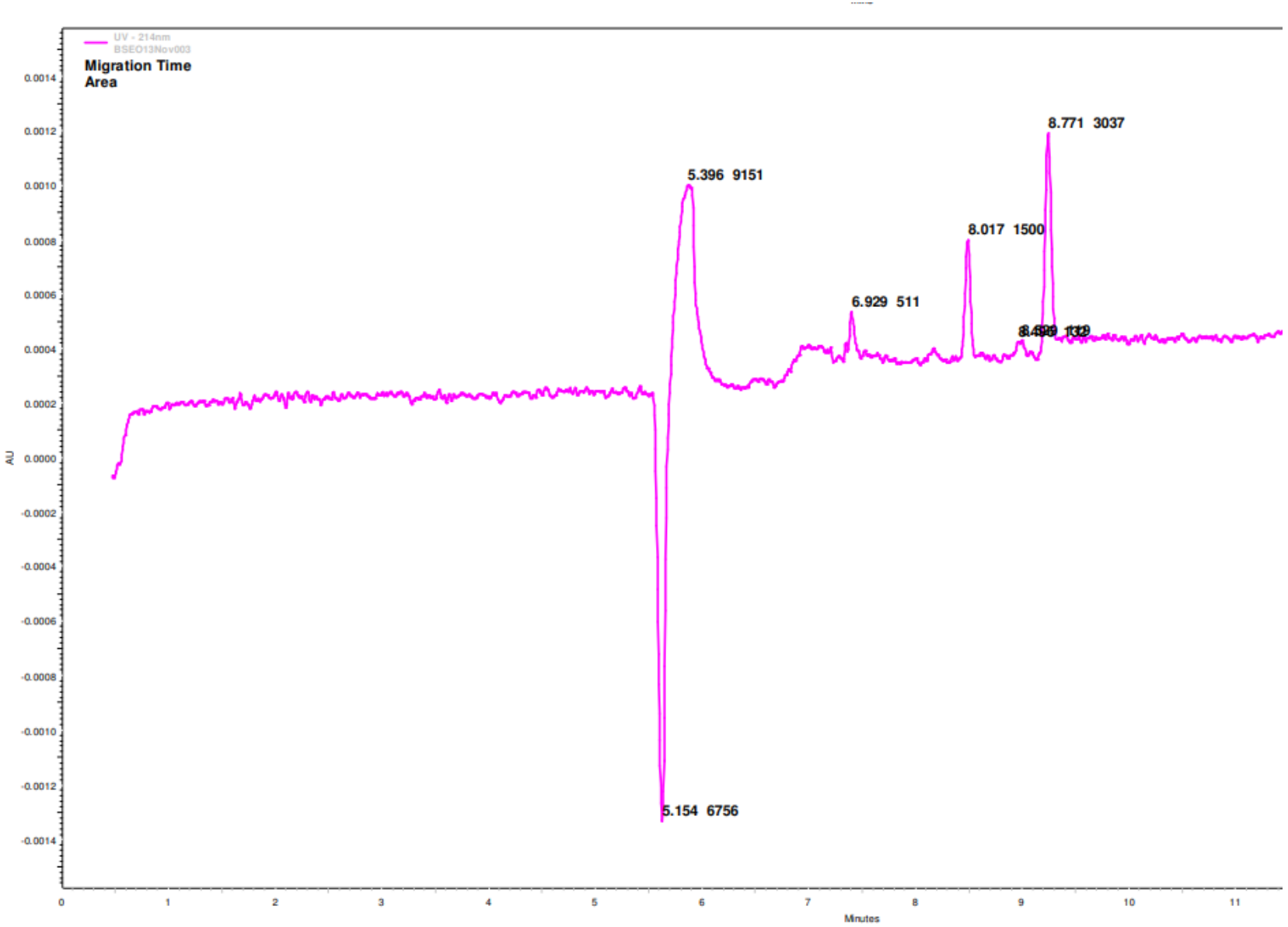

Figure A10. Electropherogram displaying analyte peak as a function of migration time for unspiked lotion sample, week 2.

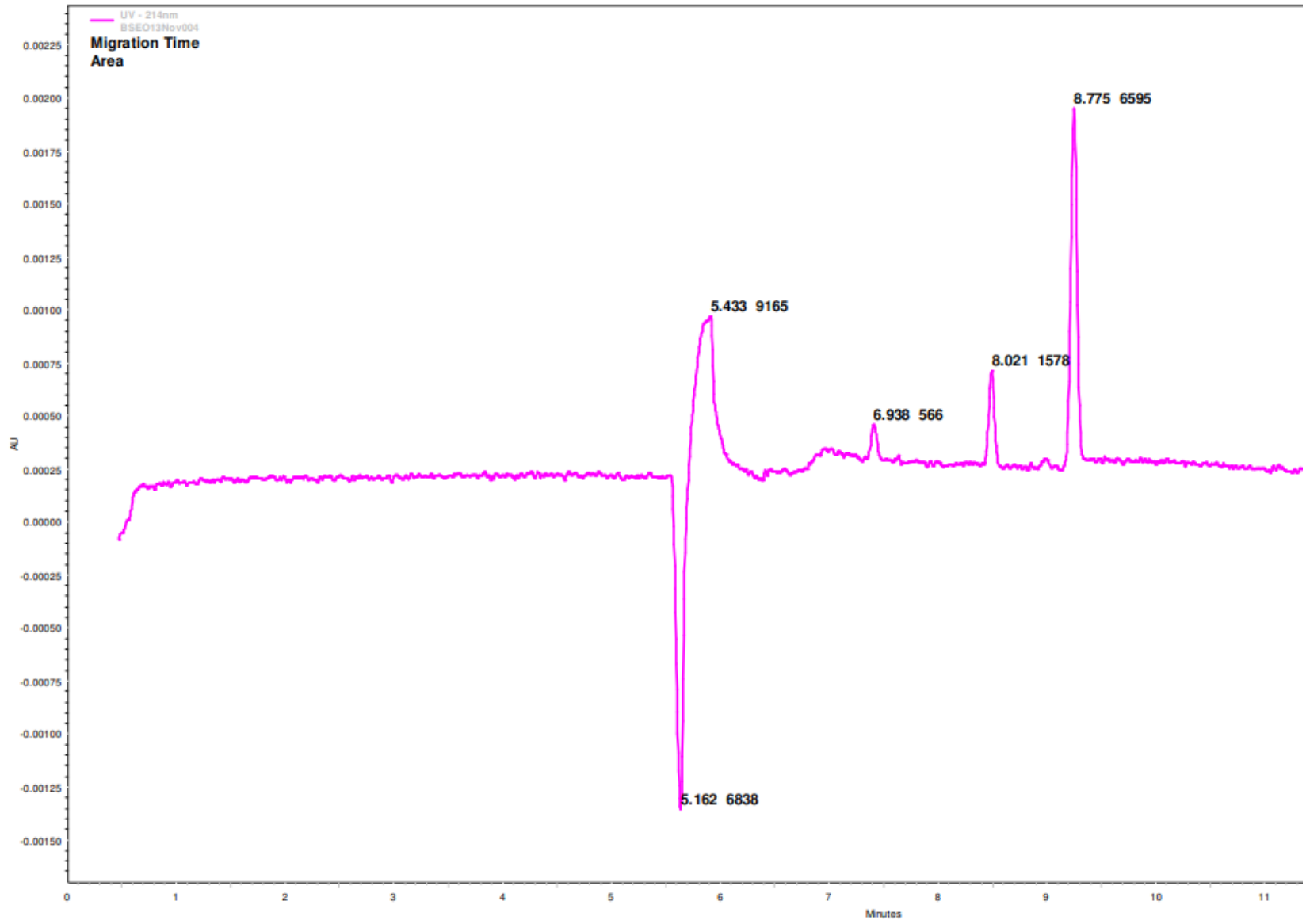

Figure A11. Electropherogram displaying analyte peak as a function of migration time for 80 % spiked lotion sample, week 2.

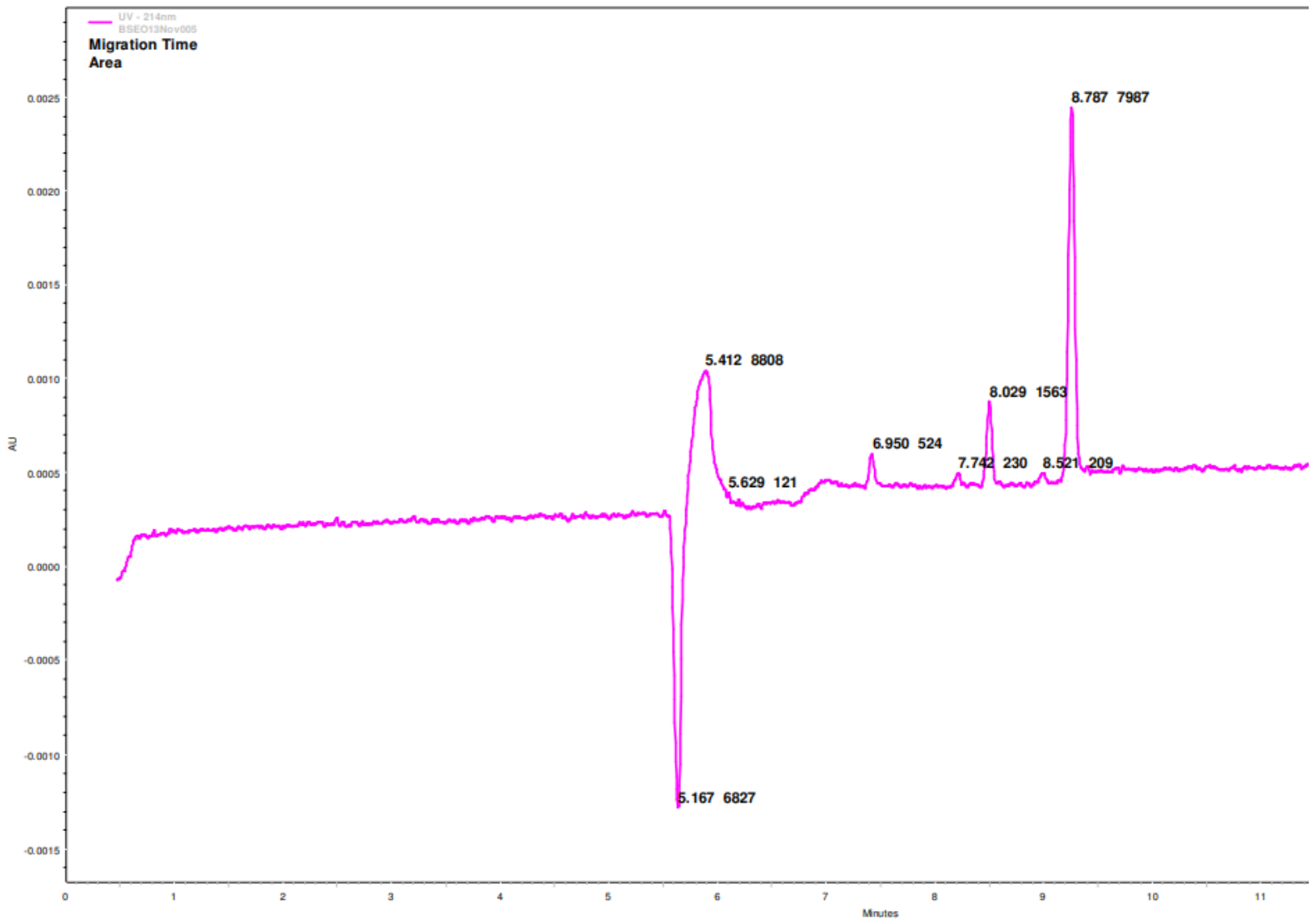

Figure A12. Electropherogram displaying analyte peak as a function of migration time for 100 % spiked lotion sample, week 2.

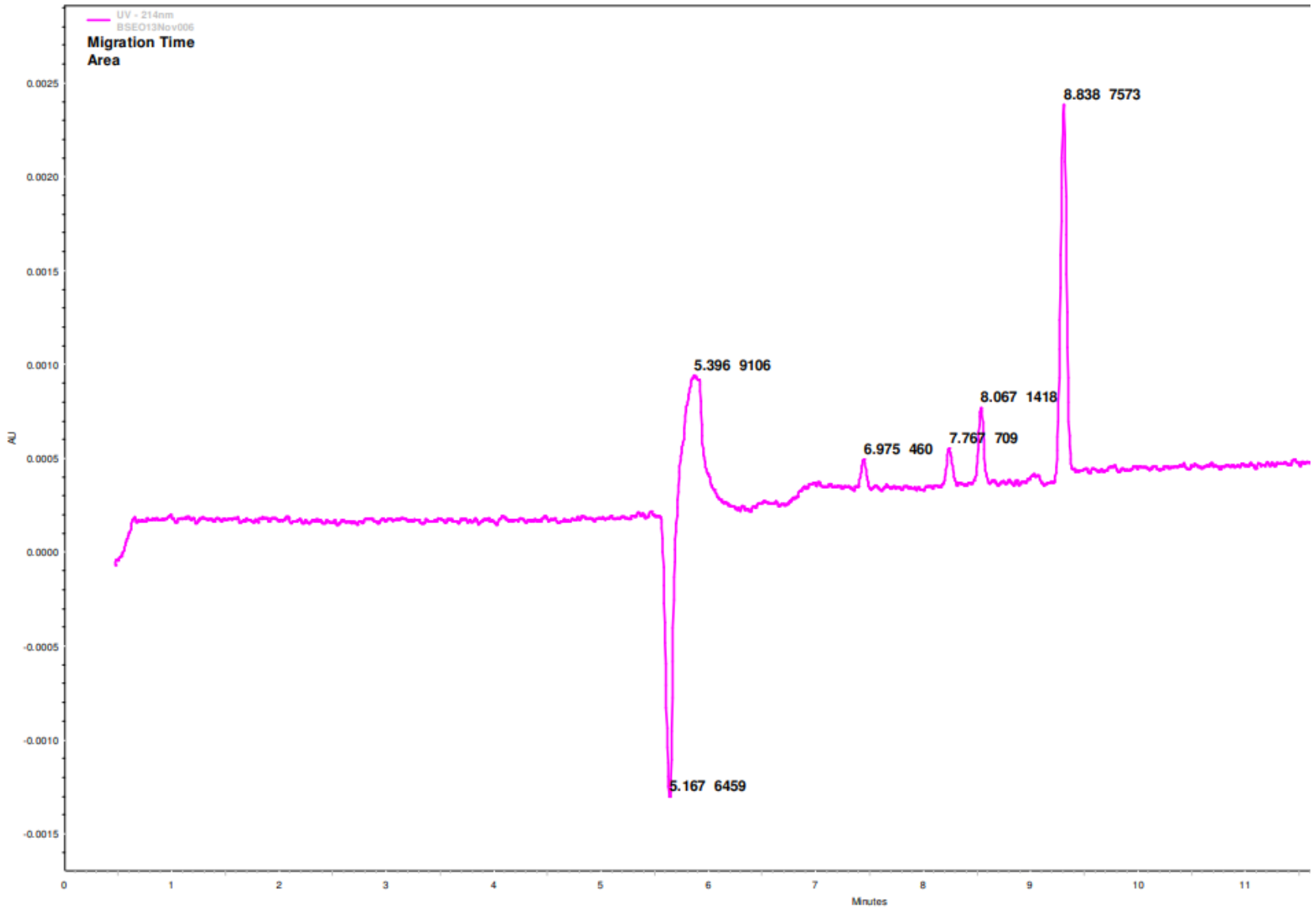

Figure A13. Electropherogram displaying analyte peak as a function of migration time for 120 % spiked lotion sample, week 2.