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Quantitation of Acetyl Hexapeptide-8 in Cosmetics by Hydrophilic Interaction Liquid Chromatography Coupled to Photo Diode Array Detection

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Abstract: Bioactive peptides are gaining more and more popularity in the research and development of cosmetic products with anti-aging effect. Acetyl hexapeptide-8 is a hydrophilic peptide incorporated in cosmetics to reduce the under-eye wrinkles and the forehead furrows. Hydrophilic interaction liquid chromatography (HILIC) is the separation technique of choice for analyzing peptides. In this work, a rapid HILIC method coupled to photodiode array detection operated at 214 nm was developed, validated and used to determine acetyl-hexapeptide-8 in cosmetics. Chromatography was performed on a Xbridge® HILIC BEH analytical column using as mobile phase a 40 mM ammonium formate water solution (pH 6.5)-acetonitrile mixture 30:70, *v/v* at flow rate 0.25 mL min^{-1}. The assay was linear over the concentration range 20 to 30 μg mL^{-1} for the cosmetic formulations and 0.004 to 0.007% (*w/w*) for the cosmetic cream. The limits of quantitation for acetyl hexapeptide-8 were 1.5 μg mL^{-1} and 0.002% (*w/w*) for the assay of cosmetic formulations and cosmetic creams, respectively. The method was applied to the analysis of cosmetic formulations and anti-wrinkle cosmetic creams.

Keywords: hydrophilic interaction liquid chromatography; chromatography; oligopeptides; acetyl hexapeptide-8; acetyl hexapeptide-3; Argireline; cosmetics

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1. Introduction

Skin aging is a biological process *influenced* by various genetic, environmental (pollution, UV radiation), hormonal and metabolic factors. For more than 20 years, bioactive ingredients have been incorporated in cosmetics to smooth out deep wrinkles, improve skin elasticity and reduce the effects of the skin aging [1]. Given that most natural processes within the body are stimulated through the interaction of peptides and proteins with their target partners, bioactive peptides incorporated in cosmetics are one of the most popular ways to reduce wrinkles and fine lines, improve skin appearance and texture, and treat decolorated skin [2]. The role of peptides is crucial in several natural processes related to skin care, such as the modulation of cell proliferation, inflammation, melanogenesis, cell migration, angiogenesis and the synthesis and regulation of proteins. Bioactive peptides are gaining more and more popularity in the research and development of cosmetic products with anti-aging effect [3]. Acetyl hexapeptide-8, also known as acetyl-hexapeptide-3, is a neurotransmitter inhibitor peptide designed from the N-terminal end of the synaptosomal-associated protein (SNAP25) [4,5]. It competitive inhibits the SNAP25 component of the said vesicle docking and fusion protein complex (SNARE) [6] which triggers the calcium- dependent neurotransmitter release into the synapse, a process necessary for muscle contraction [7–10]. Acetyl hexapeptide-8 is marketed as Argireline® [11], and it

has been efficiently used in cosmetics for smoothening the under-eye wrinkles and the forehead furrows [12–14]. After topical application at specific areas of the face, it inhibits the reactions that cause muscles to move or contract for example when forming facial expressions such as smiling or frowning [15,16]. A clinical trial of daily topical application of acetyl hexapeptide-8 in 24 patients with blepharospasm concluded that topical application of this peptide is safe and promising for prolonging the action of injectable botulinum neurotoxin therapy [17]. The quality control of cosmetic products containing bioactive peptides should be addressed under a more systematic investigation and the concentration of these peptides in cosmetics should be supported by product-specific studies. Therefore, there is a real need to set up analytical methods to quantitate the bioactive compounds in cosmetic products.

In the last decade, the increased interest in the separation of peptides has gained momentum due to the emphasis on the chromatographic separation of various proteins in proteome. High performance liquid chromatography (HPLC) has been widely used in the analysis of peptides in various fields of research and development, using different modes of separation. Nowadays, hydrophilic interaction liquid chromatography is the separation technique of choice for the analysis of peptides [18–22]. Usually, bioactive peptides incorporated in cosmetic products are hydrophilic compounds that show little or no retention on conventional RP-HPLC analytical columns. The stationary phases in HILIC are mainly polar such as silica gel, diol-, amino- or cyano- bonded and other zwitterionic packing materials and the typical mobile phases consists of mixtures of a highly polar solvent (typically water) with an organic modifier (typically acetonitrile) [23,24]. The analytes retention is increased by increasing the proportion of the organic modifier in the mobile phase [25,26]. The polar functional groups on the HILIC stationary phases absorb water (0.5%–1.0%) and this way a water-enriched layer is immobilized between the mobile and the stationary phase, especially when the water content of the mobile phase is less than 40% [27]. In HILIC the more hydrophilic analytes are eluted later than the less polar compound. In the literature several works have been published on peptide separation using HILIC columns [28–30], but only a few studies were dedicated to peptide quantitation in cosmetics [31–33]. In one of these publications, ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) using a TSK-gel Amide-80® HILIC analytical column was used to quantitate acetyl hexapeptide-8 in cosmetics after solid phase extraction procedure [34]. The TSK-gel Amide-80® HILIC analytical column was also used in LC-MS/MS methods developed to evaluate transdermal absorption of acetyl-hexapeptide-8 [35,36]. Even though the abovementioned LC-MS/MS approaches are selective and sensitive for the quantitation of acetyl-hexapeptide-8 in various matrices, there is a real need for the development of a reliable analytical method without the need for specialized equipment that can be used in routine analyses.

In this work a rapid and sensitive hydrophilic interaction liquid chromatographic method with photodiode array (PDA) detection was developed and validated for the quantitation of acetyl-hexapeptide-8 in cosmetics. The appropriate stationary phase, pH, buffer concentration and mobile phase composition, was thoroughly investigated prior to method validation. The combination of HILIC with PDA detection provides an accurate, repeatable and robust method for the quantitative analysis of cosmetic products. To the best of our knowledge, this is the first report of a HILIC-PDA method for the quantitation of acetyl-hexapeptide-8 in cosmetics. In this work a Xbridge®-HILIC BEH analytical column has been used and combined with a rapid and simple sample pretreatment. All the above in combination with a short run time of less than 10 min, makes the proposed HILIC-PDA method suitable for the routine quality control of cosmetics.

2. Materials and Methods

2.1. Chemical and Reagents

HPLC grade solvents were bought from Sigma-Aldrich (St. Louis, MO, USA). Analytical reagent grade ammonium formate was acquired from Alfa-Aesar (Haverhill, MA,

USA). HPLC grade water was produced by means of a Synergy® UV water purification system (Merck Millipore, MA, USA). Whatman nylon membrane filters with pore size 0.45 μm and diameter 47 mm were purchased from GelmanSciences (Northampton, UK). Hydrophobic polytetrafluorethylene (PTFE phobic 13 mm, pore size 0.22 μm) syringe filters were acquired from Novalab SA, Athens, Greece representative of RephiLe Bioscience Ltd., Europe.

Acetyl hexapeptide-8 (97% pure), acetyl-Glu-Glu-Met-Gln-Arg-ArgNH2 was kindly provided from Cellco Chemicals Ltd. (Athens, Greece) distributor in Greece of Caregen Co. Ltd. (Gyeonggi, Korea).

Acetyl hexapeptide-8 formulation, namely Argireline peptide solution C®, was donated from N. Krallis S.A. distributor in Greece of Lipotec S.A., Spain containing aqua, 0.05% *w/w* acetyl hexapeptide-8 and 0.05% *w/w* 1,2 octanediol.

The anti-wrinkle cosmetic cream containing 0.005% *w/w* acetyl hexapeptide-8 was produced in the Laboratory of Chemistry-Biochemistry-Cosmetic Science, Department of Biomedical Sciences, University of West Attica in Athens, Greece by using the Argireline peptide solution C®. The excipients present in anti-wrinkle cream are aqua, xalifin-15, propylene glycol, sabowax FX-65, squalene, butylated hydroxyl toluene (BHT) and germall 115. Placebo cream containing only the excipients, without acetyl hexapeptide-8, was also prepared for validation purposes.

2.2. Stock and Calibration Standard Solutions

Acetyl hexapeptide-8 stock standard solution at 500 μg mL^{-1} was prepared in acetonitrile-water mixture (60:40, *v/v*). The stock standard solution was further diluted in acetonitrile-water mixture (60:40, *v/v*) to prepare mixed working standard solutions. The solutions were stored in amber bottles at 4 °C and remained stable for two months.

For the quantitation of acetyl hexapeptide-8 in Argireline peptide solution C® (formulation), the calibration samples were prepared at concentration levels 20, 23, 25, 28 and 30 μg mL^{-1} in water/acetonitrile mixture (30:70, *v/v*). Quality control (QC) samples were also prepared at concentration levels 20 and 25 μg mL^{-1}.

For the quantitation of acetyl hexapeptide-8 in the anti-wrinkle cosmetic cream the calibration spiked cream samples at concentration levels 0.004, 0.0045, 0.005, 0.006 and 0.007 *w/w* were prepared by spiking placebo cream with appropriate dilutions of acetyl hexapeptide-8 stock standard solution. Quality control (QC) samples were also prepared in a similar manner at concentration levels 0.004, 0.005 and 0.007% *w/w*.

2.3. Sample Preparation Procedure

2.3.1. Cosmetic Formulation

An accurately weighted amount (0.5 g) of Argireline peptide solution C® is transferred at a 10 mL volumetric flask and diluted to volume with water/acetonitrile (30:70, *v/v*). A portion of this solution is then analyzed by the proposed HILIC-PDA method for the quantitation of acetyl hexapeptide-8.

2.3.2. Cosmetic Cream

An accurately weighted amount (0.1 g) of cosmetic cream is mixed with 200 μL of acetonitrile-water mixture (60:40, *v/v*) and 800 μL of 30% 40 mM ammonium formate water solution in acetonitrile. The mixture is shaken for 2 min and then centrifuged at 18,000× *g* for 30 min, at ambient temperature. The upper layer is then filtered through a PTFE hydrophobic syringe filter prior to HILIC-PDA analysis.

2.4. HILIC-PDA

The HPLC-PDA analytical instrument used in this work is consisting of a Waters 717 plus autosampler, a column temperature oven, a Waters 1515 isocratic pump and a Waters 996 photodiode array detector (Milford, MA, USA). The Empower software (Milford, MA, USA) was used for the data acquisition. The chromatographic eluent was monitored

over the wavelengths 200 to 400 nm and extracted chromatograms at λ 214 nm were used for data analysis. An Xbridge®-HILIC BEH guard column (20 × 2.1 mm, 3.5 µm) in line with an Xbridge®-HILIC BEH analytical column (2.1 × 150 mm, 3.5 µm) were used for the chromatography. The mobile phase was composed of 30% 40 mM ammonium formate aqueous solution in acetonitrile and pumped at a flow rate of 0.25 mL min^{-1}. Prior to the chromatography the mobile phase was filtered through a 0.22 µm nylon membrane filter, Membrane Solutions (Kent, WA, USA) and degassed under vacuum. Samples were injected via a 10 µL injection loop and acetyl hexapeptide-8 was quantitated in cosmetic products with a chromatographic run time of 10 min.

2.5. Method Validation and Application to the Analysis of Real Samples

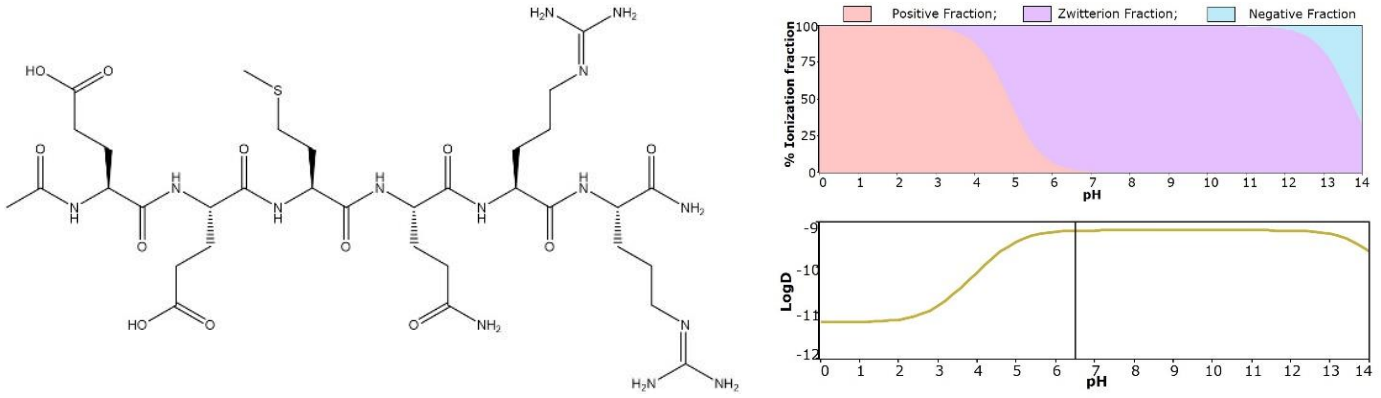
The HILIC-PDA method was validated in terms of linearity, limit of detection, limit of quantitation, intra-day and inter-day precision and overall accuracy [37]. The method was applied to the analysis of various batches of a cosmetic formulation namely Argireline peptide solution C® and to the analysis of various batches of an anti-wrinkle cosmetic cream. To evaluate the linearity, linear regressions were used to construct the calibration graphs after the analysis calibration standards and spiked cream samples at five different concentration levels. Peak area measurements were used for quantitation of acetyl hexapeptide-8. The % coefficient of variations (%CVs) and the % relative recovery were calculated to evaluate precision (intra- and inter-day) and overall accuracy, respectively.

3. Results and Discussions

3.1. HILIC-PDA Method Development

Chromatography

Acetyl-hexapeptide-8 consists of a six amino acids chain acetylated at the N-terminal residue, N-acetyl-Glu-Glu-Met-Gln-Arg-ArgNH_2, as shown in Figure 1a. The ionization fraction and the LogD values of acetyl-hexapeptide-8 as a function of pH were calculated by the ADME boxes software ver. 3.0.3 built 45, Pharma Algorithms Ltd. (Toronto Canada) and the results are presented in Figure 1b.



(**a**) (**b**)

Figure 1. Acetyl hexapeptide-8 (**a**) Chemical structure; (**b**) Plots of the ionization fraction (**top**) and the LogD values (**bottom**) as a function of pH.

LogD values of acetyl hexapeptide-8 versus pH are less than -9, indicating that this compound is highly hydrophilic (Figure 1b, bottom). HILIC is the analytical technique of choice for the chromatographic analysis of polar compounds, and it was therefore used in the present work. The retention mechanism in HILIC is based on several types of interactions such as adsorption, partition, electrostatic, hydrogen bonding and reversed-phase interactions [38–40]. Greater retention is achieved when more than 70% of organic modifier (e.g., acetonitrile) is used in the mobile phase. The chromatographic conditions were optimized to achieve adequate retention and optimum peak shape of acetyl-hexapeptide-8.

To find the optimal mobile phase composition we examined various combinations of aqueous buffer solutions and acetonitrile with changed content of each component. The detection wavelength was set to 214 nm, because at this wavelength acetyl heptpeptide-8 shows satisfactory absorption. The flow rate was set to 0.25 mL min^{-1} and the experiments were conducted at ambient temperature.

The XBridge®-HILIC BEH analytical column used in this work consists of BEH particles. Some accessible free silanol groups on the surface of these BEH particles are responsible for electrostatic interactions. The addition of aqueous salt solutions in the HILIC mobile phase eluents reduces the electrostatic interactions between the stationary phase and the analyte [41]. Plot of the logk values of acetyl hexapeptide-8 as a function of the concentration of ammonium formate is presented in Figure 2a. Ammonium formate concentration was modified from 2.5 to 100 mM with a constant aqueous component of the mobile phase eluent at 30%, *v/v* and a constant pH at 6.5. Under these conditions the free silanol groups on the surface of these BEH particles are negatively charged and acetyl hexapeptide-8 is in zwitterion form. The results showed that by increasing the concentration of ammonium formate the retention of acetyl hexapeptide-8 is slightly decreased up to 40 mM and then increased up to 100 mM. These findings indicate that the retention mechanism of acetyl hexapeptide-8 in XBridge®-HILIC BEH analytical column is complex and comprises both hydrophilic partition with secondary electrostatic interactions. From these experiments, we concluded that by using a 40 mM ammonium formate concentration in the mobile phase peak symmetry and plate numbers are improved and the analyte is adequately retained and well separated from the solvent front.

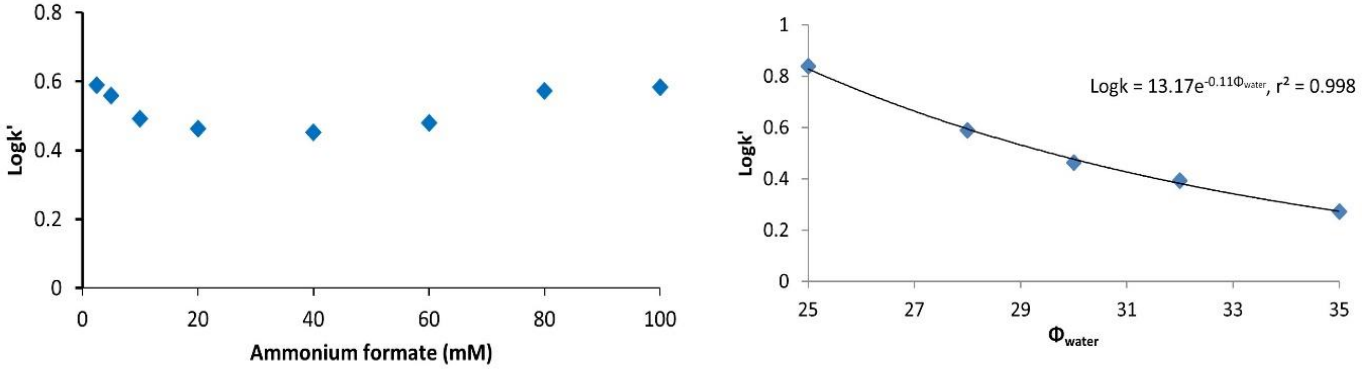


Figure 2. Plots of logk values for acetyl hexapeptide-8 as a function of: (**a**) the concentration of ammonium formate, and (**b**) the percentage of the water content of the mobile phase.

The chromatography of acetyl hexapeptide-8 was also explored by using various mobile phases where the concentration of ammonium formate in the whole mobile phase was kept constant at 12 mM, while the percentage of water, Φ_{water} varied from 25% to 35%. As shown in Figure 2b, the logk values of the peptide decrease exponentially with increasing Φ_{water}, implying a complex retention mechanism for this analyte on the selected HILIC column. The optimum mobile phase composition consists of 30% 40 mM ammonium formate water solution (pH 6.5) in acetonitrile. As shown in Figure 3, acetyl hexapeptide-8 is eluted at 8.15 min and the proposed HILIC-PDA method allows the isocratic elution of acetyl hexapeptide-8 within 10 min.

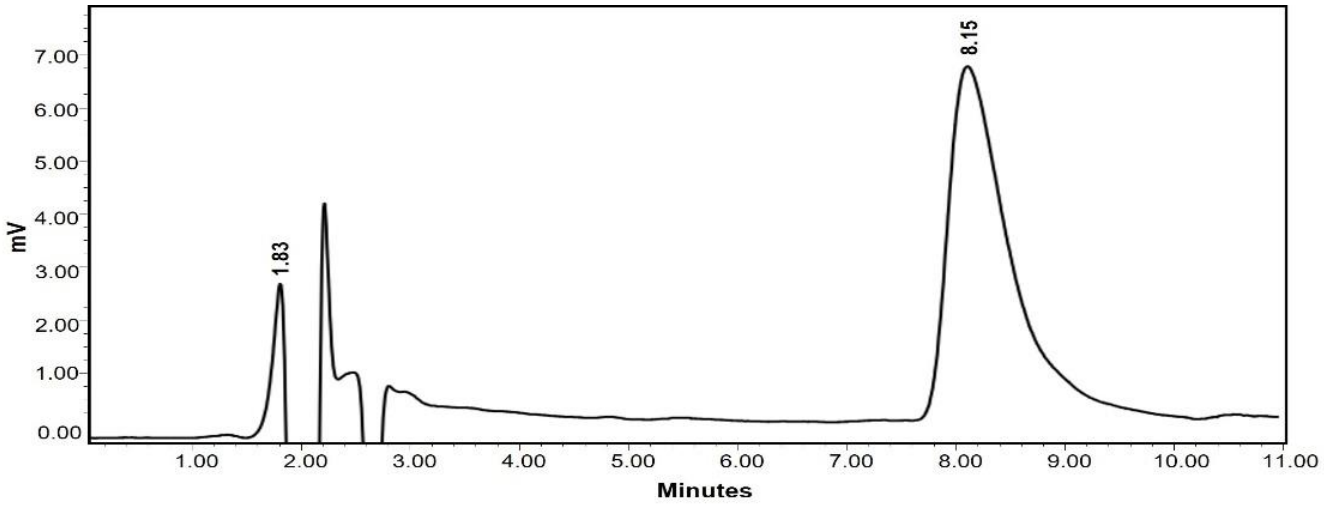


Figure 3. HPLC-PDA chromatogram of a quality control sample of acetyl hexapeptide-8 prepared in water/acetonitrile (30:70, *v/v*) at 25 µg mL^{-1}. Chromatographic conditions: BEH XBridge®-HILIC analytical column; mobile phase: 40 mM ammonium formate aqueous solution/acetonitrile (30:70, *v/v*), flow rate 0.25 mL min^{-1} at 214 nm.

3.2. Statistical Analysis of Data

3.2.1. Selectivity

The selectivity of the HILIC-PDA method to the analysis of Argireline peptide solution C® (cosmetic formulation) is demonstrated in Figure 4, where a chromatogram obtained from the analysis of the cosmetic formulation (red spiked line) is superimposed to a chromatogram of a quality control sample of acetyl hexapeptide-8 prepared in water/acetonitrile (30:70, *v/v*), both samples contain the analyte at 25 µg mL^{-1} (grey line).

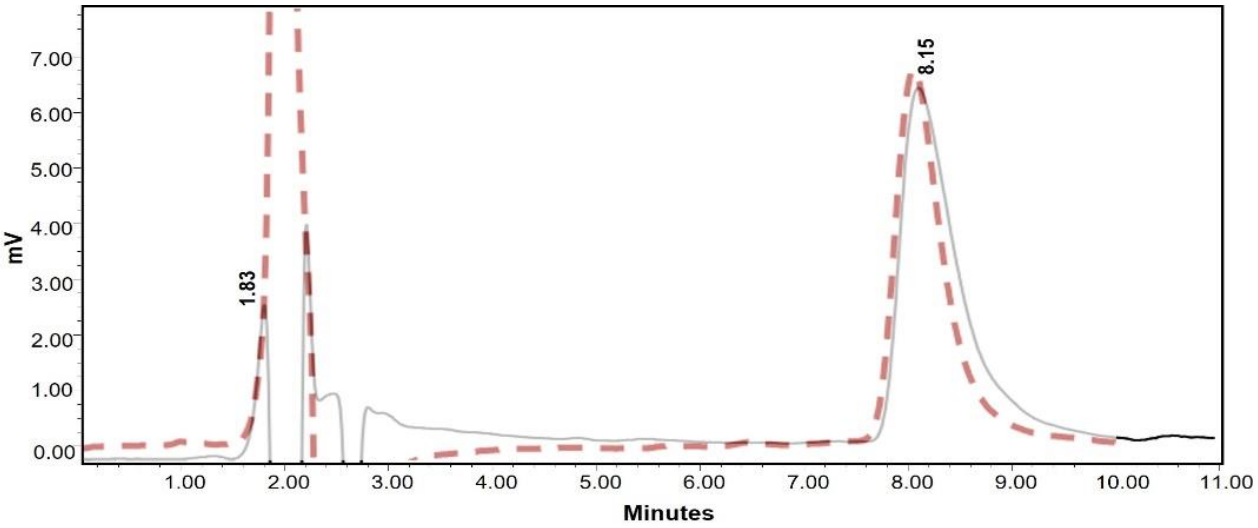


Figure 4. HPLC-PDA chromatogram obtained from the analysis of Argireline peptide solution C® (red spiked line) superimposed to a chromatogram of a quality control sample of acetyl hexapeptide-8 prepared in water/acetonitrile 30:70, *v/v* (grey line), both samples contain the analyte at 25 µg mL^{-1}. Chromatographic conditions: BEH XBridge®-HILIC analytical column; mobile phase: 40 mM ammonium formate aqueous solution/acetonitrile (30:70, *v/v*), flow rate 0.25 mL min^{-1} at 214 nm.

Moreover, the selectivity of the HILIC-PDA method to the analysis of cosmetic creams incorporated with acetyl hexapeptide-8 is demonstrated in Figure 5, where a chromatogram obtained from the analysis of a placebo cream sample (black line) is superimposed to a

chromatogram of a cream sample obtained after the sample preparation described in Section 2.3.2 containing acetyl hexapeptide-8 at 0.005% *w/w* (blue line).

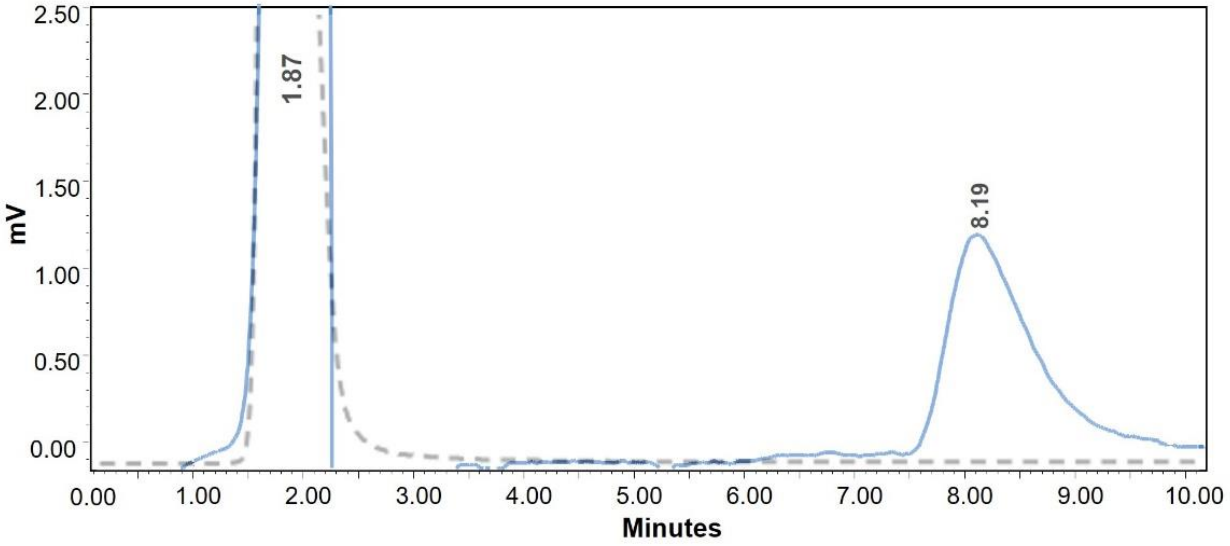


Figure 5. HPLC-PDA chromatogram obtained from the cosmetic cream (blue line) superimposed to a chromatogram of a black cream sample (grey spiked line). Chromatographic conditions: BEH XBridge®-HILIC analytical column; mobile phase: 40 mM ammonium formate aqueous solution/acetonitrile (30:70, *v/v*), flow rate 0.25 mL min^{-1} at 214 nm.

3.2.2. Linearity Data

For the quantitation of acetyl hexapeptide-8 in the cosmetic formulation (Argireline peptide solution C®) the calibration curves have been constructed at the range of concentrations 20 to 30 μg mL^{-1}. The peak area signal of the peptide, S, versus the corresponding concentrations, C exhibited linear relationships and the results of a typical calibration curve are shown in Table 1. A Student's *t*-test was also performed to evaluate whether the intercept of the regression equation was significantly different from the theoretical zero value. The test was based on the estimation of the experimental t-value, $t_{experimental}$ = a/Sa, where a is the intercept and Sa is the standard deviation of the intercept of the regression equation, and on the comparison of $t_{experimental}$ with the theoretical t-value. tp. The results presented in Table 1 indicate that the intercept of the regression equation does not differ from the theoretical zero value.

Table 1. Linearity data for the quantitation of acetyl hexapeptide-8 in Argireline peptide solution C® (formulation) as assessed by the HILIC-PDA method.

Concentration Range (μg mL^{-1})	20–30
Regression Equation	S = 18.08 (±0.64) × 10^3 C − 20 (±16) × 10^3
Correlation Coefficient, R	0.998
Standard Error of Estimation, Sr	5072
Limit of Detection, LOD (μg mL^{-1})	0.5
Limit of Quantitation, LOQ (μg mL^{-1})	1.5
$t_{experimental}$: a/S_a [1]	1.24
tp, f = 3, *p* = 0.05 [2]	4.30

[1] $t_{experimental}$ = experimental t-value; a = intercept; Sa standard deviation of the intercept; [2] tp = theoretical t-value; f = degrees of freedom; *p* = *p*-value.

For the quantitation of acetyl hexapeptide-8 in cosmetic creams, calibration curves were constructed after the analysis of spiked cream samples over the concentration range 0.004 to 0.007 *w/w*. The results of a typical calibration curve are presented in Table 2. In all cases correlation coefficient is greater than 0.994 indicating linear relationships between the peak area signal of the analyte, S and the corresponding concentrations, C. A Student's

t-test was also performed in analogous manner, and the results (Table 2) indicate that the intercept of the regression line is not significantly different from zero and thus there is no interference from the cream matrix.

Table 2. Linearity data for the quantitation of acetyl hexapeptide-8 in cosmetic cream as assessed by the HILIC-PDA method.

Concentration Range (% *w/w*)	0.004–0.007
Regression Equation	S = 1105 (±41) × C + 0.71 (±0.23)
Correlation Coefficient, r	0.998
Standard Error of Estimation, Sr	0.098
Limit of Detection, LOD (% *w/w*)	6.8×10^{-4}
Limit of Quantitation, LOQ (% *w/w*)	0.002
$t_{experimental}$: a/S_a [1]	3.10
tp, f = 3, *p* = 0.05 [2]	4.30

[1] $t_{experimental}$ = experimental t-value; a = intercept; Sa standard deviation of the intercept; [2] tp = theoretical t-value; f = degrees of freedom; *p* = *p*-value.

Limit of detection (LOD) and limit of quantitation (LOQ) values for acetyl hexapeptide-8 were calculated as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively. This was achieved by the analysis of dilute solutions of the peptide at known concentration prepared by the appropriate sample preparation procedure [42]. LOD and LOQ values for acetyl hexapeptide-8 in cosmetic formulation and in cosmetic cream are reported in Table 1 and in Table 2, respectively.

3.2.3. Accuracy and Precision

Precision and accuracy were evaluated by one-way analysis of variance (ANOVA) and the results are presented in Table 3. The total precision was between 1.74 to 4.34 for the cosmetic formulation and 2.46 to 3.53% for acetyl hexapeptide-8 in cosmetic cream. The total accuracy was between 98.9 to 99.8% for the analyte in cosmetic formulation and 99.3 to 101.6% for the quantitation in cosmetic cream.

Table 3. Accuracy and precision data of the HILIC-PDA method for the quantitation of acetyl hexapeptide-8 in cosmetic formulation and cosmetic creams (*n* = 3 runs in 5 replicates).

Matrix	**Concentration Levels**		
Cosmetic Formulation			
Added concentration (μg mL^{-1})	20		25
Overall mean	19.95 (±0.91)		24.74 (±0.42)
Intraday CV(%)[1]	4.76		1.66
Total precision CV(%) [1]	4.37		1.74
Total accuracy Er%[2]	99.8		98.9
Cosmetic cream			
Added concentration (% *w/w*)	0.004	0.005	0.007
Overall mean	39.7 (±0.82) × 10^{-4}	50.62 (±0.39) × 10^{-4}	69.71 (±0.79) × 10^{-4}
Intraday, CV(%) [1]	2.41	3.33	3.11
Total precision, CV(%) [1]	2.46	3.35	3.53
Total accuracy, Relative Recovery (%) [2]	99.3	101.2	99.6

[1] Coefficient of variation; [2] Relative recovery percentage.

3.3. Application to the Analysis of Real Samples

The proposed method was applied to the analysis of three batches of Argireline peptide solution C® labelled to contain 0.05% *w/w* acetyl hexapeptide-8, and three batches of anti-wrinkle cosmetic cream labelled to contain 0.005% *w/w* of the peptide. Results obtained from the analysis of real cosmetic products are presented in Table 4.

Table 4. Quantitation of acetyl hexapeptide-8 in cosmetic formulation and cosmetic cream.

Cosmetic Product	% Label Claim (±SD) [1] (n = 5)	% CV [2]
Cosmetic formulation		
Batch No F1	0.0511 (±0.0015)	2.93
Batch No F2	0.0507 (±0.0011)	2.16
Batch No F3	0.0491 (±0.0016)	3.25
Cosmetic cream		
Batch No C1	0.00491 (±0.00018)	3.7
Batch No C2	0.00501 (±0.00013)	2.6
Batch No C3	0.00509 (±0.00021)	4.1

[1] Standard deviation; [2] Coefficient of variation.

The % recovery for the quantitation of acetyl hexapeptide-8 by the proposed HILIC-PDA method is ranged from 98.2 to 102.2 % in cosmetic formulation and from 98.2 to 101.8% in cosmetics creams.

4. Conclusions

There is a real need to set up analytical methods to quantitate the active compounds in cosmetic products incorporated with bioactive peptides in low content. In this work, a HILIC-PDA method was developed and validated for the determination of acetyl hexapeptide-8 in cosmetics. Over the past two decades the use of biopeptides in cosmetic products is increasingly expanded. The developed method takes full advantage of the benefits of HILIC leading to efficient retention of acetyl hexapeptide-8 with less matrix effect. Validation results demonstrate that the proposed method allows for the quantitation of acetyl hexapeptide-8 in both cosmetic formulations and cosmetics creams. The simplicity of sample preparation procedure and the short chromatographic run time of less than 10 min gives the method the capability for high sample throughput and it can be used to support quality control of cosmetic products containing low content of acetyl hexapeptide-8. There is no doubt that HILIC chromatography enables the determination of bioactive peptides in cosmetic products without the need for specialized detection methods. The proposed method could be extended for future applications in the analysis of various bioactive peptides used for cosmetic purposes.

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