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Development of an electrokinetic chromatography method for the rapid enantiomeric determination of 5-hydroxytryptophan. Application to the analysis of dietary supplements

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5-Hydroxytryptophan is a chiral non-proteinogenic amino acid that plays a key role in the metabolic and neurological balance of an organism, as it is the precursor of serotonin. Dietary 5-hydroxytryptophan supplementation based on *Griffonia simplicifolia* seed extracts is widely used to enhance positive emotion recognition. Since the L-form of this amino acid exhibits the desired biological activity, and legal regulations do not allow the presence of the D enantiomer in dietary supplements, their quality control requires the development of chiral methodologies. This work describes the development of a rapid electrokinetic chromatography method for the enantiomeric determination of 5-hydroxytryptophan and its application to the analysis of a dietary supplement. The developed strategy was based on the use of 1.75% (w/v) sulfated-γ-cyclodextrin in 100 mM formate buffer (pH 2.2) as a separation medium, a short-end sample injection, and an uncoated fused-silica capillary with an effective length of 8.5 cm (total length of 48.5 cm). Under the optimized conditions, the enantiomeric separation of 5-hydroxytryptophan was achieved in less than 3 min with a resolution of 4.6. The analytical characteristics of the developed methodology were evaluated, showing a good performance in terms of linearity (R > 0.985), precision (RSD <6.0% for migration times and <8.6% for peak areas), accuracy (90 ± 4%) and LOQs (0.42 and 048 mg L^{-1} for D and L-5-hydroxytryptophan, respectively), and it was successfully applied to the quality control of a *Griffonia simplicifolia* dietary supplement rich in L-5-hydroxytryptophan.

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1. Introduction

5-Hydroxytryptophan (5-HTP) is a non-proteinogenic amino acid synthesized in an organism through the hydroxylation of L-tryptophan (Trp), a reaction catalyzed by the enzymes tryptophan hydroxylase 1 (TPH1) and tryptophan hydroxylase 2 (TPH2). The TPH1 isoform, predominantly expressed in gastrointestinal enterochromaffin cells, facilitates peripheral 5-HTP availability through its release into the circulatory system. In contrast, TPH2 is expressed in peripheral neurons of the mesenteric plexus and within the raphe nuclei of the central nervous system, where 5-HTP serves as a precursor in serotonergic neurotransmission.[1,2] The decarboxylation of 5-HTP is essential for the production of serotonin, a key neurotransmitter that regulates vital biological processes such as sleep, circadian rhythm, appetite, mood, and learning.[3,4] In addition, 5-HTP has acquired increasing interest from a clinical perspective due to its involvement in neuropsychiatric and gastrointestinal disorders. In this framework, 5-HTP supplementation appears as a treatment for low serotonin level diseases such as fibromyalgia,[5,6] Parkinson's disease,[7–9] depression,[10–12] obesity,[13,14] gut health[15–17] and insomnia.[18–20] Moreover, the antioxidant and anti-inflammatory properties attributed to 5-HTP suggest its potential use as an analgesic and in the treatment of inflammatory diseases.[21–23] To obtain 5-HTP for its use as a dietary supplement, different sources have been explored, including microbial and chemical synthesis, as well as natural extracts. Among these, the extract from *Griffonia simplicifolia* has been reported to exhibit the highest concentration of 5-HTP.[24–26]

An important feature of 5-HTP is its chiral nature, as it exists in two enantiomeric forms. Among them, only the L-5-HTP enantiomer exhibits biological activity due to its interaction with enantioselective biological receptors. This fact, together with current regulations that specify that only the L-form, and not the racemate, is allowed in dietary supplements,[27,28] highlights the relevance of developing chiral analytical methodologies for the enantiomeric determination of 5-HTP.

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Several studies published in the literature reported the achiral analysis of 5-HPT in a variety of matrices using different analytical techniques. For instance, high-performance liquid chromatography (HPLC) coupled to mass spectrometry,[29–32] fluorescence detection,[33,34] or electrochemical detection[35,36] was employed for the determination of 5-HTP in urine,[29] cerebrospinal fluid,[30] plasma,[32,33,35] rat gastric mucosa[34] and sea slug ganglia.[36] Capillary electrophoresis (CE) has also been successfully used, coupled to MS,[37] laser-induced native fluorescence,[38,39] and multiphoton-excited fluorescence detection,[40] for the achiral determination of 5-HPT in human plasma,[37] urine,[38] single cells,[39] and rat neuronal extract.[40] In the field of food science, 5-HTP was determined along with other compounds in mushrooms and *G. simplicifolia* by HPLC-UV,[25,41] beverages by HPLC-MS/MS,[42] coffee by HPLC with fluorescence detection after its derivatization with *o*-phthalaldehyde,[43] and in chocolate by capillary liquid chromatography-MS.[44] Moreover, a methodology using CE-UV was described to carry out the quality control of 5-HTP-based dietary supplements.[45] Nevertheless, all these studies were based on achiral methodologies.

To the best of our knowledge, until now, only three studies have described the enantiomeric determination of 5-HPT in standard samples by HPLC. Thus, Smith and Pirkle reported an HPLC-UV methodology using a chiral column, which allowed the separation of 5-HPT enantiomers after derivatization with 3,5-dinitrobenzoyl,[46] whereas Slijkhuis *et al.* employed a conventional HPLC column with a circular dichroism detector for the identification of 5-HPT enantiomers in standard samples.[47] On the other hand, Liu *et al.* achieved the partial separation of 5-HTP enantiomers by using ligand-exchange chromatography based on the use of a chiral stationary phase with chitosan and $CuSO_4$.[48]

Although CE is already a well-established separation technique in the field of chiral separation, mainly in the format of electrokinetic chromatography (EKC), due to its inherent properties such as versatility, high-resolution power, and high separation efficiency,[49] its potential for the separation of 5-HPT enantiomers has scarcely been explored. In fact, as far as we know, just one study based on the use of ligand exchange CE with *N*-(2-hydroxy-octyl)-L-4-hydroxyproline as a chiral selector and copper as a metal ion has been employed to achieve the enantioseparation of 5-HTP in a standard sample in 15 min with a resolution value of 2.6.[50]

Considering all the above-mentioned points, the main goal of this work was to develop a simple and fast methodology enabling the chiral separation of 5-HTP by EKC. With this aim, cyclodextrins (CDs) as chiral selectors and strategies to decrease the analysis time were employed. After the optimization of different experimental variables to find the most adequate results in terms of resolution and analysis time, the analytical characteristics of the developed methodology were evaluated. Then, the EKC method was applied, for the first time, to the purity and quality control of an L-5-HTP-based dietary supplement.

2. Materials and methods

2.1. Reagents and samples

Analytical grade quality reagents were employed, and the ultrapure water used in the preparation of all solutions was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Formic acid (98%), sodium hydroxide and sulfated-β-CD (S-β-CD, DS 7–11) were provided by Sigma Aldrich (St Louis, MO, USA). Sulfated-γ-CD (S-γ-CD, DS 14), sulfated-α-CD (S-α-CD, DS 12.8), and phosphate-γ-CD (P-γ-CD, DS 3.5) were from Cyclolab (Budapest, Hungary). Racemic D/L-5-HTP and L-5-HTP standards were purchased from ThermoFisher (Kandel, Germany). The dietary supplement based on *Griffonia simplicifolia* seed extracts containing 100 mg of L-5-HTP per capsule was acquired from a web page of dietary products.

2.2. CE conditions

Experiments were conducted using an Agilent 7100 capillary electrophoresis (CE) system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD). System control and data acquisition were performed *via* 3D-CE ChemStation software provided by Agilent Technologies. Analyses were carried out employing uncoated fused-silica capillaries (50 μm I.D., 362.8 μm O.D.) with a total length of 48.5 cm and an effective length of 8.5 cm from Polymicro Technologies (Phoenix, AZ, USA). The background electrolyte (BGE) was 1.75% (w/v) S-γ-CD in the separation buffer (100 mM formate buffer at pH 2.2). The electrophoretic separation was achieved using a separation voltage of 30 kV, a temperature of 25 °C, a short-end sample injection at −50 mbar for 4 s, and a detection wavelength of 220 nm with a bandwidth of 4 nm.

New capillaries were conditioned, using a pressure of 1 bar, as follows: 30 min of 1 M sodium hydroxide, 5 min of ultrapure water, and 60 min of buffer. Each day, the capillary was conditioned sequentially with 0.1 M sodium hydroxide (10 min), ultrapure water (5 min), buffer (30 min), and BGE (10 min), each step performed under a pressure of 1 bar. Between successive injections, the capillary was rinsed with BGE for 2 min.

2.3. Preparation of BGEs and samples

The separation buffer was prepared by diluting formic acid in ultrapure water to a final concentration of 100 mM (pH 2.2). BGEs were prepared by dissolving an appropriate amount of each CD in the buffer. Before their use, BGEs were sonicated to avoid air bubbles.

Stock solutions of D- and L-5-HTP were prepared separately by dissolving accurately weighed amounts of each compound in ultrapure water to achieve a final concentration of 10 mM. These stock solutions were stored at 4 °C until their dilution to the final concentrations before analyses.

For the analysis of the L-5-HTP-based dietary supplement, the contents of six capsules were accurately weighed and homogenized to ensure sample uniformity. Then, based on the

labeled L-5-HTP content of the supplement, an appropriate amount of the powder was accurately weighed and dissolved in ultrapure water to prepare a 1 mg mL^{-1} sample solution using ultrasonication for 15 min to ensure complete dissolution. This solution was kept at 4 °C until its use.

2.4. Evaluation of the analytical characteristics of the method

Linearity was evaluated at six different concentrations of standard racemic 5-HTP solutions, ranging from 2.5 to 25 mg L^{-1} of each enantiomer, which were injected in triplicate on two consecutive days. For calibration by the standard addition method, four increasing concentrations of L-5-HTP were added to a solution of the dietary supplement prepared at a concentration of 12.5 mg L^{-1} of L-5-HTP. Precision was measured based on instrumental repeatability, method repeatability, and intermediate precision by analyzing a standard solution containing 12.5 mg L^{-1} of D and L-5-HTP or a dietary supplement solution containing 12.5 mg L^{-1} of L-5-HTP. The instrumental repeatability was determined from six consecutive injections of each solution. The method repeatability was evaluated by injecting in triplicate three replicates of each solution on the same day, and the intermediate precision was determined from three replicates of each solution that were injected in triplicate on three consecutive days. The recovery was evaluated by adding four known amounts of L-5-HTP (ranging from 25 to 100% of the nominal concentration) to the dietary supplement containing L-5-HTP at a nominal concentration of 12.5 mg L^{-1}. Limits of detection (LODs) and quantification (LOQs) were calculated as three times and ten times the signal-to-noise ratio (S/N), respectively.

2.5. Data treatment

Data acquisition (peak areas, migration times and resolution values) was performed using the 3D-CE Chemstation software from Agilent Technologies. This information was treated and statistically analyzed using Microsoft Excel 365 and STATGRAPHICS Centurion XVII. For electropherogram composition figures, Origin 8.0 software was used.

3. Results and discussion

3.1. Development of an EKC methodology for the enantiomeric separation of 5-HTP

The indole ring present in the chemical structure of 5-HTP serves as a chromophore, allowing its detection in the UV region. Although 5-HTP exhibits a characteristic absorption band at approximately 274 nm, a higher absorbance is observed at 220 nm; therefore, UV detection was set at 220 nm with a bandwidth of 4 nm. According to its pK_a values (pK_a(Cα-COOH) = 2.15, pK_a(Cα-NH_3^+) = 9.18), 5-HTP is positively charged at low pH (Fig. S1).[51] Therefore, the use of an acidic medium containing anionic CDs represents a promising strategy for the enantioseparation of 5-HTP by EKC-UV. A previous study performed by our research group demonstrated the potential of negatively charged CDs in 100 mM formate buffer (pH 2.2) for the enantioselective separation of tryptophan,[52] using a separation voltage of −30 kV and a temperature of 25 °C. Considering the structural similarities between tryptophan and 5-HTP, the experimental conditions established in our previous work were selected as initial conditions to evaluate the discrimination power of different anionic CDs for the chiral separation of 5-HTP. Under these conditions, P-γ-CD, S-γ-CD, S-β-CD, and S-α-CD at a concentration of 1.25% (w/v) were evaluated as chiral selectors (see Table S1). Fig. 1 shows the electropherograms obtained for the separation of 5-HTP enantiomers using each of the four CDs. As shown in this figure, the use of P-γ-CD enabled the chiral separation of 5-HTP with a resolution value of 1.7, although with a long analysis time (>40 min), whereas the other three CDs achieved the separation with resolution values ≥2.5 in less than 20 min. Among these three CDs, S-γ-CD provided the highest resolution (R_s = 8.8) in less than 15 min. The differences observed in terms of resolution values and migration times are mainly related to analyte–chiral selector interactions, which may vary depending on the characteristics of the different CDs employed as chiral selectors. Considering the results obtained, S-γ-CD was selected for further analyses.

The effect of the concentration of S-γ-CD on the chiral separation was then investigated in the range of 0.75% (w/v) to 1.75% (w/v), since the concentration of the chiral selector can considerably affect the enantioseparation. Fig. 2 and Table S2 show how the increase in CD concentration leads to improved resolution while the migration time decreases slightly (from 11.8 to 9.4 min). A concentration of 1.75% (w/v) was selected as the most suitable as it provided the shortest migration time. Under these conditions, a solution containing different con-

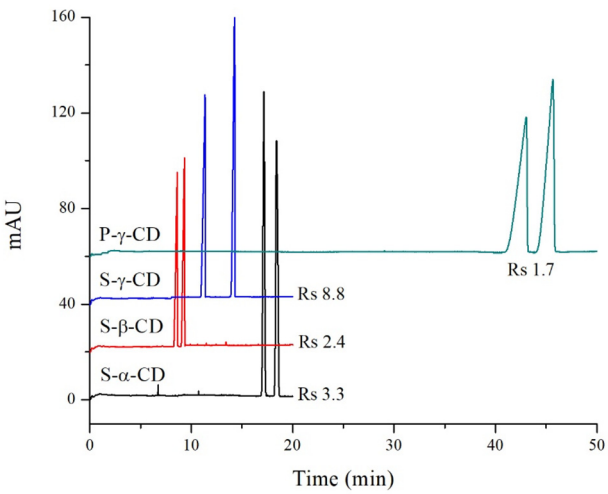

Fig. 1 Screening of different anionic CDs at a concentration of 1.25% (w/v) for the separation of 5-HTP enantiomers (5 mM). Experimental conditions: uncoated fused-silica capillary, 50 μm I.D. × 58.5 cm (50 cm effective length); 100 mM formate buffer (pH 2.2); applied voltage −30 kV; temperature 25 °C; injection by pressure, 50 mbar for 4 s; UV detection at 220 nm.

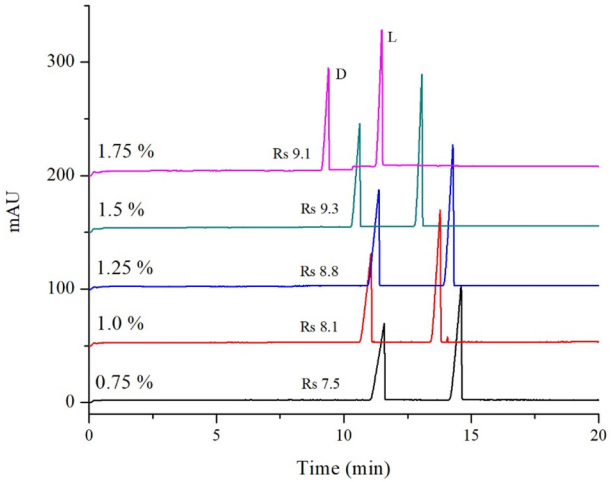

Fig. 2 Effect of the S-γ-CD concentration on the separation of 5-HTP enantiomers. Other conditions are as in Fig. 1.

centrations of each 5-HPT enantiomer was analyzed to determine their migration order. The results obtained demonstrated that the first-migrating enantiomer was D-5-HTP and it is the desirable situation to avoid the overlapping of the peak of the major enantiomer with that of the enantiomeric impurity.

Considering the high resolution obtained, the effects of voltage and temperature were investigated with the aim of reducing the analysis time (see Tables S3 and S4). A decrease in the separation voltage to values of −25 and −20 kV resulted in longer migration times and higher resolution values, as can be observed in Fig. 3A. Bearing in mind that the aim was to reduce the analysis time, −30 kV was fixed as the separation voltage. Subsequently, the influence of temperature on the separation was evaluated for values of 20, 25, and 30 °C. An increase in temperature led to a reduction in both analysis time and resolution values, although the latter remained above 8.6 in all cases (see Fig. 3B). Considering the results obtained, a temperature of 25 °C was chosen due to its higher resolution value (9.1) and shorter migration time (9.4 min).

Under the optimized conditions, the enantiomeric resolution was sufficiently high (R_s 9.1) to evaluate other approaches to shorten the analysis time, while maintaining a resolution of at least 2.5. As a first approach, the capillary length was reduced by 10 cm (effective length of 40 cm and total length of 48.5 cm), resulting in the separation of 5-HTP enantiomers in 7.2 min with a resolution value of 7.5 (see Fig. 4 and Table S5). Subsequently, the capillary length was shortened by another 10 cm (effective length of 30 cm and total length of 38.5 cm); however, in this case, the current generated inside the capillary was too high (>120 μA), which led to system instability. The second approach employed to achieve the rapid chiral separation of 5-HTP was to use a short-end injection (8.5 cm effective length). To achieve this, the injection was performed by applying a pressure of −50 mbar for 4 seconds, and a reverse polarity (*i.e.*, 30 kV) was used. As Fig. 4 shows, the use

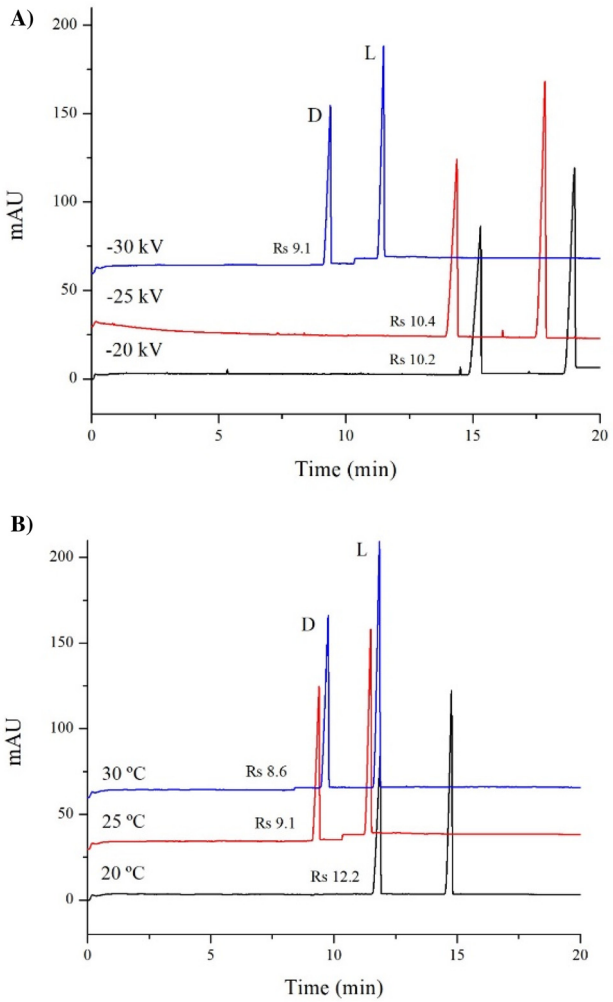

Fig. 3 Effect of separation voltage (A) and temperature (B) on the enantiomeric separation of 5-HTP with a concentration of 1.75% of sulfated-γ-CD. Other conditions are as in Fig. 1.

of this approach enabled a fast separation of 5-HTP enantiomers (in less than 3.0 min) with a high resolution (R_s = 4.6).

3.2. Analytical characteristics of the developed EKC method

Analytical characteristics, such as linearity, precision, accuracy, and the limits of detection (LOD) and quantification (LOQ), of the developed method were evaluated in accordance with the International Council on Harmonization (ICH) guidelines Q2 (R2)[53] to demonstrate the suitability of the developed methodology. The data obtained for the evaluation of the different figures of merit are presented in Table 1.

Linearity was established with an external calibration method using six standard racemic 5-HTP solutions, ranging from 2.5 to 25 mg L^{-1} of each enantiomer. Correlation coefficients higher than 0.985 were obtained, and the data fit properly to a linear model, as indicated by the *p*-values of the ANOVA test, which were higher than 0.05 at a 95% confidence level. In addition, the confidence intervals for the intercepts

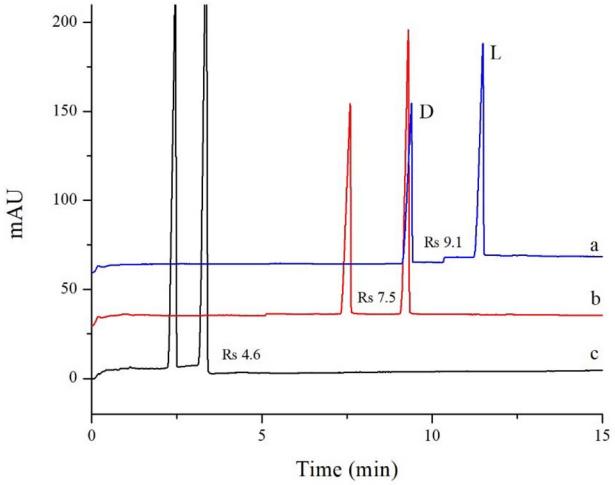

Fig. 4 Electropherograms corresponding to the chiral separation of 5-HTP enantiomers using (a) an uncoated fused-silica capillary, 50 μm I. D. × 58.5 cm (50 cm effective length), injection by pressure, 50 mbar for 4 s, and a separation voltage of −30 kV; (b) an uncoated fused-silica capillary, 50 μm I.D. × 48.5 cm (40 cm effective length), injection by pressure, 50 mbar for 4 s, and a separation voltage of −30 kV; and (c) an uncoated fused-silica capillary, 50 μm I.D. × 48.5 cm (8.5 cm effective length), injection by pressure, 50 mbar for 4 s, and a separation voltage of +30 kV. EKC conditions: temperature 25 °C; UV detection at 220 nm. Other conditions are as in Fig. 3.

included zero, whereas those for the slopes excluded it. A response relative factor of 1.1 was obtained for the 5-HTP enantiomers, calculated as the slope ratio of D-5-HTP to L-5-HTP, which is within the 0.8–1.2 range specified by the European Pharmacopoeia.[54] This result means that the percentage of D-5-HTP can be established from the ratio between the areas of both enantiomers.

To evaluate matrix interferences, the standard addition calibration method was performed by adding four known concentrations of L-5-HTP to the dietary supplement prepared at a concentration of 12.5 mg L^{-1} of L-5-HTP. Statistical analysis of the confidence intervals obtained for the slopes of both external standard and standard addition calibration methods at a 95% confidence level demonstrated that they did not differ significantly (p value >0.05), so there are no matrix effects, and the external calibration method can be employed to carry out the quantitative analysis of 5-HTP enantiomers in dietary supplements.

Precision of the developed EKC method was evaluated in terms of instrumental repeatability, method repeatability, and intermediate precision. All the data obtained in these analyses are shown in Table 1.

RSD values obtained for instrumental repeatability were below 1.6% for migration time and 3.3% for peak areas. For method repeatability, RSD values were lower than 1.3 and 5.0% for migration times and peak areas, respectively. Finally, in the evaluation of the intermediate precision, RSD values lower than 6.0% for the migration times and 8.6% for the peak areas were obtained.

Table 1 Analytical characteristics of the developed EKC method for the chiral analysis of 5-HTP

	D-5-HTP	L-5-HTP
External standard calibration method[b]		
Linear range	2.5–25 mg L^{-1}	2.5–25 mg L^{-1}
Slope ± $t{\cdot}s_b$	2.9 ± 0.6	2.7 ± 0.7
Intercept ± $t{\cdot}s_b$	−0.2 ± 8.2	7 ± 9
R	0.989	0.985
p-Value of ANOVA[c]	0.06	0.09
Standard addition calibration method[d]		
Linear range	—	0–12.5 mg L^{-1}
Slope ± $t{\cdot}s_b$	—	3.2 ± 1.0
R	—	0.984
p-Value of ANOVA[c]	—	0.06
Matrix interferences (p-value of t-test)[e]		0.45

Precision	Concentration level	RSD (%)	RSD (%)
Instrumental repeatability (n = 6)[f]	12.5 mg L^{-1}	t, 0.5; A, 1.4	t, 0.6; A, 3.0 t, 1.6[a]; A, 3.3[a]
Method repeatability (n = 9)[g]	12.5 mg L^{-1}	t, 0.6; A, 3.0	t, 0.6; A, 3.8 t, 1.3[a]; A, 5.0[a]
Intermediate precision (n = 9)[h]	12.5 mg L^{-1}	t, 3.8; A, 8.0	t, 6.0; A, 8.6 t, 6.0[a]; A, 5.3[a]
Accuracy (% mean recovery)[i]		—	90 ± 4
LOD (mg L^{-1})[j]		0.13	0.15
LOQ (mg L^{-1})[k]		0.42	0.48

t: time; A, peak area. [a] Data corresponding to the dietary supplement. [b] Six standard solutions at different concentrations injected in triplicate on two consecutive days. [c] p-Value from ANOVA to confirm the adequacy of the linear models to describe the experimental data. [d] Four known amounts of L-5-HTP were added to a solution of the dietary supplement containing a nominal concentration of 12.5 mg L^{-1}. [e] p-Value of the t-test (ANOVA) for the comparison of the slopes of external standard and standard addition methods at a confidence level of 95%. [f] A standard solution or the dietary supplement solutions injected six times. [g] Three standard solutions or dietary supplement solutions injected three times on the same day. [h] Three standard solutions or dietary supplement solutions injected three times on three different days. [i] Mean recovery of nominal concentration of the samples (12.5 mg L^{-1}) after the addition of known enantiomer standard concentrations (25, 50, 75 and 100% of nominal concentration value). [j] Obtained experimentally for an S/N ratio = 3. [k] Obtained experimentally for an S/N ratio = 10.

The accuracy of the developed method was assessed through the mean recovery obtained after adding four known amounts of L-5-HTP (ranging from 25 to 100% of the nominal concentration) to the dietary supplement containing L-5-HTP at a nominal concentration of 12.5 mg L^{-1}. A recovery value of 90 ± 4% was obtained.

The LODs and LOQs for both 5-HTP enantiomers were experimentally determined considering a signal-to-noise ratio of 3 and 10. LODs of 0.13 and 0.15 mg L^{-1} were obtained for D- and L-5-HTP, respectively, whereas the LOQs were 0.42 mg L^{-1} for D-5-HTP and 0.48 mg L^{-1} for L-5-HTP.

3.3. Quantitative analysis of a 5-HTP-based dietary supplement

Once the feasibility of the developed method for the enantiomeric separation of 5-HTP was demonstrated, it was applied to

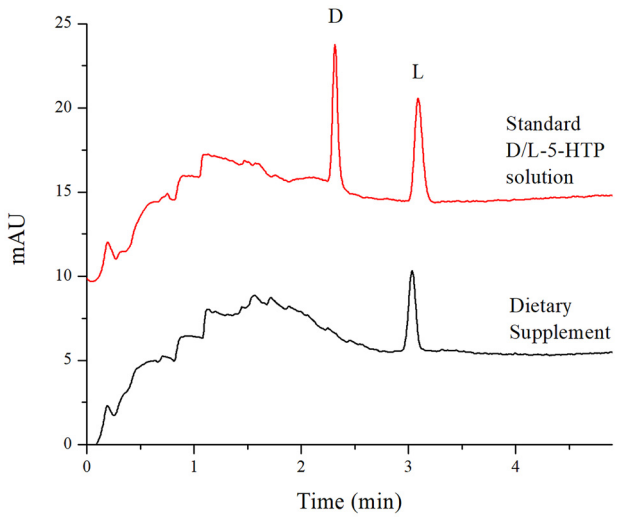

Fig. 5 Electropherograms corresponding to the chiral separation of 5-HTP in a standard solution containing 25 mg L^{-1} of racemic 5-HTP and to the analysis of a dietary supplement containing 12.5 mg L^{-1} of 5-HTP. EKC conditions are as in Fig. 4c.

the analysis of a 5-HTP-based dietary supplement. Fig. 5 presents the electropherograms corresponding to a racemic 5-HTP standard solution and the dietary supplement solution.

As can be observed in this figure, an appropriate selectivity was obtained since there was no evidence of interferences from other components present in the dietary supplement. The result obtained for the content of L-5-HTP, after considering the recovery, corresponds to a percentage of 97 ± 4% with respect to the labeled amount of L-5-HTP. Moreover, as can be seen in Fig. 5, D-5-HTP could not be detected in the sample, which means that this enantiomer was not present in the sample or its amount was below 1.0% with respect to L-5-HTP (calculated according to the LOD for D-5-HTP and the nominal concentration injected for L-5-HTP). This fact reveals that the racemic mixture of 5-HTP was not employed to prepare the dietary supplement evaluated in this work, as established by legal regulations. Even though the developed methodology was applied to only one dietary supplement, the good results obtained in this study demonstrate its potential for routine quality control of dietary supplements.

4. Conclusions

This work presents the first enantiomeric separation of 5-HTP by EKC. The EKC-UV methodology developed combined the use of a formate buffer at pH 2.2 containing 1.75% S-γ-CD as a chiral selector with the use of a capillary with an effective length of 8.5 cm (total length of 48.5 cm) and a strategy of short-end injection, to achieve the enantiomeric separation of 5-HTP in less than 3 min with a resolution of 4.6. These results improve upon those previously reported in the literature since, compared with LECE, the proposed EKC method enables faster chiral analysis with higher resolution. Moreover, compared with HPLC, this methodology does not require a chiral column, which, together with the low volumes of reagents and samples needed, reduces both the economic and environmental impact, respecting the principles of green analytical chemistry.

The evaluation of the analytical characteristics of the developed method demonstrated its suitability and good performance for the enantiomeric analysis of 5-HTP. The application of the proposed EKC method to the quantitative analysis of L-5-HTP in the dietary supplement revealed that the content of L-5-HTP agreed with the labeled one (97 ± 4% with respect to the labeled amount). In addition, the enantiomeric impurity D-5-HTP was not detected at the LOD level in the analyzed supplement demonstrating that legal regulations were fulfilled. The present work constitutes the first application of the chiral separation of 5-HTP to the analysis of a dietary supplement since in the previous studies using LECE and HPLC, only 5-HTP standard solutions were analyzed. Overall, the results obtained in this work highlight the high potential of EKC as an analytical tool to carry out the routine quality control of dietary supplements rich in L-5-HTP.

Author contributions

S. A.-R.: investigation, methodology, formal analysis, validation, data curation, visualization, writing – original draft, writing – review & editing. M. L. M.: conceptualization, methodology, validation, data curation, visualization, resources, supervision, writing – original draft, writing – review & editing, project administration, funding acquisition. M. C.-P: conceptualization, methodology, validation, formal analysis, data curation, visualization, resources, supervision, writing – original draft, writing – review & editing, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5an01100k.

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