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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Figure S1. Molecular structures of L-5-hydroxytryptophan and all the CDs evaluated as chiral selectors.

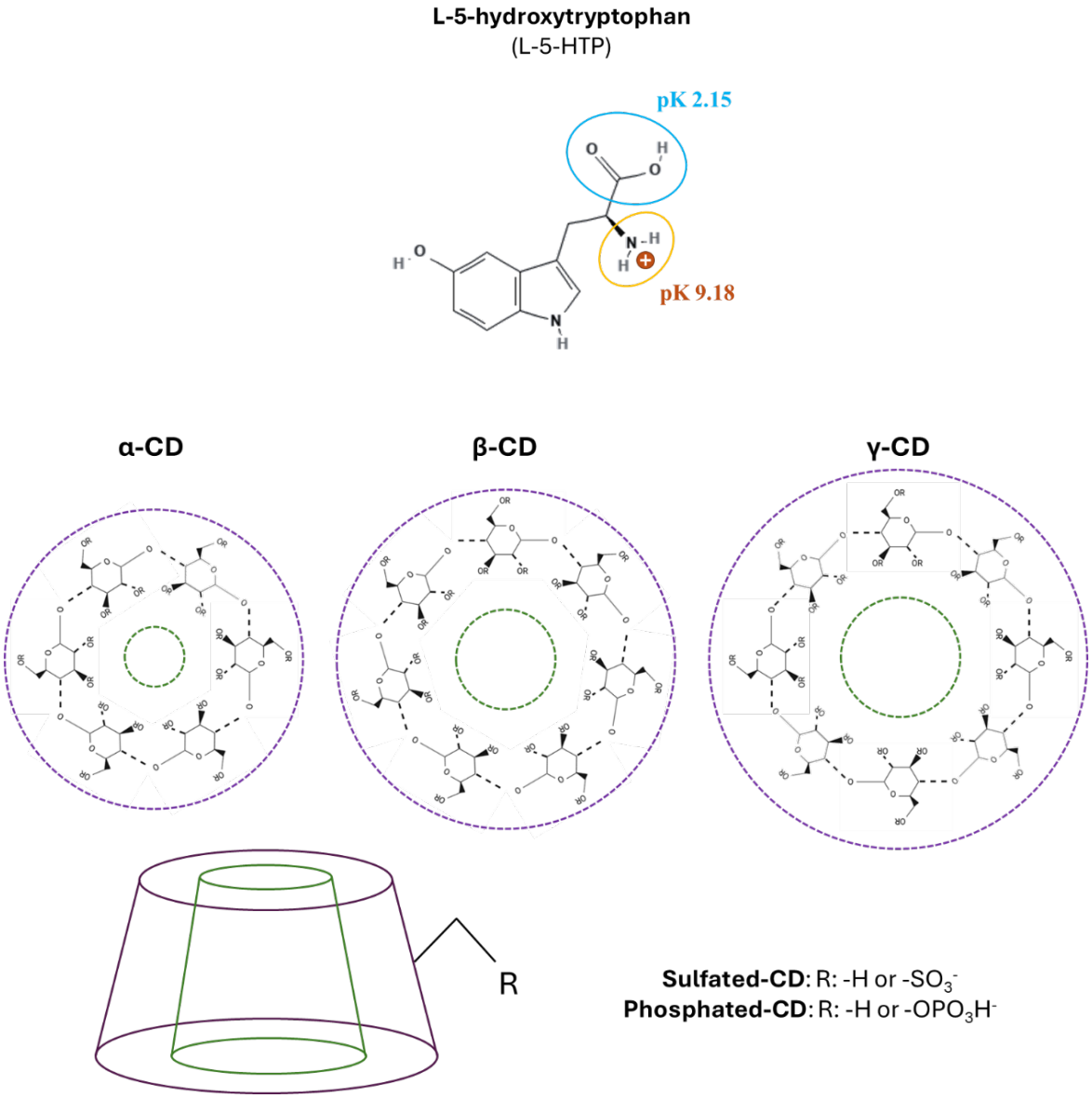

Table S1. Migration times and resolution values of 5-HTP enantiomers employing different CDs as chiral selectors.

CD	Concentration (%)	t_1	t_2	Rs	Mt_1	Mt_2	MRs
	1.25	17.2	18.4	3.4	16.4	17.6	3.3
S-α-CD	1.25	16.4	17.5	3.2			
	1.25	15.8	17.0	3.2			
	1.25	8.9	9.6	2.4	8.6	9.3	2.4
S-β-CD	1.25	8.6	9.3	2.5			
	1.25	8.4	9.1	2.5			
	1.25	37.9	40.0	1.6	40.4	42.7	1.7
P-γ-CD	1.25	40.1	42.5	1.7			
	1.25	43.0	45.7	1.8			
	1.25	12.1	15.2	9.0	11.7	14.7	8.8
S-γ-CD	1.25	11.7	14.7	8.8			
	1.25	11.4	14.3	8.5			

t_1: migration time of the first enantiomer (min), t_2: migration time of the second enantiomer (min), Rs: resolution between the enantiomers, Mt_1: average of first enantiomer migration times of three analysis, Mt_2: average of second enantiomer migration times, MRs: average of resolutions of three analysis with the same conditions. CE conditions: uncoated fused-silica capillary, 50 µm I.D. x 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.

Table S2. Migration times and resolution values obtained in the separation of 5-HTP enantiomers using different concentrations of S-γ-CD.

Concentration (%)	t_1	t_2	Rs	Mt_1	Mt_2	MRs
0.75	12.2	15.4	7.8	11.8	15.0	7.5
	11.8	14.9	7.4			
	11.6	14.6	7.3			
1.00	11.5	14.3	8.1	11.3	14.0	8.1
	11.2	14.0	8.3			
	11.1	13.8	8.1			
1.25	12.1	15.2	9.0	11.7	14.7	8.8
	11.7	14.7	8.8			
	11.4	14.3	8.5			
1.50	11.1	13.7	9.4	10.9	13.4	9.3
	10.9	13.3	9.3			
	10.6	13.1	9.2			
1.75	9.6	11.8	9.3	9.4	11.5	9.1
	9.4	11.5	9.4			
	9.2	11.3	8.6			

t_1: migration time of the first enantiomer (min), t_2: migration time of the second enantiomer (min), Rs: resolution between the enantiomers, Mt_1: average of first enantiomer migration times of three analysis, Mt_2: average of second enantiomer migration times, MRs: average of resolutions of three analysis under the same conditions. CE conditions: uncoated fused-silica capillary, 50 µm I.D. x 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.

Table S3. Migration times and resolution values obtained for 5-HTP enantiomers using different separation voltages.

Voltage (kV)	t_1	t_2	Rs	Mt_1	Mt_2	MRs	Current (μA)
-20	15.8	19.8	10.4	15.4	19.1	10.2	40
	15.3	19.0	10.1				
	15.1	18.6	10.1				
-25	14.4	17.8	10.5	14.0	17.4	10.4	50
	14.0	17.3	10.1				
	13.6	16.9	10.5				
-30	9.6	11.8	9.3	9.4	11.5	9.1	70
	9.4	11.5	9.4				
	9.2	11.3	8.6				

t_1: migration time of the first enantiomer (min), t_2: migration time of the second enantiomer (min), Rs: resolution between the enantiomers, Mt_1: average of first enantiomer migration times of three analysis, Mt_2: average of second enantiomer migration times, MRs: average of resolutions of three analysis under the same conditions. CE conditions: uncoated fused-silica capillary, 50 μm I.D. x 58.5 cm (50 cm effective length); 100 mM formate buffer (pH 2.2) with 1.75 % S-γ-CD; temperature 25 ˚C; injection by pressure, 50 mbar for 4 s; UV detection at 220 nm.

Table S4. Migration times and resolution values obtained in the separation of 5-HTP enantiomers using different concentrations of S-γ-CD.

Temperature (˚C)	t_1	t_2	Rs	Mt_1	Mt_2	MRs
20	12.1	15.0	12.3	12.0	14.9	12.2
	11.9	14.8	12.0			
	11.9	14.9	12.4			
25	9.6	11.8	9.3	9.4	11.5	9.1
	9.4	11.5	9.4			
	9.2	11.3	8.6			
30	9.7	11.9	8.8	9.5	11.6	8.6
	9.6	11.6	8.6			
	9.3	11.2	8.4			

t_1: migration time of the first enantiomer (min), t_2: migration time of the second enantiomer (min), Rs: resolution between the enantiomers, Mt_1: average of first enantiomer migration times of three analysis, Mt_2: average of second enantiomer migration times, MRs: average of resolutions of three analysis under the same conditions. CE conditions: uncoated fused-silica capillary, 50 µm I.D. x 58.5 cm (50 cm effective length); 100 mM formate buffer (pH 2.2) with 1.75 % S-γ-CD; temperature 25 ˚C; injection by pressure, 50 mbar for 4 s; UV detection at 220 nm.

Table S5. Variation of the migration time and resolution of 5-HTP enantiomers as a function of the capillary length and type of injection.

Sample injection	Effective capillary length (cm)	t_1	t_2	Rs	Mt_1	Mt_2	MRs
Normal injection	40	7.6	9.3	7.8	7.3	8.9	7.5
		7.2	8.8	7.3			
		6.9	8.5	7.3			
Normal injection	30	2.0	2.7	3.3	2.0	2.7	3.4
		2.1	2.7	3.3			
		2.0	2.7	3.7			
Short-end injection	8.5	2.5	3.6	4.3	2.6	3.5	4.6
		2.6	3.6	4.7			
		2.6	3.9	4.7			

t_1: migration time of the first enantiomer (min), t_2: migration time of the second enantiomer (min), Rs: resolution between the enantiomers, Mt_1: average of first enantiomer migration times of three analysis, Mt_2: average of second enantiomer migration times, MRs: average of resolutions of three analysis under the same conditions. CE conditions: uncoated fused-silica capillary, 50 µm I.D; 100 mM formate buffer (pH 2.2) with 1.75 % S-γ-CD; temperature 25 ˚C; applied voltage -30 kV; conditions for injection or short-end injection: 50 mbar for 4 s; UV detection at 220 nm.