

Capillary electrophoresis–contactless conductivity detection for routine screening of PDE-5 inhibitor adulterants in sexual-enhancement supplements

Thi Anh Huong Nguyen¹, Khanh Hung Nguyen¹, Quoc Anh Hoang¹, Cao Son Tran², Thi Trang Vu², Viet Chien Dinh², Thi Phuong Quynh Le^{3,4}, Thi Thuy Duong⁵, Anh Phuong Vu⁶, Thi Ngoc Mai Pham¹, Bach Pham^{1*}, and Thi Hong Hao Le^{1,2*}

¹ *Department of Analytical Chemistry, Faculty of Chemistry, University of Science, Vietnam National University-Hanoi - 19 Le Thanh Tong, Hoan Kiem ward, Hanoi, Vietnam*

² *National Institute for Food Control, 65 Pham Than Duat, Phu Dien ward, Hanoi, Viet Nam*

³ *Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet road, Nghia Do ward, Hanoi 10000, Vietnam*

⁴ *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet road, Nghia Do ward, Hanoi 10000, Vietnam.*

⁵ *Institute of Science and Technology for Energy and Environment, Vietnam Academy of Science and Technology, Doi Can road, Nghia Do ward, Hanoi 10000, Vietnam.*

⁶ *Poison Control Center, Bach Mai Hospital, 78 Giai Phong road, Hanoi 10000, Viet Nam*

Correspondence should be addressed to:

Bach Pham (phamgiabach@hus.edu.vn)

Thi Hong Hao Le (haolth@nifc.gov.vn)

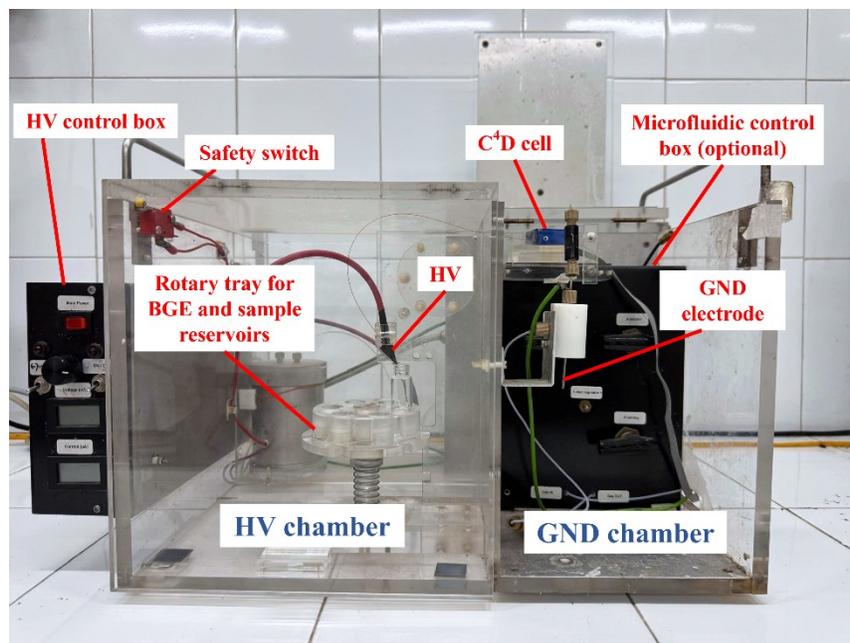


Figure S1. A photo of our typical CE-C⁴D setup. HV: high voltage, GND: ground electrode

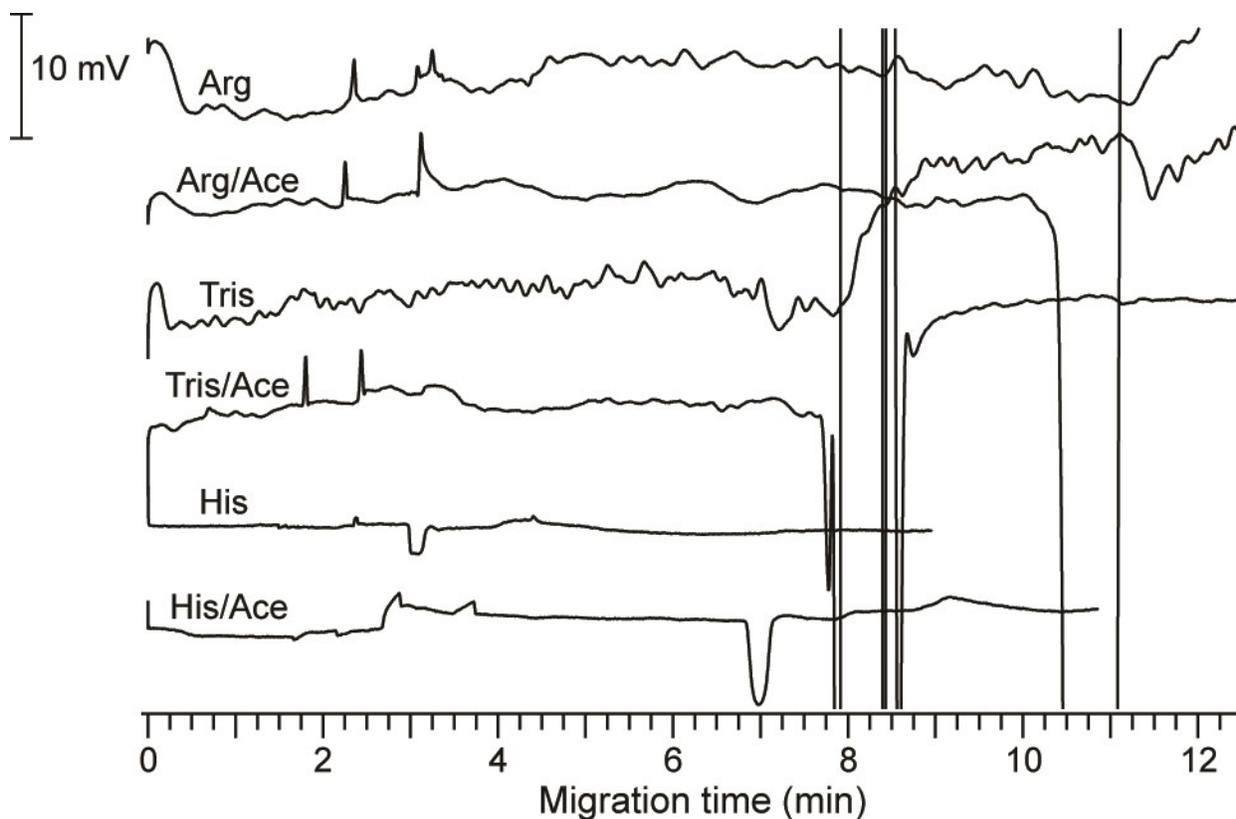


Figure S2. Preliminary CE-C⁴D screening of single-component and acetate-based BGEs for evaluation of baseline stability and overall electropherographic behavior. The tested BGEs were: (1) 20 mM Arg (pH 10.50), (2) 20 mM Arg/Ace (pH 6.00), (3) 20 mM Tris (pH 10.19), (4) 20 mM Tris/Ace (pH 6.00), (5) 20 mM His (pH 7.50), and (6) 20 mM His/Ace (pH 6.00). Experimental conditions: separation voltage 18 kV; hydrodynamic injection for 30 s at 20 cm; fused-silica capillary, 60 cm × 50 μm I.D.; effective length, 35 cm.

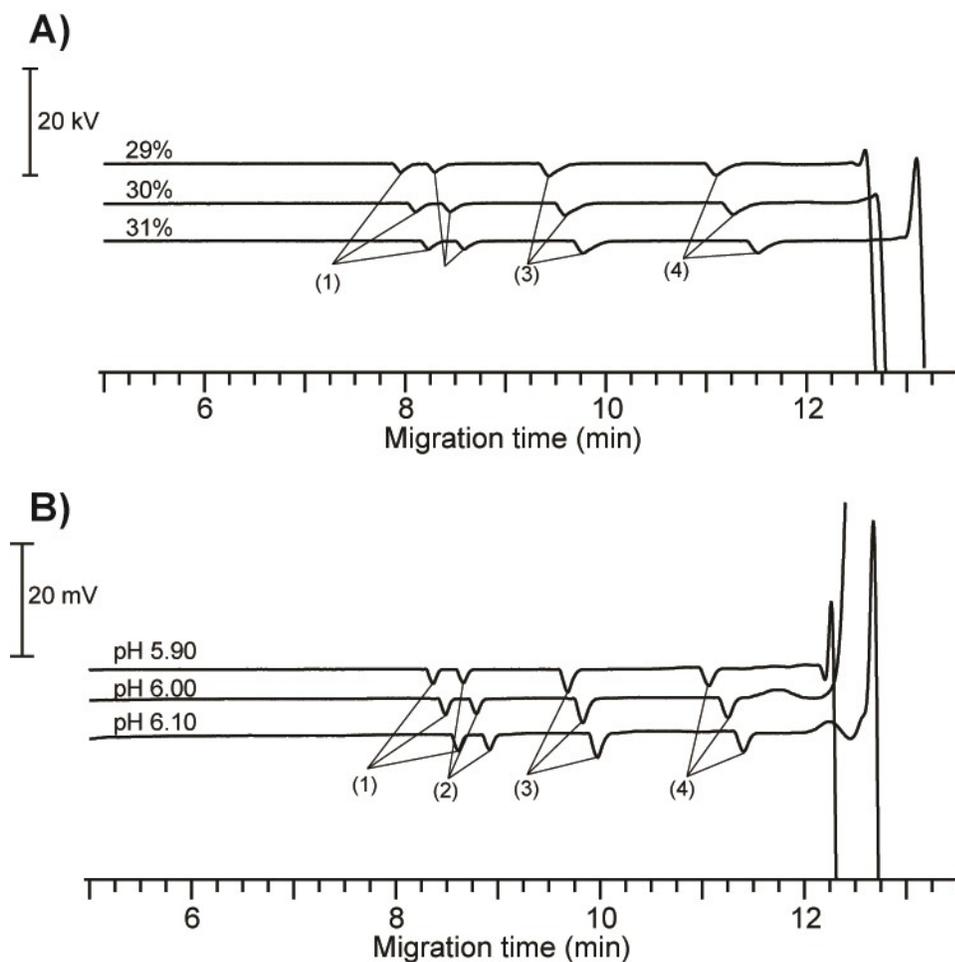


Figure S3. Representative CE-C⁴D electropherograms used for robustness evaluation of the optimized method under small deliberate variations in (A) ACN content and (B) pH. A mixed standard solution containing sildenafil, homosildenafil, hydroxyhomosildenafil, and avanafil (10 ppm each) was analyzed under the optimized conditions, except for the indicated variations in ACN content or pH. Peak assignments: (1) homosildenafil, (2) sildenafil, (3) hydroxyhomosildenafil, and (4) avanafil. Experimental conditions: Arg/MES/ACN background electrolyte; separation voltage 18 kV; hydrodynamic injection for 90 s at 20 cm; fused-silica capillary, 60 cm × 50 μm I.D.; effective length, 35 cm.

Table S1: LC-MS/MS operating conditions

Parameter	Condition
LC-MS/MS system	LC-MS/MS system consisting of a Shimadzu HPLC 20 AXL and an AB Sciex 6500 triple quadrupole mass spectrometer (Applied Biosystems)
Ionization mode	ESI(+)
Analytical column	Acquity BEH C18, 100 mm × 2.1 mm, 1.7 μm (Waters)
Guard column	Acquity BEH C18 pre-column, 5 mm × 2.1 mm, 1.7 μm (Waters)
Flow rate	0.30 mL min ⁻¹
Column temperature	Room temperature
Injection volume	10 μL
Mobile phase A	0.1% formic acid in water
Mobile phase B	Acetonitrile
Elution mode	Gradient
Gradient program	0–1.0 min, 90% A / 10% B; 1.0–5.0 min, linear change to 10% A / 90% B; 5.0–8.0 min, 10% A / 90% B; 8.0–8.5 min, linear change to 90% A / 10% B; 8.5–12.0 min, 90% A / 10% B
Curtain gas (CUR)	20 psi
Collision gas (CAD)	7 psi
ionspray voltage (IS)	5500 V
Source temperature (TEM)	300 °C
Ion source gas 1 (GS1)	20 psi
Ion source gas 2 (GS2)	10 psi
Entrance potential (EP)	10 V

Table S2: Multiple reaction monitoring (MRM) transitions and optimized parameters

Analyte	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	CE (eV)	Use
Sildenafil	475.2	283.1	41	Quantifier
		100.0	45	Qualifier
Homosildenafil	489.1	283.0	41	Quantifier
		99.1	71	Qualifier
Hydroxyhomosildenafil	504.9	487.2	33	Quantifier
		129.1	41	Qualifier
Avanafil	484.1	375.1	36	Quantifier
		155.1	53	Qualifier

Table S3 : Sample information used in this study

Sample No.	Sample state	Package	Ingredients
M1	Powder	20 packages per box × 3 g/package	Tribulus terrestris extract, Balanophora sp extract, Morinda officinalis extract, Tribulus terrestris, Cordyceps militaris powder
M2	Tea powder	15 packages per box × 4 g/package	Garcinia cambogia extract, Panax ginseng extract, Hoodia gordonii extract, Green tea extract, Lotus leaf extract, Terminalia chebula, Terminalia bellirica, Areca catechu extract, Lactose
M3	Capsule	6 blister packs × 10 capsules × 500 mg/capsule	Oyster extract, Rhizoma curculiginis extract, Cordyceps militaris powder, Talc
M4	Capsule	570 mg/capsule × 60 capsules/box	L-arginine hydrochloride, Panax ginseng, Epimedium brevicornum, Cordyceps sinensis, Eurycoma longifolia, Cistanche deserticola, Tribulus terrestris, Zinc gluconate, Gelatin, Magnesium stearate, Talc, Ascorbic acid
M5	Capsule	500 mg/capsule × 50 capsule/box	Cordyceps and multivitamins
M6	Capsule	500 mg/capsule × 30 capsule/box	Cordyceps and multivitamins
M7	Medicinal alcohol	350 mL bottle	Curculigo orchioides, Tribulus terrestris, Morinda officinalis, Epimedium
M8	Tablet	500 mg/tablet × 60 tablets/box	Eurycoma longifolia extract, Herba epimedii extract, Cornstarch, Aerosil, Magnesium stearate, Talc

Table S4: One-factor-at-a-time (OFAT) optimization workflow for the CE-C⁴D method, including investigated parameters, tested levels, fixed conditions, and selected conditions.

Optimization step	Parameter investigated	Tested levels	Conditions kept constant	Evaluation criteria	Selected condition
1	BGE system	Tris/MES, Arg/MES, His/MES	pH 6.00; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Separation of four analytes, especially resolution of homosildenafil/sildenafil; peak area; peak shape	Arg/MES
2	Organic modifier in BGE	Tris/MES/ACN, Arg/MES/ACN, His/MES/ACN	pH 6.00; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Resolution, peak area, migration time, peak symmetry	Arg/MES/ACN
3	ACN content	20% (v/v), 30% (v/v), 40% (v/v)	20 mM Arg/MES; pH 6.00; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Resolution of homosildenafil/sildenafil, signal intensity, peak symmetry, run time	30% (v/v) ACN
4	Arg concentration	10 mM, 20 mM, 30 mM	Arg/MES with 30% ACN; pH 6.00; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Peak area, peak shape, resolution, EOF interference	20 mM Arg
5	pH	5.50, 6.00, 6.50	20 mM Arg/MES with 30% ACN; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Resolution, peak area, migration time, interference from EOF	pH 6.00
6	Effective length	25 cm, 35 cm, 45 cm	20 mM Arg/MES with 30% ACN; separation voltage 18 kV; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.	Resolution, signal intensity, analysis time	35 cm
7	Separation voltage	16 kV, 18 kV, 20 kV	20 mM Arg/MES with 30% ACN; pH 6.00; injection 30 s at 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Run time, peak area, peak shape, resolution	18 kV
8	Injection time	30 s, 90 s, 150 s	20 mM Arg/MES with 30% ACN, pH 6.00; separation voltage 18 kV; injection height 20 cm; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Signal intensity, resolution, run time, EOF overlap	90 s
9	Injection height	10 cm, 20 cm, 30 cm	20 mM Arg/MES with 30% ACN, pH 6.00; separation voltage 18 kV; injection time 90 s; capillary 60 cm × 50 μm I.D.; effective length 35 cm	Signal intensity, resolution, peak shape	20 cm

Table S5: Effects of background electrolyte (BGE) composition on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

BGE	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
Tris/MES	6.63 ± 0.03	8.48 ± 0.73	6.80 ± 0.03	7.83 ± 0.88	7.40 ± 0.04	17.51 ± 0.86	8.08 ± 0.05	13.89 ± 0.62	1.59 ± 0.07
Arg/MES	6.62 ± 0.04	9.51 ± 0.36	6.82 ± 0.05	9.17 ± 0.05	7.60 ± 0.06	19.99 ± 0.44	8.45 ± 0.07	19.33 ± 0.15	1.34 ± 0.10
His/MES	6.77 ± 0.03	4.32 ± 0.21	6.96 ± 0.03	3.63 ± 0.23	7.69 ± 0.04	9.10 ± 0.10	8.46 ± 0.05	8.51 ± 0.38	1.77 ± 0.02
Tris/MES/ACN	8.61 ± 0.04	5.93 ± 0.68	8.99 ± 0.05	8.87 ± 0.64	10.35 ± 0.04	18.79 ± 1.55	12.51 ± 0.02	15.96 ± 0.65	1.92 ± 0.16
Arg/MES/ACN	7.83 ± 0.12	11.67 ± 0.39	8.14 ± 0.12	11.36 ± 0.21	9.28 ± 0.13	26.24 ± 0.17	11.22 ± 0.16	25.60 ± 0.31	2.52 ± 0.04
His/MES/ACN	8.52 ± 0.05	2.69 ± 0.27	8.90 ± 0.07	3.08 ± 0.34	10.28 ± 0.07	8.34 ± 0.08	12.54 ± 0.08	10.18 ± 0.23	3.34 ± 0.16

Table S6: Effects of percentage of acetonitrile on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

Percentage of ACN	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
20%	6.74 ± 0.16	8.54 ± 0.38	6.98 ± 0.18	8.34 ± 0.47	7.71 ± 0.21	17.47 ± 0.26	8.56 ± 0.24	17.25 ± 0.51	2.62 ± 0.02
30%	7.25 ± 0.14	10.04 ± 0.5	7.57 ± 0.15	9.95 ± 0.39	8.44 ± 0.18	20.21 ± 0.22	9.47 ± 0.22	19.70 ± 0.19	2.95 ± 0.07
40%	8.26 ± 0.25	12.00 ± 0.22	8.75 ± 0.27	11.60 ± 0.29	9.94 ± 0.35	25.52 ± 0.23	11.43 ± 0.46	20.96 ± 0.59	3.68 ± 0.23

Table S7: Effects of Arg concentration on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

Arg concentration	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
10 mM	6.55 ± 0.09	9.25 ± 0.37	6.85 ± 0.09	8.73 ± 0.18	7.60 ± 0.11	17.86 ± 0.08	8.44 ± 0.13	14.71 ± 0.19	2.47 ± 0.18
20 mM	7.25 ± 0.14	10.04 ± 0.5	7.57 ± 0.15	9.95 ± 0.39	8.44 ± 0.18	20.21 ± 0.22	9.47 ± 0.22	19.70 ± 0.19	2.95 ± 0.07
30 mM	8.77 ± 0.32	10.81 ± 0.56	9.20 ± 0.34	10.45 ± 0.88	10.48 ± 0.42	22.00 ± 1.65	12.11 ± 0.52	21.08 ± 0.25	3.41 ± 0.10

Table S8: Effects of BGE pH value on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

pH	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
5.50	-	-	-	-	8.26 ± 0.11	16.04 ± 0.09	9.65 ± 0.15	17.83 ± 0.65	0.00 ± 0.00
6.00	8.18 ± 0.21	10.94 ± 0.78	8.47 ± 0.21	10.45 ± 0.88	9.60 ± 0.22	23.38 ± 1.12	11.61 ± 0.29	23.08 ± 1.23	2.22 ± 0.08
6.50	8.49 ± 0.05	18.25 ± 0.38	9.15 ± 0.05	17.99 ± 0.23	10.70 ± 0.09	36.33 ± 0.50	12.34 ± 0.19	30.35 ± 3.22	4.29 ± 0.06

- : not detected

Table S9: Effects of effective length on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

Effective length	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
25 cm	6.49 ± 0.07	7.57 ± 0.37	6.68 ± 0.07	7.01 ± 0.42	7.29 ± 0.10	13.84 ± 0.55	8.35 ± 0.11	13.02 ± 0.47	2.08 ± 0.13
35 cm	7.95 ± 0.10	11.56 ± 0.46	8.26 ± 0.11	11.61 ± 0.24	9.45 ± 0.15	25.67 ± 0.76	11.37 ± 0.19	25.22 ± 0.72	2.45 ± 0.07
45 cm	9.97 ± 0.16	14.10 ± 0.34	10.52 ± 0.16	14.63 ± 0.52	11.86 ± 0.20	27.19 ± 1.02	13.28 ± 0.28	6.73 ± 1.54	3.49 ± 0.11

Table S10: Effects of separation voltage on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

Separation voltage	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
16 kV	8.95 ± 0.02	12.13 ± 0.13	9.34 ± 0.02	11.83 ± 0.08	10.66 ± 0.01	25.30 ± 0.10	12.53 ± 0.00	23.53 ± 0.09	2.85 ± 0.02
18 kV	7.98 ± 0.03	11.43 ± 0.34	8.34 ± 0.03	11.31 ± 0.40	9.53 ± 0.04	23.57 ± 0.30	11.22 ± 0.06	21.80 ± 0.25	2.77 ± 0.06
20 kV	7.14 ± 0.00	9.79 ± 0.45	7.45 ± 0.01	9.63 ± 0.51	8.51 ± 0.01	18.63 ± 0.46	10.00 ± 0.02	17.39 ± 0.30	2.74 ± 0.03

Table S11: Effects of injection time on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements (n=3).

Injection time	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
30 s	8.11 ± 0.10	11.15 ± 0.39	8.48 ± 0.11	10.75 ± 0.11	9.61 ± 0.13	22.36 ± 0.51	11.08 ± 0.17	19.82 ± 0.72	3.16 ± 0.03
90 s	7.71 ± 0.18	34.07 ± 0.25	8.03 ± 0.19	32.11 ± 0.96	8.62 ± 0.75	64.33 ± 0.41	10.16 ± 0.30	53.85 ± 2.57	2.07 ± 0.15
150 s	6.36 ± 0.07	42.48 ± 1.30	6.55 ± 0.07	38.91 ± 1.88	7.08 ± 0.08	90.02 ± 0.89	7.75 ± 0.10	79.10 ± 1.49	1.27 ± 0.04

Table S12: Effects of injection height on migration times and peak areas of the four analytes (30 ppm each), and on the resolution between homosildenafil and sildenafil. Values are means of three replicate measurements.

Injection height	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
10 cm	9.00 ± 0.17	15.77 ± 0.88	9.38 ± 0.19	15.26 ± 0.28	10.65 ± 0.25	31.56 ± 0.72	12.35 ± 0.35	28.52 ± 1.17	2.81 ± 0.32
20 cm	7.71 ± 0.18	34.07 ± 0.25	8.03 ± 0.19	32.11 ± 0.96	8.62 ± 0.75	64.33 ± 0.41	10.16 ± 0.30	53.85 ± 2.57	2.07 ± 0.15
30 cm	7.30 ± 0.14	47.51 ± 0.34	7.54 ± 0.15	41.55 ± 0.45	8.21 ± 0.17	90.29 ± 0.71	9.10 ± 0.20	76.27 ± 0.49	1.39 ± 0.04

Table S13: Robustness evaluation of the optimized CE-C⁴D method under small deliberate variations in ACN content. Data are presented as mean ± SD of replicate measurements (n = 3). The concentration of each analyte was 10 ppm.

Percentage of ACN	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
29	7.97 ± 0.03	9.84 ± 0.04	8.31 ± 0.03	9.61 ± 0.15	9.44 ± 0.03	21.36 ± 0.31	11.12 ± 0.03	20.47 ± 0.33	2.02 ± 0.04
30	8.10 ± 0.04	9.80 ± 0.47	8.47 ± 0.03	9.91 ± 0.40	9.60 ± 0.04	21.83 ± 0.24	11.29 ± 0.04	21.09 ± 0.29	2.00 ± 0.03
31	8.23 ± 0.01	9.92 ± 0.25	8.60 ± 0.01	9.96 ± 0.10	9.78 ± 0.01	21.55 ± 0.10	11.52 ± 0.01	21.40 ± 0.74	2.01 ± 0.08
RSD (%)	1.37	2.80	1.49	2.89	1.46	1.41	1.49	2.61	2.09

Table S14: Robustness evaluation of the optimized CE-C⁴D method under small deliberate variations in pH. Data are presented as mean ± SD of replicate measurements (n = 3). The concentration of each analyte was 10 ppm.

pH	homosildenafil		sildenafil		hydroxyhomosildenafil		avanafil		Peak Resolution between homosildenafil and sildenafil
	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	Migration time (min)	Area (mV·s)	
5.90	8.37 ± 0.02	11.74 ± 0.33	8.66 ± 0.02	11.68 ± 0.40	9.69 ± 0.02	24.31 ± 0.91	11.07 ± 0.02	20.58 ± 0.39	2.48 ± 0.03
6.00	8.50 ± 0.01	12.29 ± 0.20	8.80 ± 0.01	12.16 ± 0.21	9.84 ± 0.01	24.94 ± 0.23	11.27 ± 0.02	21.43 ± 0.22	2.59 ± 0.03
6.10	8.65 ± 0.03	12.69 ± 0.10	8.96 ± 0.03	12.57 ± 0.10	9.99 ± 0.02	26.17 ± 0.23	11.46 ± 0.05	22.21 ± 0.80	2.60 ± 0.02
RSD (%)	1.48	3.76	1.48	3.71	1.36	3.79	1.52	3.93	2.46

Table S15: LC-MS/MS confirmation of surrogate blank matrix M1, showing the absence of sildenafil, homosildenafil, hydroxyhomosildenafil, and avanafil. Measurements were performed in eight replicate runs (n = 8).

	Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (ng/mL)	Standard Query Status	Use Record	Record Modified	Calculated Concentration (ng/mL)	Accuracy (%)
1	M1	Unknown	2.20e+003	5.66e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A
2	M1	Unknown	2.95e+003	4.51e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A
3	M1	Unknown	2.04e+004	3.98e+003	N/A	N/A		<input type="checkbox"/>	0.00	N/A
4	M1	Unknown	1.24e+004	1.78e+003	N/A	N/A		<input type="checkbox"/>	0.00	N/A
5	M1	Unknown	4.97e+002	2.93e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A
6	M1	Unknown	1.42e+003	2.39e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A
7	M1	Unknown	2.52e+003	9.33e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A
8	M1	Unknown	4.47e+002	2.42e+002	N/A	N/A		<input type="checkbox"/>	0.00	N/A

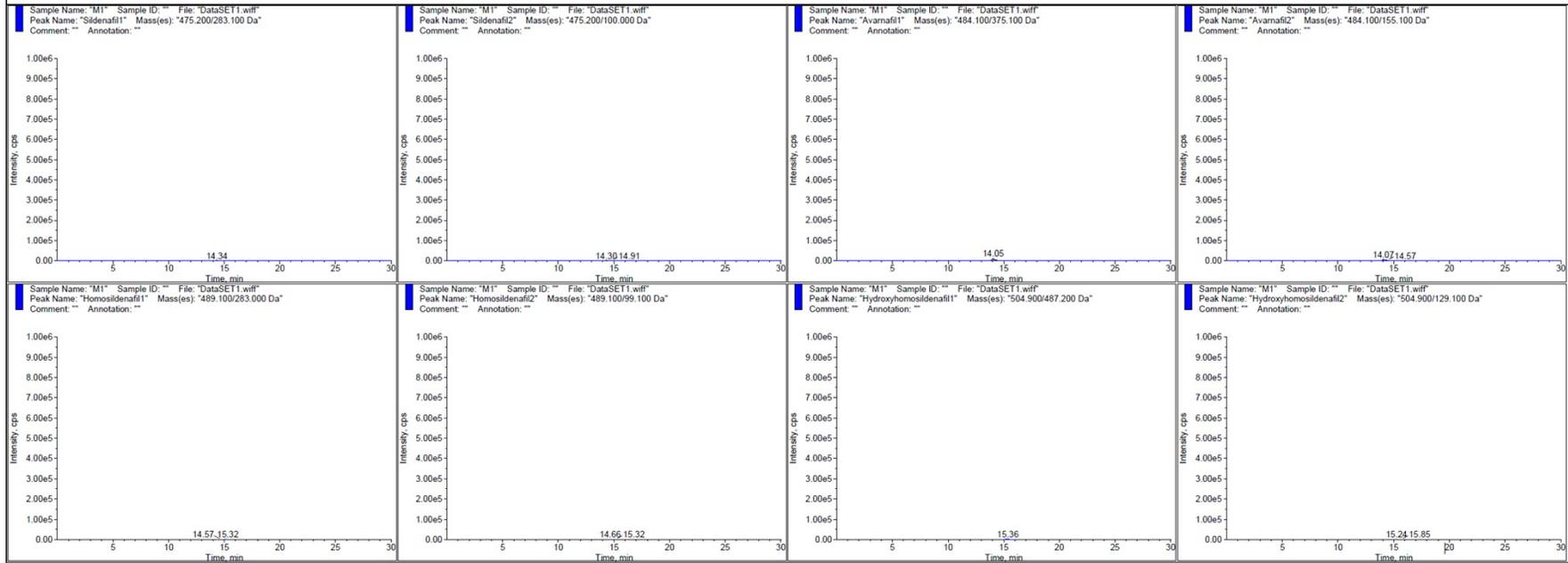


Table S16: Assessment of method specificity based on migration times and peak areas. Values are means of three replicate measurements (n=3).

Peak	Migration time (min)			RSD (%)	Peak area (mV·s)			RSD (%)
	Blank solution	Spiked blank solution	Standard solution		Blank solution	Spiked blank solution	Standard solution	
homosildenafil	-	8.28 ± 0.04	8.30 ± 0.02	0.17	-	11.07 ± 0.08	10.87 ± 0.14	1.29
sildenafil	-	8.54 ± 0.03	8.56 ± 0.02	0.17	-	10.33 ± 0.29	10.30 ± 0.14	0.21
hydroxyhomosildenafil	-	9.36 ± 0.03	9.38 ± 0.04	0.15	-	19.78 ± 0.40	19.86 ± 0.13	0.29
avanafil	-	10.41 ± 0.03	10.44 ± 0.05	0.20	-	16.80 ± 0.46	16.18 ± 0.04	2.66

- : not detected

Table S17: Reproducibility (between-day performance) and recovery of the analytes in the surrogate blank matrix.

Analyte	Concentration (ppm)	Peak area (mV·s)						SD	RSD (%)	Recovered concentration (ppm)	Recovery (%)	Mean recovery (%)
		1 st day	2 nd day	3 rd day	4 th day	5 th day	Mean					
Homosildenafil	5.00	4.80	4.93	4.88	4.61	5.08	4.86	0.17	3.56	4.76	95.24	101.61
	25.00	22.93	22.82	25.50	24.43	23.24	23.78	1.15	4.85	25.71	102.83	
	50.00	47.48	50.16	49.98	46.98	49.36	48.79	1.47	3.01	53.39	106.77	
Sildenafil	5.00	4.80	5.01	4.83	4.62	4.97	4.85	0.15	3.19	4.92	98.40	100.93
	25.00	21.34	22.57	24.60	23.56	23.21	23.06	1.21	5.24	25.22	100.88	
	50.00	46.06	48.44	48.07	43.85	47.86	46.86	1.91	4.09	51.75	103.51	
Hydroxyhomosildenafil	5.00	10.53	10.66	10.37	10.09	10.12	10.35	0.25	2.41	5.39	107.75	106.25
	25.00	49.38	49.48	46.50	49.36	49.85	48.91	1.36	2.79	26.55	106.20	
	50.00	95.60	98.04	97.71	93.80	94.90	96.01	1.82	1.90	52.40	104.79	
Avanafil	5.00	7.47	7.11	6.56	6.63	6.37	6.83	0.45	6.60	5.14	102.75	102.90
	25.00	40.53	43.01	43.02	45.62	46.21	43.68	2.29	5.24	24.85	99.41	
	50.00	96.71	94.72	99.68	97.94	94.87	96.78	2.10	2.17	53.27	106.53	

Table S18: The signals of four analyte at limit of quantification level

Analytes	Concentration (ppm)	Peak height (mV)	Noise (mV)	S/N
Avanafil	2.37	0.705	0.070	10.067
Sildenafil	2.87	0.711		10.157
Homosildenafil	2.77	0.758		10.833
Hydroxyhomosildenafil	1.96	0.730		10.424

Table S19: Repeatability and recovery of the analytes in the surrogate blank matrix

Analyte	Concentration (ppm)	Peak area (mV·s)						SD	RSD (%)	Recovered concentration (ppm)	Recovery (%)	Mean recovery (%)
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean					
Homosildenafil	5.00	4.95	4.88	5.03	4.98	4.97	4.96	0.05	1.10	4.87	97.50	104.22
	25.00	24.52	24.43	24.52	25.61	24.69	24.75	0.49	1.97	26.78	107.12	
	50.00	49.28	49.18	49.54	49.48	49.36	49.37	0.15	0.30	54.02	108.05	
Sildenafil	5.00	4.85	4.85	5.11	5.00	4.93	4.95	0.11	2.22	5.03	100.67	103.23
	25.00	23.67	22.98	22.98	23.39	24.64	23.53	0.68	2.91	25.75	103.01	
	50.00	47.86	47.51	48.32	48.35	47.86	47.98	0.35	0.74	53.01	106.01	
Hydroxyhomosildenafil	5.00	10.48	10.33	10.50	10.46	10.44	10.44	0.07	0.64	5.44	108.72	106.49
	25.00	49.60	48.29	49.25	49.30	49.68	49.22	0.55	1.13	26.72	106.88	
	50.00	92.83	96.54	95.17	96.40	94.90	95.17	1.49	1.57	51.94	103.87	
Avanafil	5.00	7.04	6.79	7.20	7.01	7.02	7.01	0.15	2.08	5.24	104.72	102.24
	25.00	43.00	39.94	42.18	45.31	44.87	43.06	2.17	5.04	24.52	98.09	
	50.00	92.82	94.20	94.42	95.39	94.87	94.34	0.96	1.02	51.96	103.92	