

Supplementary Material

Electrochemical profiling and LC-MS characterization of synthetic cathinones: from methodology to detection in forensic samples

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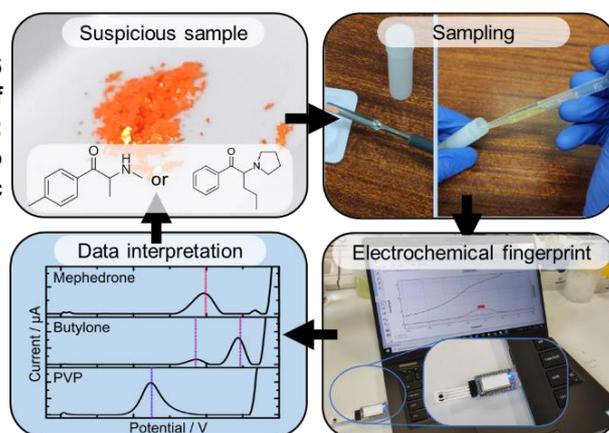
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A novel on-site testing approach for the classification of synthetic cathinones in seizures is presented.

The concept is based on electrochemical methods that uses screen-printed electrodes for the determination of the electrochemical profile.

The method is assessed with common cutting agents, and finally validated with 26 street samples from forensic laboratories.

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Tables

Table S1. Adulterants/cutting agents and their proportions in seized samples found in literature.

Adulterant/ cutting agent	Frequency (%) in seizures	NPS purity	References
Caffeine	13.1	-	[1-7]
Procaine	-	-	[1,7]
Lidocaine	5.9	-	[4-7]
Benzocaine	-	-	[1,6,7]
Paracetamol	-	-	[8]
Phenacetin	-	-	[7,9]
Creatine	18	69±19	[7,10]
Monosodium glutamate	19	40 ±17	[7,10]
Sucrose	8	57±10	[7,10]
Lactose	-	-	[3,5]

Table S2. Analytical techniques for synthetic cathinones detection.

NPS	Method	Electrode	LOD	Linear range / $\mu\text{g mL}^{-1}$	Sample Solution	Ref
MC,			44.5 $\mu\text{g mL}^{-1}$	16–200	PBS pH 12	
4-MMC	CV (oxidation peak)	SPE	39.8 $\mu\text{g mL}^{-1}$	16-350	PBS pH 2	[11]
4-MEC			84.2 $\mu\text{g mL}^{-1}$	16-350	PBS pH 2	
4-MMC	CV (reduction peak)	Bi/Hg film	11.8 $\mu\text{g mL}^{-1}$	100–400	Acetate buffer pH	[12]
4-MEC		SPE	11.6 $\mu\text{g mL}^{-1}$	100–400	4.3	
4-MMC	CV (oxidation peak)	Coin	0.6 $\mu\text{g mL}^{-1}$	0.01 – 0.1	Acetate buffer pH	[13]
4-MEC			0.5 $\mu\text{g mL}^{-1}$	0.01 – 0.1	8.5	
C	DPV (reduction peak)	MIP / graphene	NH_2 -3.3 pg mL^{-1}	$4.9 \times 10^{-6} - 9.8 \times 10^{-3}$	PBS pH 6, 5.0 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$,	[14]
MC			8.9 pg mL^{-1}	$1.5 \times 10^{-5} - 1.1 \times 10^{-2}$	0.2 M KCl	
4-MC	DPV (oxidation peak)	SPE	4.0 $\mu\text{g mL}^{-1}$	10-250	PBS pH 7	[15]
4-MMC-R	DPV (reduction peak)		3.6 $\mu\text{g mL}^{-1}$	5-300	PBS pH 3	
C						
MDEC	SWV	Aptamer-modified Au	-	-	Tris-HCl pH 7.4	[16]
Others						
4-MMC	SWV (oxidation peak)	MIP/polytyramine/f-MWCNT@AuNPs	0.2 $\times 10^{-3} \text{ mL}^{-1}$	$0.2 \times 10^{-3} - 1.8 \times 10^{-3}$	$\text{K}_3[\text{Fe}(\text{CN})_6]$, 5.0 mM KCl 0.1 M	[17]
	CV (oxidation peak)	GCE		$1.8 \times 10^{-3} - 17.7 \times 10^{-3}$		
MDEC	DPV (oxidation peak)	BDD	0.7 $\mu\text{g mL}^{-1}$	2.3-15.6	H_2SO_4 5.0 M	[18]
	SWV (oxidation peak)					
MDMC	PT	ISM	62.0 $\mu\text{g mL}^{-1}$	63-853	NaCl 1×10^{-3} M	[19]
Others						
4-MMC	CV (reduction peak)	DME	-	-	Boric acid	[20]
	DPP (reduction peak)				Citric acid	
					Phosphate	
4-MMC	AM (oxidation peak)	SPE	14.7 $\mu\text{g mL}^{-1}$	50-500	Acetate pH 4.3	[21]
4-MEC			9.4 $\mu\text{g mL}^{-1}$			
MDPV	AdSDPV	SPE	0.14 $\mu\text{g mL}^{-1}$	0.4-27.5	BR pH 6	[22]
MET	SWV	MIP/SPAuE	0.23 $\mu\text{g mL}^{-1}$	0.4-10.4	PBS pH 7.4	[23]

Abbreviations: AdSDPV: adsorptive stripping differential pulse voltammetry; AM: amperometry; AuNPs: gold nanoparticles; BDD: boron-doped diamond electrode; BR: Britton Robison buffer; C: cathinone; DME: dropping mercury electrode; DPP: differential pulse polarography; DPV: differential pulse voltammetry; GCE: glassy carbon electrode; ISM: ion-selective membrane; MC: methcathinone; 4-MC: nor-mephedrone or 4-methylcathinone; 4-MMC: 4-methylmethcathinone or mephedrone; 4-MMC-R: dihydromephedrone or 4-methylephedrine; 4-MEC: 4-methyl-N-ethylcathinone; MDEC: 3,4-methylenedioxy-N-ethylcathinone or ethylone; MDPV: 3,4-methylenedioxypropylvalerone; MET: Methyloxy; MIP: molecular imprinted polymer; MWCNT: multi-walled carbon nanotubes; NPS: new psychoactive substance; PBS: phosphate buffer saline; PT: potentiometry; SPAuE: screen-printed gold electrodes; SPE: graphite screen-printed electrode; SWV: square-wave voltammetry; Tris: tris(hydroxymethyl)aminomethane.

Table S3. Overview of the mephedrone oxidation products observed in the LC-QTOFMS analysis and their corresponding information.

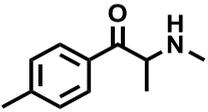
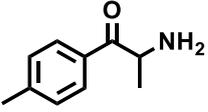
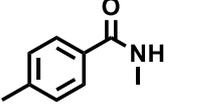
Compound	Retention time (min)	Measured m/z [M+H] ⁺	Theoretical m/z [M+H] ⁺	Diff (ppm)	DBE	Score	Chemical formula	Structure
MEP	5.76	178.1247	178.1226	11.63	5	82.03	C ₁₁ H ₁₅ NO	
M1	5.48	164.1068	164.1070	1.17	5	99.8	C ₁₀ H ₁₃ NO	
M2	6.73	150.0910	150.0913	2.28	5	99.32	C ₉ H ₁₁ NO	

Table S4. Overview of the methylone oxidation products observed in the LC-QTOFMS analysis and their corresponding information.

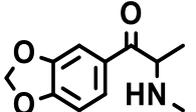
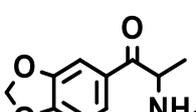
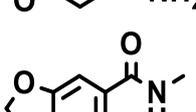
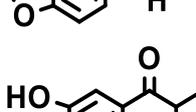
Compound	Retention time (min)	Measured m/z [M+H] ⁺	Theoretical m/z [M+H] ⁺	Diff (ppm)	DBE	Score	Chemical formula	Structure
MET	3.99	208.0981	208.0968	6.18	6	92.79	C ₁₁ H ₁₃ NO ₃	
T1	3.75	194.0812	194.0812	0.16	6	100	C ₁₀ H ₁₁ NO ₃	
T2	4.75	180.0651	180.0655	2.34	6	99.08	C ₉ H ₉ NO ₃	
T3	2.16	196.0966	196.0968	1.13	5	99.76	C ₁₀ H ₁₃ NO ₃	

Table S5. Overview of the 4-Cl- α -PVP oxidation products observed in the LC-QTOFMS analysis and their corresponding information.

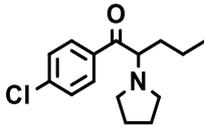
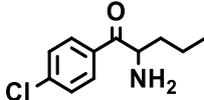
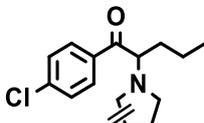
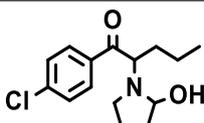
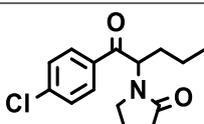
Compound	Retention time (min)	Measured m/z [M+H] ⁺	Theoretical m/z [M+H] ⁺	Diff (ppm)	D B E	Score	Chemical formula	Structure
4-Cl- α -PVP	6.06	266.1308	266.1306	-0.68	6	99.87	C ₁₅ H ₂₀ ClNO	
P1	5.34	212.0832	212.0837	2.22	5	98.98	C ₁₁ H ₁₄ ClNO	
P2	6.16	264.1143	264.1150	2.54	7	98.27	C ₁₅ H ₁₈ ClNO	
P3	6.55	282.1244	282.1255	4.03	6	95.45	C ₁₅ H ₂₀ ClNO ₂	
P4	8.51	280.1089	280.1099	3.52	7	96.51	C ₁₅ H ₁₈ ClNO ₂	

Table S6. Composition of the real samples analyzed by the forensic laboratory at NICC (i.e., gas chromatography-mass spectrometry) and comparison of the analysis with the electrochemical portable device. Red color indicates positive for Class I of SC. Purple color indicates positive for Class II of SC. Blue color indicates positive for Class III of SC.

Sample	GC-MS	Abbreviation	Electrochemical method
Cs 1	Alpha-Pyrrolidinopropiophenone	a-PPP	P
Cs 2	N- Ethylpentylone	NEP	P
Cs 3	4-fluoro-alpha pyrrolidinohexanophenone	F-PHP	P
Cs 4	4-Methyl-alpha-ethylaminopentiophenone	4-MEAP	P
Cs 5	N-ethylhexedrone	NEH	P
Cs 6	3-Methylmethcathinone	3-MMC	P
Cs 7	Mephedrone	MEP	P
Cs 8	Mephedrone	MEP	P
Cs 9	Mephedrone	MEP	P
Cs 10	Cathinone	CAT	FN
Cs 11	4-Chloromethcathinone	4-CMC	P
Cs 12	Pyrrolidinovalerophenone	PVP	P
Cs 13	Chloroethcathinone	CEC	P
Cs 14	4-chloro-alpha-PVP	Cl-PVP	P
Cs 15	2-methylaminoindane	2-MAI	FP
Cs 16	Pentedrone	PEN	P
Cs 17	N-ethylhexedrone	NEH	P
Ws 1	4-Fluoro-methcathinone	4-FMC	P
Ws 2	3-Fluoro-methcathinone	3-FMC	P
Ws 3	Ethcathinone	ETC	P
Ws 4	Mephedrone	MEP	P
Ws 5	Buphedrone	BUP	P
Ws 6	Butylone	BUT	P
Ws 7	Methylenedioxypropylone	MDPV	P
Ws 8	Methylone	MET	P
Ws 9	Naphyrone	NAP	P

P=true positive; N=true negative; FP=false positive; FN=false negative.

Figures

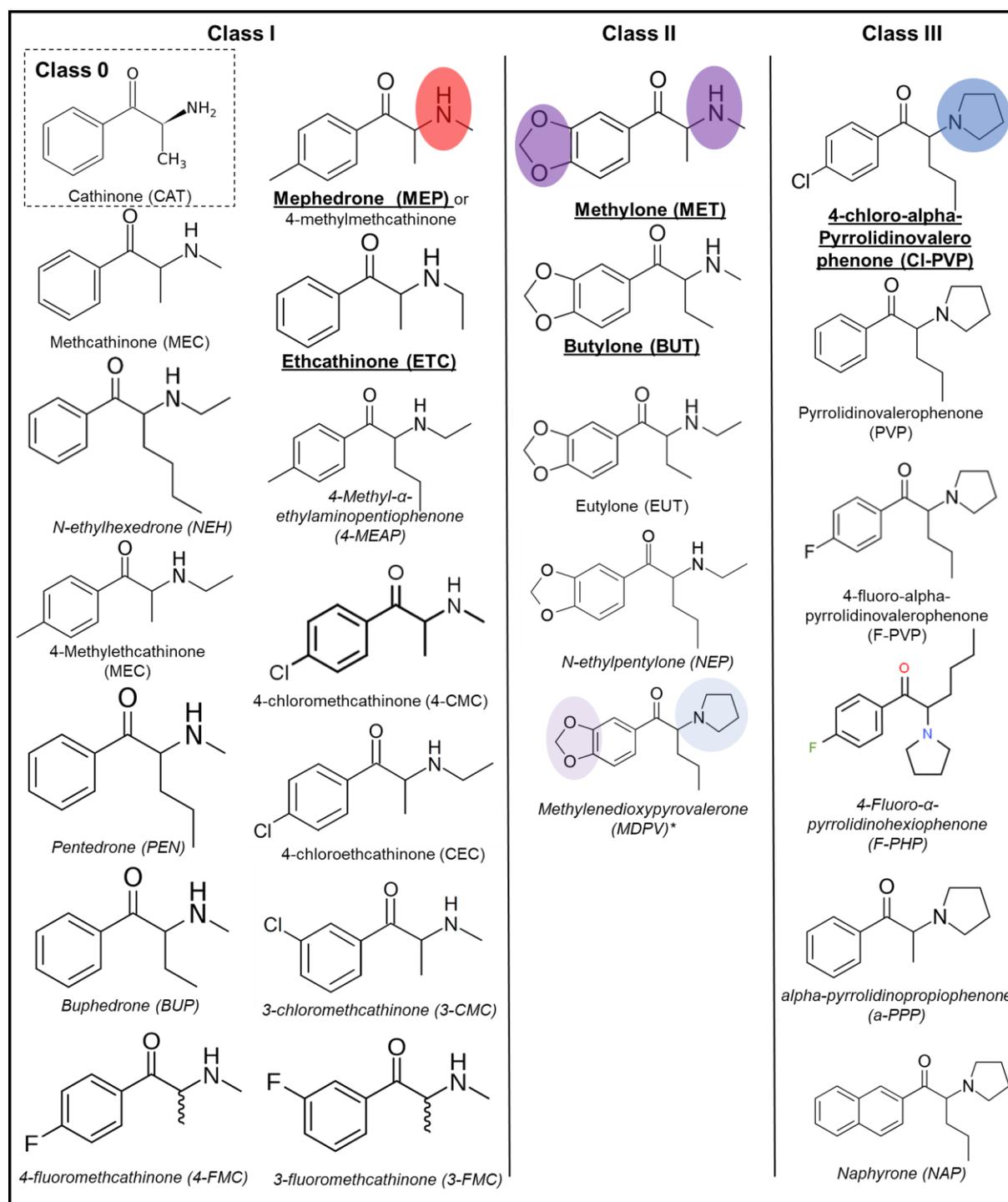


Figure S1. Chemical structure of synthetic cathinones (SCs). SCs in **bold** indicate the compounds thoroughly studied in this work. SCs in *italics* indicate SC found in confiscated and street samples. SCs are distributed in three different classes according to the oxidation molecules (highlighted in blue) that yield the electrochemical response. * Methylendioxypropylone shares groups from Class II and Class III.

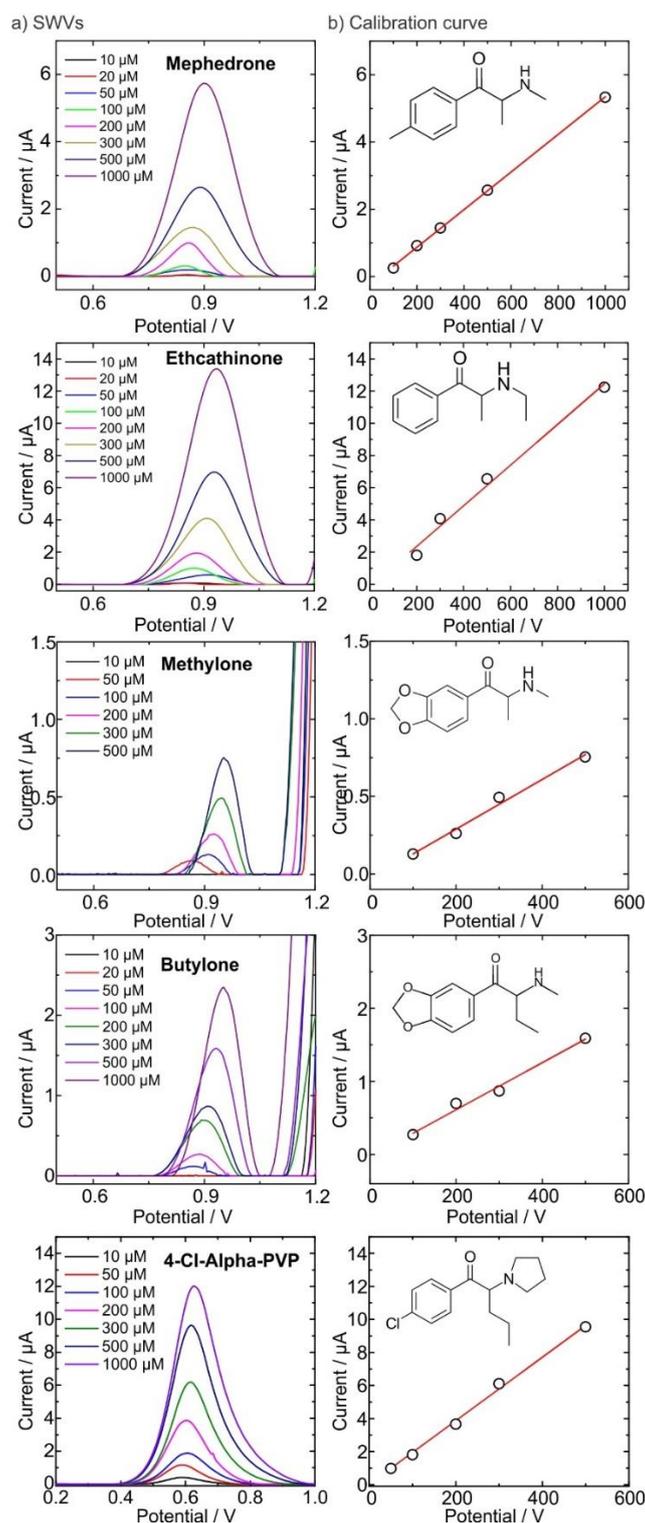


Figure S2. Analytical performance of SC (MEP, ETC, MET, BUT and CI-PVP, from top to bottom) in PBS pH 12 from 10 to 1000 μM at SPE: a) SWVs of increasing concentration of SC, and b) corresponding calibration plot.

The analysis leads to: (a) MEP a slope of $5.6 \mu\text{A mM}^{-1}$ ($E_p=0.90 \text{ V}$), from 100-1000 μM and a limit of detection (LOD) of $27.8 \mu\text{M}$; (b) ETC a slope of $12.6 \mu\text{A mM}^{-1}$ ($E_p=0.92 \text{ V}$), from 200-1000 μM and a LOD of $157.2 \mu\text{M}$; (c) MET a slope of $1.6 \mu\text{A mM}^{-1}$ ($E_p=0.91 \text{ V}$), from 100-500 μM and a LOD of $79.2 \mu\text{M}$; (d) BUT a slope of $3.2 \mu\text{A mM}^{-1}$ ($E_p=0.91 \text{ V}$), from 100-500 μM and a LOD of $65.3 \mu\text{M}$; and (e) CI-PVP a slope of $19.2 \mu\text{A mM}^{-1}$ ($E_p=0.62 \text{ V}$), from 50-500 μM and a LOD of $39.9 \mu\text{M}$.

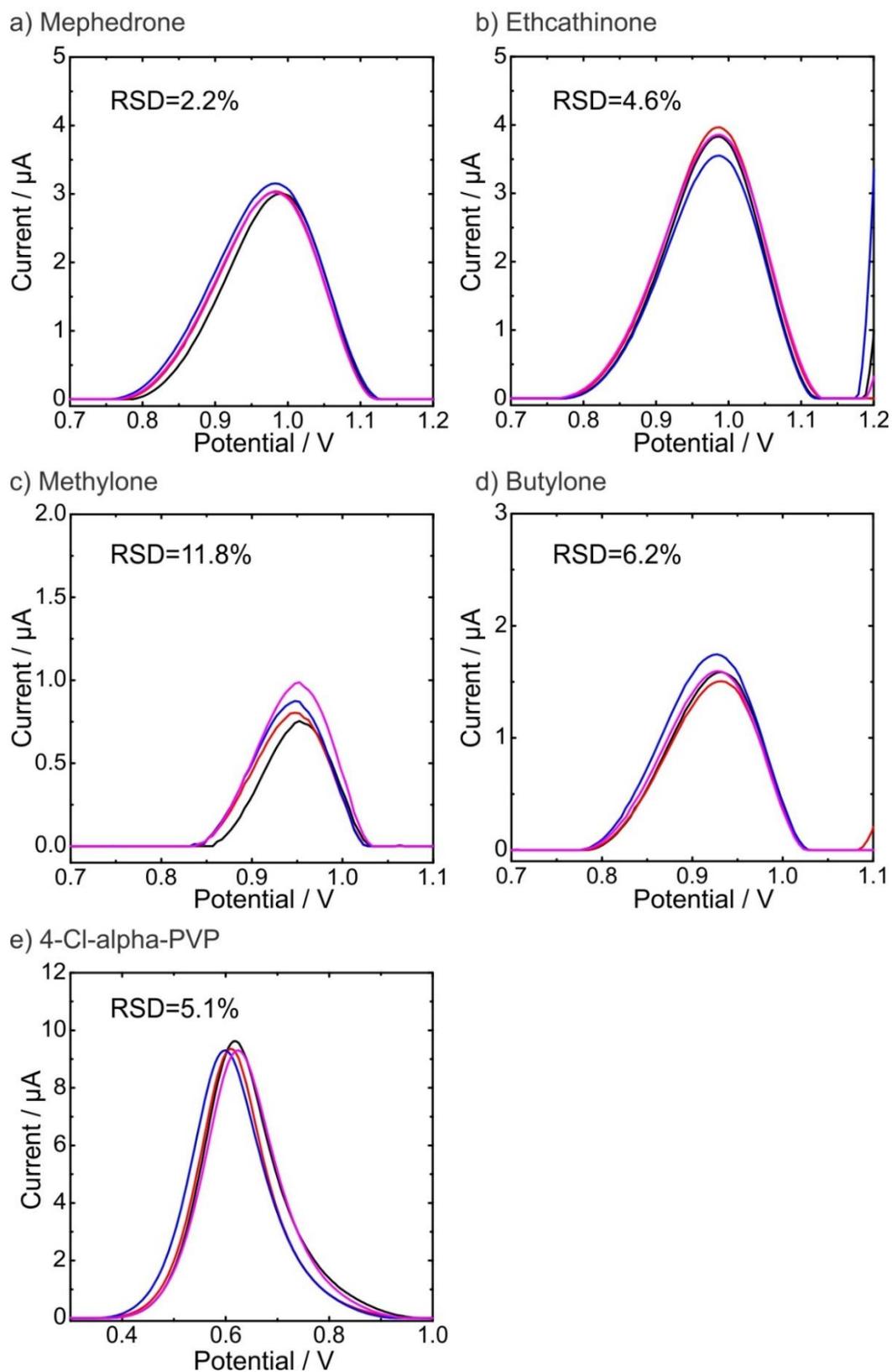


Figure S3. Intraday reproducibility studies by SWV of synthetic cathinones 0.5 mM at different SPE pH 12, N=4: a) mephedrone, b) ethcathinone (0.25 mM), c) methydone, d) butylone, and e) 4-Cl-alpha-PVP.

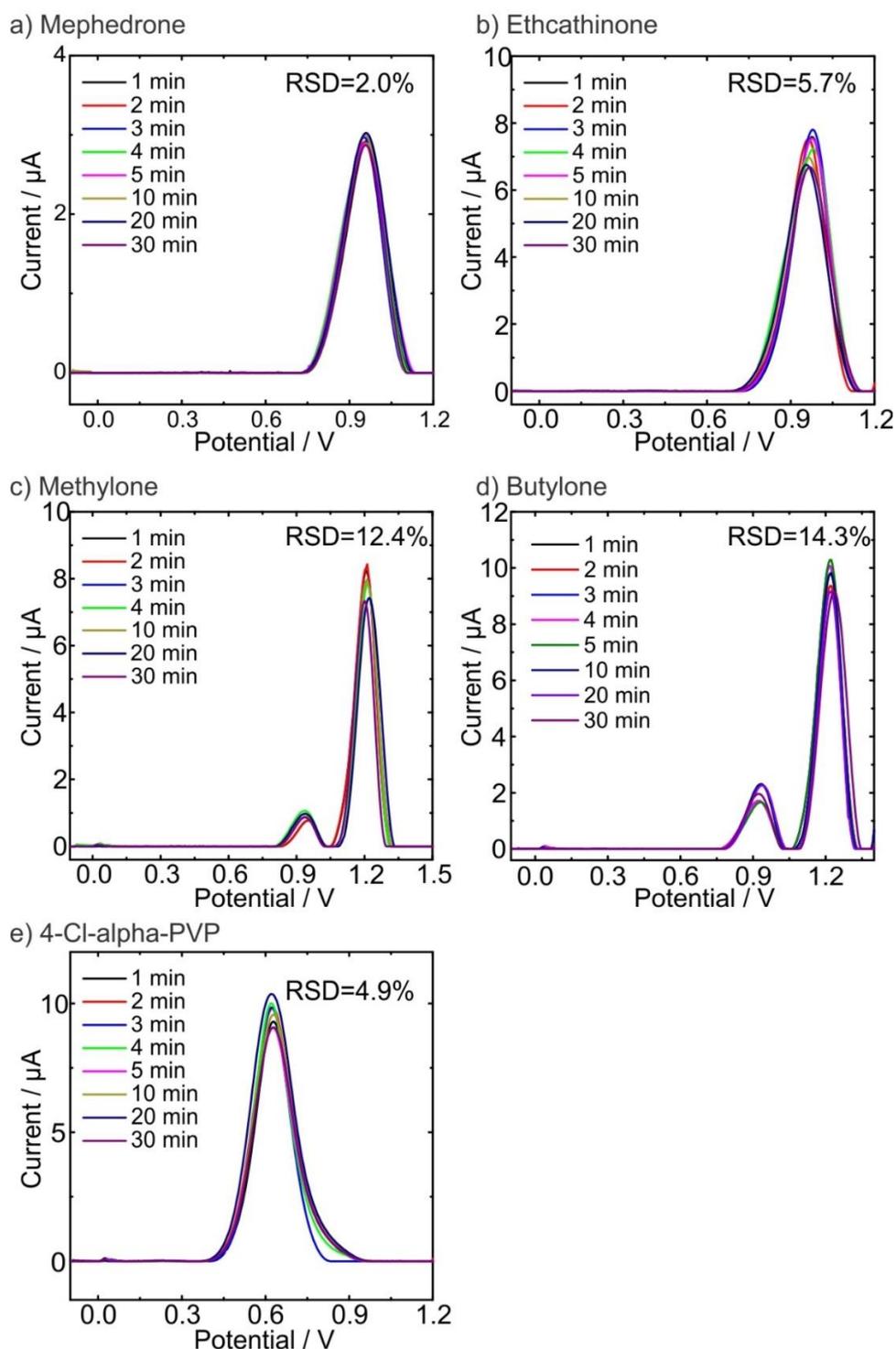


Figure S4. Stability study of synthetic cathinones at 0.5 mM by SWV with moving average correction in PBS pH 12 through time (from 1 min to 30 min): a) mephedrone, b) ethcathinone, c) methylone, d) butylone, and e) 4-Cl-alpha-PVP.

Stability study: measurements from 1 to 30 min after sample preparation were performed, showing high reproducibility: (a) MEP $I_p=2.9\pm0.1$ μA (RSD= 2.0%, N=8) at $E_p=0.95$ V; (b) ETC $I_p=7.2\pm0.4$ μA (RSD= 5.7%, N=8) at $E_p=0.97$ V; (c) MET $I_p=0.8\pm0.1$ μA (RSD= 12.4%, N=8) at $E_p=0.94$ V; (d) BUT $I_p=2.0\pm0.3$ μA (RSD= 14.3%, N=8) at $E_p=0.93$ V; and (e) Cl-PVP $I_p = 9.6\pm0.5$ μA (RSD= 4.9%, N=8) at $E_p=0.62$ V. Thus, negligible degradation of any SC through time in pH 12 was observed, therefore no risks are associated with the on-site detection.

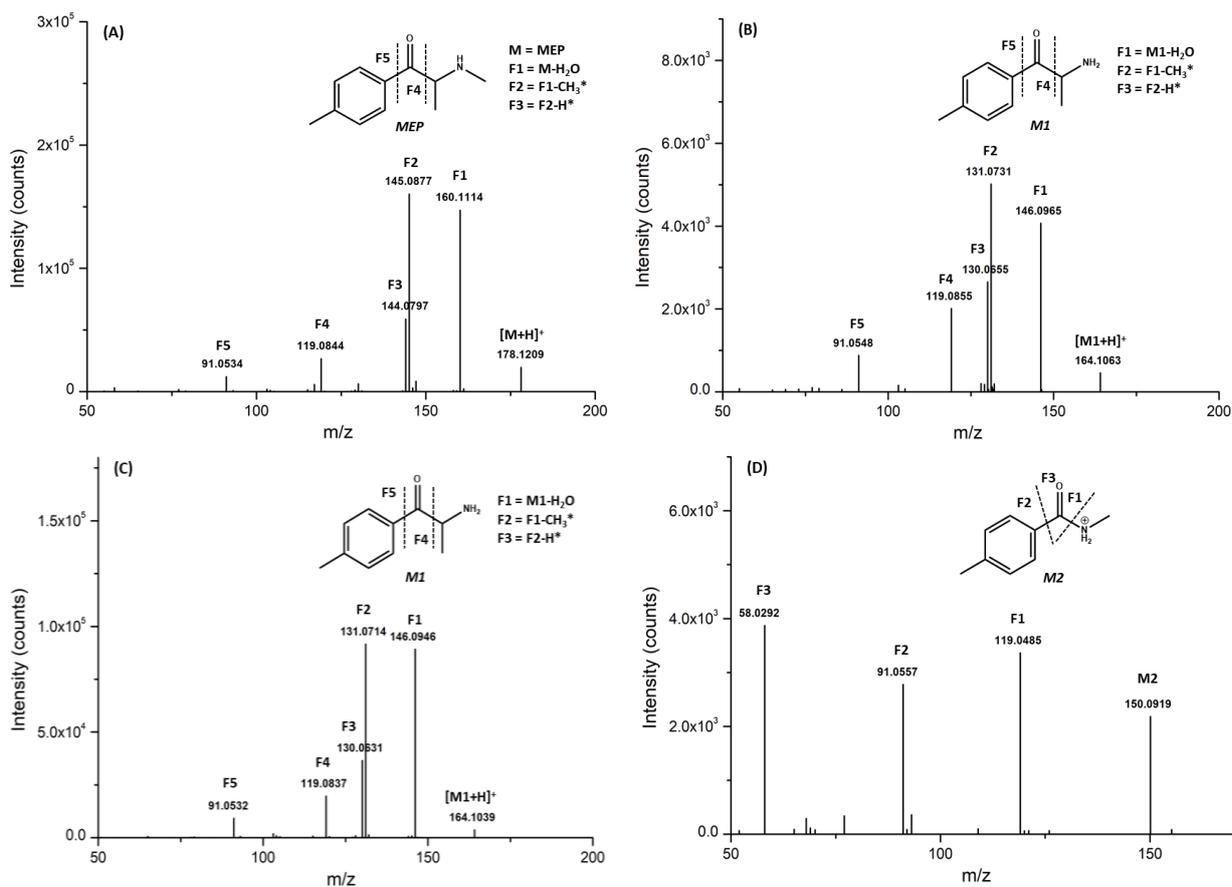


Figure S5. MS/MS spectra of (A) mephedrone (m/z 178.1226), (B) nor-mephedrone (standard, m/z 164.1063), (C) oxidation product M1 (m/z 164.1068) and (D) oxidation product M2 (m/z 150.0910).

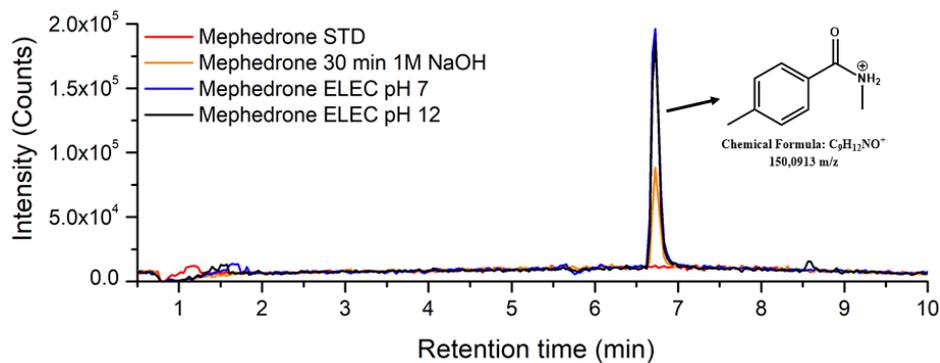


Figure S6. Extracted ion chromatograms (m/z 150.0913) of 20 ng μL^{-1} solutions of mephedrone standard (red), mephedrone degradation sample in 1 M NaOH solution (orange) and electrolysis samples in pH 7 (blue) and pH 12 (black).

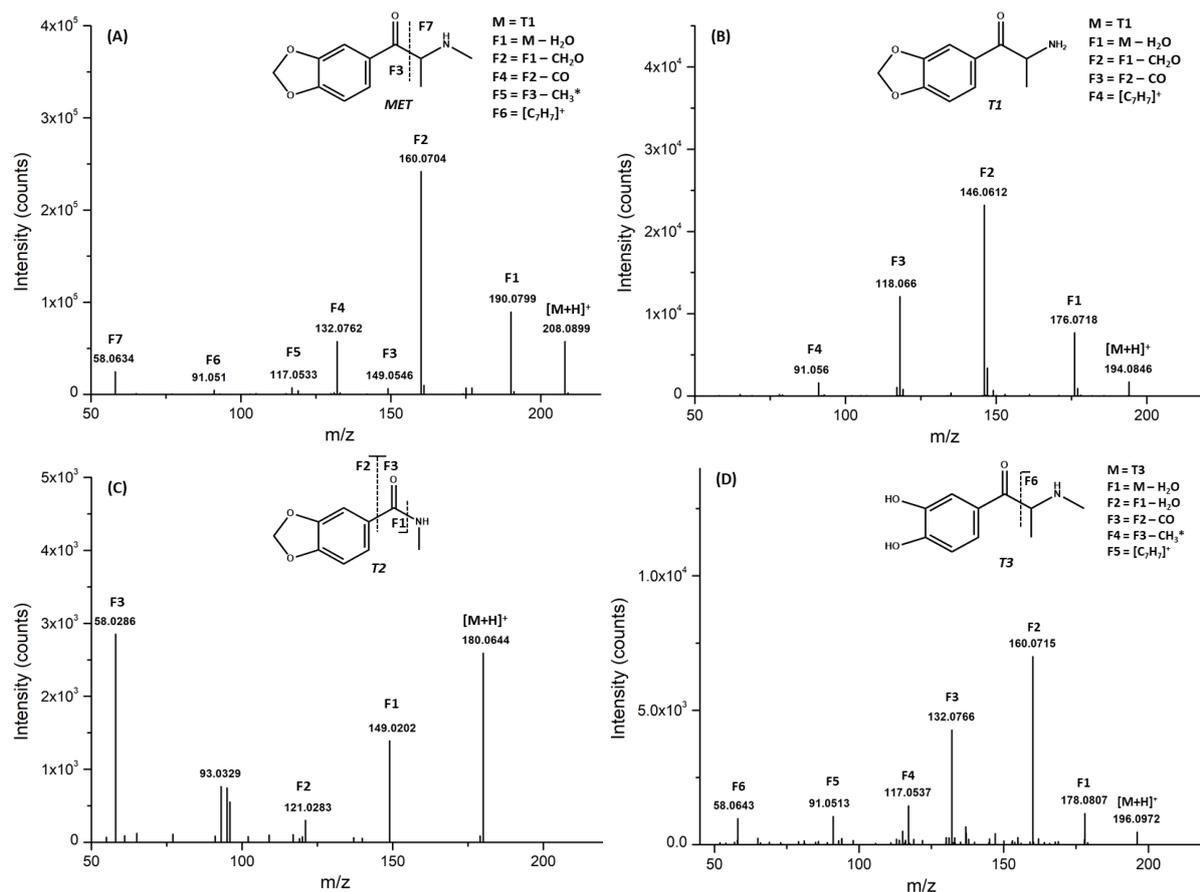


Figure S7. MS/MS spectra of (A) methylene (m/z 208.0981), (B) oxidation product T1 (m/z 194.0812), (C) oxidation product T2 (m/z 180.0651) and oxidation product T3 (m/z 196.0966).

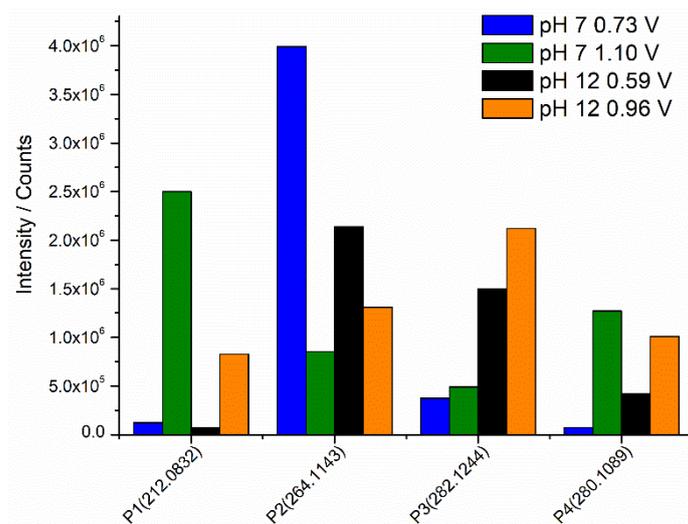


Figure S8. Intensities of the oxidation products observed for 4-Cl- α -PVP in pH 7 and 12.

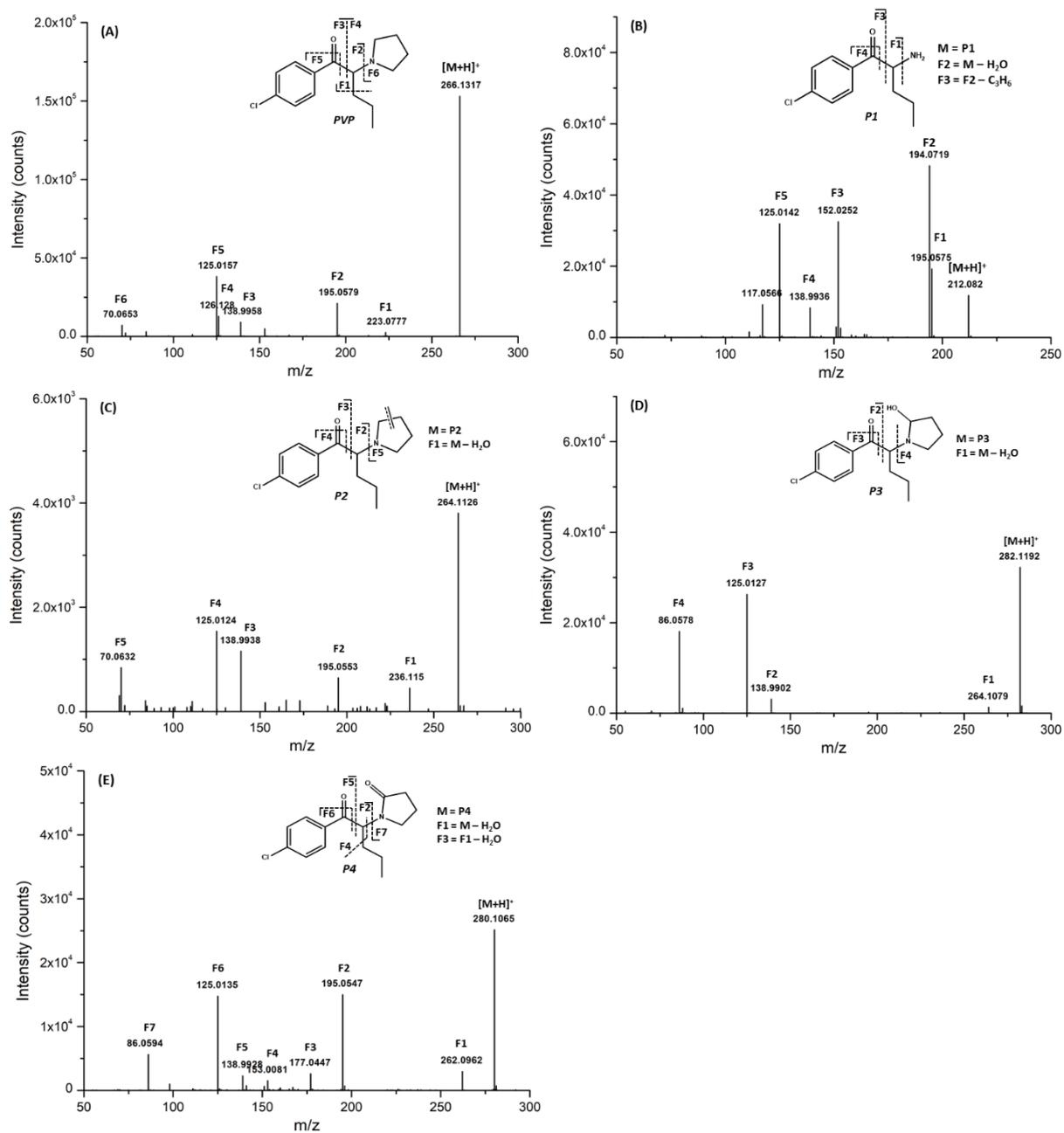


Figure S9. MS/MS spectra of (A) 4-chloro- α -PVP (m/z 266.1308), (B) oxidation product P1 (m/z 212.0832), (C) oxidation product P2 (m/z 264.1143), (D) oxidation product P3 (m/z 282.1244) and (E) oxidation product P4 (m/z 280.1089).

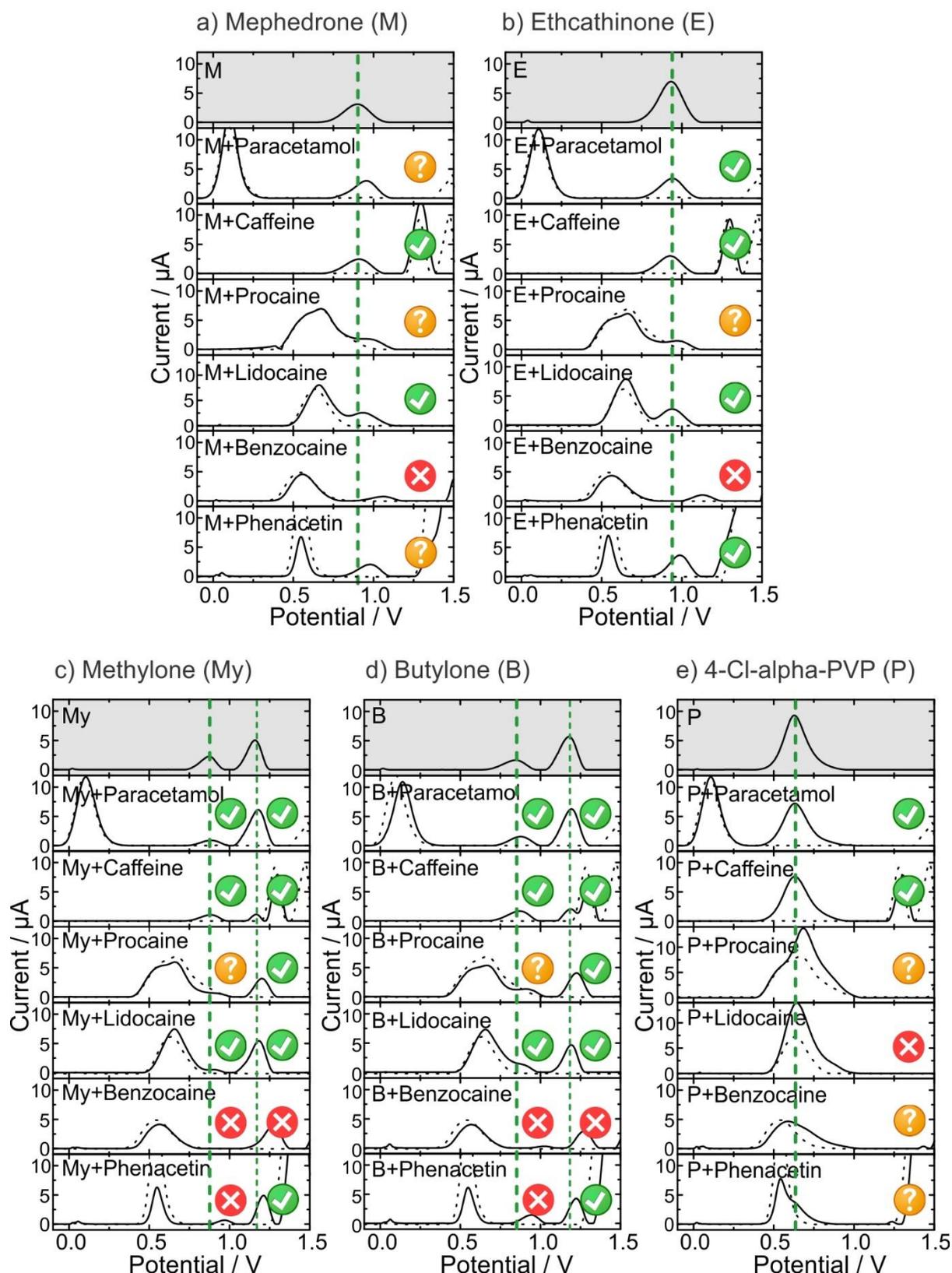


Figure S10. SWV analysis of binary mixtures (equimolar concentrations, 0.5 mM) with common adulterants in 20 mM PBS 100 mM KCl pH 12 at SPE: a) mephedrone (M), b) ethcathinone (E), c) methylone (My), d) butylone (B), and e) 4-Cl-alpha-PVP (P). The dotted SWVs display the electrochemical profile of the pure compounds. The dashed line indicates where the oxidation peak signal of SC is located.

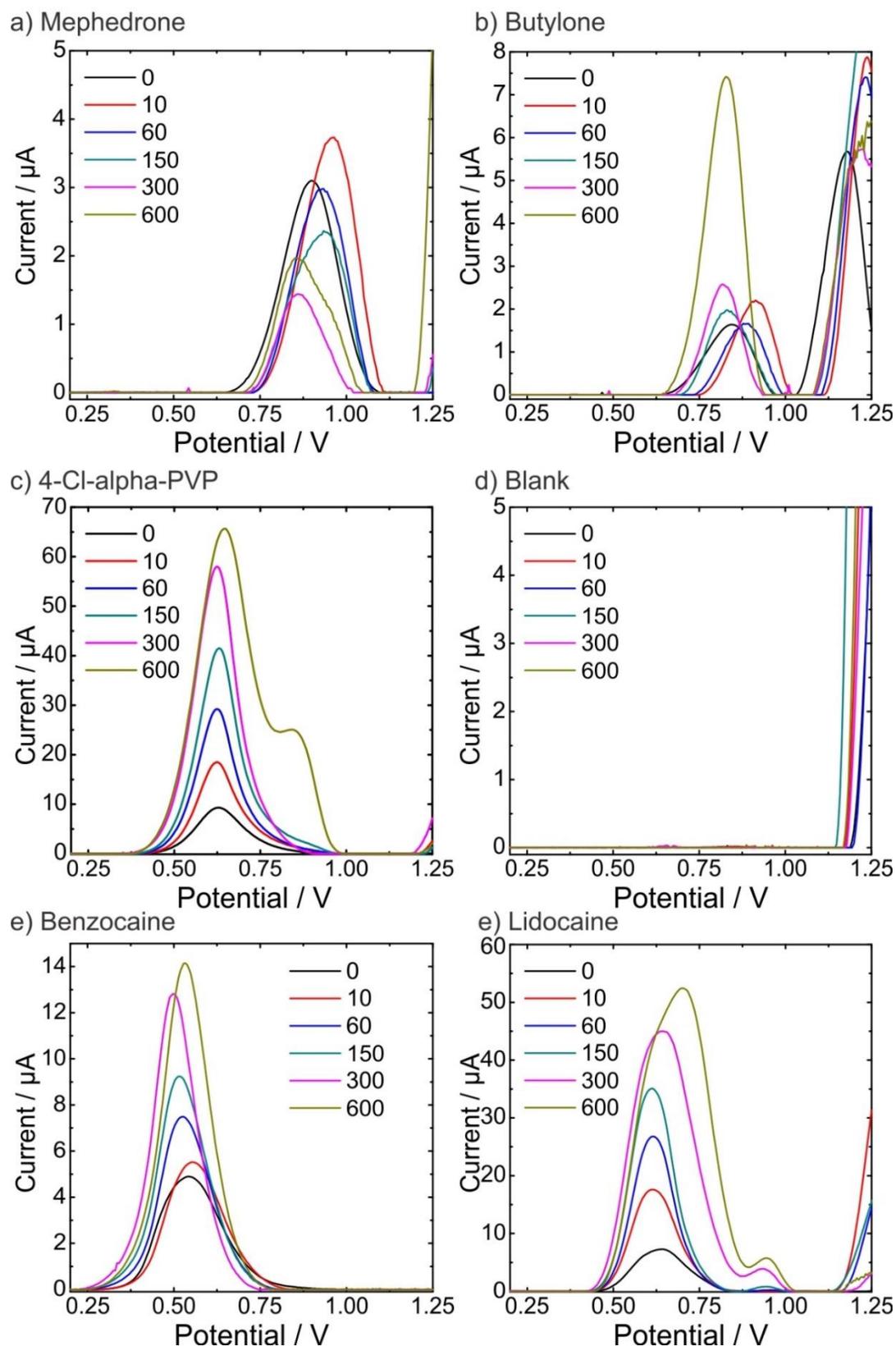


Figure S11. Effect of the cathodic pretreatment (-0.8V) on the SWV signal at different time: a) in 0.5 mM Mephedrone; b) 0.5 mM Butylone; c) 0.5mM 4-Cl-alpha-PVP; d) on the background signal; e) in 0.5 mM Benzocaine; and e) in 0.5 mM lidocaine. All tests are performed in PBS pH 12.

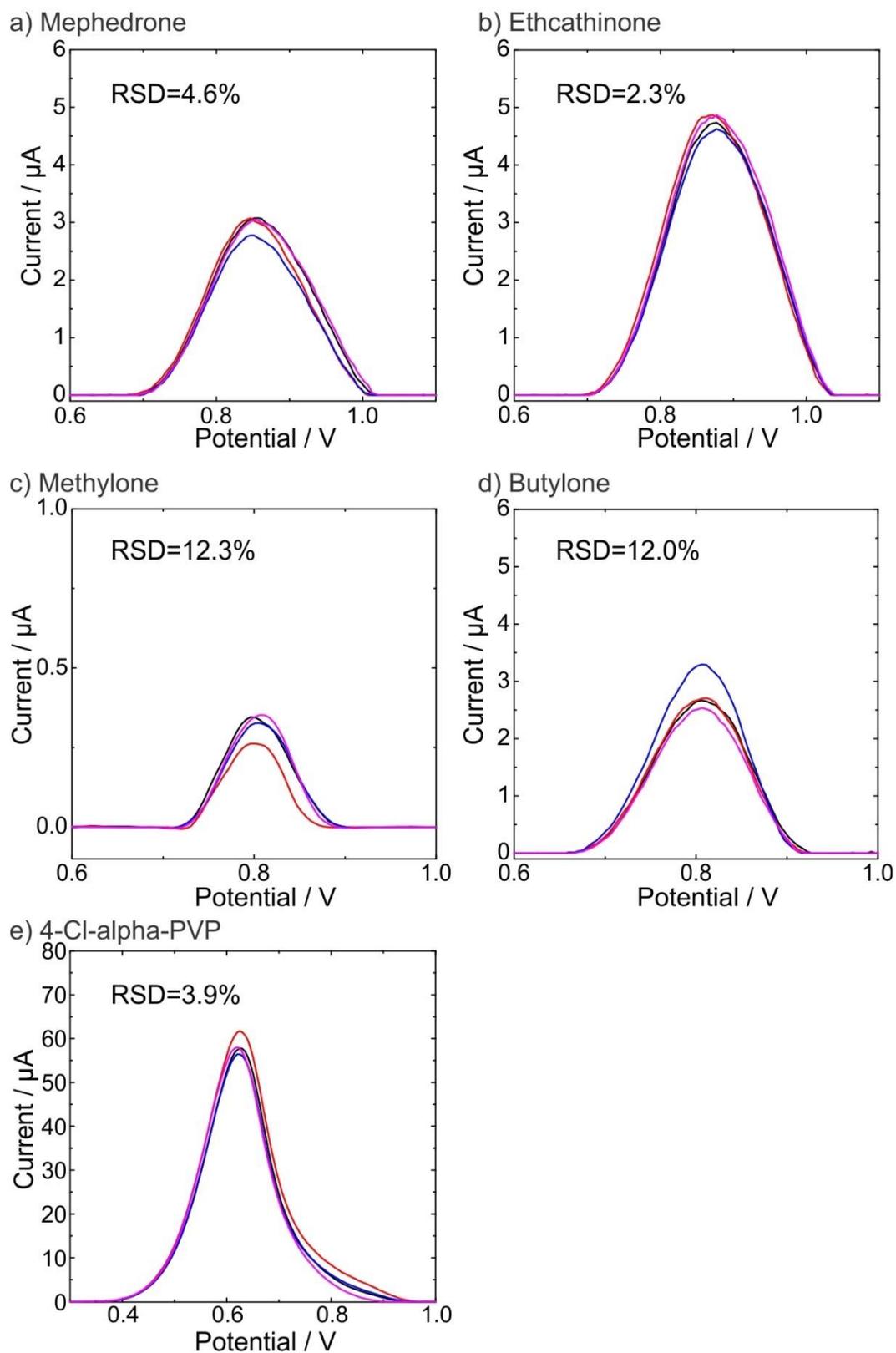


Figure S12. Reproducibility studies after cathodic pretreatment (-0.8V, 300s) by SWV of synthetic cathinones 0.5 mM at SPE pH 12, N=4: a) mephedrone, b) ethcathinone, c) methylone, d) butylone, and e) 4-Cl-alpha-PVP.

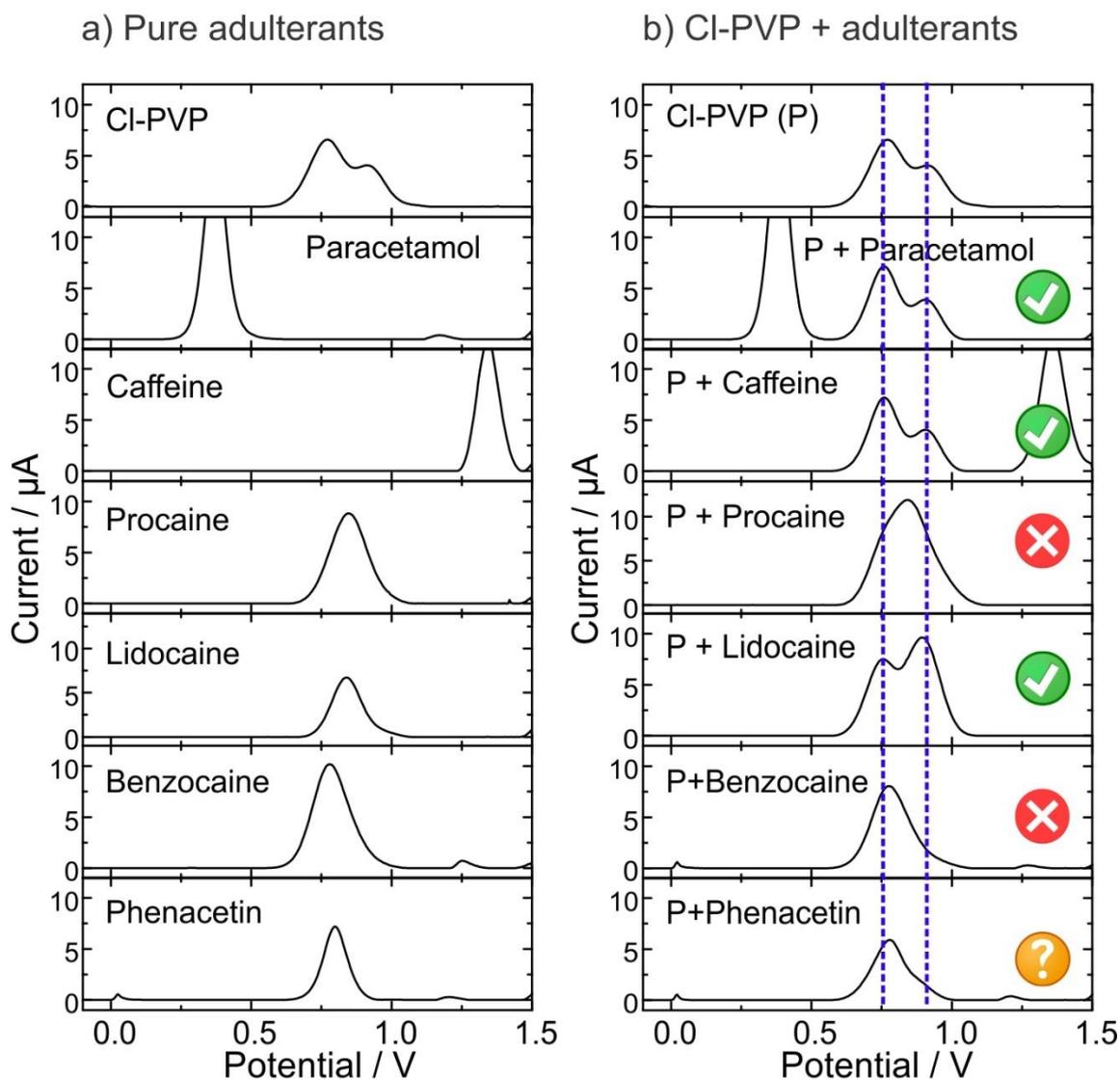


Figure S13. SWV analysis of binary mixtures (equimolar concentrations, 0.5 mM) of 4-Cl- α -PVP (CI-PVP) with common adulterants in 20 mM PBS 100 mM KCl pH 7 at SPE. a) Pure adulterants electrochemical fingerprint; b) binary mixture electrochemical profile. The dashed line indicates where the oxidation peak signal of SC is located.

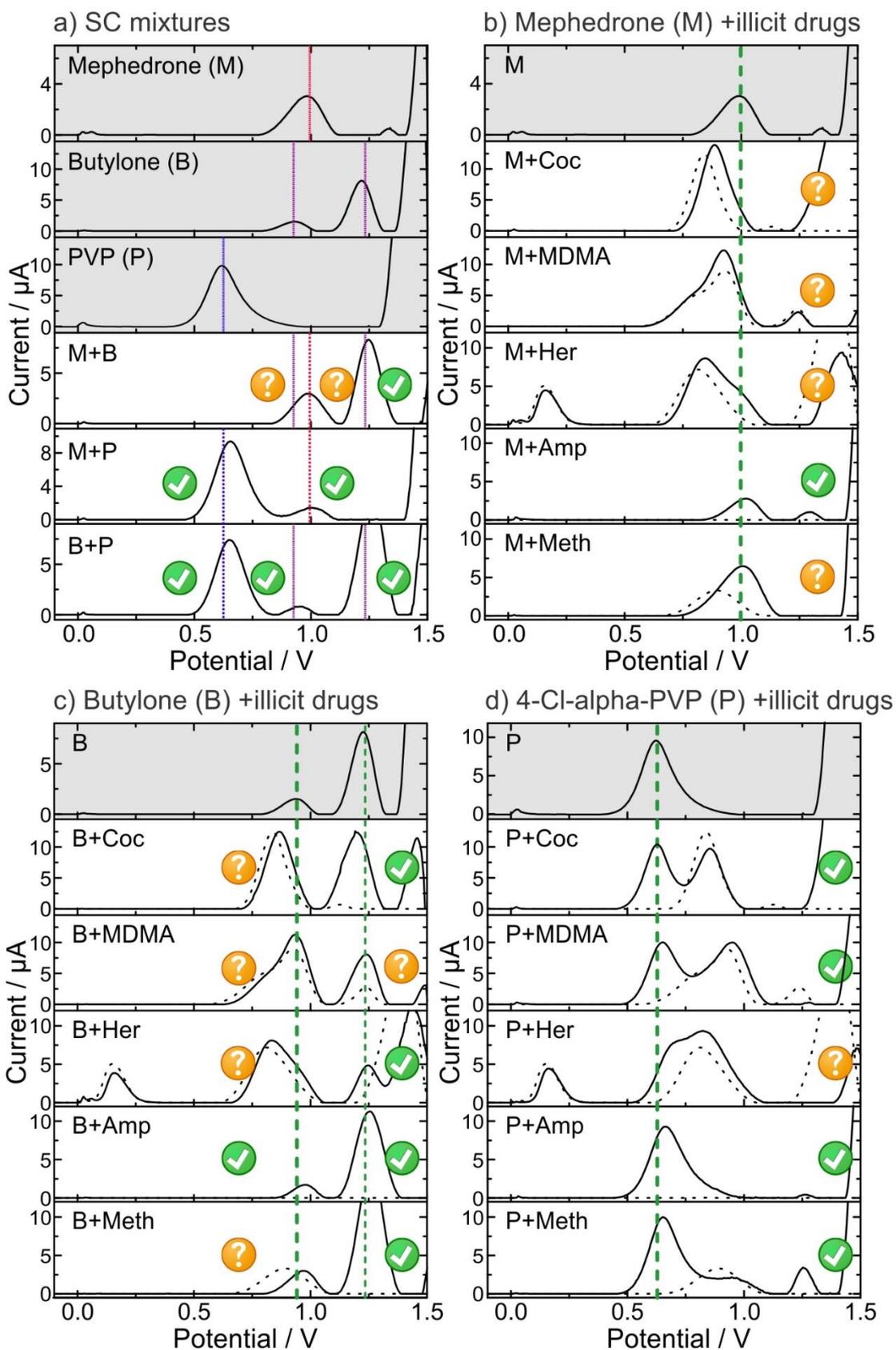


Figure S14. SWV analysis of binary mixtures (equimolar concentrations, 0.5 mM) with other illicit drugs in 20 mM PBS 100 mM KCl pH 12 at SPE: a) SCs, b) mephedrone, c) butylone, and d) 4-Cl-alpha-PVP. The dashed SWVs display the electrochemical profile of the pure compounds. The dotted line indicates where the oxidation peak signal of SC is located.

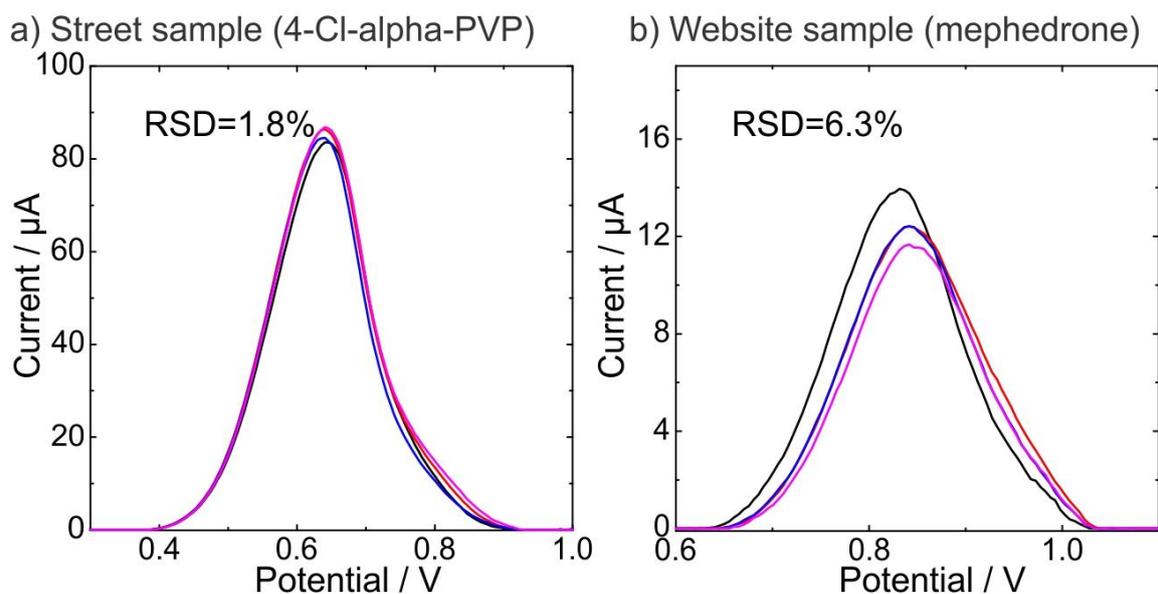


Figure S15. Reproducibility studies after cathodic pretreatment (-0.8V 300s) by SWV of street samples at SPE pH 12, N=4.

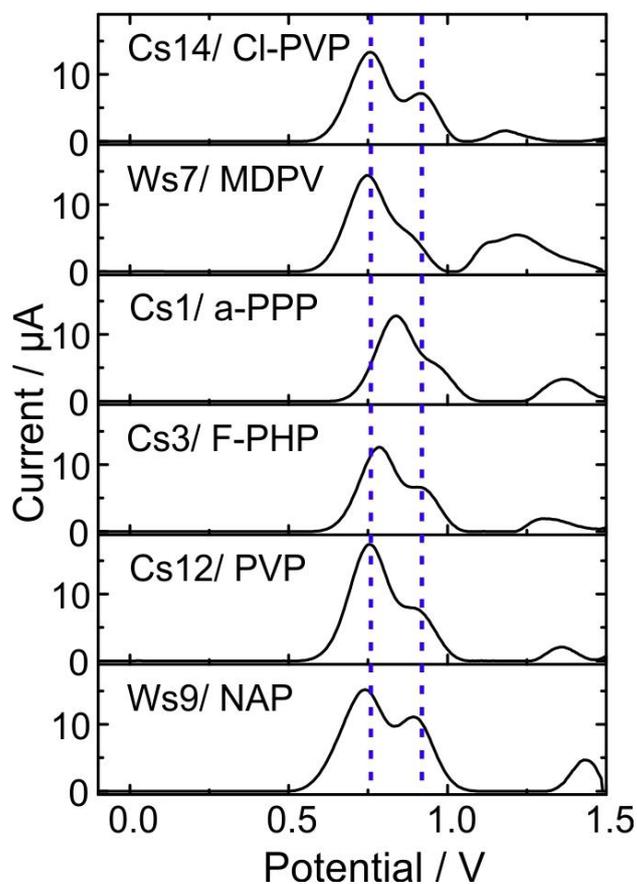


Figure S16. Electrochemical profile of real samples of SC-III at SPE in PBS pH 7. Table S6 indicates the main compound of the sample.

References

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