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Electrooxidation of New Synthetic Cannabinoids: Voltammetric Determination of Drugs in Seized Street Samples and Artificial Saliva

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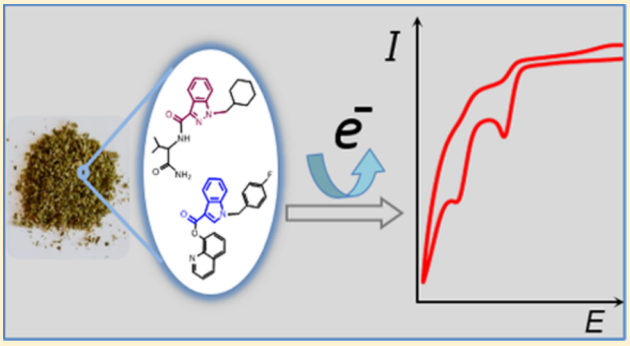
Supporting Information

ABSTRACT: The electrochemical sensing of new psychoactive substances, synthetic cannabinoids (SCs), commonly marketed under the trade name "Spice" is explored for the first time. The electrooxidative transformations of 11 new indole and indazole SCs which are currently the predominant illicit smoking mixtures on the drug market is performed using cyclic and differential pulse voltammetry with various commercially available electrodes (Pt, GC, Bdd). It is found that SCs exhibit voltammetric responses that can be used for their detection in smoking mixtures and artificial saliva with limits of detection in the nanomolar range. The indole-based SCs exhibited an anodic peak at ~1.5 V (vs Ag/Ag^+) and ~1.2 V (vs Ag/AgCl) in acetonitrile and artificial saliva, respectively, and the indazoles exhibited corresponding peaks at ~1.7 V and ~1.5 V. The voltammetric procedure was evaluated by prescreening of SCs in 12 confiscated street samples that were also independently analyzed by GC-MS and LC-MS techniques. A good agreement between the three analytical protocols was found. Voltammetry provides a tool for the prescreening of synthetic cannabinoid derivatives in seized materials and biological samples.



Synthetic cannabinoids (SCs) is a collective term which is frequently used to address a diverse group of drug substances, generally having little chemical resemblance to classical cannabinoids produced by the *Cannabis sativa* plant. They are unified by this terminology, owing to their affinity toward the CB1 and CB2 cannabinoid receptors in the central and peripheral nervous systems.[1]

Although, many SCs were investigated and developed legitimately as therapeutic agents over the past 40 years, it has been proven difficult to separate their beneficial properties from unwanted psychoactive effects.[2] Thus, the majority of these compounds were not incorporated in medical treatment. SCs found their way to the recreational drug market, where they were first detected in 2008.[3] These drugs are usually impregnated on plant leafs and sold in designated "smart shops" and via the Internet as incense or room odorizers. Of these products, the trade name "Spice" or "K2" is perhaps the best well-known.[4,5] Although herbal mixtures often bear the intentionally misleading disclaimer "not for human consumption", spice-type products are being smoked as an alternative to cannabis to produce similar (and often more potent) effects.[6] Numerous cases of poisonings, as well as fatalities, were linked to the use of SCs.[7−9] Consequently, many of these substances were banned by legislation worldwide.[10] However, the demand and popularity of "Spice" among the general public and especially teens, remain high as they are attracted by the high accessibility, low price, and perceived legality of such compounds as compared with other drugs of abuse.[9,11,12]

In order to maintain their profitable operation, despite legal measures, distributors of SCs bring to market new compounds each year.[13,14] These are prepared by chemically modifying existing SCs and are aimed at replacing banned compounds, as well as delivering enhanced psychoactive effects. As such, they are often termed "designer drugs".[15,16]

The newest generation of SCs currently dominating the drug market are *N*-alkyl indole-3-carbonyl derivatives (referred to here as "indole-based SCs") and *N*-alkyl indazole-3-carbonyl analogues (referred to as "indazole-based SCs").Their prevalence can be attributed to higher potency and ease of synthesis compared to the other classes of compounds.[17] Scheme 1 delineates several common structural features of such compounds. All newly detected SCs in Israel belong to the indole- and indazole-based SCs. These substances were also reported globally, including in countries such as Japan, United States, Russia, Germany, Great Britain, Spain, The Netherlands, Australia and many others.[14] These new SC structures include naphthoylindoles (AM-2201, JWH-018), cyclopropylindoles (XLR-11); analogues obtained by substitution of the indole ring moiety for indazole (like naphthoylindazole THJ-2201), and also ester- (FDU-PB-22, FUB-PB-22, 5F-PB-22) or amide-type (AB-FUBINACA, AB-CHMINACA, AB-PINACA, 5F-AMB) analogues (see Scheme 1). Beside these structures,

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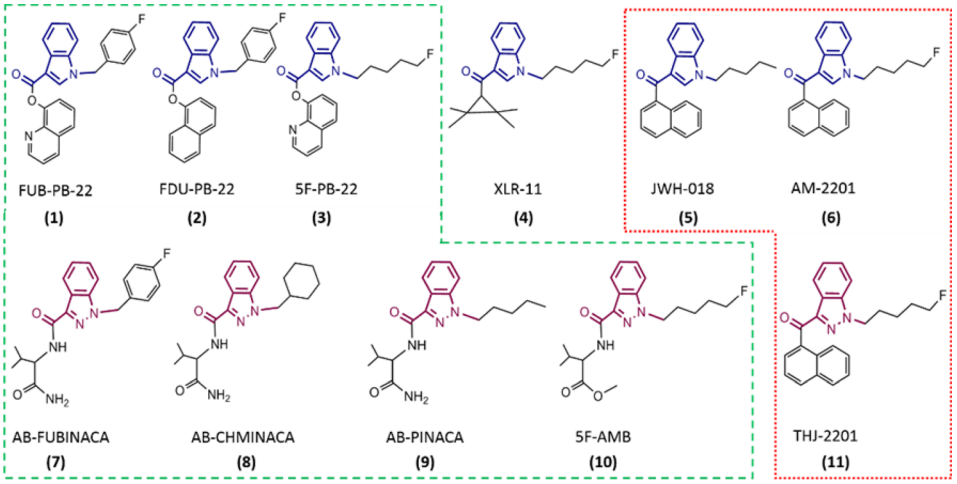
Scheme 1. Structural Formulae with Trivial Names of Indole- and Indazole-Based SCs Currently Found in Smoking Mixtures[a]



[a]JWH-018 is shown as an "ancestor" of present indole-based cannabinoids, though nowadays it is seldom found in smoking mixtures. The dotted line shows naphthoyl-indoles/indazoles; the dashed line shows amide- or ester-type analogues. XLR-11 belongs to cyclopropylindole family.

adamantyl-, phenylacetyl-, and benzoyl-indoles/indazoles SCs, as well as many others may be found. The chemically changed drugs are skillfully tailored to pass through traditional screening analyses and, as a consequence, dozens of new compounds of the indole and indazole groups are brought to market each year.[18−20]

The United Nations Office on Drugs and Crime have recommended methods for detection and identification of SCs in seized materials.[17] As for many other new psychoactive substances, the gold standards for forensic analysis are GC-MS and LC-MS techniques.[21,22] High-resolution LC-MS techniques such as LC-QTOF-MS allow for accurate determination of the molecular weight of the compounds. GC-EI-MS, on the other hand, is more routinely used for drug identification by forensic laboratories because of the inherent fragmentation pattern, which allows for comparison to drug spectral libraries. However, a common drawback of EI ionization is the small abundance of molecular ion usually encountered with SCs having in their structure naphthoyl-, benzoyl- and cyclopropyl-moieties. Moreover, regular EI ionization does not provide an adequate molecular ion for amide- or ester-type analogues of synthetic cannabinoids.[18,19] Overcoming this drawback involves complementing GC-EI-MS with techniques such as LC-QTOF-MS or GC-CI-MS (chemical ionization method). Although GC-MS and LC-MS methods are invaluable for conclusive identification of emerging drugs of abuse, the instrumentation used is inherently expensive and bulky and is not fit for field analysis.

Hence, the development of simple screening methods for synthetic cannabinoid (SC) detection and quantification in seized and biological materials is still urgently required. TLC, IR, RAMAN, and ion mobility spectroscopy are a few of the screening techniques developed for that end.[22] Colorimetric detection represents another screening technique, and a number of color tests for SC detection have been recently developed.[23,24] Although it is possible to detect synthetic cannabinoids by these color tests in herbal blends, they suffer from low sensitivity, making this technique inapplicable for SC detection in body fluids. Immunochemical methods, on the other hand, provide low limits of detection (higher sensitivity) allowing detection of SCs in urine. Thus, commercially available immunoassay kits, such as Drug-Check K2/Spice Test gives positive results for older types of synthetic cannabinoids, such as JWH-018. Unfortunately, newer designer indole and indazole drugs such as AB-FUBINACA, AB-CHMINACA, and AB-PINACA cannot be detected due to the high specificity of immunoassay kits.[24,25] Hence, there is a need for alternative general screening methods that would be sensitive enough for SC detection not only in smoking mixtures but also in body fluids, as well as applicable for a wide range of emerging SC structures. Electrochemistry is known to be an advantageous analytical tool that is adaptable to in-the-field devices, due to its portability, and can exhibit high sensitivity and selectivity toward many target analytes. Thus, for example, Smith et al. has recently developed and verified a new electrochemical protocol for determination of cathinones, a group of psychoactive substances commonly abused alongside SCs.[26] The development of analytical techniques based on electrochemistry would be highly beneficial for forensic applications, though nowadays to the best of our knowledge there is no electrochemical analytical screening technique for synthetic cannabinoid detection. In this work, the electrochemical sensing of the largest group of psychoactive substances—indole- and indazole-based SCs—is explored for the first time.

■ EXPERIMENTAL SECTION

All chemicals used were of analytical grade and were used as received from Sigma-Aldrich without further purification. All solutions were prepared with deionized water of resistivity no less than 18 MΩ cm. The 11 synthetic cannabinoid chemicals (FUB-PB-22, FDU-PB-22, 5F-PB-22, XLR-11, AM-2201, JWH-018, AB-FUBINACA, AB-CHMINACA, AB-PINACA, 5F-AMB, THJ-2201) in the form of crystalline powders, were provided by the Department of Identification and Forensic Science of the Israeli Police and were used as reference standards (formal chemical names and molecular formulas of the synthetic cannabinoid standards are presented in Table S1 of the SI).

Twelve street samples provided by the Department of Identification and Forensic Science of the Israeli Police, were received as dry herb leaves in zip-lock bags. LC-MS, GC-MS,

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and electrochemical analysis were performed independently to quantify the chemical composition of the street samples. For the analysis of street samples, 150 mg of herbal mixture from every batch was placed in a separate glass vial. Ten milliliters of acetonitrile was added to every vial and vortexed for 2 min. Then the extracts were filtered and subjected to GC-MS, LC-MS, and electrochemical testing. Details of the experimental protocol for both GC-MS and LC-MS methods can be found in the Supporting Information.

Voltammetric measurements were carried out using a CHI 750B potentiostat/galvanostat and controlled by CHI760B Electrochemical Workstation software. Tetrabutylammonium perchlorate (TBAP, ≥ 99.0% purity, Sigma-Aldrich) was used as a supporting electrolyte. A conventional three-electrode cell with Ag/AgCl (sat. KCl; BASI Inc.) or Ag/Ag^+ (0.01 M $AgNO_3$, 0.1 M TBAP in CH_3CN; BASI Inc.) reference and a platinum wire counter was used. Boron-doped diamond (Bdd), glassy carbon (GC), and platinum (Pt) electrodes were used as the working electrodes. Both GC and Pt electrodes were homemade with a diameter of 2 mm, and the Bdd electrode (Windsor scientific, D-531-SA) had a diameter of 3 mm. Working electrodes were cleaned before each run. GC working electrode was polished with diamond paste (6 μm) and rinsed with ethanol and deionized water. The Pt electrode was rinsed with deionized water and ethanol, kept in piranha solution for 15 min, and finally rinsed with deionized water. The Bdd electrode was pretreated galvanostatically in 0.5 M H_2SO_4 solution. A cathodic pretreatment was performed by application of 25 mA/cm^2 for 60 s, and the anodic pretreatment comprised the cathodic treatment followed by application of −25 mA/cm^2 for 180 s. The artificial saliva was prepared according to ref 27, and the components of the artificial saliva are detailed in Table S2 of the SI. The pH of saliva was adjusted to 5 with a few drops of dilute nitric acid or sodium hydroxide solutions.

RESULTS AND DISCUSSION

Electrochemical Oxidation of SCs. The electro oxidative detection of six indole-based SCs (**1**−**6**) and five indazole-based SCs (**7**−**11**) (Scheme 1) in acetonitrile solution was investigated by cyclic voltammetry (CV) using Pt, GC, and Bdd working electrodes. Figure 1 depicts the respective voltammograms in a 0.01 M TBAP/CH_3CN solution for 1 mM concentration of the indole-based synthetic cannabinoids **1** and **6**; and for the indazole-based SCs **10** and **11**. The Bdd electrode exhibited the highest sensitivity for all analytes, followed by the Pt electrode. However, the Pt electrode exhibited a shift of the oxidation peaks to less positive potentials, which is beneficial from the analytical point of view. The GC electrode showed the lowest sensitivity toward all analytes. The other SC compounds (**2**−**5**; **7**−**9**) showed similar electrochemical behavior, and comparison of their electro-oxidation data are summarized in Table 1.The complete voltammograms of all tested SCs (**1**−**11**) are presented in Figures S1 and S2 in the SI.

The electrooxidation process likely involves electron abstraction from the indole ring moiety as reported for other substituted indoles.[28−31] The peak oxidation potentials for indole-based SCs are around ∼1.5 V (by DPV measurements) and are close to the reported oxidation potential of indole-5-carboxylic acid[29] (∼1.46 V, see Table 1). For indazole SCs, a first oxidation wave is observed at higher potentials (∼1.7 V), which can be explained by the fact that unmodified indazole has a higher oxidation potential than indole (∼1.43 V vs ∼1.15 V,

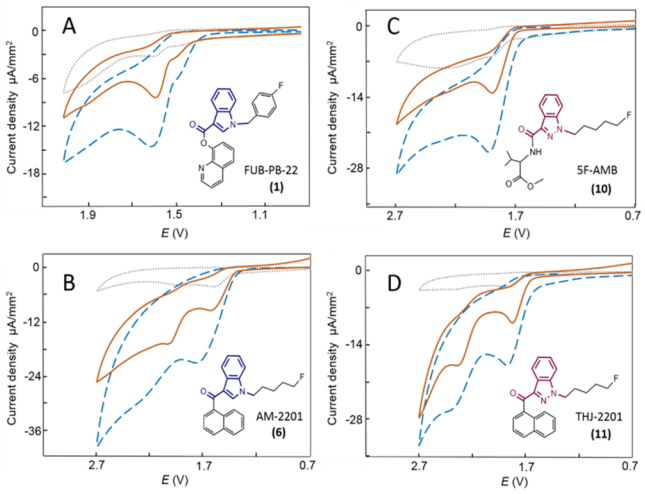

Figure 1. Cyclic voltammograms recorded using Bdd (dashed line), Pt (solid line), and GC (dotted line) electrodes in 0.01 M TBAP/CH_3CN solution for 1 mM of **A:** FUB-PB-22 (**1**); **B:** AM-2201 (**6**); **C:** 5F-AMB (**10**); **D:** THJ-2201 (**11**). Scan rate, 100 mVs^{-1}. All potentials are reported vs Ag/Ag^+ reference.

Table 1. Electrochemical Oxidation Potentials of Synthetic Cannabinoids in Acetonitrile

synthetic cannabinoids		$E_{1/2}$[a] (V)	E_p^{DPV}[b] (V)	R.f.[c] (μA/1 mM)
indole-based	(**1**) FUB-PB-22	1.61	1.57	12.5
	(**2**) FDU-PB-22	1.54	1.47	10.8
	(**3**) 5F-PB-22	1.56	1.54	9.06
	(**4**) XLR-11	1.39	1.33	10.3
	(**5**) JWH-018	1.50	1.44	9.52
	(**6**) AM-2201	1.48	1.45	9.50
	average R.f.			10.3 ± 1.25
indazole-based	(**7**) AB-FUBINACA	1.88	1.82	9.58
	(**8**) AB-CHMINACA	1.82	1.75	9.20
	(**9**) AB-PINACA	1.82	1.75	10.8
	(**10**) 5F-AMB	1.75	1.71	12.8
	(**11**) THJ 2201	1.70	1.67	9.62
	average R.f.			10.4 ± 1.46
	indole		1.15	
	indole-5-carboxylic acid		1.46*	
	indazole		1.43	

[a]Half wave potential measured by CV at 100 mV s^{-1}. [b]Oxidation potential peak measured by DPV. [c]Response factor−oxidation current peak height for 1 mM solution of analyte (measured by DPV). EC measured in 0.01 M TBAP/CH_3CN solution on Pt electrode vs Ag/Ag^+. *Peak oxidation potential measured by linear sweep voltammetry on Pt electrode in 0.1 M $LiClO_4/CH_3CN$ solution, taken from ref 29.

Table 1). Thus, for analogous structures **6** and **11** obtained by substitution of the indole ring moiety for indazole, the potential difference of the first oxidation wave is ∼230 mV which is comparable to the potential difference between the oxidation peaks of indole and indazole (∼280 mV).

In case of SCs containing naphthalene or quinoline moieties, a second nonreversible oxidation wave was observed (see Figure 1B,D), which is attributed to the oxidation of the naphthalene or quinoline functionalities;[32,33] this particular

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oxidation wave is known to occur at higher potentials than that of indole or indazole.

Next, the effect of the scan rate on electrochemical oxidation of **6** and **11** was explored for 1 mM solutions of the analytes in 0.01 M TBAP/CH_3CN solution. In all cases, a plot of peak height against the square root of the scan rate was found to be linear, indicating a diffusional process. The dependencies are shown only in the SI since they were linear and could be adequately described by the expressions: Ip = $-2.78\ \nu^{0.5} - 32.0$ with correlation coefficient, $R^2 = 0.95$ for **6** and Ip = $-2.44\ \nu^{0.5} - 17.6$; $R^2 = 0.99$ for **11**, where Ip is given in μA and scan rate ν in mV s^{-1}. However, the peak potentials were shifted slightly to more positive values with increasing scan rate indicating sluggish kinetics. The corresponding square root dependencies are delineated in Figures S3 and S4 in the SI.

Now, attention is turned toward exploring the electroanalytical aspects by studying the differential pulse voltammetry (DPV) of synthetic cannabinoids. All DPV voltammograms reported herein were obtained using the following optimized parameters: 70 mV for the pulse amplitude, 4 mV for increment potential and 50 ms pulse width. Figure 2 shows DPV

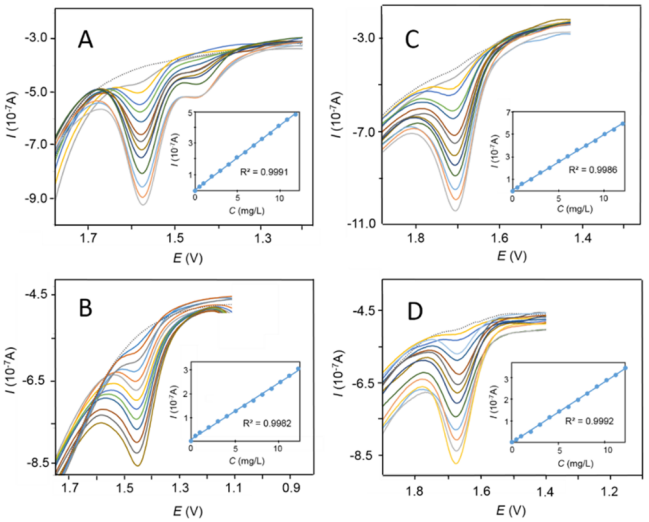

Figure 2. DPV curves for different concentrations of the analyte (concentration range: 0.5–12 mg/L, blank–dotted line) in 0.01 M TBAP/CH_3CN solution at Pt electrode for **A:** FUB-PB-22 (**1**); **B:** AM-2201 (**6**); **C:** 5F-AMB (**10**); **D:** THJ-2201 (**11**). Inserts show calibration curves in the form of oxidation peak height vs analyte concentration. Potentials are recorded vs Ag/Ag^+.

voltammograms of the SCs **1**, **6**, **10**, and **11** over the range 0.5–12 mg/L. The corresponding calibration plots demonstrated linear responses: Ip = 0.404 C + 0.044, for **1**; Ip = 0.252 C + 0.011, for **6**; Ip = 0.492 C + 0.045, for **10**; Ip = 0.312 C + 0.0242, for **11** with correlation coefficients, R^2 equal 0.9991, 0.9982, 0.9986 and 0.9992, respectively. The analyte concentration, C is expressed in mg/L and Ip in 10^{-7}A. The limits of detection (LOD at $3\sigma/S$) were found to correspond to 0.28 mg/L for **1**, 0.37 mg/L for **6**, 0.23 mg/L for **10**; and 0.35 mg/L for **11**. For the rest of the synthetic cannabinoids, the limits of detection are in the range of 0.23–0.84 mg/L (see Table 2). The limits of detection reported herein are sufficient for SC detection in street samples, as drug content in seized materials was reported to be in the range of 0.3–966.6 mg/g;[34] they are comparable, and in some cases lower, than the LODs obtained by electrochemical methods for other classes of psychoactive

Table 2. Comparison of Electrochemical Data for Synthetic Cannabinoid Oxidation in Artificial Saliva and Acetonitrile Solution (ACN)

	SALIVA		ACN	
synthetic cannabinoids	E_p^{DPV} (V)	LOD (μg/L)	E_p^{DPV} (V)	LOD (mg/L)
(**1**) FUB-PB-22	1.26	26	1.57	0.28
(**2**) FDU-PB-22	1.21	28	1.47	0.30
(**3**) 5F-PB-22	1.25	56	1.54	0.84
(**4**) XLR-11	1.15	20	1.33	0.35
(**5**) JWH-018	1.16	50	1.44	0.45
(**6**) AM-2201	1.17	30	1.45	0.37
(**7**)AB-FUBINACA	1.55	42	1.82	0.50
(**8**)AB-CHMINACA	1.46	45	1.75	0.56
(**9**) AB-PINACA	1.46	42	1.75	0.30
(**10**) 5F-AMB	1.41	63	1.71	0.23
(**11**) THJ 2201	1.43	33	1.67	0.35

compounds. Thus, for example, LODs for cathinones were reported to be in the range of 11.60–28.61 mg/L (measured by cyclic voltammetry[26]) and for Δ^9-tetrahydrocannabinol the detection limits were reported to be in the range of 0.3–5.65 mg/L (measured by square-wave voltammetry[35]). Note that this is the first report on electroanalytical detection and quantification of SCs.

SC Detection in Artificial Saliva. Drug detection in oral fluids is known to be of great interest to the police. Roadside drug testing or rapid analysis in detention facilities can rely on saliva samples because the test is noninvasive, and it removes the inconveniences related with handling of urine samples.

Electrochemical detection of SCs in artificial saliva was feasible only with the Bdd electrode due to the high oxygen evolution overpotential of Bdd electrodes.[36] An electrochemical method was developed for the determination of SCs at a Bdd electrode using differential pulse voltammetry (DPV). The sensitivity of the DPV measurements was significantly improved by using a predominantly oxygen-terminated Bdd electrode which could be obtained by an anodic pretreatment. It was shown that the Bdd surface terminal groups (i.e., whether composed predominantly of hydrogen (referred here as H-Bdd) or oxygen (referred to as O-Bdd) terminating groups) may strongly influence the electrochemical activity of this electrode toward redox species. Surface termination of Bdd electrodes may be modulated by appropriate electrochemical pretreatments.[37−39] Here, these pretreatments were carried out galvanostatically, as described in the Experimental Section; the use of an anodic current (inducing oxygen evolution) leads to the prevalence of the O-Bdd form, whereas a cathodic current (with hydrogen evolution) leads to the prevalence of the H-Bdd form. The effect of surface termination on the Bdd electrode DPV response in the oxidation of **1** is shown in Figure 3. Clearly, the O-Bdd electrode exhibits higher electrochemical activity for the oxidation of **1** compared with the H-Bdd electrode. The magnitude of the peak current obtained for **1** oxidation on the O-Bdd electrode is more than twice that of H-Bdd. Additionally, another positive effect of the anodic pretreatment was demonstrated; the anodic peak potential obtained with the O-Bdd electrode (~1.26 V vs Ag/AgCl) is displaced to less-positive values by approximately 30 mV, indicating lower overpotential and favorable electrocatalysis by O-Bdd. All determinations reported herein were carried out using an anodically pretreated Bdd electrode.

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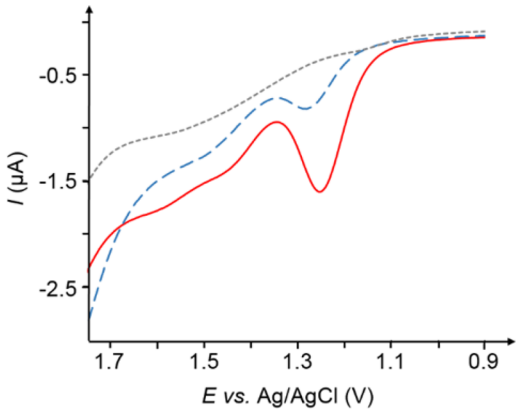

Figure 3. DPV curves for 1 mg/L of **1** in artificial saliva using anodically (solid line) and cathodically (dashed line) pretreated Bdd electrode. The dotted curve represents the blank. Cathodic pretreatment: 25 mA cm^{-2} for 60 s; anodic pretreatment, first 25 mA cm^{-2} for 60 s and then −25 mA cm^{-2} for 180 s. DPV parameters: pulse amplitude, 50 mV; sample width, 17 ms; pulse width, 50 ms; pulse period, 500 ms; increment potential 4 mV; quiet time, 2 s.

However, a cathodic pretreatment was always carried out prior to the anodic treatments in order to clean the electrode surface.[39] Figure 4 shows DPV curves of an anodically

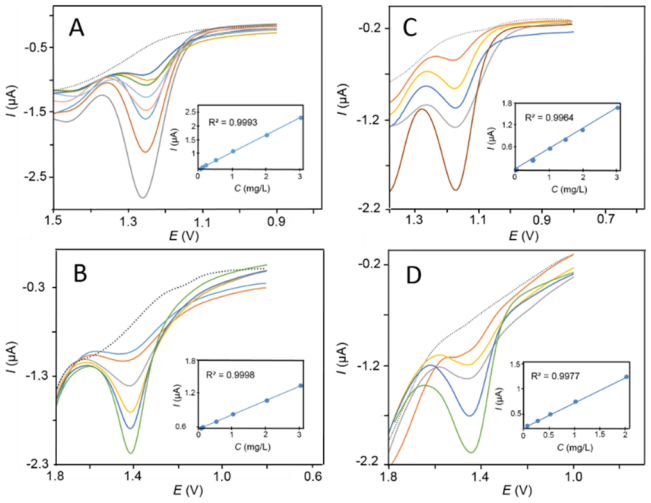

Figure 4. DPV curves on anodically pretreated Bdd electrode for different concentrations of analyte in artificial saliva − **A:** FUB-PB-22, concentrations: 0.05, 0.1, 0.2, 0.5, 0.8, 1, 2, 3 mg/L; **B:** 5F-AMB, concentrations: 0.05, 0.1, 0.5, 1, 2, 3 mg/L; **C:** AM-2201, concentrations: 0.5, 1, 1.5, 2, 3 mg/L; **D:** THJ-2201, concentrations: 0.05, 0.25, 0.5, 1, 2 mg/L; blank − dotted line. Insets show calibration curves in the form of oxidation peak height vs analyte concentration. Potentials are recorded vs Ag/AgCl.

pretreated Bdd electrode for different concentrations of the indole SCs **1**, **6** and the indazoles **10** and **11** in artificial saliva. Linear analytical curves were obtained: Ip = 0.631 *C* + 0.459 for **1**; Ip = 0.557*C* + 0.013, for **6**; Ip = 0.263*C* + 0.571, for **10**; Ip = 0.503*C* + 0.266 for **11**; where Ip is given in μA and the analyte concentration, *C*, is expressed in mg/L. The detection limits (at S = 3σ) were found to be 0.26 μg/L for **1**; 0.30 μg/L for **6**; 0.63 μg/L for **10**; and 0.33 μg/L for **11**. Table 2 shows a comparison of the first wave oxidation potentials and the LODs obtained using a Bdd electrode in saliva with the results obtained with a Pt electrode in acetonitrile for the 11 studied SCs. It can be seen that the Bdd electrode in saliva has a much higher sensitivity and provides lower detection limits, and electrooxidation occurs at less positive potentials. The detection limits are usually in the nanomolar range (see Table 2).

As far as it could be verified, this is the first electroanalytical method proposed for determination of synthetic cannabinoids in saliva.

Interferences. Some psychoactive substances like cathinones are known to be adulterated ("cut") with other mask compounds, like caffeine, benzocaine, and so on;[40,41] however, SCs are usually found in smoking mixtures as a single ingredient or as a mixture of several (usually two) synthetic cannabinoids.[34]

In the extracts of the 12 studied street samples, no other additives except for SCs were detected by GC-MS/LC-MS analysis (see representative chromatograms in Figure S5 in the SI). Figure 5A shows the electrochemical response of an extract

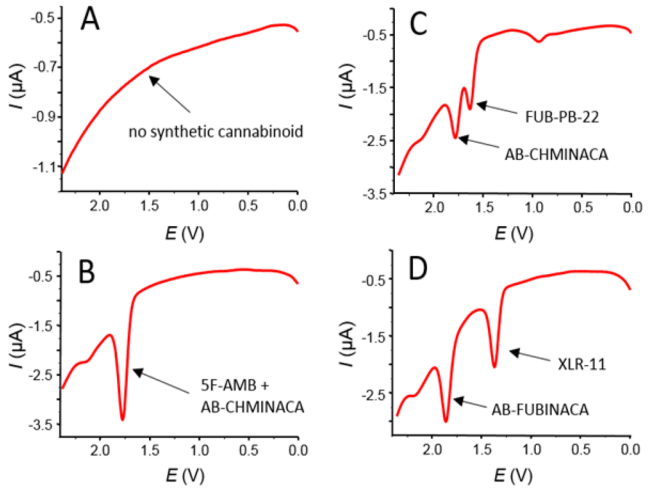

Figure 5. DPV response of herbal plant extract that did not contain any SCs (**A**); herbal plant that contained two indazole-based SCs (**B**); one indole-, one indazole-based SC (**C** and **D**). Potentials are recorded vs Ag/Ag^{+}.

from an unspiked herbal material (smoking mixture that did not contain any synthetic cannabinoid chemicals). As can be seen, there are no oxidation peaks in this voltammogram, and the same holds for all 12 field samples that were examined in this study, wherein we obtained either a single or two clean peaks, or there were no oxidation peaks in the relevant potential window.

As was shown in Table 1, the different indole-based SCs have peak oxidation potentials in a rather narrow range of ∼1.33−1.57 V (by DPV measurements), and the same holds for the indazole-based SCs whose peak oxidation potential is always within the range ∼1.67−1.82 V. Figure 5B demonstrates the electrochemical response of a mixture of two indazole-based SCs with similar oxidation potentials: 5F-AMB (∼1.71 V) and AB-CHMINACA (∼1.75 V). The individual peaks are not resolved, and they merge together giving a single oxidation peak at 1.73 V. On the contrary, in Figure 5C and Figure 5D, the separation of peaks could be clearly seen between indole- and indazole-based SCs. Consequently, whereas the distinction between indole- and indazole-based SCs can be easily attained by electrochemical studies, it is impossible to differentiate between SCs within the same group on the basis of electrochemistry alone. Thus, the proposed electrochemical

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Table 3. Direct Comparison between Quantification Data Collected from LC-ESI-QTOF-MS, GC- SMB-EI-QQQ-MS and by the Electrochemical Protocol for the Analytical Quantification of Synthetic Cannabinoids in Seized Street Samples

sample no.	identified SC	LC-MS % wt	GC-MS % wt	electrochemical % wt
1	THJ-2201	1.57 (±0.045)	1.60 (±0.072)	1.70 (±0.025)
2	5F-AMB	0.91 (±0.028)	1.00 (±0.039)	0.90 (±0.014)
3	FUB-PB-22/AB-CHMINACA	0.10 (±0.0031)/0.37 (±0.011)	0.12 (±0.0046)/0.40 (±0.015)	0.09 (±0.0011)/0.45 (±0.0054)
4	AB-CHMINACA	2.04 (±0.067)	1.78 (±0.083)	2.13 (±0.023)
5	FUB-PB-22	0.37 (±0.011)	0.40 (±0.016)	0.44 (±0.0065)
6	5F-AMB	2.45 (±0.067)	2.51 (±0.093)	2.19 (±0.030)
7	FUB-PB-22	4.03 (±0.11)	4.23 (±0.15)	4.37 (±0.079)
8	5F-AMB/AB-CHMINACA	0.52 (±0.016)/0.40 (±0.013)	0.58 (±0.024)/0.51 (±0.021)	0.80 (±0.017)
9	5F-AMB	2.33 (±0.066)	2.06 (±0.087)	2.10 (±0.038)

protocol cannot be applied for distinction between different synthetic cannabinoids. However, it is very useful for screening purposes prior to conclusive mass spectrometry or chromatographic analysis. Note that at the screening stage, there is usually no need to identify the exact indole or indazole drug, as their presence is sufficient for confiscation of the smoking mixture or other suspected material until conclusive analytical studies are carried out in the laboratory.

Table 1 presents the electrochemical response factor expressed as the peak height divided by the concentration for each of the analytes. It can be seen that the average response factor for the indole group of studied SCs is 10.3 ± 1.25 μA/mM and for all the indazole SCs it is 10.4 ± 1.46 μA/mM. Thus, even without conclusive identification of the individual SCs, it is possible to determine their concentration with satisfactory accuracy based on the average response factor, because the relative standard deviation of the response factors within a group is less than 14.0%. Because conclusive identification is pertinent for forensic applications, MS identification of the peak is mandatory, and this would allow somewhat higher analytical accuracy even without quantitative MS analysis. In fact, direct infusion of the extract to the ESI-MS would be sufficient to obtain a conclusive analytical determination.

Field Samples. *Analysis of Seized Street Samples by LC-MS and GC-MS Techniques.* LC-MS and GC-MS analyses were used for confirmative identification of SCs in street samples, as these methods are considered the "gold-standard" for SCs identification and determination.[17,42,43] Although the high-resolution LC-ESI-QTOF-MS technique allows accurate determination of the molecular weight of the target compounds, electro-ionization mass spectrometry (EI-MS) provides fragmentation fingerprinting, which by comparison to spectral libraries can provide conclusive identification. The GC-SMB-QQQ-MS, the technique used in this study, incorporates cold electro-ionization mass spectrometry by supermolecular beam, SMB,[44] which provides enhanced molecular mass peak,[45] and triple quadrupole analyzer configuration, which allows multiple reaction monitoring. Both features, however, are not pertinent for confirmatory analysis. Both LC-MS and GC-MS techniques provide identification and quantification of analyzed SCs. The analytical parameters (retention times, ions used for quantification, and LODs) of GC-MS and LC-MS techniques for the tested SCs (**1**−**11**) are summarized in Table S3 in the SI.

Twelve batches of herbal mixtures that were suspected to contain synthetic cannabinoids were confiscated from the local market by the Israeli Police and were kindly provided to us. The obtained herbs acetonitrile extracts were subjected to comparative LC-MS and GC-MS analysis. Preliminary LC-MS and GC-MS analysis indicated that three batches did not contain any SCs, whereas nine batches did contain SC drugs. These nine batches were labeled: Samples 1−9; two of these samples (Sample 3 and Sample 8) contained two SCs each, while all others contained only one SC (see Table 3). It is important to note that all nine tested samples contained only synthetic cannabinoids, without any adulterants (e.g., caffeine or benzocaine), as indicated by the chromatograms obtained for these samples. Validated LC-MS and GC-MS methods were used to quantify SCs in the seized smoking mixtures. Standard solutions demonstrated calibration curves with linear responses (R^2 = 0.999) over a 5−25 mg/L range. The limits of detection for SCs were determined as being in the range of 0.009−1.062 mg/L for GC-MS and 0.001−0.296 mg/L for LC-MS. The concentration of SCs in the extracts was calculated from the calibration curve, and drug content in the herbal mixtures was found to be in the range 1.0−42.3 mg g^{-1}, or 0.10−4.23% wt (see Table 3).

Application of the Electroanalytical Protocol. A weighed portion of supporting electrolyte (TBAP) corresponding to a concentration of 0.01 M in acetonitrile was added to the extracts of 12 street samples, after which the electrochemical protocol was applied. The three samples that according to GC-MS and LC-MS did not contain any SCs did not show any oxidation peaks in the relevant potential window. Sample 3 showed two oxidation peaks corresponding to two synthetic cannabinoids − FUB-PB-22 and AB-CHMINACA. Sample 8 showed one oxidation peak, corresponding for both 5F-AMB and AB-CHMINACA, SC concentration was determined as if the extract contained only one compound AB-CHMINACA. The rest of the tested samples showed one oxidation peak corresponding to a single SC. For SCs identified by GC-MS and LC-MS techniques in street samples (FUB-PB-22, 5F-AMB, THJ-2201, AB-CHMINACA), calibration standards were prepared in 0.01 M TBAP/CH_3CN solution. Corresponding analytical curves were obtained using the oxidation peak currents observed at 1.57 V (FUB-PB-22), 1.71 V (5F-AMB), 1.67 V (THJ-2201), and 1.82 V (AB-CHMINACA) (vs Ag/Ag^+), demonstrating a linear response (R^2 = 0.999) over the concentration range of 5−20 mg/L. The analyte concentration in the extract was determined from the calibration curves, and the drug content in the herbal mixture was derived. Table 3 outlines a comparison between quantification analysis via LC-MS, GC-MS, and the electrochemical analysis. Good agreement between the analytical approaches is evident. The average relative difference between data obtained by LC-MS, GC-MS, and electrochemical method is 12%, with the maximum relative difference being only 18%.

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CONCLUSIONS

In the present work, electrochemical sensing of synthetic cannabinoids was explored for the first time. It was shown that direct electrochemical oxidation of SCs gives meaningful voltammetric signatures both in nonaqueous and aqueous buffer solutions, thus allowing SC detection in seized materials and biological samples. The proposed electroanalytical protocol was applied for the determination of SCs in a dozen seized street samples and was independently verified by LC-MS and GC-MS techniques, demonstrating excellent agreement between the different tools. The various indole and indazole-based SCs exhibit response factors of the same magnitude, which allows determination of the total indole and total indazole SC family by a single analysis. However, this benefit comes at a price: electrochemistry cannot identify unequivocally the SC involved, and the molecular identification power still resides mainly in MS techniques. This method, however, is beneficial for screening purposes and thus may be applied in-the-field or in the laboratory screening for preliminary quantification of common synthetic cannabinoids found in real street or biological samples prior to conclusive mass spectrometry or chromatographic analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b00368.

Additional information as noted in text (PDF)

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Notes

The authors declare no competing financial interest.

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