Electrochemical Strategies for the Screening of Synthetic Cannabinoid BB-22 (QUCHIC) within a Toxicological Specimen

Anaam Ameen, Kelly Brown,* and Lynn Dennany\footnote{E-mail: lynn.dennany@strath.ac.uk}

WestCHEM Department of Pure & Applied Chemistry
University of Strathclyde, Technology & Innovation Centre, Glasgow G1 1RD, United Kingdom

*Electrochemical Society Member.

Abstract

Synthetic Cannabinoids (SC) are amongst the most prevalent class of novel psychoactive substances (NPS) which have emerged. The increase in hospitalisation and fatalities as a result of the prevalence of these SC and their abuse, highlights the necessity for the rapid and reliable screening for the substance within toxicological samples. Being able to rapidly screen for these substances would aim to improve overdose triage and ultimately improve treatment administration. Not only this but
it would have further advantages for in-field applications such as the identification of drivers under the influence. To this avail we investigate the ability of a simple electrochemical screening strategy for the detection of SC, BB-22 and its primary metabolite BB-22-3-Carboxyindole. Assessment of the feasibility of the strategy for implementation into toxicological sample analysis was performed through detection not only under ideal electrolyte conditions (down to 5 µM for BB-22 and 2 µM for its metabolite) but also within the complex biological matrix of human pooled serum (between 200 and 700 µM), a relevant matrix it would likely encounter if employed within this field.
Introduction

Synthetic Cannabinoids (SC) are a distinct and popular class of novel psychoactive substances (NPS) which possess an affinity toward the CB1 and CB2 cannabinoid receptors.\textsuperscript{1-5} Despite their familiar name SC do not structurally or chemically resemble the active component $\Delta^9$-THC found within the \textit{Cannabis sativa} plant.\textsuperscript{7} Instead these species were categorised due to their similar effects upon the bodies cannabinoid receptors.\textsuperscript{2-5} SC, like many NPS, were initially developed from failed pharmaceutical drug campaigns, with expired patents providing clandestine chemists with the necessary information to synthesis these highly potent synthetic designer drugs.\textsuperscript{6, 7} The popularity of NPS, can mainly be attributed to their initially wide and unrestricted availability. However, this coincided with an increase in the number of associated hospitalisation and deaths resultant from the unknown or unexpected toxicity of these compounds.\textsuperscript{4, 8-10} Synthesised within a lab setting there is no knowledge concerning the pharmacology and potency of these compounds. Yet with users often ignorant to their increased risk or falsely assume their safety given their legality, high street presence and colloquial terminology of “legal highs” \textsuperscript{11, 12} SC terminology gifts them with a false perception of safety, with many users associating them with the \textit{Cannabis sativa} plant assuming similar effects and toxicity to that of $\Delta^9$-THC. Unfortunately, this is not the case. Unlike $\Delta^9$-THC, SC behave as full agonists toward the CB1 and CB2 receptors whereas $\Delta^9$-THC behaves as a partial agonist.\textsuperscript{2, 3, 13} This receptor affinity increases their potency and hence psychoactive effects upon the user.\textsuperscript{13, 14}
SC were first reported on the global drug market in 2008\textsuperscript{15}, and quickly became popular amongst users. The next decade saw their rapid growth, with a huge variety widely available, through minor structural modifications. Frequently impregnated upon herbal material, SC were sold under the guise of decorative potpourri or room incense to evade detection and subsequent seizure.\textsuperscript{16, 17} The increase in use and resultant deaths seen a world wide increase in legislative controls. Yet their popularity remains.\textsuperscript{18-21}

The increase in hospitalisation following consumption of these substances have made it crucial that SC can be rapidly identified. Of course gold standard identification lies in the realms of the hyphenated chromatographic mass spectrometry systems, and remain the primary technique recommended by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).\textsuperscript{22} Recent advancements in these chromatographic techniques have addressed some of the concerns regarding the employment of chromatographic techniques for screening protocols.\textsuperscript{23-25} Yet one significant hurdle remains, their huge cost and laboratory restricted specialised equipment. Their complexity also renders them unsuited for use by non-specialised personnel such as medical staff or hospital technicians who may have some training but not specialised in these analytical techniques.\textsuperscript{26, 27}

One technique shows promise for the employment as screening methodologies and has proven such in the screening of biological matrices for illicit substances is electrochemistry.\textsuperscript{28-32} Electrochemistry satisfies a number of criteria implemented for the employment of sensors within the biomedical detection field. Instrumentation
used for electrochemistry is now portable, electrodes disposable and operationally simple, what’s more they are significantly lower in cost than spectroscopic alternatives such as Raman. These advantageous characteristics are only highlighted by the large number of works which have employed electrochemistry for the detection of a range of NPS. Interestingly despite a wider acceptance of electrochemical techniques as viable alternatives for NPS screening, to date SC have received little attention in this regard. Currently only one prior work on their electrochemical detection is available. This limited investigation of the electrochemical techniques for the analysis of SC is surprising given their structural functionalities indicating their high suitability for such methods. To this extent we investigate not only the ability to detect SC via a simple electrochemical method utilising differential pulse voltammetry (DPV), but also the ability to directly detect these species within the toxicological samples it would likely encounter. The ability to rapidly screen for these SC in toxicological samples would aid in a number of areas including triaging potential overdose patients or determining substance abuse of a user behind the wheel. Through interrogation of the indole based SC, BB-22 and its primary metabolite BB-22-3-Carboxyindole under both ideal conditions and within human pooled serum assessment of the technique was made. Performance of this analysis will ultimately lay the initial foundation for the feasibility of electrochemical techniques for the screening of SC within biological matrices through simulated samples which would be encountered during screening for the use of these substances.
Experimental

Reagents and materials

Lithium perchlorate (LiClO₄), and human pooled serum were purchased from Sigma Aldrich. HPLC grade acetonitrile (ACN) was purchased from VWR chemicals. 1-(cyclohexylmethyl)-1H-indole-3-carboxylic acid 8-quinoliny1 ester (BB-22) and its corresponding metabolite, BB-22-3-Carboxyindole, were all purchased from LGC Ltd. All chemicals were used as received. All organic phase solutions were prepared in ACN, with all aqueous solutions prepared in Milli-Q (18 mΩ cm⁻¹).

Instrumentation

All electrochemical measurements were performed utilising the PalmSens EmStat Blue potentiostat, controlled by the PSTrace software. Lithium perchlorate was used as the supporting electrolyte within organic phase measurements. The traditional three-electrode cell with a 3 mm diameter glassy carbon (GC) working electrode, a silver wire reference electrode and a platinum counter electrode were utilising throughout. Working electrodes were prepared prior to measurement via polishing with a alumina slurry (1.0 µm - 0.3 µm) felt pad and rinsed with deionised water and ACN prior to each measurement.
Results and Discussion

_Electrochemical Behaviour of Parent Drug_

With the electrochemical behaviour of synthetic cannabinoids (SC) having rarely been studied it was essential to establish their own electrochemical behaviour prior to that of their metabolites. Indeed, the identification of the consumption of illicit substances can often rely upon the identification of both the parent compound and their corresponding metabolites. In the case of BB-22, its high potency results in minimal concentrations of the parent compound within a user's circulating blood with concentrations typically reported between 94 to 97 ng/L from 5 hours after consumption, with even lower levels down to 60 ng/L observed within serum, identified via hyphenate chromatographic techniques. As such, should an electrochemical method be considered for the reliable detection of SC toxicity within patient samples, it must display the same sensitivity as reported by the gold standard of the hyphenated mass spectrometry techniques. We have previously reported the electrochemical behaviour of BB-22, as a result of its indole and quinoline functionalities giving rise to its dual peak oxidation processes (see Figure 1). The earlier anodic peak observed at ~ 1.36 V (vs Ag) can be attributed to the oxidation of the C2 position of the heterocyclic pyrrole moiety contained within the indole group. While the later anodic peak at ~ 1.47 V (vs Ag) is the result of electron abstraction from the nitrogen of the heterocyclic ring of the quinoline functionality.
To ensure the proposed methodology met the reliability required for implementation into a clinical environment for patient testing, a number of criteria are typically required. This includes a reproducibility and repeatability below the specified limit of 5%. Here a reproducibility of 0.56% and a repeatability across days of 0.74% was achieved. Both these values are well below the typical analytical limit of 5% and as such are ideal for implementation within the clinical environment. These significantly low RSD values indicate a reliability for the detection of BB-22 in-line or indeed improved upon with those described for LC-MS methodologies, despite the significantly simplified instrumentation required for electrochemical methods.

With confidence in the reliability of the designed methodology, it was then necessary to assess the detection ranges achievable. With the reported ranges for the detection of BB-22 within patient samples between 0.16 to 0.24 nM, and LOD for LC-MS methods in the pM ranges it becomes necessary to established where electrochemical methodologies can operate. Assessment of the detection ranges achievable via our proposed electrochemical methodology was performed with the linear detection range presented within Figure 2. Here we observed a linear range across 10 to 100 µM, with a R² coefficient of 0.994. It was noted upon decreasing the concentration of BB-22 present, the initial peak related to the indole oxidation process was lost (see Figure S1). This is not surprising given the intensity difference noted between the two oxidation peaks observed in Figure 1. As such, focus was placed upon the monitoring of the second oxidation peak, concerned with the quinoline oxidation, with the potential range investigated shortened to encompass
this. The detection limit for BB-22 was established at 5 µM (see Figure S2), with no peak differentiation from the baseline achieved below this concentration. Although this methodology provided initial promise, achieving high repeatability and reproducibility, the detection ranges achievable were significantly higher than those reported within patient fatalities.

**Electrochemical Behaviour of BB-22 Metabolite**

The high potency of BB-22, ultimately results in only minimal concentrations present within a users circulating blood. BB-22 is rapidly metabolised following ingestion, with up to 10 metabolites produced as a result of the ester hydrolysis leading to quinolinyl loss. The primary metabolite reported is that of BB-22-3-Carboxyindole (Figure 3 inset), with hydrolysis of the linker, accounting for 90% of the metabolites formed. All further metabolites are derived from BB-22-3-Carboxyindole via hydroxylation and/or glucuroniation. This pattern is also witnessed for the metabolism of other structurally similar SC containing the carboxylate linker, including PB-22, 5F-PB-22 and FUB-PB-22, whose ester hydrolysis resulted in the loss of a side chain. This similarity in metabolism pathway has seen the identification of the same metabolites across different SC species. As such it would be more beneficial to combine parent drug detection with at least one metabolite for species identification. However, with urine analysis, often the preliminary matrix utilised for drug detection, comes as a limitation for SC detection, with little to no parent drug found within urinary analysis. The similarity of the metabolites produced across a
range of SC, does on one hand limit the identification of individual SC but does provide the ability to provide a suitable screening method for a range of different SC based upon an identical metabolite production.

To this end we assessed the ability to utilise our electrochemical methodology for metabolite detection. The electrochemical behaviour of the metabolite was assessed under the same conditions as the parent drug. This revealed a single oxidation peak at \( \sim 1.36 \) V vs Ag, see Figure 3. This singular peak contrasts the dual peak behaviour observed for the parent drug. This is not entirely unexpected given the metabolite arises from ester hydrolysis and the subsequent removal of the quinoline functional group, which is attributed to the second anodic peak seen at \( \sim 1.47 \) V vs Ag in the parent drug. As such, the indole functionality responsible for the earlier peak in parent compound is maintained and hence is seen here. The mechanism responsible for this peak is shown within scheme 1, and is the result of the oxidation of the C2 position of the pyrrole group within the indole moiety, as is the case in many structurally similar SC.

The detection range was once again assessed and revealed a similar trend to the parent compound, with a linear range from 10 to 80 µM with an \( R^2 \) coefficient of 0.997, see Figure 4. The detection limit was assessed and found to be lower than that of the parent compound at a detection limit of 2 µM observed (refer to Figure S3).
Although again, this methodology demonstrated initial promise, the reported levels for the detection of BB-22-3-Carboxyindole lie within the nM regions of 3 to 147 nM in serum and 0.5 to 83 nM in urine.\textsuperscript{45} These are again significantly below the detection limit demonstration within this contribution. However, despite this it is worth note that the detection of SC via electrochemical methods is to date limited and as such this demonstration of a simple detection method is indeed a significant step toward the required screening methodologies for these substances. It is also important to highlight that although the concentrations reported here for the detection of BB-22 and its primary metabolite are outwith the ranges observed within recorded patient samples, but are within the ranges encountered within typical street samples.

Detection with Biological Matrices

To determine the viability of the proposed methodology for the screening of the consumption of SC, it is essential to assess the feasibility of the electrochemical method within biological matrices. To do this, assessment was made via the spiking of BB-22-3-Carboxyindole into pooled human serum. In order enhance the portability and ease of use all measurements where made directly without any purification and separation strategies. Of course by negating these processes we open up the possibility for increased interferents from the biological matrix components themselves. Direct electrochemical analysis of biological matrices has revealed a number of interfering species present. Most notably the naturally present amino acids, uric acid, lactic acid and creatinine, all of which have been reported to
undergo oxidation directly at a range of different electrode surfaces.\textsuperscript{47-55} This has meant typically a threshold must be established above which signals can be attributed to the presence of additional analyte species and not just those intrinsic to the biological matrix.\textsuperscript{29, 31, 56} What’s more, in addition to these naturally present species, additional interferents are likely to arise in the form of other illicit substances, SC are rarely found as a single species but typically contain a range of different SC structures. However, with all structures representing a controlled substance the inability to distinguish between multiple SC or indeed interference from those metabolites other than that of BB-22, will not bare a significant detriment upon this screening technique. This is furthered when consideration is given to the identical metabolites produced by a range of different SC species. Further problems arise in the shape of commonly encountered species including prescription medications, caffeine and nicotine. The consequence of the presence of these species within a sample must indeed be thoroughly assessed going forward to understand the potential impact these non-illicit substances may have upon the proposed electrochemical screening methodology. Where it becomes essential for both criminal and healthcare environments that false positives are not drawn.

Unsurprisingly analysis of the blank serum matrix revealed a small intrinsic peak of the matrix itself around the same potential region of the metabolite at $\sim 1.1\, V$ (vs Ag). As such it is essential to establish this as a threshold value below which any peaks observed are not considered significant. The current observed for the serum matrix was observed at 0.04 $\mu$M (see Figure 5). In order to establish a reliable
threshold value it would of course require the analysis of a range of different serum samples, from a wide ranging population of volunteers. Analysis of a range of concentrations of the BB-22 metabolite via a standard addition method within the serum matrix revealed a detection limit of 200 µM, below which we could not distinguish between the intrinsic serum matrix and that containing the metabolite. Increasing concentrations from 200 to 700 µM of the metabolite were added to the matrix and revealed a linear relationship with current intensity, see Figure 6. This result can be considered a promising outcome, with the ability to directly detect the metabolite within serum without the requirement for sample purification or separation, via a simple standard electrochemical cell with the widely available glassy carbon electrode. However, it is obvious that the detection ranges we observed here are significantly greater than those attributed to fatalities or hospitalisations associated with BB-22 (with reported concentrations of 3 to 147 nM of the metabolite found within patient fatalities, approximately 1000 lower than the µM ranges achieved here). As such, this does mean that in this current format our methodology is not ready for translation into the toxicological field. However, we believe it does present a significant step forward in the potential employment of electrochemistry for the toxicological screening of SC. Indeed, further sensitivity much be coined prior to employment but this is not currently consider an insurmountable challenge.
Conclusion

The huge variety and initial availability of SC ultimately cumulated in the increase in hospitalisation and fatalities of users. The falsely perceived safety and incorrect assumption of their similarity to the traditional illicit substance, cannabis, is often attributed to these increased overdose incidents. Unlike their name sake, SC possess a higher potency and toxicity, as a consequence of their full agonist actions upon the cannabinoid receptors. With an increase in the prevalence of SC and the subsequent hospitalisations it is becoming apparent that methods for the rapid toxicological screening of these substances is needed to triage users or to determine the substance a user may have consumed prior to driving. To this end, we report here a reliable and simplistic electrochemical method for the detection of SC BB-22 and its primary metabolite BB-22-3-Carboxyindole. Utilising the basic electrochemical cell set up with the widely available glassy carbon electrodes, detection of BB-22 and its metabolite was achieved. BB-22 was detected down to concentrations of 5 µM, whilst this was slightly lower for its metabolite at 2 µM. Of more note however was the ability to detect the metabolite species directly within human pooled serum. This was achieved without the need for separation or purification strategies, commonly necessary for the analysis of these sample types. This is a significant step toward the develop of toxicological screening strategies for SC. However, this it is not without its limitations. Although offering a reliable and simplistic methodology suitable for translation outwith a laboratory environment and suited for use by non-specialised personnel, in its current format the methodology is only suited toward the detection of high concentrations. Typically, the concentration ranges found within these
toxicological samples lie within the nM region, however our current methodology operates in the µM region under ideal conditions and mM region within the complex matrix. As such, moving forward it is necessary to build upon the current strategy through the employment of alternative surface modification strategies\textsuperscript{57, 58} or tailored recognition complexes\textsuperscript{59, 60}, previously shown to improve electrode sensitivity, in order to induce a higher sensitivity than currently witnessed upon the bare electrode surface used here.
Acknowledgments

The authors would like to thank the State of Kuwait for financially supporting this research. All data underpinning this publication are openly available from the University of Strathclyde KnowledgeBase:

https://doi.org/10.15129/352f9a13-69bb-4531-a792-1776a30e1973
References

1. J. Huffman and L. Padgett, Current medicinal chemistry, 2005, 12, 1395-1411.
33. L. R. Cumba, A. V. Kollipooulos, J. P. Smith, P. D. Thompson, P. R. Evans, O. B. Sutcliffe, D. R. do Carmo and C. E. Banks, Analyst, 2015, 140, 5536-5545.
**Figure 1**: DPV of 1 mM BB-22 (purple) in LiClO$_4$ as the supporting electrolyte (yellow) upon a GC working electrode across a potential region of $1.2 \leq E \geq 1.65$ V vs Ag at a scan rate of 100 mV s$^{-1}$. 

*Figures*
Figure 2: DPV of varying concentrations of BB-22 from 10 to 100 µM in LiClO₄ as the supporting electrolyte upon a GC working electrode across a potential region of 1.3 ≤ E ≥ 1.65 V vs Ag at a scan rate of 100 mV s⁻¹. Inset shows the linear relationship of concentration with average peak current across triplicate measurements. Error bars represent ±1SD.
**Figure 3**: DPV of 1 mM BB-22-3-carboxylindole (yellow) in LiClO₄ as the supporting electrolyte (purple) upon a GC working electrode across a potential region of 1.2 ≤ E ≥

**Figure 4**: DPV of varying concentrations of BB-22-3-carboxylindole from 10 to 80 µM in LiClO₄ as the supporting electrolyte upon a GC working electrode across a potential region of 1.3 ≤ E ≥ 1.65 V vs Ag at a scan rate of 100 mV s⁻¹. Inset shows the linear relationship of concentration with average peak current across triplicate measurements.

Error bars represent ±1SD.
Figure 5: DPV analysis of serum (blue) and 0.2 mM BB-22-3-Carboxyindole (pink) in LiClO₄ as the supporting electrolyte upon a GC working electrode across a potential region of 1.0 ≤ E ≥ 1.2 V vs Ag at a scan rate of 100 mV s⁻¹.

Figure 6: DPV of varying concentrations of BB-22-3-Carboxyindole from 0.3 to 0.7 µM in human serum upon a GC working electrode across a potential region of 0.9 ≤ E ≥ 1.2 V vs Ag at a scan rate of 100 mV s⁻¹. Inset shows the linear relationship of concentration with average peak current across triplicate measurements. Error bars represent +/- 1SD.
Scheme 1: Proposed mechanism for the electro-oxidation of BB-22-3-Carboxyindole at C2 position of the pyrrole group of the indole moiety