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Voltammetric determination of Δ^9-THC in glassy carbon electrode: An important contribution to forensic electroanalysis

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ABSTRACT

A new voltammetric method for the determination of Δ^9-tetrahydrocannabinol (Δ^9-THC) is described. The voltammetric experiments were accomplished in N-N dimethylformamide/water (9:1, v/v), using tetrabutylammonium tetrafluoroborate (TBATFB) 0.1 mol/L as supporting electrolyte and a glassy carbon disk electrode as the working electrode. The anodic peak current was observed at 0.0 V (vs. Ag/AgCl) after a 30 s pre-concentration step under an applied potential of −1.2 V (vs. Ag/AgCl). A linear dependence of Δ^9-THC detection was obtained in the concentration range 2.4–11.3 ng/mL, with a linear correlation coefficient of 0.999 and a detection limit of 0.34 ng/mL. The voltammetric method was used to measure the content of Δ^9-THC in samples (hemp and hashish) confiscated by the police. The elimination of chemical interferences from the samples was promptly achieved through prior purification using the TLC technique, by employing methanol/water (4:1, v/v) as the mobile phase. The results showed excellent correlation with results attained by HPLC.

1. Introduction

1.1. Illicit drugs

Illicit drugs are chemical substances that affect the central nervous system. They cause changes in behavior and often culminate in addiction. The drugs can be divided according to their action, namely depressants (barbiturates, alcohol, and opiates), stimulants (nicotine, cocaine, crack cocaine, and amphetamines), hallucinogens (lysergic acid, LSD), impairing drugs (hemp derivatives), and mixed-acting drugs (ecstasy) [1–9].

1.2. Forensic analysis of hemp

Analyses of cannabinoids and other hemp constituents have been performed by chromatographic techniques such as GC–MS, HPLC, and LC–MS [11]. Therefore, it has been possible to analyze several samples of plants from different cultures grown in tropical and temperate regions of the planet, which has aided establishment of their chemical signature by testing procedures based on the determination of inorganic constituents such as copper, zinc, cadmium, manganese, iron, and yttrium. Analyses of the various samples have furnished information about the characteristics of the soil where *Cannabis sativa* was cultivated [12].

In forensic laboratories, the presumptive tests utilized for the detection of hemp can be accomplished by chromatographic, spectrometric or colorimetric techniques. The colorimetric tests employed for the identification of Δ^9-THC such as the assay involving the reagent Fast Blue B Salt $C_{14}H_{12}Cl_2N_4O_2$. $ZnCl_2$ [12] consist of a coupling reaction that generates a deep red or purple chromophore with the active ingredients of the substance of forensic interest. Hemp analysis often includes the TLC technique and the Fast Blue B Salt as the reagent. The latter chemical is useful because it produces a different color upon reaction with Δ^9-THC and the two other target cannabinoids in the plant extract. The double salt Fast Blue BB Salt ($C_{17}H_{18}N_3O_3Cl{\cdot}1/2ZnCl_2$) can also be employed. This protocol is widely utilized by police forces in various countries.

When chemical analyses are applied for the identification of drugs in forensic science, there is a compelling need for

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confirmation of the nature of the analyzed material. In this context, two or more different methods are commonly utilized. The Department of Justice of the United States follows the classification of several analytical methods provided by the Scientific Working Group for the Analysis of Seized Drugs – SWGDRUG [13], which considers three categories, namely A, B, and C, depending on the specificity of the method. According to these standards [13], the forensic chemical analysis of a given analyte is only deemed irrefutable when two chemical analyses are conducted, namely a class A and a complementary one (A, B, or C). Alternatively, three chemical analyses can be carried out, more specifically two class B and a complementary one (B or C).

1.3. Electroanalytical methodologies

Electroanalytical techniques have often been used for the analysis of a wide range of substances of industrial, pharmacological, and biomedical interest [14–16,23]. Voltammetry, for instance, consists in the application of a linear ramp of electrical potential between two electrodes (working and auxiliary) immersed in a liquid solution containing an analyte, with subsequent measurement of the produced electric current [20]. This current signal is of the transient type, and the intensity of the peak current can be directly related to the analyte concentration. In this context, it is possible to apply the potential scan for the oxidation (anodic scan) or reduction (cathodic scan) of the analyte. When both scans (anodic and cathodic) are reported in the same graph, the voltammetric modality is called cyclic voltammetry (CV). However, when the monitoring of a specific scan (anodic or cathodic, according to the properties of the analyte) is desired, the modality is known as linear sweep voltammetry (LSV).

Besides furnishing qualitative and quantitative information, electrochemical methods can provide important information about surface reaction mechanisms, which can aid understanding of the pathogenic and physiological mechanisms of several drugs [17–20], including cocaine [18,19,21] and metabolites such as benzoylecgonine [17]. Furthermore, these methods are easy to automate and can be applied in field research and in vivo analysis, as in the case of microelectrodes [17,19,22,23]. In terms of practicality, it is worth mentioning that voltammetric techniques can be carried out on micropotentiostats that cost US$ 1000.00, with a completely disposable three-electrode system that cost US$ 1.00, for example. Additionally, almost all the electrochemical techniques can run in portable equipments that weigh less than 1 kg and can be directly used in crime scenes or other kinds of field.

Although there are several studies on the analysis of substances of forensic interest by electrochemical techniques, there is a lack of works on the direct analysis of Δ^9-THC by conventional electrodes. Actually, there is only one work reporting on the electrochemical analysis of Δ^9-THC [10], which employs an indirect determination of this analyte by measuring the decrease of amino-diphenylphenol in solution. Thus, here we have investigated the electrochemical behavior of Δ^9-THC during its direct analysis (qualitative and quantitative) by cyclic voltammetry using a glassy carbon working electrode.

2. Experimental

2.1. Reagents and samples

Seized hemp and hashish samples were provided via a partnership between this research group and the criminal experts of the laboratory of toxicological analysis – Institute of Criminalistics – city of Ribeirão Preto – São Paulo, Brazil. These samples were analyzed by means of the colorimetric test using the Fast Blue B Salt as reagent. Results attested to the presence of Δ^9-THC. The standard sample of Δ^9-THC (Cerrilliant®) contained 1000 mg/L of this substance in methanol. For electrochemical analyses, dimethylformamide (DMF) acquired from Merck was employed. The supporting electrolyte solution was prepared with TBATFB, by addition of 25 mL DMF to a 100 mL volumetric flask containing 0.66 g TBATFB. In order to remove electroactive oxygen from the solution, the latter had been previously purged with nitrogen gas for 15 min. Deionized water was added before the start of the experiment, giving a supporting electrolyte solution in DMF/water at a 9:1 (v/v) ratio. The final TBATFB concentration was 0.1 mol/L.

Note: all stock solutions were provided in mol/L unities. The final results were also reported in g/L unities.

2.2. Preparation of the standard solution

The preparation of the standard Δ^9-THC solutions was accomplished by dilution of a 1000 mg/L standard Δ^9-THC solution at different volumes. For voltammetric analysis, 1 mL of a 10 mg/L Δ^9-THC standard solution was evaporated using a rotary evaporator Waterbath BUCHI-B-480 for removal of the methanol present in the solution. Next, 25 mL DMF were added, resulting in a Δ^9-THC concentration of 1.3 μmol/L.

2.3. Preparation of the samples

One gram of the seized drug samples was weighed and transferred to 250 mL flasks. Each bottle received 100 mL methanol and was placed in an ultrasonic bath for 10 min. Later, with the aid of a funnel and a filter paper, the extract was filtered, and the resulting filtrate had a green color. The filtered solution was placed in a capped dark glass container, in order to await the evaporation step. The sample solvent was removed in the equipment, which was submitted to moderate rotation at 65 °C. The total time elapsed for solvent evaporation was approximately 5 min.

2.4. Analysis by cyclic voltammetry and linear sweep

Cyclic voltammetry and linear sweep voltammetry were carried out using a μAutoLabIII potentiostat coupled to a microcomputer. To this end, a 5.0 mL conventional electrochemical cell was employed, using a Metrohm glassy carbon disk as the working electrode, an Ag/AgCl (saturated aqueous KCl) reference electrode, and a spiralized platinum auxiliary electrode. The potential scans were performed between −1.2 V and 0.9 V, at a speed of 100 mV s^{-1}, which comprised the entire working range of the utilized solvent. The total time elapsed for each complete voltammetric scan in this work range was 38 s.

2.5. Predicted separation of the Δ^9-THC present in the samples by TLC

Considering that there are about 60 different species of cannabinols in the plant, a pre-purification step was proposed, in order to prevent their interference. To this end, solutions containing hemp and hashish were subjected to a chromatographic run on TLC containing a commercial UV (254 nm) developer, using methanol/water 8:2 (v/v) as the mobile phase. A conventional UV box was used to locate the spot containing the analyte, and its scraping was performed on the chromatographic silica. The scraped silica containing the analyte was conditioned by dilution in DMF solution containing the support electrolyte. The resulting solution was filtered, in order to eliminate the silica, and the filtrate was analyzed on the potentiostat. The total time elapsed for this experimental stage was also approximately 5 min.

2.6. HPLC analysis

Chromatographic analysis of the hemp samples was carried out on a Shimadzu HPLC apparatus. For this purpose, a Rheodyne Model 7725 injection valve with a 20 μL sample loop was employed. A Shimadzu Shim-pack C18 (250 mm × 6.0 mm I.D., 5 mm) reverse-phase column was applied for all the measurements, using a guard column Shimadzu Shim-pack C18. Prior to use, the mobile phase solvents had been filtered through a 0.45 mm filter (Millipore Milex) and degassed by an ultrasonic device coupled to the HPLC system. An isocratic program was used for the HPLC analyses, which involved wavelength detector set at 224 nm and a flow rate of 1.0 mL/min. All the reagents were analytical grade, and all the aqueous solutions were prepared with deionized water. Results from the determination of Δ^9-THC in seized samples were compared with those obtained by the chromatographic method.

3. Results and discussion

3.1. Voltammetric analysis of Δ^9-THC

The choice of DMF/water 9:1 (v/v) as solvent and of TBATFB 0.1 mol/L as the supporting electrode proved to be effective in the potential range −1.2 to 0.9 V (vs. Ag/AgCl). Unwanted Faradaic peak currents were not observed when the potential cycle was applied (Fig. 1). Δ^9-THC has a well-defined irreversible anodic peak current at 0.0 V, as shown in Fig. 2. This can be attributed to the oxidation of the phenol group [24–26] in an electrochemical process involving one electron [27], as presented in Fig. 3.