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Water-compatible imprinted pills for sensitive determination of cannabinoids in urine and oral fluid

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ABSTRACT

A novel molecularly imprinted solid phase extraction (MISPE) methodology followed by liquid chromatography tandem mass spectrometry (LC–MS/MS) has been developed using cylindrical shaped molecularly imprinted pills for detection of Δ^9-tetrahydrocannabinol (THC), 11-nor-Δ^9-tetrahydrocannabinol carboxylic acid (THC-COOH), cannabinol (CBN) and cannabidiol (CBD) in urine and oral fluid (OF). The composition of the molecular imprinted polymer (MIP) was optimized based on the screening results of a non-imprinted polymer library (NIP-library). Thus, acrylamide as functional monomer and ethylene glycol dimethacrylate as cross-linker were selected for the preparation of the MIP, using catechin as a mimic template. MISPE pills were incubated with 0.5 mL urine or OF sample for adsorption of analytes. For desorption, the pills were transferred to a vial with 2 mL of methanol:acetic acid (4:1) and sonicated for 15 min. The elution solvent was evaporated and reconstituted in methanol:formic acid (0.1%) 50:50 to inject in LC–MS/MS. The developed method was linear over the range from 1 to 500 ng mL^{-1} in urine and from 0.75 to 500 ng mL^{-1} in OF for all four analytes. Intra- and inter-day imprecision were <15%. Extraction recovery was 50–111%, process efficiency 15.4–54.5% and matrix effect ranged from −78.0 to −6.1%. Finally, the optimized and validated method was applied to 4 urine and 5 OF specimens. This is the first method for the determination of THC, THC-COOH, CBN and CBD in urine and OF using MISPE technology.

1. Introduction

According to the United Nations 2014 World Drug Report, cannabis remains the most widely used illicit substance in the world [1]. Urine is the primary matrix used for the detection of cannabis intake, since cannabinoids can be detected for up to four days in light smokers and even after several months in chronic smokers [2]. The primary urinary compound for testing purposes is the major metabolite of THC, THC-COOH, excreted mainly as glucuronic-acid conjugate (THC-COOH-glucuronide). Positivity is set at concentrations above 15 ng mL^{-1}, eliminating the possibility of false positive due to passive inhalation [3] but providing no information about drug administration or exposure [4]. CBN and CBD are minority compounds of cannabis and rarely analyzed in urine, but they have been recently proposed as biomarkers of recent cannabis intake [5–7].

In recent years, oral fluid (OF) monitoring has been increased [8,9], because it is a better indicator of recent use than urine monitoring [10,11]. In addition, the use of OF for testing has the advantage of a non-invasive, simple and observed specimen collection, which reduces the opportunity for adulteration [9,11,12]. On the other hand, disadvantages of OF testing include small sample volume, especially after cannabis smoking and low concentrations requiring high sensitivity [8]. THC is the primary target in OF for detecting cannabis intake. The use of OF raises the probability of false positives in the test results due to THC's presence at concentrations exceeding 4 ng mL^{-1} in samples taken from passive smokers. Simultaneously, the metabolite THC-COOH was never detected in this kind of population [8,13–16]; though it can be detected in

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chronic daily cannabis smokers OF up to 4 weeks of abstinence, thus, the detection of this cannabinoid in OF may not reflect recent use [17]. Also in OF, CBD and CBN have been proposed as markers of recent consumption [8,18,19]. Therefore, the simultaneous determination of THC, THC-COOH, CBD and CBN in OF allows the correct interpretation of cannabis intake by eliminating the possibility of passive inhalation and providing markers of recent cannabis smoking.

Traditionally, cannabinoids in urine were subjected to basic or enzymatic hydrolysis for cleavage of glucuronic acid fraction in order to increase the sensitivity of the analysis, normally, by chromatography tandem mass spectrometry (GC–MS/MS) [20,21] or LC–MS/MS [2,6,22,23]. Solid phase extraction (SPE) is then usually selected as a clean-up treatment. Moreover, when GC–MS/MS is employed, a derivatization process prior to injection is required to improve the quantification [20,24]. When the matrix is OF, highly sensitive analytical methods are required as THC-COOH it is present at low pg mL^{-1} concentrations. Only a few methods with the sensitivity required are available [17–19]. These methods were developed using GC–MS/MS [25], 2D-GC–MS [19,24,26] or LC–MS/MS [8,9,27–30], but in most of them, tedious sample treatments are involved and derivatization of the extract to increase the sensitivity is required. Only two recent papers describe LC–MS/MS methodologies without a derivatization step [8,9]. Previously, this team published the first method to determine the THC and its primary metabolite in urine and OF using MISPE coupled to LC–MS/MS [31] with preemptive hydrolysis of urine specimens but no derivatization of the extract for both matrices. The use of MIPs as SPE sorbent allowed a simple, rapid, effective and selective extraction compare to traditional SPE due to the fact that they are materials prepared in the presence of a target analyte (template) or closely related species (mimic) that serves as a mould for the formation of complementary binding-sites. In this report, the MIP was synthesized using catechin as a mimic-template, achieving a LOQ of 1 and 2.5 ng mL^{-1} for THC and THC-COOH in OF and urine, respectively. In order to improve the MISPE procedure and enhance the sensitivity of the methodology, new formulations of MIP were investigated.

Traditional combinatorial screening approaches were previously employed successfully to predict the binding properties and optimize the composition of MIPs [32–35]. Nevertheless, these methods are tedious and too time-consuming to routine applications. Pre-polymerization studies conducted using molecular models or spectroscopic techniques could help to optimize the polymer synthesis by analysing the interactions at a molecular level in the pre-polymerization mixture [36–38], but these methods have experimental limitations since they do not consider the influence of the cross-linking agent, the polymerization step or the interfering compounds in real samples. The use of a new MIP design methodology based on a screening library of NIPs, could overcome these limitations [39–43], since it is based on the premise 'if a NIP shows binding properties toward a target molecule, the corresponding MIP will show a significant imprinting effect'. The presence of the template molecule in the pre-polymerization mixture acts to improve binding properties that already exist in the NIP [41].

Herein, a non-imprinted polymer (NIP) library was developed with monomers capable of forming high affinity complexes with the template and cross-linkers with different degrees of functionality and rigidity. Their maximum 'water-compatibility' [40,44] should be also considered since urine and OF are aqueous media and traditionally, MIPs exhibit diminished aqueous performance when compared to that observed in hydrophobic organic solvents [45]. Thus, the binding capacity of the NIPs was analysed in polar and apolar conditions to value non-specific and specific interactions respectively. The NIP with the highest affinity to THC based on the ratio of binding capacities in both media was selected and this monomer-cross-linker composition was used to prepare the MIP. The MISPE procedure was optimized regarding to the type of elution solvent. The novel MISPE-LC–MS/MS method for the determination of THC, THC-COOH, CBD and CBN in urine and OF specimens was fully validated. Finally, the method was applied to the analysis of 5 OF and 4 urine specimens.

2. Experimental

2.1. Chemicals and reagents

2.1.1. Reagents involved in polymer preparation, binding evaluation, optimization of the procedure and HPLC-PDA analysis

Triethylene glycol dimethyl ether (TRIGLYME), poly (vinylacetate) (PVAc, Mw = 100 g mol^{-1}), 2-hydroxiethyl-methacrylate (HEMA), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TRIM), trimethylolpropane triacrylate (TMPTA), (+)-Catechin hydrate (CATE), acetaminophen (AAP), and *N*,*N*-dimethylformamide (DMF) were obtained from Sigma–Aldrich (Steinheim, Germany). Acrylamide (AM), 2,2-azobisisobutyronitrile (AIBN), caffeine (CAF) and ammonium hydroxide solution (25% in water) were supplied from Fluka (Buchs, Switzerland). The functional monomer 4-vinylpyridine (4-Vpy) was purchased from ACROS organics (Geel, Belgium). Ethanol (EtOH) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany) and acetic acid glacial (AcOH) from Panreac (Barcelona, Spain), while formic acid (98–100%) reagent grade and acetonitrile (ACN) as mobile phase were from Riedel-de Haën (Seelze, Germany) and BDH Prolabo chemicals (Briare, France) respectively. Pure THC (Dronabinol) was obtained from LGC Standards (Barcelona, Spain). Water was purified using a Milli Q Ultrapure water-purification system (Millipore, Bedford, MA, USA).

2.1.2. Reagents involved in the validation of the MISPE-LC–MS/MS methodology

THC, THC-COOH, CBN and CBD at 1 mg mL^{-1} in methanol, and THC-d_3, THC-COOH-d_3, CBN-d_3 and CBD-d_3 at 0.1 mg mL^{-1} in methanol were purchased from Cerilliant™ (Round Rock, TX, USA). Methanol Chromasolv® LC–MS from Sigma Aldrich (St. Louis, USA), formic acid (98–100%) reagent grade and acetic acid (99.8%) reagent grade from Scharlau (Sentmenat, Spain), and acetonitrile LC–MS from Panreac (Castellar del Vallès, Spain). Water was purified with a Milli Q water system (Millipore, Le-Mont-sur-Lausanne, Switzerland). Salivette® oral fluid collection devices were from Sarstedt (Nümbrecht, Germany).

2.2. Instrumentation

2.2.1. HPLC-PDA analysis for binding evaluation

HPLC-PDA analysis were performed using a Waters 2695 (Waters, Mildford, MA, USA) with gradient pump and automatic injector. Chromatographic experiments were carried out using a stainless steel column packed with SunFire™ C_{18} (150 mm × 3.0 mm, 3.5 μm) (Waters). Isocratic elution was performed with formic acid 0.1% (0.1% formic acid in deionized water): ACN (20:80) as the mobile phase at a flow rate of 0.5 mL min^{-1} and injection volume of 20 μL. Column oven temperature was set at 30 °C. Detection was carried out using a photodiode array detector (PDA, model 996 UV) set in the range of 200–400 nm. Output signals were monitored and integrated using a personal computer operated under the Empower 2 software (Waters). Wavelength of 235 nm for THC analysis was selected as output PDA signals.