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Pressurized liquid extraction for the determination of cannabinoids and metabolites in hair: Detection of cut-off values by high performance liquid chromatography–high resolution tandem mass spectrometry

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ABSTRACT

Hair analysis has become a routine procedure in most forensic laboratories since this alternative matrix presents clear advantages over classical matrices; particularly wider time window, non-invasive sampling and good stability of the analytes over time. There are, however, some major challenges for the analysis of cannabinoids in hair, mainly related to the low concentrations of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), that is the major metabolite. In this study a fast, accurate and sensitive method for the determination of cannabinol, cannabidiol, THC and THC-COOH in hair has been developed. The extraction of analytes from hair (50 mg) is based on an automated pressurized liquid extraction (PLE) using water modified with the surfactant sodium dodecyl sulphate as eluent phase. PLE extract is then cleaned up by SPE using polymeric reversed phase cartridges Strata XL before the injection in the HPLC–HRMS/MS system. Chromatographic conditions obtained with a fused-core column allowed a good separation of the analytes in less than 4 min. The whole procedure has been validated according to SWGTOX guidelines. The LLOQs obtained for THC-COOH and the other analytes were respectively 0.1 and 2 pg/mg. To the best of our knowledge, this is the first LC–MS/MS based method that allows the detection of THC-COOH in hair at values lower than the cut-off.

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1. Introduction

Hair analysis has become a routine procedure in most forensic laboratories since this alternative matrix presents clear advantages over classical biological matrices; particularly wider time window, non-invasive sampling and stability [1]. However, cannabinoids analysis in hair is still not straightforward. Major challenges arise from (i) low concentration of Δ^9-tetrahydrocannabinol (THC) and even lower amounts of the main metabolite 11-nor-9-carboxy-THC (THC-COOH; expected concentration in the fg/mg range) [2–4]; (ii) the necessity to develop procedures taking into account only real use excluding environmental contamination as cannabis smoke can condensate on the hair surface and can be easily adsorbed, [5]; (iii) the time consuming steps needed for extraction and derivatization: in fact, gas chromatography coupled with tandem mass spectrometry GC–MS(/MS) is still the technique of choice for the analysis of cannabinoids in hair.

The determination of THC-COOH has been shown to be crucial to distinguish between passive drug exposure and active consumption since this molecule is an exclusive product of metabolism and can be considered as marker of drug abuse.

Other cannabinoids, i.e. cannabinol (CBN) and cannabidiol (CBD) are often quantified as well, since it has been reported that the sum of the major cannabinoids may provide a better indication of drug use than THC alone [6]; however, their presence alone cannot fully exclude passive exposure. Recently, in order to identify external contamination, the detection of a specific marker, i.e. THCA-A has been proposed; this non-psychoactive precursor of THC is the main cannabinoid in fresh plant material and it is not significantly incorporated into hair after cannabis intake [7].

To date, the techniques generally used to reach the cut-off of 0.2 pg/mg recommended by the Society of Hair Testing (SoHT)

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[8] for THC-COOH are GC–MS/MS using either electron impact (EI) ionization mode [9,10] or negative ion chemical ionization (GC–NCI–MS–MS) that allows a further increase of the sensitivity [2–4] and GC/GC–MS [11]. Liquid chromatography coupled with mass spectrometry (LC–MS/MS) in cannabinoids hair analysis has only been recently reported [12–14], and THC-COOH was included in few studies. The LOQ obtained in one work was significantly higher than the cut-off value and thus the method can be applied only for chronic use studies [15]; in the other paper the LOQ reported, obtained by using a MS^3 experiment, was exactly at the cut-off [16].

The most used approach for sample preparation is based on digestion in alkaline medium using NaOH followed by liquid/liquid extraction (LLE) [2–4,9,13,15–19], solid phase extraction (SPE) [10,11,20], solid phase micro extraction (SPME) [21–25] or solid phase dynamic extraction (SPDE) [26,27]. Enzymatic digestion [28] and direct methanol extraction [5,7,29] have been also reported but require longer times, up to 5 h. A drawback of NaOH digestion is that the stability of the analytes might be affected during the digestion procedure, for example CBD is not stable under the severe conditions of alkaline digestion [19]. Large volume of organic solvents are, instead, used for LLE procedure and the reproducibility is poor.

Pressurized liquid extraction (PLE) has reached an increasing attention in the last years since it showed significant advantages over competing techniques as time saving, solvent use, automation and efficiency [30]. Initially the technique has been mainly focused on environmental samples [31]. Other applications included food samples [32] and biological matrices such as tissues [33]; we have recently demonstrated the potential of PLE for the extraction of illicit drugs from hair, allowing automation of the extraction and a significant reduction of total analysis time [34].

The aim of the present work was to develop a fast and accurate method for the determination of CBN, CBD, THC and THC-COOH in hair. The extraction of analytes from hair is based on an automated PLE using water modified with the surfactant sodium dodecyl sulphate as eluent phase. The PLE eluent is then cleaned-up by SPE that allows both the reduction of matrix effect and the enrichment of the analytes which is particularly useful for the detection of THC-COOH. The chromatographic conditions obtained with a fused-core column allowed a good separation of the analytes in less than 5 min. The whole procedure has been validated according to SWGTOX guidelines. To the best of our knowledge, this is the first LC–MS/MS based method that allows the detection of THC-COOH in hair at lower values than the cut-off.

2. Experimental

2.1. Standard and reagents

THC, THC-COOH, CBD, CBN drugs standards and THC-d_3 and THC-COOH-d_3 internal standards were purchased from LGC standard (Sesto San Giovanni, Milan, Italy). The purity of the reference compounds was ≥99%. All standards were provided at a concentration of 1 mg mL^{-1} with the exception of ISs that were at a concentration of 0.1 mg mL^{-1}. Individual stock solutions were prepared in methanol at 0.1 mg mL^{-1}, except for the ISs which were diluted at 1 μg mL^{-1}. Working standard mixtures were prepared by appropriate dilution of the standards in methanol. All solutions were stored at −20 °C in the dark. Ultrapure water, formic acid, acetonitrile and methanol used to prepare the mobile phases were of HPLC grade and were purchased from Fisher Scientifc (Fair Lawn, NJ, USA). Sodium dodecyl sulphate (SDS) and sodium hydroxide were from Sigma–Aldrich. Ultrapure water used for sample preparation was produced by a Milli-Q Plus apparatus from Millipore (Bedford, MA, USA).

2.2. External decontamination procedure

Crudely cut hair (≈1 cm) was placed in a 50 mL Falcon cone tube in 5 mL of phosphate buffer (0.1 M, pH 6). The mixture was vortexed for one minute and, after removal of aqueous buffer, it was sequentially washed with isopropanol (5 mL) and dichloromethane (5 mL). The last wash was collected in a vial and evaporated under a gentle stream of N_2; the residue was reconstituted with 1 mL of 10 mM formic acid in methanol and was stored for further analysis in order to assess the performance of the procedure.

2.3. Pressurized liquid extraction

Fifty milligrams of hair sample, cut into 1–2 mm segments, were homogenized with diatomaceous earth (Sigma–Aldrich, Milan, Italy) by means of a mortar. The sorbent was previously powdered and washed with the same PLE extraction conditions used for hair sample. The mixture was then placed in a 1 mL pressure resistant stainless steel cell that was sealed at both ends with glass-fiber filters. Void volumes in the cell were filled up with diatomaceous earth and 25 μL of methanol containing the ISs at a concentration of 2 ng mL^{-1} for THC-COOH-d_3 and 20 ng mL^{-1} for THC-d_3 were added. PLE was carried out performing a single extraction cycle using as extraction solvent a 90:10 (v/v) water–methanol mixture containing SDS 25 mM. The extraction conditions were: pressure, 100 bar; temperature, 150 °C; preheat time, 1 min; heat time, 7 min; static time, 5 min; flush volume, 0%; purge time, 60 s. The PLE extract (5–6 mL) was automatically collected in glass vial with caps solvent resistant (PTFE) septa.

2.4. Solid phase extraction

PLE extract was collected into a graduated tube and centrifuged at 6000 × g for 5 min at 25 °C. Five mL were then cleaned up by SPE using polymeric reversed phase cartridges Strata XL 30 mg/1 mL from Phenomenex (Torrance, CA, USA). The cartridge, installed on vacuum system (Visiprep), was previously conditioned with 1 mL of methanol and 1 mL of water–methanol (90:10 v/v). After loading, the cartridge was washed with 5 mL of water–methanol (90:10 v/v) and 3 mL of water–methanol (50:50 v/v); the analytes were then eluted using 1 mL of methanol. The eluate was directly injected in the HPLC–HRMS/MS system (6 μL).

2.5. NaOH digestion

For comparative purposes three authentic positive hair samples were pretreated with alkaline digestion as well. The digestion was carried out according with an existing method described in literature with slight modifications [15]. Briefly, 50 mg of decontaminated sample were transferred into an amber glass vial and 25 μL of ISs solution was added. The samples were then subjected to digestion in 1 mL of 2.5 M NaOH at 60 °C for 25 min; after cooling to room temperature, the solution was neutralized with formic acid. The mixture obtained was extracted by vortex mixing using 3 mL ethyl acetate. After centrifugation the organic supernatant was separated, evaporated to dryness under a gentle flow of nitrogen and the residue was finally reconstituted in 200 μL of methanol. 10 μL were then injected in the HPLC–HRMS/MS system.

2.6. HPLC–HRMS/MS analysis

HPLC–HRMS/MS analysis was performed on a Dionex UltiMate® 3000 Rapid Separation LC system from Thermo Fisher Scientific (San Jose, CA, USA) coupled to a Thermo Scientific Q Exactive Mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

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