Simultaneous identification and quantitative determination in urine of the more significant metabolites of synthetic cannabinoids JWH-018, JWH-073, JWH 122 and JWH-250 using authentic references and deuterated isotopologues as internal standards

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Introduction

Synthetic cannabinoids (SC) are substances displaying a high affinity for cannabinoid receptor CB1 and represent the psychoactive agents in herbal mixtures called "Spice" or "K2". Because of its great potential of abuse, several SC are banned in many countries, otherwise the detection of their illegal intake is complicated by the fact that the urine give negative results in commonly used drug screening procedures. Additionally due to rapid metabolic transformation, the native SC are not detectable in urine samples and then the analytical methods must be based on the identification and quantification of their metabolites.

Background

The indole derived cannabimimetics undergo extensive metabolism: the N-alkyl mono-hydroxylated compounds, their glucuronides and the terminally carboxylic acid seem to be the main metabolites. The correct identification of these metabolites in the urine samples requires the use of authentic standards obtained by synthesis. In the case of JWH-018 and JWH-073 all three major metabolites were identified by comparison with authentic standards,[1-4] while in the case of JWH-122 and JWH-250 only for some metabolites the reference standards were available.[5,6]

Aim of the work

The aim of our study is to set-up, using synthesised reference standards, a LC-MS/MS method for routine screening procedures to assess the intake of JWH-018, JWH-073, JWH 122 and JWH-250. The method should permit the simultaneous identification of the most significant metabolites of each cannabinoid with the sensitivity, precision and accuracy assured by the use of deuterated internal standards.

Methods

The urine were hydrolysed with 3% HCl at 90-95°C for 60 min[7,8] and extracted with n-hexane-ethyl acetate (9/1 v/v).

The LC-MS/MS analysis was performed in positive mode in multiple reaction monitoring (two transitions for each analyte) on an API 4000 Triple Quadrupole Mass Spectrometer (ABSciex) equipped with a Acquity C-18 HSS T3 (100x2 mm, 1.8 µm, Waters) with isocratic elution (55% of 10 mM $HCOONH_4$ containing 0.1% HCOOH and 45 % of ACN) at 45°C and at flow rate of 0.4 mL/min.

Results and Discussion

All the synthesized compounds were fully characterized with regard to the structure and purity, by ^1H, ^{13}C NMR and GC-MS (after esterification with CH_2N_2 for the carboxylic acid metabolites).

For sample treatment, the recovery of extraction of the metabolites at different pH (1,3,5,9 and 10) was evaluated showing a complete recovery at pH 1-5, while at higher pH the recovery of carboxylic acid metabolites is unacceptably low. The method showed linearity (0.5-100 ng/mL), reproducibility and accuracy (ranged between -15% and + 15%).

The application of the method shows that the most abundant metabolite for all SC evaluated is the (ω-1)-OH derivative and that the metabolites are present in the urine for 2 days, with a peak of concentrations at 2-7 hours after the intake.

After ingestion of JWH-018, in addition to ω-OH and ω-COOH metabolites (40-50 and 30-45% of (ω-1)-OH respectively) were also detected JWH-073ω-COOH (15-25% in the first 2-7 h and 40-50% after 24 h) and JWH-073(ω-1)-COOH (10-20% in the first 2-7 h) which becomes the main metabolite after 24 hours. These results are consistent with those of previous studies[4,9,10] in which JWH-073ω-COOH was detected in urine samples known to have been administered with JWH-018 only. They support also the recent observation[11] that JWH-073(ω-1)-COOH (JWH-072 *N*-propanoic acid) is the predominant metabolite found in urine samples probably collected after adequate time from the assumption of JWH-018.

In the case of JWH-073, besides the most abundant (ω-1)-OH metabolite, were detected the ω-COOH metabolite (40-60%of (ω-1)-OH in the first 2-7 h and 80-120% after 24 h) and then ω-OH (20-40% of (ω-1)-OH).

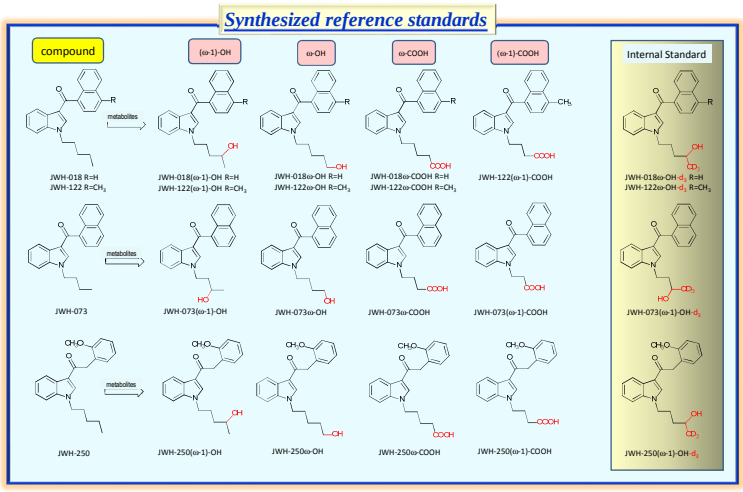
In the case of JWH-250, the second abundant metabolite was ω-COOH (15-25% of (ω-1)-OH derivative), while the ω-OH metabolite was present in smaller amount (<4% of (ω-1)-OH).

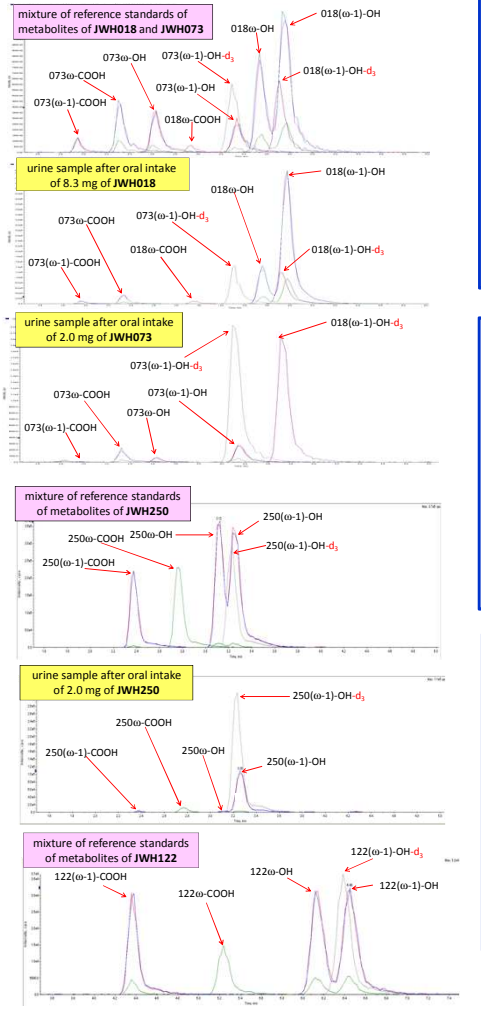
Conclusions

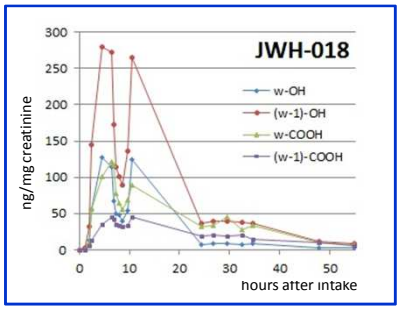
In this study, the simultaneous determination of major metabolites in urine samples after intake of JWH-018, JWH-073 and JWH-250 was performed.

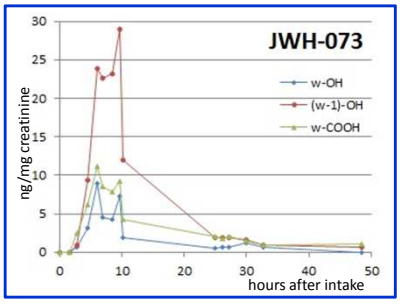
The estimation of profiles of metabolite concentrations after intake versus urine collection times, can be of help in to interpret the observed[4,11] variation of concentrations among different metabolites in authentic urine specimens obtained from drug offenders.

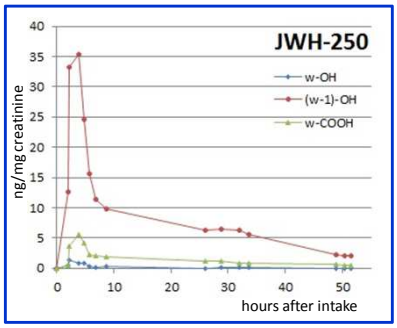
Synthesized reference standards











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