Elsevier Editorial System(tm) for Journal of Chromatography A

Manuscript Draft

Manuscript Number:

Title: Development of a micro-solid-phase extraction molecularly imprinted polymer technique for synthetic cannabinoids assessment in urine followed by liquid chromatography - tandem mass spectrometry

Article Type: Full length article

Keywords: synthetic cannabinoids, molecularly imprinted polymer porous membrane-protected micro-solid phase extraction, urine, high performance liquid chromatography tandem mass spectrometry

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Dear Editor,

We send to you our manuscript titled “Development of a micro-solid-phase extraction molecularly imprinted polymer technique for synthetic cannabinoids assessment in urine followed by liquid chromatography - tandem mass spectrometry” for publication in Journal of Chromatography A, if it is possible. The current paper is an unpublished work and it has not been submitted for publication elsewhere.

The current research describes for the first time the synthesis of molecularly imprinted polymers (MIPs) for synthetic cannabinoids (SCs), and the development of a micro-solid phase extraction (μ-SPE) procedure in which the adsorbent (MIP particles) is enclosed inside a cone shaped device made of polypropylene. Several SCs classes (AM, JWH, and RCS classes) are simultaneously pre-concentrated and further determined by high performance liquid chromatography – tandem mass spectrometry.

Waiting your news

Best regards,

Dr. Antonio Moreda-Piñeiro (corresponding author)

Dr. Sabina Strano-Rossi (corresponding author)

Highlights:

> Molecularly imprinted polymer (MIP) for synthetic cannabinoids (SCs) assessment in urine

> μ-SPE (cone shaped) device based on porous membrane-protected containing MIP

> Fast SCs (AM, JWH, and RCS classes) and metabolites determination by reverse phase HPLC-MS/MS

Development of a micro-solid-phase extraction molecularly imprinted polymer technique for synthetic cannabinoids assessment in urine followed by liquid chromatography - tandem mass spectrometry

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Abstract

Several molecularly imprinted polymers (MIPs) have been synthesized for the first time using various synthetic cannabinoids (JWH007, JWH015 and JWH098) as template molecules. Ethylene dimethacrylate (EDMA) was used as a functional monomer for all cases. Similarly, divinylbenzene (DVB) and 2,2´-azobisisobutyronitrile (AIBN) were used as cross-linker and initiator, respectively. The prepared MIPs have been fully characterized and

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evaluated as new selective adsorbents for micro-solid phase extraction (μ-SPE) of synthetic cannabinoids in urine. The developed MIP-μ-SPE devices consisted of a polypropylene (PP) porous membrane containing the adsorbent for operating in batch mode (porous membrane-protected micro-solid phase extraction), which allowed a fast and integrated extraction-cleanup procedure. High performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) was used for quantifying the analytes after MIP-μ-SPE. The best performances were obtained for MIPs prepared from JWH015 as a template. Optimum loading conditions were found to be urine pH of 5.0 and adsorption time of 8.0 min under mechanical (orbital-horizontal) stirring at 100 rpm. The composition of the eluting solution consisted of 75:20:5 heptane/2-propanol/ammonium hydroxide. The elution was assisted by ultrasounds (37 kHz, 325 W) for 8.0 min. In addition, studies regarding selectivity have also been addressed for several drugs of abuse under optimized loading/adsorption conditions. Validation of the method showed good precision and analytical recovery by intra-day and inter-day assays (RSD values lower than 7 and 10% for intra-day and inter-day precision, and within the 83-100% range for intra-day and inter-day analytical recovery).

Keywords: synthetic cannabinoids, molecularly imprinted polymer porous membrane-protected micro-solid phase extraction, urine, high performance liquid chromatography tandem mass spectrometry.

1. Introduction

As stated in the recent World Drug Report 2017 by the United Nations Office on Drugs and Crime (UNODC), the global market for new psychoactive substances (NPSs) continues to expand, and the range of drugs available on the market has probably never been wider [1]. The data reported for 2016 show that the major identified NPSs were cathinones and several

new synthetic cannabinoids (SCs) [1]. SCs share certain parts of the molecular structure of drugs obtained from natural products. The psychoactive power of these new substances is therefore similar, or even higher, than that exhibited by similar substances of natural origin. However, these new substances have certain structural differences when compared to natural drugs; thus, they go unnoticed during illicit drug market control and also during routine toxicological-forensic analysis.

Recent literature dealing with the detection of these substances in clinical samples shows the need for using highly sensitive techniques such as mass spectrometry [2,3]. However, the development of analytical methodologies that favor the extraction and pre-concentration of these substances from complex biological matrices is needed [2]. Selectivity obtained by the available pre-concentration methods is a key factor for enhancing the performances of modern mass spectrometry-based techniques. This is quite important mainly when using electrospray ionization (ESI) sources because of the existence of the matrix effect (changes in the ionization process of the drug due to endogenous matrix components) which results in ion enhancement (gain in signal) or suppression (loss of signal) affecting the precision, accuracy and robustness of the method [2]. Regarding Biological specimen analysis, conventional sample pre-treatments have been proposed for isolating/pre-concentrating SCs [4-11]. Conventional liquid-liquid extraction (LLE) [4,6-9] and solid phase extraction (SPE) using commercially available cartridges [5] have been proposed for urine analysis. Moreover, recent developments have been based on dispersive liquid-liquid microextraction (DLLME) [10,11]. Molecularly imprinted polymers (MIPs) have emerged as highly selective adsorbents for both SPE and μ-SPE procedures. These artificial materials emulate the high selectivity of the antibody-antigen and enzyme systems [12], but the preparation (synthesis) is quite easier and more straightforward than the production methods required for natural receptors. In addition, the cost of synthesizing MIPs is almost negligible when compared to antibody-

antigen and enzyme reagents. After preparing an MIP using a specific template, the artificially generated recognition sites in synthetic MIPs are able to specifically rebind a target molecule in preference to other closely related compounds [13]. The technology of MIPs has been used extensively for separation applications, and several recent reviews deal with the selective pre-concentration separation of environmental organic and inorganic compounds, and also natural products and/or contaminants in foodstuffs [13-16]. There are few applications, however, addressing the assessment of illicit drugs in clinical/forensic samples. Recent developments have dealt with cocaine and metabolites assessment [17-20], and also with natural cannabinoids [21,22] in urine and plasma.

The aim of the current work has been the synthesis and characterization of MIPs for the recognition of twenty SCs (mainly AM and JWH class), and the development of a µ-SPE for urine analysis. To the best of our knowledge, this research is the first development regarding MIPs synthesis and evaluation for synthetic cannabinoids.

Several templates (three structurally different synthetic cannabinoids) were used for MIPs synthesis. Capabilities of the three synthesized MIPs were evaluated for recognizing and pre-concentrating the template molecules, as well as other related molecules so that most synthetic cannabinoids can be simultaneously assessed. The developed MIP-µ-SPE (batch mode) novel approach was based on using polypropylene (PP) porous membrane-protected technology [23]. This µ-SPE format, first proposed by Basheer et al. [24], allows the target to diffuse freely through the membrane's pores and to be retained by the solid sorbent phase; whereas, the sample's concomitants diffusion (large biomolecules typically present in clinical specimens) is hampered by the membrane.

2. Materials and methods

2.1. Instrumentation

Determinations were performed with an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with a Jet Stream electrospray ionization source operating in Multiple Reaction Monitoring (MRM) mode, and equipped with an Agilent 1290 Infinity system (Agilent Technologies) consisting of a binary pump with an integrated vacuum degasser, a high performance well-plate autosampler, and a thermostatted column module. Chromatographic separations were performed with a Kinetex 2.6µ C18 100 Å reverse phase column (100 mm length × 2.10 mm i.d., 5.0 µm particle diameter) from Phenomenex (Torrance, CA, USA). A Boxcult temperature-controlled chamber (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for MIP synthesis. A multi mixer 717 (Asal Srl, Milano, Italy) was used for MIP-µ-SPE (loading stage). The cone-shaped PP envelope containing MIP beads was heat-sealed with a TN1010 heat-sealer from Siemens (Munich, Germany). An ELMA Transonic 450/H ultrasonic cleaner bath (Carlo Erba, Milano, Italy) with programmable temperature and time and ultrasound frequency of 37 kHz was used for analyte elution from the µ-SPE device. Other laboratory devices were: a field emission scanning electron microscope Ultra Plus (Zeiss Oberkochem, Germany), a Spectrum Two FT-IR-UATR spectrometer from Perkin Elmer (Waltham, MA, USA), a Basic20 pH–meter with a glass–calomel electrode (Crison, Barcelona, Spain), a Reax 2000 mechanical stirrer (Heidolph, Kelheim, Germany), a vacuum pump (Millipore Co., Bedford, MA, USA), an oven model 207 from Selecta (Barcelona, Spain), a VLM EC1 metal block thermostat and N_2 sample concentrator from VLM (Leopoldshöhe-Greste, Germany), and a R-210 rotavapor equipped with a B-491 heating bath and a V-740 vacuum pump (Büchi Laboryechnik AG, Flawil, Switzerland).

2.2. Reagents

Ultrapure water 18 MΩcm of resistivity (Milli-Q purification device) from Millipore Co. (Bedford, MA, USA). The following SCs were supplied by LGC Standards (Milano, Italy):

JWH007, JWH015, JWH098 (these three SCs were also used for MIP synthesis), AKB 48 (APINACA), AM694, AM2201, AM2233, CB13, JWH018, JWH019, JWH030,JWH073, JWH081, JWH122, JWH147, JWH203, JWH210, JWH250, JWH 251, JWH302, JWH307, JWH398, RCS4, RCS8, JWH073 N-(3-hydroxibutyl) metabolite, JWH073 4-butanoic acid metabolite, JWH0734-Hydroxyindole metabolite, JWH0735-Hydroxyindole metabolite JWH018 4-hydroxyindole metabolite, JWH018N-Pentanoic acid metabolite, JWH250 5-Hydroxyindole metabolite JWH250 N-pentanoic acid metabolite, and JWH210-d_9 (internal standard). Methanolic solutions (1.0 or 0.1 mg mL^{-1}) of SCs were prepared from pure standards. Other drugs (methanolic solutions at 1 or 0.1 mg mL^{-1}, from LGC Standards) used for cross-reactivity studies were: buprenorphine (BUP), Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (Δ^9-THC-COOH), 11-hydroxy-Δ^9-tetrahydrocannabinol (Δ^9-THC-OH), cannabinol (CBN), cannabidiol (CBD), amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 1,3-benzodioxolyl-N-methylbutanamine (MBDB), benzoylecgonine (BEG), cocaine (COC), cocaethylene (CE), ecgonine methyl ester (EME), morphine, O-6-monoacetylmorphine (O-6-MAM), codeine, methylone, butylone, cathinone, ethylone, methylenedioxypyrovalerone (MDPV), pentylone, naphyrone, flephedrone, 3,4-dimethylmethcathinone (3,4-DMMC), and methcathinone (ephedrone). MIP particles were synthesized by using divinylbenzene-80 (DVB) from Sigma-Aldrich (Steinhelm, Germany), and ethylene dimethacrylate (EDMA) and 2,2´-azobisisobutyronitrile (AIBN) from Fluka (Buchs, Switzerland). ACCUREL® PP membrane was from Membrana (Wuppertal, Germany). Acetonitrile and methanol (supragradient HPLC grade), heptane, ammonium acetate, neutral alumina, and sodium hydroxide were from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was from BDH (Poole, UK). Toluene, 2-propanol, ammonium hydroxide, and acetic acid 96%

(m/m) were from Panreac (Barcelona, Spain). Other consumables were: Durapore 0.20 µm membrane filters (Millipore), cellulose extraction thimbles (Filtros Anoia, Barcelona, Spain), and 0.20 µm cellulose acetate syringe filters (LLG, Meckenheim, Germany).

2.3. Urine samples

Ten drug-free urine samples obtained from laboratory staff volunteers were used for method optimization. For all cases, urine samples were collected in clean sealed polyethylene vials and kept at -20°C when necessary.

2.4. Synthesis of MIP particles

Liquid synthetic cannabinoids standards of JWH-007, JWH-015 and JWH-098 were used as templates for the synthesis of three MIPs. For each MIP synthesis, 108 µL of JWH-007, JWH-015 and 116 µL of JWH-098 were mixed with 67.3 µL of EDMA and 25 mL of porogen (acetonitrile/toluene 3:1) into 30 mL glass test tubes. The mixture was then stirred for 5 min and kept in the dark overnight to allow the self-assembly of the template and monomer. DVB (1.25 mL) and AIBN (0.091 g) were then added to the pre-polymerization mixture, and after stirring for 1 min the mixture was purged with argon for 5 min and immediately sealed, and placed in a low-profile roller (33 rpm on its long axis) inside a temperature-controllable incubator chamber (the temperature was ramped from room temperature to 60ºC during 2 h, and then maintained at 60ºC for a further 24 hours). DVB was previously treated to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing approximately 0.5 g of neutral alumina. Similarly, AIBN was purified by crystallization at -20°C after dissolving the reagent in methanol at 50–60°C.

Once polymerization was finished, the synthesized material was vacuum filtered, washed with acetonitrile, and oven-dried overnight at 40ºC. Non-imprinted polymers (NIPs) were

also prepared as shown above, but without adding the templates. The NIPs were then subjected to the same washing pre-treatment described above.

2.5. Template removal procedure

Templates were removed from synthesized MIPs by subjecting approximately 300 mg of dried MIP to Soxhlet extraction (two 20-hour cycles with 200 mL of methanol/water/acetic acid, 85:10:5). With this treatment, negligible drug concentrations were found in the washing solutions when performing HPLC-MS/MS analysis. The MIP beads were then rinsed with ultrapure water, and finally, the MIP particles inside the cellulose thimble were oven-dried at 40°C for 12 hours before use.

2.6. Preparation of the MIP-µ-SPE device

The MIP – porous membrane protected µ-SPE device was prepared with a PP membrane using a cone-shape design with only one seal on the upper end [17]. This configuration (4.0 cm in height) prevents degradation of the heat seal by the solvents typically used during the elution stage. An amount of 50 mg of prepared MIPs was placed into one of the closed-end folds before heat-sealing. Before use, each MIP-µ-SPE device was conditioned by sonication with 5 mL of 0.1M/0.1M KH_2PO_4/NaOH buffer solution (pH 5.0) for 10 min. The MIP-µ-SPE devices were stored soaked in the buffer solution.

2.7. MIP-µ-SPE procedure

Urine (1.0 mL) was spiked with the internal standard (JWH-210 d_9) at a concentration of 10 µg L^{-1}) into 25 mL flasks. The pH was adjusted at 5.0, and the conditioned MIP-µ-SPE device was placed into the buffered sample. The flasks were shaken (orbital – horizontal stirring) at 100 rpm for 8 min (ten flasks can be simultaneously treated). After analyte retention, the MIP-µ-SPE device was removed with tweezers and rinsed with 2 mL of 0.1M/0.1M KH_2PO_4/NaOH buffer solution at pH 5.0 (orbital – horizontal shaking of 100 rpm for 10 min). Analyte elution was then performed by placing the MIP-µ-SPE device into a

198 flask containing 2 mL of 75:20:5 heptane/2-propanol/ammonium hydroxide mixture, and
199 elution was assisted by ultrasounds (37 kHz) for 8 min. After MIP-µ-SPE removal, the eluate
200 was evaporated to dryness (stream of N_2, 40°C), and re-dissolved with 50 µL of 2 mM
201 ammonium acetate in methanol (pre-concentration factor of 20). The prepared MIP-µ-SPE
202 devices can be reused for 30-35 retention/eluting cycles without losing the retention
203 efficiency.

204 **2.8. Liquid chromatography-tandem mass spectrometry measurement**

205 Gradient elution (Table 1) was required for resolving several targets. Mobile phase A
206 consisted of 0.1% formic acid in ultrapure water, whereas mobile phase B was 0.1% formic
207 acid in methanol. MRM was used for data acquisition. Optimized ion source potentials and
208 collision energies for each MRM transition (at least two precursor ion→product ion
209 transitions for each analyte), are listed in Table 2. Electrospray source parameters are also
210 listed in Table 2.

211 Standard addition curves were prepared in duplicate by spiking 1.0 mL of drug-free urine
212 samples with the deuterated analogue (10 µg L^{-1}JWH-210 d_9), and analyte standards (5.0, 10,
213 15, and 20 µg L^{-1} each). This offers analyte concentrations within the 0 – 400 µg L^{-1} range
214 and a JWH-210 d_9 concentration of 200 µg L^{-1} in the reconstituted extract (pre-concentration
215 factor of 20). A typical chromatogram is shown in Figure 1.

216

217 # 3. Results and discussion

218 **3.1. MIP characterization**

219 As shown in the scanning electron microscopy (SEM) photographs (Figure S1,
220 supplementary section) MIPs and NIPs consisted of agglomerates of spherical particles. MIPs
221 exhibit smaller beads than NIPs (approximately 5.0-7.0 µm), while NIP beads are 10 µm in
222 size. The smallest bead sizes are those of the MIP-based JWH015.

FT-IR spectra were quite similar for all MIPs (after template removal) and NIPs (Figure S2, supplementary section). This is because there are no specific functional groups in the template molecules, and bands attributed to the template overlap with those exhibited by the monomer and cross-linker (present in both MIP and NIP). Figure S2 shows some characteristic bands in the FT-IR such as ~2900 cm^{-1} (C-H stretch), ~1450 cm^{-1} (C-H bending), and ~1731cm^{-1} (C=O stretch). In addition, three weak bands (1486, 1510, and 1599 cm^{-1}) related to C=C stretch, and ~1100 and ~1160 cm^{-1} (C-O stretch) can also be observed.

3.2. Optimization of MIP-µ-SPE conditions

3.2.1. Preliminary experiments.

Preliminary studies were performed mainly to find the most favorable pH (acid or alkaline) for target adsorption during the loading stage, and also the composition of the eluting solution for an efficient elution. These studies were performed using an MIP prepared with JWH-098 as a template. As previously reported for common cannabinoids using MIP-µSPE [22], retention is improved when buffering the sample at acid pHs; whereas, elution is more efficient at alkaline pHs. Therefore, drug-free urine (1 mL) spiked with all targets (10 μL^{-1} each) and buffered at pH 6.0 were subjected to MIP-µ-SPE under non-optimized conditions. The literature regarding LLE shows the effectiveness of an organic solvent such as hexane/ethyl acetate (7:1) [4], ether/n-hexane/ethyl acetate (7:1:2) [9], chlorobutane/2-propanol (7:3) [8], and chlorobutane [6]. Based on these proposals, less toxic solvents such as heptane and 2-propanol have been selected for eluting the retained SCs. In addition, since SCs are retained onto the MIP particles at acid pHs, the eluting solution was alkalinized by using a low proportion of ammonium hydroxide. Therefore, elution was first performed using (75:20:5) heptane/2-propanol/ammonium hydroxide (pH 8.0) as eluting solution. As with

other developments based on MIP-µ-SPE, analyte desorption was found to be enhanced by assisting the elution with ultrasounds.

3.2.2. Effect of urine pH on loading conditions. MIPs comparison

First, the influence of urine pH (5.0, 6.0, 7.0 and 8.0, fixed with variable volumes of 0.1 M/0.1M KH_2PO_4/NaOH buffer solutions at each pH) on target retention was evaluated using drug-free urine samples (1.0 mL) spiked with 10 µg L^{-1} of each analyte and deuterated analogues, and using MIP synthesized with JWH-098 as a template. Other loading variables were fixed at 200 rpm for the orbital-horizontal stirring speed, 25°C for temperature, and 10 min for loading time; whereas elution was performed by sonication with 2 mL of heptane/2-propanol/ammonium hydroxide (75:20:5) for 10 min. As shown in Figure 2 (experiments in duplicate), analyte retention for all synthetic cannabinoids was favored at acid pHs (the highest analytical recoveries in Figure 2), thus a pH of 5.0 was finally chosen.

After selecting a pH of 5.0 for buffering urine samples before loading with MIP JWH-098, a set of experiments (urine pH fixed at 5.0, and other variables as shown above) was performed with all prepared MIPs, and also with NIPs. Figure 3 shows results for synthetic cannabinoids retention when using MIPs derived from JWH-015, JWH-098, and JWH-007 as templates (and also NIPs). It can be seen that the best retention properties for all synthetic cannabinoids is achieved with the MIP prepared when using JWH-015 as a template. The better retention/recognition properties of MIP-based JWH015 can be attributed to the smaller particle size of the MIP beads than in the case of MIP-based JWH007 and MIP-based JWH098 (SEM images from Figure S1, supplementary data). Negligible responses were obtained when using NIPs for all cases. These findings show that interaction between synthetic cannabinoids with MIP particles occurs through the generated recognition cavities. Further experiments have been therefore performed using the MIP prepared with JWH-015.

3.2.3. Effect of shaking speed and time on loading conditions

The effect of the loading time (Figure 4a) was evaluated by fixing the remaining variables at convenient values (pH 5.0, 25°C, and 200 rpm for shaking speed; and sonication with 2 mL of 75:20:5heptane/2-propanol/ammonium hydroxide for 10 min for elution). Analyte retention was gradually enhanced from 4 to 8 min; however, a slight decrease in target retention was observed when using longer extraction times. Therefore, a loading time of 4 min was finally selected.

After fixing the loading time at a low value (4 min), the effect of the shaking speed on analyte retention (Figure 4b) shows that the highest analytical recoveries were obtained when using low stirring speeds, thus 100 rpm was selected. These findings are quite similar to those previously reported when using porous-membrane – protected μ-SPE [17,18,22] and also when using SPE procedures based on magnetic MIPs [19,20], hollow-fiber microextraction [25], and electro-mediated microextraction [26]. This is attributed to back-diffusion phenomena (high speeds and long contact time between MIP particles and analytes appear to promote back-diffusion phenomena).

3.2.4. Elution conditions

Elution conditions were studied using heptane/2-propanol/ammonium hydroxide as an eluting solution along with ultrasounds assistance. Variables affecting the loading stage were fixed at the convenient values reported above, and the effect of the percentage of ammonium hydroxide in the eluting solution was first tested. As shown in Figure 5a, higher target recoveries were obtained when using low ammonium hydroxide percentages (2 and 5%); whereas, elution worsened when increasing the ammonium hydroxide percentage (and also eluting solution pH) above 5%. Variations in the heptane/2-propanol proportion did not lead to significant changes in the target recoveries, and the best performances were therefore established using 75:20:5heptane/2-propanol/ammonium hydroxide (pH of 12.5).

Finally, the effect of the elution time (sonication time) is plotted in Figure 5b. Times higher than 4 min were needed for eluting most of the synthetic cannabinoids, and an eluting time of 8 min was finally selected. Short times for elution, as well as for loading, imply fast adsorption/desorption kinetics, which results appealing for performing the sample pre-treatment in short times. Optimized eluting conditions have led to analytical recoveries close to 100% for the thirty SCs. Improvements (high analytical recoveries) were mainly obtained for JWH019, JWH98, JWH122, and JWH210, SCs which showed poor recoveries at the beginning of the optimization (Figures 2, 3, and 4).

3.3. Cross-reactivity and imprinting effect

Recognition capacities (imprinting effect and selectivity) of the prepared material were evaluated through several parameters such as extraction efficiency (analytical recovery), distribution ratio (D) and selectivity coefficient ($S_{JWH015/D}$), defined as shown in Table 3. A solution (1 mL) containing all synthetic cannabinoids at a concentration level of 10 µg L^{-1}, as well as other substances such as natural cannabinoids and metabolites (Δ^9-THC-COOH, and Δ^9-THC-OH), cocaine and metabolites, opioids, amphetamines, and cathinones (also at 10 µg L^{-1}), was subjected to the MIP-µ-SPE procedure in triplicate. The same experiments were also performed using NIP instead of MIP. Results after HPLC-MS/MS assessment for synthetic cannabinoids (program optimized in this research), and also for other compounds (optimized HPLC-MS/MS not given) show analytical recoveries (extraction efficiencies) close to 100% for all synthetic cannabinoids, from 75% (JWH 203) to 90% (JWH 015). Regarding NIP experiments, extraction efficiencies within the 7–30 % range were obtained. This proves that SCs interact with the MIP recognition cavities of MIP particles, and pre-concentration is attributed to the imprinting effect of the MIP material.

Table 3 also lists high distribution ratios, and hence selectivity coefficients close to the unity for JWH-015 (template molecule), and also for other SCs. These findings imply that the

prepared material is selective for SCs (JWH and RSC classes). Regarding other drugs/metabolites, including natural cannabinoids (Δ^9-THC, CBN, and CBD) and metabolites(Δ^9-THC-COOH, and Δ^9-THC-OH), low extraction efficiencies were obtained (extraction efficiency lower than 20 and 30% when using MIP, and NIP, respectively, Table 3). This implies that the interaction of these drugs with MIP particles (and also with NIP particles) occurs through surface adsorption. In general, high selectivity coefficients were obtained for other drugs/metabolites (Table 3). We can thus conclude that MIP offers imprinting properties (and is highly selective) for SCs.

The developed porous membrane-protected MIP-μ-SPE demonstrated to efficiently extract all the SCs studied, pertaining to the classes of benzoylindoles, naphtoyl indoles, phenylacetyl indoles, including those with a slightly different chemical structure, such as AKB 48, a second-generation SC that shares an adamantly indazole structure.

This is of fundamental importance considering the variability of the SC market, as new compounds are continuously being synthesized and are appearing on the illicit market [1]. Preparative methods for their purification and pre-concentration from biological samples, coupled to sensitive analytical methods such as LC-MS/MS that give the possibility to screen for a huge variety of SCs, is therefore an invaluable analytical tool.

3.4. Method validation

Although the method has been developed for all SCs, validation (LODs, linearity, precision and analytical recovery) has been performed only on selected SCs due to their large number and similar structures.

3.4.1. Calibration. Matrix effect

Several external calibrations (n=4) and standard addition calibrations (n = 7) obtained in several days were used to check the existence of the matrix effect. Standard addition curves were prepared in duplicate as shown in section 2.8, using 1.0 mL of drug-free urine samples

346 spiked with analytes within the 5.0 – 20 µg L^{-1} range (concentrations ranging from 100 to
347 400 µg L^{-1} after pre-concentration). Table 4 lists the slopes as an average value ± standard
348 deviation. A significant decrease in slope calibrations was obtained when using the standard
349 addition technique with respect to external calibrations. The matrix effect is therefore
350 important, and analysis must be performed using the standard addition technique.

351 **3.4.2. Limit of detection/quantification**

352 The limit of detection (LOD) was established through the 3σ criterion (σ is the standard
353 deviation of eleven measurements of a blank), while the low limit of quantification (LLOQ),
354 and the limit of quantification (LOQ) were established through the 5σ and 10σ criterion [27],
355 respectively. Calculated LODs, LLOQ, and LOQs, referring to the original urine sample, are
356 listed in Table 4. Good sensitivity was achieved, LOQ from 0.106µg L^{-1} (JWH073 5OH) to
357 2.49µg L^{-1} (JWH019).

358 **3.4.3. Precision and accuracy**

359 Precision and analytical recovery (accuracy) were evaluated by intra-day and inter-day
360 assays. Intra-day precision and analytical recovery required the preparation of three standard
361 addition graphs (drug-free urine spiked with analytes from 0 to 20 µg L^{-1}) in three different
362 days. The first standard addition graph was obtained by replicating the lowest analyte
363 concentration level (5.0µg L^{-1}) seven times; whereas, the other analyte concentration levels
364 were replicated twice. Similarly, second and third standard addition calibrations were
365 obtained by replicating the intermediate (10 µg L^{-1}) and the highest (20 µg L^{-1}) analyte
366 concentration levels seven times (the remaining analyte concentration levels were replicated
367 twice). Table 5 shows that good intra-day precision (RSD values lower than 5%), and
368 analytical recovery (values within the 80-120% range) were assessed.

369 Similarly, inter-day precision was established by preparing seven standard addition
370 calibrations in seven different days (each analyte concentration level in duplicate). RSD

values lower than 8% (Table 6) allow us to conclude that good inter-day precision was achieved. In addition, analytical recoveries close to 100% (Table 6) were obtained, which implies good inter-day accuracy.

In addition to the high SCs recognition capacity offered by the prepared adsorbents, the developed MIP-µ-SPE has been found to be highly reliable (precise and accurate) and sensitive. These features make the proposed sample pre-treatment procedure appealing for assessing SCs, including as yet unknown SCs.

Conclusions

Several MIPs for SCs recognition have been synthesized for the first time and have been applied for selective SCs pre-concentration from urine samples. The developed porous membrane-protected MIP-µ-SPE has been shown to be a convenient, cost-effective and fast sample pre-treatment method because several MIP-µ-SPE devices can be used simultaneously (the capacity of the orbital-horizontal shaker allowed the simultaneous performance of at least twenty MIP-µ-SPE devices, increasing the throughput of the method when compared to MEPS and on-line SPE approaches). The conical MIP-µ-SPE proposed in the current development avoids heat-sealing damage, and hence loss of MIP particles, because the heat-seal in the conical µ-SPE device is not in contact with heptane during the elution stage. The PP membrane for containing MIP particles also acts as a barrier and prevents the interaction of large biomolecules from urine with the MIP sorbent. Therefore, urine samples can be directly subjected to the MIP-µ-SPE process, and previous dilution/protein separation/centrifugation stages are not required. After urine pH adjustment (5.0), SCs are selectively retained onto MIP particles enclosed in a PP membrane (the loading stage takes only 8 min). Retained analytes are easily released using heptane/2-propanol/ammonium hydroxide (75:20:5) as an eluent, under sonication (37 kHz) for 8 min.

The use of an MIP as an adsorbent for µ-SPE has shown to offer selective enrichment of SCs from urine samples. The high selectivity obtained together with a pre-concentration factor of 20 and the high sensitivity of HPLC-MS/MS, makes the proposed methods reliable and appealing methodologies for NPSs assessment in complex samples.

Acknowledgements

The authors wish to thank the *Dirección Xeral de I+D – Xunta de Galicia* (Project numbers 10CSA209042PR and 6RC2014/2017 Galician Competitive Research Group). These programs are co-funded by FEDER (EU). This study was developed in part within the framework of the project NEPRA – New Psychoactive Substances and Road Accidents funded by the Italian Antidrug Policies Department.

Table 1. HPLC gradient program

	Kinetex 2.6µ C18 100 Å reverse phase column (100 mm length × 2.10 mm i.d., 5.0 µm particle diameter)
Injection volume / µL	20
Mobile phase composition	Gradient: 0.1% formic acid in water(A); 0.1% formic acid in methanol (B)
Mobile phase composition gradient	0.0–1.0 min: 55 → 45 % A, 45 → 55 %B 1.0–4.0 min: 45 → 20% A, 55 → 80 %B 4.0–5.0 min: 20% A, 80 %B 5.0–6.0 min: 20 → 15% A, 80 → 85 %B 6.6–7.0 min: 15 → 10 % A, 85 → 90 %B 7.0–8.0 min: 10 → 5 % A, 90 → 95 %B 8.0–9.0 min: 5 → 0 % A, 95 → 100 %B 9.0–11.5 min: 0 % A, 100 %B 11.5–12.0 min: 0 → 10 % A, 100 → 90 %B
Mobile phase flow rate	0.40 mL min^{-1}

Table 2. ESI source operating conditions and acquisition MS/MS parameters

Compound	Precursor ion (m/z)	Product ion (m/z)	Dwell (V)	Fragmentor (V)	Collision energy (V)	Cell accelerator voltage (V)	Polarity
AKB48	366	135	10	135	20	7	positive
AKB48	366	107	10	135	50	7	positive
AKB48	366	93	10	135	60	7	positive
AM2201	360	155	10	135	10	7	positive
AM2201	360	232	10	135	10	7	positive
AM2201	360	127	10	135	10	7	positive
AM2233	459	112	10	135	10	7	positive
AM2233	459	362	10	135	10	7	positive
AM2233	459	98	10	135	10	7	positive
AM694	436	231	10	135	30	7	positive
AM694	436	292	10	135	30	7	positive
AM694	436	203	10	135	40	7	positive
CB13	369	155	10	135	10	7	positive
CB13	369	299	10	135	10	7	positive
CB13	369	171	10	135	10	7	positive
JWH007/JWH019	356	127	10	135	50	7	positive
JWH007/JWH019	356	228	10	135	20	7	positive
JWH007/JWH019	356	155	10	135	20	7	positive
JWH015/JWH073	328	127	10	135	50	7	positive
JWH015/JWH073	328	200	10	135	18	7	positive
JWH015/JWH073	328	155	10	135	22	7	positive
JWH018	342	127	10	135	55	7	positive
JWH018	342	155	10	135	10	7	positive
JWH018	342	144	10	135	45	7	positive
JWH018 4OH	358	127	10	130	55	7	positive
JWH018 4OH	358	155	10	130	25	7	positive
JWH018 4OH	358	230	10	130	30	7	positive
JWH018 NCOOH	372	155	10	130	25	7	positive

JWH018 NCOOH	372	144	10	130	40	7	positive
JWH018 NCOOH	372	127	10	130	60	7	positive
JWH018 5OH/JWH073 NCOOH	358	127	10	130	55	7	positive
JWH018 5OH/JWH073 NCOOH	358	155	10	130	25	7	positive
JWH018 5OH/JWH073 NCOOH	358	144	10	130	40	7	positive
JWH030	292	155	10	135	30	7	positive
JWH030	292	164	10	135	30	7	positive
JWH073 4OH	344	127	10	130	55	7	positive
JWH073 4OH	344	155	10	130	40	7	positive
JWH073 4OH	344	144	10	130	40	7	positive
JWH073 5 OH	344	127	10	130	55	7	positive
JWH073 5 OH	344	155	10	130	40	7	positive
JWH073 5 OH	344	216	10	130	25	7	positive
JWH081	372	185	10	135	20	7	positive
JWH081	372	214	10	135	20	7	positive
JWH081	372	144	10	135	45	7	positive
JWH098	386	185	10	135	20	7	positive
JWH098	386	228	10	135	20	7	positive
JWH098	386	157	10	135	50	7	positive
JWH122	356	169	10	135	18	7	positive
JWH122	356	214	10	135	18	7	positive
JWH122	356	141	10	135	18	7	positive
JWH147	382	127	10	135	50	7	positive
JWH147	382	254	10	135	20	7	positive
JWH147	382	155	10	135	50	7	positive
JWH302	336	121	10	135	20	7	positive
JWH302	336	214	10	135	20	7	positive
JWH302	336	188	10	135	10	7	positive
JWH250	336	121	10	135	20	7	posit

							ive
JWH250	336	130	10	135	38	7	posit ive
JWH250	336	200	10	135	18	7	posit ive
JWH203	340	125	10	135	40	7	posit ive
JWH203	340	214	10	135	30	7	posit ive
JWH203	340	144	10	135	40	7	posit ive
JWH210	370	214	10	135	30	7	posit ive
JWH210	370	183	10	135	25	7	posit ive
JWH210	370	144	10	135	45	7	posit ive
JWH250 5OH	352	121	10	135	25	7	posit ive
JWH250 5OH	352	186	10	135	10	7	posit ive
JWH250 5OH	352	91	10	135	55	7	posit ive
JWH251	320	105	10	135	20	7	posit ive
JWH251	320	214	10	135	20	7	posit ive
JWH251	320	144	10	135	45	7	posit ive
JWH307	386	127	10	135	50	7	posit ive
JWH307	386	258	10	135	10	7	posit ive
JWH307	386	155	10	135	10	7	posit ive
JWH398	376	189	10	135	20	7	posit ive
JWH398	376	214	10	135	20	7	posit ive
JWH398	376	161	10	135	20	7	posit ive
RCS4	322	135	10	135	50	7	posit ive
RCS4	322	107	10	135	50	7	posit ive
RCS4	322	92	10	135	50	7	posit ive
RCS8	377	91	10	135	50	7	posit ive
RCS8	377	144	10	135	50	7	posit ive
RCS8	377	121	10	135	50	7	posit ive
JWH018 NCOOH D_4	376	155	10	135	50	7	posit ive
JWH018 NCOOH D_4	376	127	10	135	50	7	posit ive
JWH073 NCOOH D_5	363	155	10	135	50	7	posit ive
JWH073 NCOOH	363	127	10	135	50	7	posit

D_5							ive
JWH210 D_9	379	223	10	135	20	7	posit ive
JWH210 D_9	379	183	10	135	20	7	posit ive

Electrospray operating conditions: gas temperature, 350°C; nebulizer gas (N_2), 40 psi; sheath gas temperature, 400°C; sheath gas flow, 12 L min^{-1}; capillary positive voltage, 4000 V; nozzle positive voltage, 2000 V.

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417 **Table 3**. Extraction efficiency (%), distribution ratios (D) and selectivity coefficients (S_{JWH}

418 $_{015}/_{D}$) of for MIP-µ-SPE and NIP-µ-SPE.

	MIP-µ-SPE		
	Extraction efficiency (%)[a]	Distribution ratio (D)[b]	Selectivity coefficient (S_{JWH} $_{015}/_{D}$)[c]
JWH015	90	9.37	---
JWH073 5OH	83	5.04	1.9
JWH203	75	3.27	3.1
JWH210	80	3.98	2.4
RCS4	85	5.66	1.7
RCS8	77	3.68	2.8
JWH122	80	4.14	2.4
JWH019	78	3.75	2.7
AM2201	75	3.02	3.2
JWH073	85	6.04	1.7
JWH073 COOH	81	5.71	2.4
JWH018 4OH	83	5.58	2.0
JWH302	81	5.02	2.3
JWH250 5OH	75	3.27	2.9
JWH098	87	8.71	1.5
JWH018	81	5.09	2.3
JWH250	86	4.04	2.3
COC	14	0.16	59.4
CE	13	0.15	64.2
EME	3	0.03	227
BEC	17	0.20	47.0
Δ^9-THC	9	0.10	98.5
Δ^9-THC-OH	11	0.12	79.4
Δ^9-THC-COOH	17	0.21	45.8
CBN	19	0.24	40.0

CBD	10	0.11	86.2
COD	11	0.13	82.1
MORF	6	0.06	175
O-6-MAM	19	0.24	39.9
4-MMC	28	0.40	23.7
BUP	16	0.18	51.6
Ethylone	17	0.21	45.4
Cathinone	15	0.18	53.0
3,4-DMMC	14	0.17	60.8
MDPV	11	0.12	78.4
Pentylone	16	0.20	48.2
Flephedrone	8	0.09	109
Methylone	12	0.14	70.9
Naphyrone	20	0.25	37.9
Methcathinone	11	0.13	77.5
Mephedrone	19	0.24	39.9
Butylone	15	0.17	55.4
MDEA	3	0.03	276
MDA	1	0.01	750
MDMA	1	0.01	873
MA	2	0.02	453
A	2	0.03	373
MBDB	3	0.03	369
		NIP-μ-SPE	
JWH015	13	0.31	---
JWH073 5OH	7	0.07	134
JWH203	30	0.45	24.5
JWH210	21	0.26	36.0
RCS4	22	0.28	35.1
RCS8	17	0.21	51.7
JWH122	11	0.13	97.5
JWH019	22	0.42	33.0

AM2201	16	0.19	51.7
JWH073	17	0.21	65.0
JWH073 COOH	31	0.47	21.3
JWH018 4OH	16	0.21	59.1
JWH302	30	0.43	23.6
JWH250 5OH	22	0.29	36.6
JWH098	20	0.33	75.0
JWH018	19	0.24	55.4
JWH250	16	0.20	74.3
COC	15	0.17	59.4
CE	16	0.20	49.3
EME	7	0.08	121
BEC	15	0.18	54.3
Δ^9-THC	7	0.08	139
Δ^9-THC-OH	24	0.34	35.4
Δ^9-THC-COOH	16	0.19	51.2
CBN	22	0.29	32.9
CBD	14	0.16	62.2
COD	13	0.15	63.4
MORF	13	0.15	66.5
O-6-MAM	18	0.22	43.2
4-MMC	18	0.21	44.0
BUP	14	0.16	61.0
Ethylone	11	0.12	85.3
Cathinone	11	0.12	89.7
3,4-DMMC	19	0.24	43.8
MDPV	14	0.16	60.3
Pentylone	16	0.20	54.8
Flephedrone	27	0.38	25.8
Methylone	9	0.11	99.0
Naphyrone	20	0.26	38.9

Methcathinone			
e	17	0.20	47.5
Mephedrone	27	0.37	27.7
Butylone	14	0.16	60.4
MDEA	3	0.04	265
MDA	3	0.03	301
MDMA	1	0.01	934
MA	1	0.02	700
A	6	0.06	209
MBDB	1	0.01	895

(a) $\% = (A_2 / A_T) \times 100$

(b) $D = (A_2 / A_1)$

(c) $S_{JWH\ 015}/_D = D_{JWH\ 015} / D_D$

A_1 = Amount of analyte in aqueous solution at equilibrium

A_2 = Amount of analyte enriched by magnetic MIP/NIP at equilibrium.

A_T = Total amount of analyteused in extraction.

$D_{JWH\ 015}$ = Distribution ratio for JWH 015 (template)

D_D = Distribution ratio for D (D = other SCs (different to the template) and other drugs/metabolites

Table 4. Mean slopes of calibration and standard addition, and LOD, LLOQ, and LOQ values

	Slope (mean ± SD)				
	Calibration[a]	Standard addition[b]	LOD (µg L^{-1}) [c]	LLOQ (µg L^{-1})[c]	LOQ (µg L^{-1}) [c]
AM2201	0.595±0.0222	1.02±0.789	0.356	0.593	1.19
AM2233	1.42±0.403	0.132±0.0736	0.0800	0.132	0.264
JWH015	44.3±4.91	3.06±1.14	0.212	0.354	0.708
JWH018	-----	2.20±1.02	0.442	0.738	1.48
JWH018 4OH	6.19±0.838	6.32±2.12	0.260	0.434	0.868
JWH019	0.396±0.0102	1.00±0.780	0.748	1.25	2.50
JWH073	16.7±2.80	0.562±0.252	0.032	0.0533	0.107
JWH 073 COOH	24.7±3.37	8.54±2.57	0.288	0.480	0.960
JWH073 5OH	10.6±1.65	0.917±0.328	0.0360	0.0600	0.120
JWH081	0.416±0.0607	3.63±1.97	0.624	1.04	2.08
JWH098	33.6±5.77	1.87±0.999	0.216	0.360	0.720
JWH122	0.558±0.0336	1.61±0.833	0.324	0.540	1.08
JWH203	16.2±2.84	1.75±1.01	0.360	0.600	1.20
JWH210	8.76±2.23	0.432±0.254	0.318	0.530	1.06
JWH250	0.501±0.0290	0.745±0.484	0.360	0.600	1.20
JWH250 5OH	8.56±1.66	1.66±0.820	0.0540	0.0900	0.180
JWH302	15.8±3.78	3.85±1.33	0.466	0.777	1.55
RCS-4	38.9±6.13	3.28±1.17	0.0740	0.123	0.246
RCS-8	19.3±4.27	0.964±0.422	0.664	1.11	2.22

(a) n=4; (b) n=7; (c) pre-concentration factor of 20

428 Table 5. Intra–day precision and intra-day analytical recovery (AR/%) of the method

	Low level (5.0 µg L^{-1})[a]		Intermediate level (10 µg L^{-1})[a]		High level (20 µg L^{-1})[a]	
	RSD (%)	AR (%)	RSD (%)	AR (%)	RSD (%)	AR (%)
AM2201	6	94±6	3	99±3	2	100±2
AM2233	6	98±5	2	99±2	1	99±1
JWH015	6	95±6	4	99±4	2	100±2
JWH018	5	94±5	1	99±2	1	99±2
JWH018 4OH	3	100±3	2	97±2	2	97±2
JWH019	4	97±4	4	99±4	2	99±2
JWH073	5	95±5	2	99±2	1	100±1
JWH073 COOH	4	98±4	4	96±4	1	100±1
JWH073 5OH	4	94±4	3	95±3	2	100±2
JWH081	6	94±6	5	97±4	3	99±3
JWH098	5	94±5	2	98±2	1	100±1
JWH122	4	95±4	3	98±3	1	99±2
JWH203	5	92±5	3	98±3	2	99±2
JWH210	5	99±5	4	98±4	4	99±3
JWH250	4	92±4	3	100±3	1	99±1
JWH250 5OH	3	96±3	2	98±2	2	100±1
JWH302	5	95±4	3	97±3	1	100±2
RCS-4	4	94±4	3	99±3	1	100±1
RCS-8	5	91±5	3	99±3	1	100±2

(a) n=7

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Table 6. Inter–day precision and inter-day analytical recovery (AR/%) of the method

	Low level (5.0 µg L^{-1})[a]		Intermediate level (10 µg L^{-1})[a]		High level (20 µg L^{-1})[a]	
	RSD (%)	AR (%)	RSD (%)	AR (%)	RSD (%)	AR (%)
AM2201	6	97±4	3	93±5	2	88±3
AM2233	8	95±8	4	93±4	2	88±2
JWH015	7	87±6	4	91±4	3	85±3
JWH018	7	88±7	4	97±4	2	89±1
JWH018 4OH	4	87±4	4	92±4	2	96±3
JWH019	5	92±4	3	97±3	4	90±4
JWH073	6	86±5	5	86±4	4	93±5
JWH073 COOH	5	88±4	3	93±3	4	96±4
JWH073 5OH	8	90±8	4	91±4	5	96±5
JWH081	7	93±6	6	93±5	5	97±5
JWH098	7	88±7	5	90±5	3	90±3
JWH122	4	90±4	4	95±4	4	91±4
JWH203	7	89±7	5	90±4	3	91±3
JWH210	5	97±5	4	98±4	4	93±3
JWH250	7	93±7	3	100±2	2	90±2
JWH250 5OH	7	91±6	5	89±4	5	100±5
JWH302	6	92±5	4	97±4	4	93±3
RCS-4	6	89±6	4	92±4	3	92±3
RCS-8	8	87±8	4	94±4	3	91±4

(a) n=7

Figure captions

Figure 1. MRM chromatogram for a drug-free urine sample spiked with 10 µg L^{-1} of selected SCs.

Figure 2. Effect of urine sample pH on the analytical recovery of SCs (use of MIP-based JWH098).

Figure 3. Analytical recovery of SCs obtained with MIP-based JWH007, MIP-based JWH015, MIP-based JWH098, and NIP.

Figure 4. Effect of the loading time (a), and orbital-horizontal shaking speed (b) on the analytical recovery of SCs (MIP-based JWH015).

Figure 5. Effect of NH_4OH percentage in the eluting solution (a), and the eluting (sonication) time (b) on the analytical recovery of SCs (MIP-based JWH015).

Figure 1

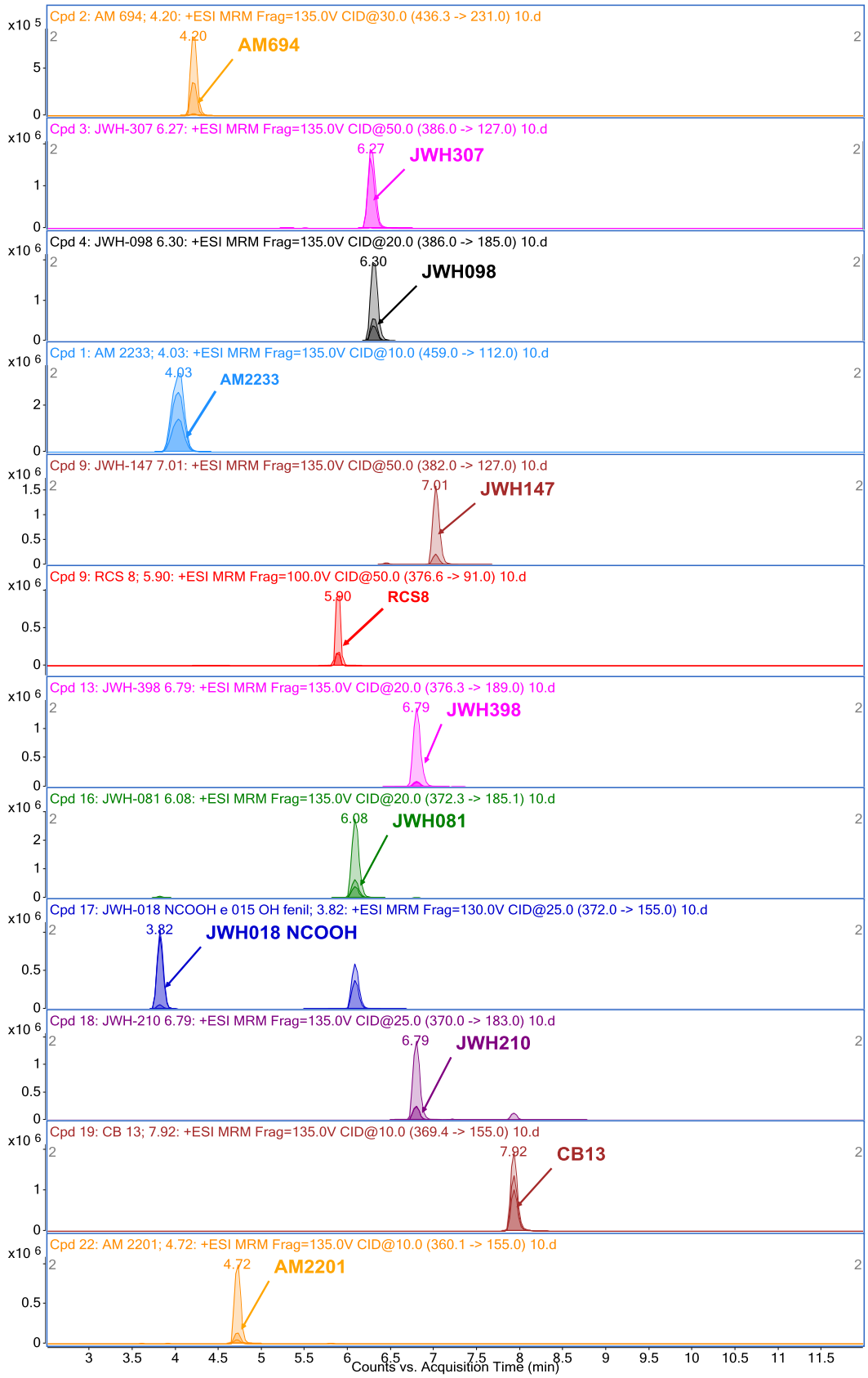
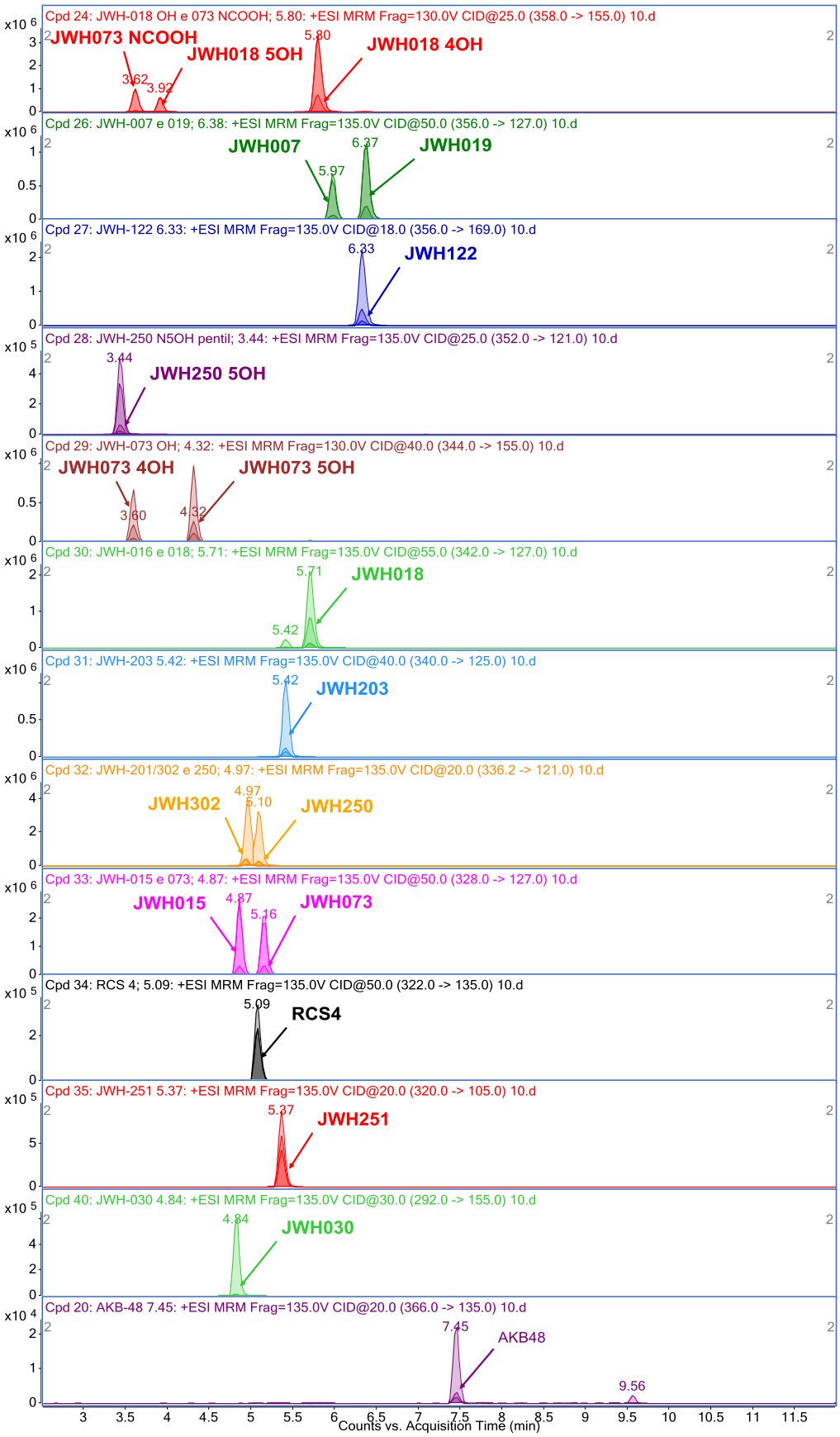
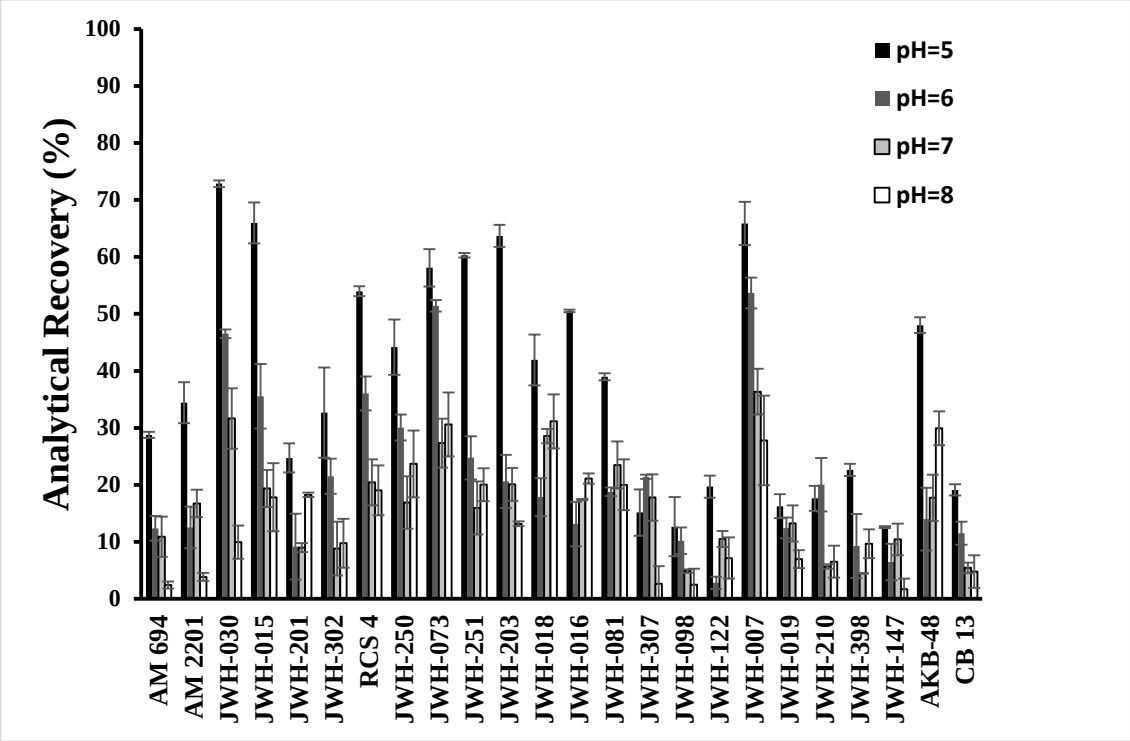

Cpd 2: AM 694; 4.20: +ESI MRM Frag=135.0V CID@30.0 (436.3 -> 231.0) 10.d
4.20
AM694
Cpd 3: JWH-307 6.27: +ESI MRM Frag=135.0V CID@50.0 (386.0 -> 127.0) 10.d
6.27
JWH307
Cpd 4: JWH-098 6.30: +ESI MRM Frag=135.0V CID@20.0 (386.0 -> 185.0) 10.d
6.30
JWH098
Cpd 1: AM 2233; 4.03: +ESI MRM Frag=135.0V CID@10.0 (459.0 -> 112.0) 10.d
4.03
AM2233
Cpd 9: JWH-147 7.01: +ESI MRM Frag=135.0V CID@50.0 (382.0 -> 127.0) 10.d
7.01
JWH147
Cpd 9: RCS 8; 5.90: +ESI MRM Frag=100.0V CID@50.0 (376.6 -> 91.0) 10.d
5.90
RCS8
Cpd 13: JWH-398 6.79: +ESI MRM Frag=135.0V CID@20.0 (376.3 -> 189.0) 10.d
6.79
JWH398
Cpd 16: JWH-081 6.08: +ESI MRM Frag=135.0V CID@20.0 (372.3 -> 185.1) 10.d
6.08
JWH081
Cpd 17: JWH-018 NCOOH e 015 OH fenil; 3.82: +ESI MRM Frag=130.0V CID@25.0 (372.0 -> 155.0) 10.d
3.82
JWH018 NCOOH
Cpd 18: JWH-210 6.79: +ESI MRM Frag=135.0V CID@25.0 (370.0 -> 183.0) 10.d
6.79
JWH210
Cpd 19: CB 13; 7.92: +ESI MRM Frag=135.0V CID@10.0 (369.4 -> 155.0) 10.d
7.92
CB13
Cpd 22: AM 2201; 4.72: +ESI MRM Frag=135.0V CID@10.0 (360.1 -> 155.0) 10.d
4.72
AM2201
Counts vs. Acquisition Time (min)

Figure 1 (Cont.)



458 **Figure 2**



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462 **Figure 3**

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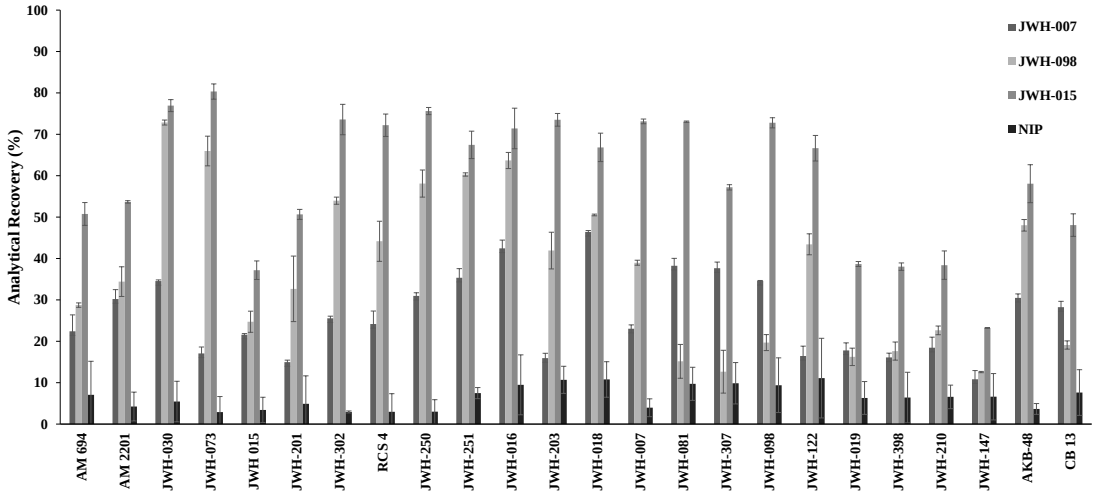

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467 **Figure 4**

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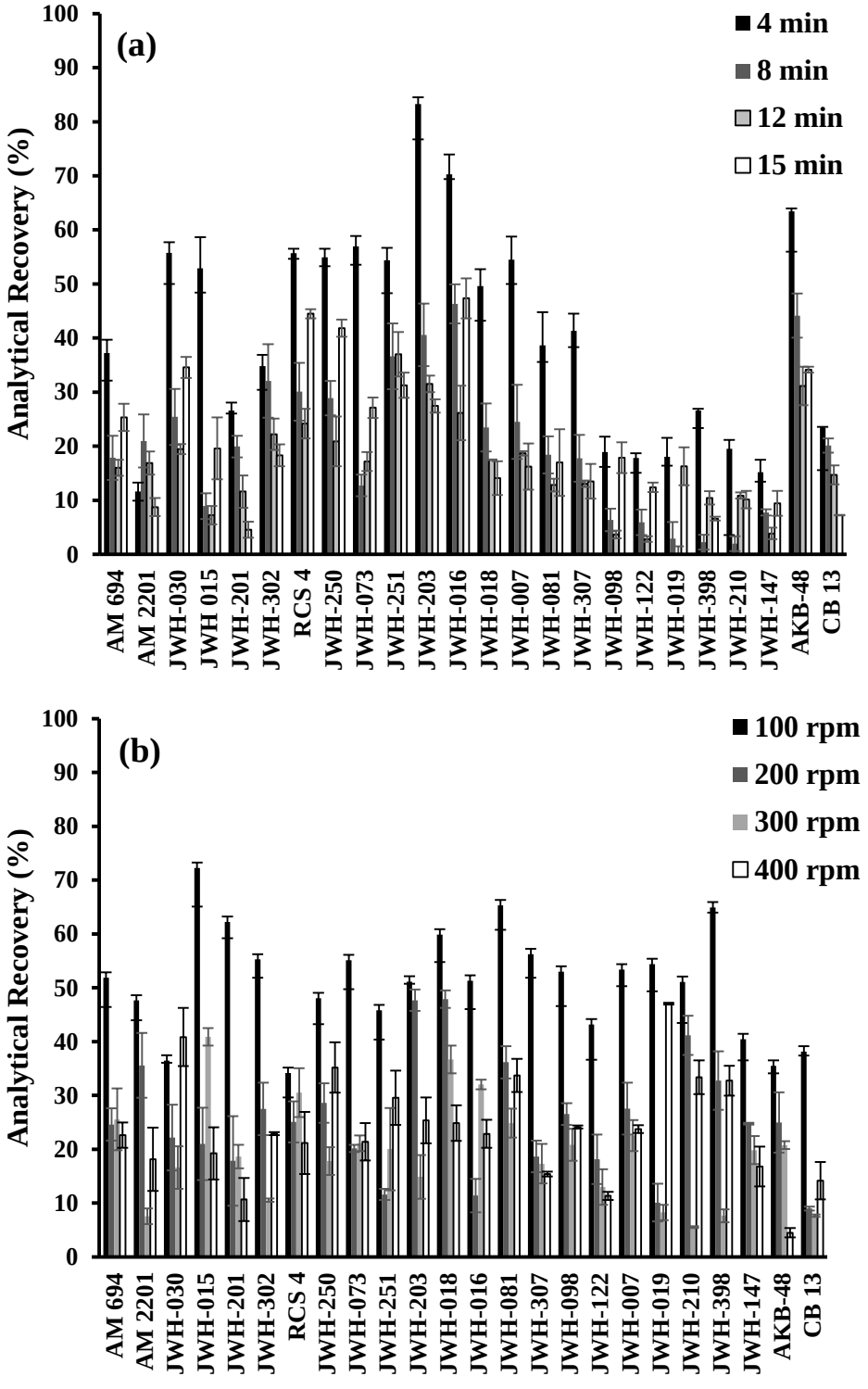

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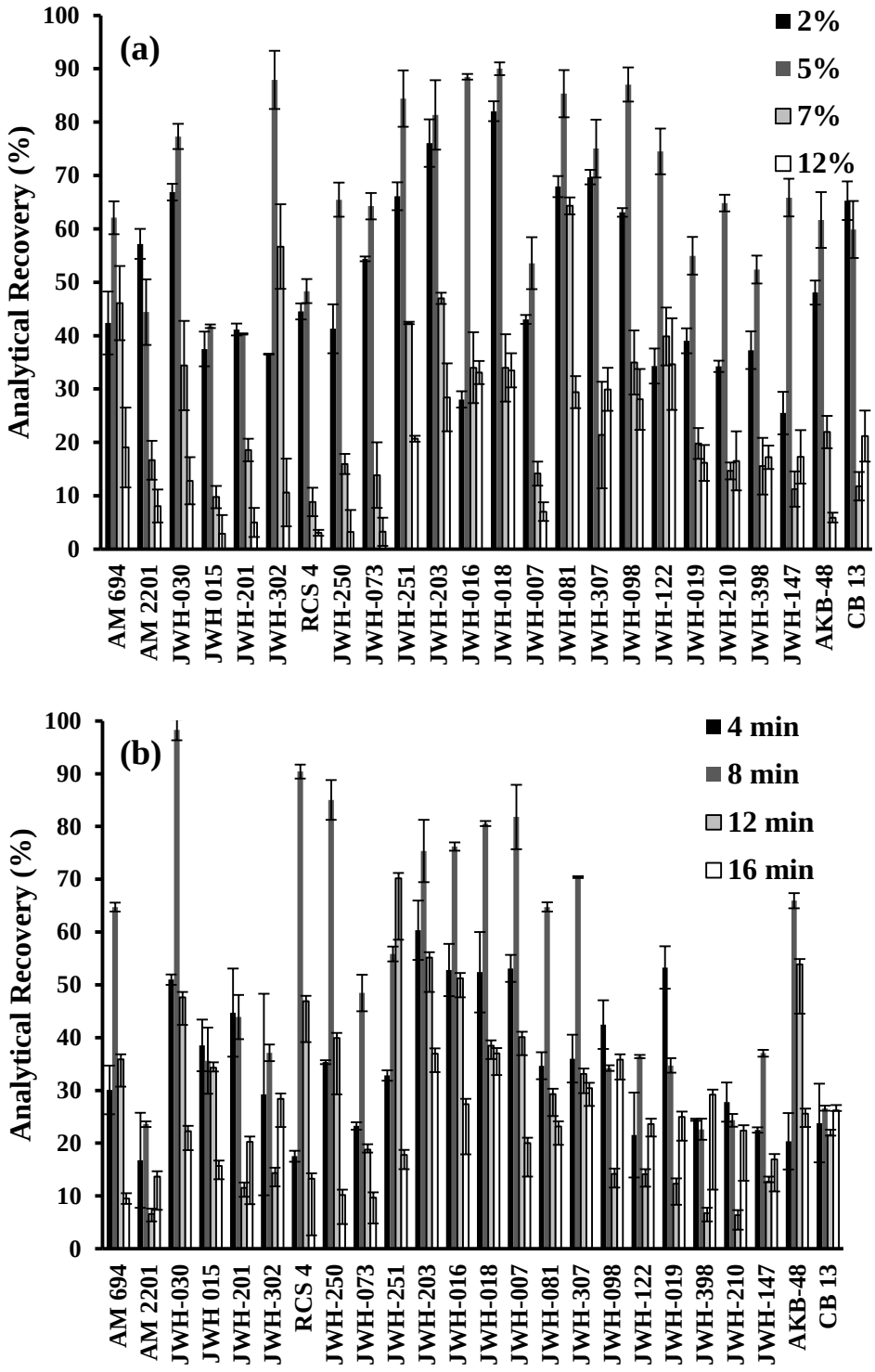
471

Figure 5


(a)
2%
5%
7%
12%
Analytical Recovery (%)
100
90
80
70
60
50
40
30
20
10
0
AM 694
AM 2201
JWH-030
JWH 015
JWH-201
JWH-302
RCS 4
JWH-250
JWH-073
JWH-251
JWH-203
JWH-016
JWH-018
JWH-007
JWH-081
JWH-307
JWH-098
JWH-122
JWH-019
JWH-210
JWH-398
JWH-147
AKB-48
CB 13
(b)
4 min
8 min
12 min
16 min
Analytical Recovery (%)

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