Toxicology Reports 5 (2018) 65–75

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Lateral flow immunoassay and enzyme linked immunosorbent assay as effective immunomethods for the detection of synthetic cannabinoid JWH-200 based on the newly synthesized hapten

Lucie Fojtíková[a,1,*], Anna Šuláková[b,1], Martina Blažková[a], Barbora Holubová[a], Martin Kuchař[b], Petra Mikšátková[b], Oldřich Lapčík[b], Ladislav Fukal[a]

[a] Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, Czech Republic

[b] Department of Chemistry of Natural Compounds, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, Czech Republic

ARTICLE INFO

Keywords:
Hapten synthesis
Synthetic cannabinoid
JWH-200
Immunomethods
ELISA
LFIA

ABSTRACT

In recent years, the use of synthetic cannabinoids (SCs) as drugs of abuse has greatly increased. SCs are associated with a risk of severe poisoning or even death. Therefore, more rapid, cost effective and reliable methods are needed, especially for the screening of drivers after traffic accidents and for detailed toxicological analysis in forensic laboratories. In this study, we developed a lateral flow immunoassay (LFIA) and an enzyme linked immunosorbent assay (ELISA) for the detection of JWH-200 in oral fluids. For this purpose a new hapten was prepared using a ten-step synthetic route. The developed immuno methods are based on antibodies obtained from rabbit immunized with synthesized hapten conjugated to carrier protein. The proposed methods are highly sensitive ($LOD_{LFIA} = 0.08 \pm 0.04\,ng\,mL^{-1}$; $LOD_{ELISA} = 0.04 \pm 0.02\,ng\,mL^{-1}$). They were applied to the quantification of JHW-200 in spiked oral fluids. The recoveries ranged from 82 to 134% for both methods. The results correlated excellently with results obtained using UHPLC–MS/MS ($R^2_{LFIA} = 0.99$; $R^2_{ELISA} = 0.99$). Our developed methods could be an important tool for analyses of JWH-200 in human oral fluids. The one-step LFIA is particularly suitable for roadside and on-site monitoring due to the rapid qualitative results it delivers, while the ELISA is especially useful for laboratory quantitative analyses of positive samples captured by LFIA.

1. Introduction

The frequent appearance of new psychoactive substances (NPS) as drugs of abuse is a matter of concern with the public. Synthetic cannabinoids (SCs) are the largest group of NPS monitored in Europe by the Early Warning System. These substances – commonly called 'Spice' are sold as 'legal' alternative to cannabis and may be marketed as 'herbal incense blends' or 'herbal mixtures' and usually labelled 'not for human consumption' in order to circumvent consumer protection and the law [1]. Their easy accessibility (especially via online shops), and impossible detectability using routine screening tests for cannabis contribute to an expansion in their abuse.

There have been numerous reports that abuse of SCs can cause a wide range of serious harms to human health (acute ischemic stroke, kidney damage, pulmonary and cardiovascular effects, and psychiatric symptoms) [1–6]. Therefore, development of simple methods that could be used for rapid determination of SCs is needed.

JWH-200, systematically named 1-[2-(morpholin-4-yl)ethyl]-3-(naphthalene-1-carbonyl)-1H-indole, is considered to be one of the most widely known SCs. A seizure of Spice adulterated with JWH-200 was first reported in 2009 by Europol but the drug soon spread out throughout Europe, North America and Japan [5,7]. JWH-200 became one of few SCs added to the list of controlled substances. That resulted in the great interest of state authorities to develop an effective analysis of this substance intended for use in the field.

Current methods used for the analysis of SCs in human fluids are

Abbreviations: BSA, bovine serum albumin; DCC, *N,N'*-dicyclohexylcarbodiimide; DIBAH, diisobutylaluminium hydride; DMF, *N,N*-dimethylformamide; ELISA, enzyme-linked immunosorbent assay; GAR, goat anti-rabbit antibody; GAR-Po, peroxidase labelled goat anti-rabbit antibody; LFIA, lateral flow immunoassay; LOD, limit of detection; NBS, *N*-bromosuccinimide; NHS, *N*-hydroxysuccinimide; NPS, new psychoactive substances; PEG, polyethylene glycol; RSA, rabbit serum albumin; RSD, relative standard deviation; SCs, synthetic cannabinoids; THC, thin layer chromatography

* Corresponding author.
E-mail address: lucie.fojtikova@vscht.cz (L. Fojtíková).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.toxrep.2017.12.004
Received 28 June 2017; Received in revised form 23 November 2017; Accepted 4 December 2017
Available online 06 December 2017

mainly based on high performance liquid chromatography or gas chromatography coupled to various selective detectors [8,9]. However, these techniques are relatively demanding with respect to costs, sample preparation, analysis times, and highly trained personnel and are unsuitable for screening analysis. On the other side, immunoassays provide an attractive alternative for rapid screening of samples. These days, enzyme-linked immunosorbent assay (ELISA) carried out in a microtiter plate is the most common technique used for immunoassays. The possibility of analysing liquid samples without any purification is one of the most outstanding advantages the immunoassays have over commonly used instrumental methods. ELISA has been successfully applied for the analysis of selected SCs mainly in urine [9–14]. Its main advantages are the possibility to analyse multiple samples simultaneously, sensitivity and the relative simplicity. However, the performance of the necessary operations including repeated incubation and washing steps and enzyme reaction for final signal generation is laborious for laboratories that are not specialized for this process. Lateral flow immunoassay (LFIA) is considered to be one of the simplest methods, which fits perfectly for on-site and roadside monitoring [15]. It combines several benefits including primarily rapidity, user-friendly format and cost-effectiveness [16,17]. On the other hand, LFIA gives only preliminary results, so it could be useful to have both, rapid and simple LFIA and also a sensitive method for quantification such as ELISA.

Herein we report the synthesis of a new hapten structurally derived from JWH-200 itself which will serve for the development of immunomethods. To the best of our knowledge, this is the first hapten bearing 1-[2-(morpholin-4-yl)ethyl]-1*H*-indole moiety used as an immunogen precursor in the detection of synthetic cannabinoids. The aims of the study are to provide the sensitive LFIA that would be as simple as possible to be applied by the state authorities for rapid roadside and on-site monitoring of JWH-200 in oral fluids and ELISA for toxicological quantitative analyses of positive samples captured by LFIA.

2. Material and methods

2.1. Material and reagents

Bovine serum albumin (BSA), rabbit serum albumin (RSA), Tween 20, polyethylene glycol (PEG), Triton X-100, *N*-hydroxysuccinimide (NHS), indole, naphthalen-1-ol, 4-(2-hydroxyethyl) morpholine, benzyl bromide, methanesulfonyl chloride, *N*-bromosuccinimide (NBS), ammonium formate, 10% palladium on carbon, sodium hydride, copper cyanide, silver nitrate and zirconium tetrachloride were purchased from Sigma-Aldrich Inc., USA. Ethyl 2-bromoacetate was obtained from Merck and *N*,*N*'-dicyclohexylcarbodiimide (DCC) was obtained from Fluka. Diisobutylaluminium hydride (DIBAH) solution in hexane, oxalyl chloride and *N*,*N*-dimethylformamide (DMF) extra dry were purchased from Acros. All the other solvents were obtained from Penta. Thin layer chromatography (TLC) was performed on Merck aluminium backed sheets coated with 60F 254 silica gel. Artificial saliva (1700-0305) was purchased from Pickering Laboratories, USA. Gold colloid nanoparticles (an average diameter of 40 nm) were purchased from BB international, UK. Goat anti-rabbit antibody (GAR) and peroxidase labelled goat anti-rabbit antibody (GAR-Po) were obtained from Nordic Immunological Laboratories, Netherlands. Nitrocellulose membranes (PRIMA 85; AE 99; AE 100) were supplied from Whatman GmbH, Germany. Other nitrocellulose membranes (HiFlow Plus HF135; HiFlow Plus HFB180), laminated card (HF000MC100), glass fiber conjugate pad (CFCP03000), cellulose fiber sample pad (CFSP173000) and absorbent pad (CFSP) were purchased from Millipore Corp., USA.

JWH-200 standard and all of the other drug standards used for cross-reactivity studies (Table 2) were obtained from Alfarma s.r.o., Czech Republic or Cayman Pharma, Czech Republic. Individual stock standard solutions containing 1 mg mL^{-1} of each compound were prepared by dissolving accurately weighted amounts in 96% ethanol and stored at −20 °C.

96-well polystyrene microtiter plates Costar 9018 were purchased from Corning Inc., USA.

2.2. Instrumentation

NMR spectra were recorded on a Varian Gemini 300 (300 MHz for ^{1}H; 75 MHz for ^{13}C) or Agilent 400-MR DD2 (400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometers. High resolution mass spectra were measured on a LTQ Orbitrap XL (Thermo Fischer Scientific) spectrometer using ESI ionization technique. Mass spectra of hapten-protein conjugates were measured on a Bruker Autoflex Speed MALDI-TOF/TOF spectrometer. Automated reverse phase chromatography was carried out using a CombiFlash Rf 200 apparatus (Teledyne ISCO) with prepacked Redisep Rf Gold C18 columns (packed with C18-reverse phase silica gel).

Microplate reader uQuant BIO-TEK was from Inc. Winooski, USA. Linomat V (Camag AG, Switzerland) and a strip cutter (Economic Cutter ZQ2000, Shanghai Kinbio Tech Co., China) were used for the preparation of immunostrips.

2.3. Buffers and solutions

2.3.1. LFIA buffers

Coating buffer (0.01 M carbonate/bicarbonate buffer pH 9.6); assay buffer (0.1 M borate buffer pH 8.8 containing Triton X-100 (1% v/v); conjugate pad buffer (0.2 M borate buffer pH 8.8 containing BSA (1% w/v); sucrose (1% w/v) and Tween 20 (1%)).

2.3.2. ELISA buffers

Coating buffer (0.01 M carbonate/bicarbonate buffer pH 9.6); assay buffer (0.01 M phosphate buffered saline (PBS), pH 7.4); wash buffer (0.01 M PBS pH 7.4 containing Tween 20 (0.05% v/v)); substrate solution for enzyme (9 mL 0.05 M citrate/phosphate buffer pH 5.0, 1 mg of TMB, 1 mL of DMSO, and 2 µL of 30% H_2O_2 (v/v)); stopping solution (2 M sulphuric acid in distilled water).

2.4. Synthesis of hapten (derivative of JWH-200)

The structure of the hapten was derived from JWH-200. The synthesis of the hapten bearing the linker with carboxylic functional group in the position 4 of the naphthalene ring was proposed and carried out (Fig. 1). Spectral data of intermediates and the final product are provided in Table 1.

2.5. 1-(Benzyloxy)naphthalene (**2**) [18]

Naphthalen-1-ol (**1**) (1442 mg, 10 mmol) was dissolved in acetonitrile (40 mL) and anhydrous potassium carbonate (2764 mg, 20 mmol) was added to the solution. After 30 min, benzyl bromide (1784 µL, 15 mmol) was gradually added. The reaction mixture was heated to reflux and stirred for 16 h. After cooling to room temperature, the mixture was diluted with ethyl acetate and washed twice with 2 M hydrochloric acid, once with water and brine. The organic layer was dried over sodium sulfate and concentrated to dryness in vacuo. The crude product was purified by column chromatography (hexane/dichloromethane 19:1) to afford 1-(benzyloxy)naphthalene (**2**) (2239 mg, 96%) as a colorless crystalline solid.

2.6. 1-(Benzyloxy)-4-bromonaphthalene (**3**) [19,20]

1-(Benzyloxy)naphthalene (**2**) (1531 mg, 6.53 mmol) was dissolved in acetonitrile (35 mL), the solution was cooled in an ice bath and NBS (1163 mg, 6.53 mmol) was added portionwise over 20 min. The reaction mixture was stirred for 3 h at 0 °C. After warming to ambient