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Screening of seized emerging drugs by ultra-high performance liquid chromatography with photodiode array ultraviolet and mass spectrometric detection

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ABSTRACT

The use of psychoactive "designer drugs" has increased rapidly due to their varying and sometimes ambiguous legal status and their ready access via the Internet and at local "headshops." A quick screening method for samples containing these substances, using ultra-high performance liquid chromatography with photodiode array UV and mass spectrometric detection (UHPLC-PDA/UV–MS), is presented. The method enables the screening of a variety of samples containing emerging/reemerging drugs, including β-keto phenethylamines (cathinone derivatives), synthetic cannabinoids/cannabimimetics, and phenethylamine derivatives. The use of dual detectors not only provides molecular weight information but also differentiates the drugs by their categories and in some cases even their subcategories. Moreover, ring positional isomers of cathinone and phenethylamine derivatives can be easily differentiated by their retention times and UV spectra.

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1. Introduction

The term "Designer drugs" was first introduced to the forensic community in the 1980s to designate non-controlled analogs of controlled substances [1,2]. Over the past few years, the use of new synthetic psychoactive designer drugs has exploded due to their easy access via the Internet and at local "head shops" [3–5]. Since most of these drugs are new and have little or no safety and toxicological data, many overdoses and bizarre effects from misuse have been reported [6–9]. Legislation has been put in place in many countries to address this problem. In July of 2012, the Synthetic Drug Abuse Prevention Act of 2012 placed 26 synthetic drugs and their analogs in Schedule I of the U.S. Controlled Substances Act [10]. Due to the vast number of potentially active compounds, it is not feasible to list every possible chemical explicitly. The inability to rapidly control such compounds provides the opportunity for illegal drug manufacturers to create new structurally similar chemicals by slightly modifying the chemical frameworks of existing illegal drugs and marketing them as "legal" alternatives, thus circumventing the law. As a result, modified versions of drugs are constantly being created and emerging into the drug abuse markets. Of particular note are the synthetic cannabinoids and cannabimimetics, which have undergone numerous changes in their structures over time [11].

Most emerging designer drugs are members of one of the following categories: Phenethylamine derivatives, cathinone derivatives, synthetic cannabinoids or cannabimimetics, piperazines, and tryptamines. As many of these new drugs are novel and have short market lifetimes, forensic laboratories face a real challenge to constantly identify unknown substances with limited reference data, lack of standard materials, or insufficient analytical capabilities.

Recent studies have reported the analyses of cathinone derivatives or synthetic cannabinoids/cannabimimetics in seized exhibits by using multiple techniques, including gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography–time of flight mass spectrometry (HPLC–TOF-MS), HPLC–MS/MS, infrared spectroscopy (IR), and/or nuclear magnetic resonance spectroscopy (NMR) [12–16]. A few researchers have also investigated designer drugs using HPLC-PDA or HPLC-PDA–MS in conjunction with GC–MS [17–20]. In one study [21], Takahashi et al. created a designer drug library including phenethylamine, tryptamine, and phenylpiperazine related compounds, by using HPLC-PDA and GC–MS data, and used it to

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identify designer drugs in purchased products. They also studied the possibility of discriminating positional isomers by using UV, retention times (RTs), and mass spectra.

Prior to drug identification, primary screening for any potential controlled substances or adulterants is necessary. Currently, GC–MS and GC-FID are the principal techniques for general forensic drug screening [22]. However, these techniques may prove inadequate for analysis of highly polar, basic phenethylamine and cathinone derivatives, or heat sensitive synthetic cannabinoids/cannabimimetics. In many cases, phenethylamine and cathinone derivatives require basic extraction in order to obtain good chromatographic performance. However, some basified cathinone derivatives undergo thermal degradation in GC injection ports [23,24]. Moreover, certain synthetic cannabinoids/cannabimimetics either break down or are converted to different compounds in GC injection ports. In contrast, liquid chromatographic techniques, such as high performance liquid chromatography (HPLC) and UHPLC, do not suffer these limitations. UHPLC offers higher speed and/or peak capacity than HPLC (but not as high as GC), and is well suited for drug screening. Additionally, many phenethylamine and cathinone derivatives undergo extensive fragmentation under EI (electron impact), and their molecular weight information is therefore either missing or difficult to discern [25]. Therefore, UHPLC–MS, which can provide either low or high resolution molecular weight information due to its soft molecular fragmentation (positive and negative electrospray ionization (ESI)), is better suited for the screening of these emerging drugs [26–28].

Another significant issue in analyzing these emerging drugs is differentiation of positional isomers of phenethylamine and cathinone derivatives. Most of these compounds cannot be fully resolved by GC without derivatization or by using special non-polar stationary phases, and their EI mass spectra are very similar or even identical [29–34]. In contrast, the majority of these compounds can be resolved by UHPLC without derivatization. For those not fully resolved by UHPLC, UHPLC–MS with ESI detection may encounter some of the same issues as GC–MS [35]; however, the UV spectra can be distinguishable for many of these compounds [36]. Therefore, UHPLC-PDA/UV can be valuable for the screening of positional isomers of phenethylamine and cathinone derivatives. Another benefit of UV detection is that although a UV spectrum cannot provide unequivocal structural information, it can offer valuable information in predicting the presence or absence of certain functional groups, which can provide clues to the structure of an unknown. In this study, a comprehensive UHPLC-PDA/UV–MS method (using a novel single gradient system) was developed as a preliminary screening tool for the detection of a wide variety of emerging drugs, including positional isomers, in drug seizures.

2. Experimental

2.1. Chemicals and reagents

Drug standards were obtained from the reference collection of this laboratory. Marshmallow plant material and testing samples were also from the same laboratory. High-purity, deionized water was obtained from a PURLAB Ultra Mk2 (ELGA LabWater Global Operations, UK). HPLC grade methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). Puriss p.a. grade formic acid was purchased from Sigma Aldrich (St. Louis, MO, USA)

2.2. Instrumentation

The UHPLC-PDA/UV–MS system consisted of a Waters Acquity UPLC liquid chromatograph coupled with a Waters photo diode array (PDA) and a Waters Single Quadrupole Detector (SQD) (Milford, MA, USA). Empower 3.0 was used for the overall instrument control, data acquisition, and processing. The chromatographic separation was performed on a Waters BEH phenyl column (150 mm × 2.0 mm, 1.7 μm) at 35 °C. The sample compartment was maintained at 5 °C. The mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The separation was carried out on a gradient elution program as follows: 5–10%B (0–5.0 min), 10–50%B (5.0–10.0 min), 50–70%B (10.0 –15.0 min), 70–75%B (15.0–17.0 min), and up to 90%B at 17.1 min with a 1.4 min hold. The column was re-equilibrated for 3 min between runs, giving a total run time of 21.5 min. The flow rate was 0.35 mL/min. The injection volume was 1 μL with partial loop injection (partial needle overflow mode with a 5 μL loop). The PDA detection was set from 210 to 400 nm. For ES^+ MS detection, the following parameters were used: The ion source temperature was 150 °C; nitrogen was used as the desolvation gas at a flow rate of 600 mL/min and at 400 °C; the capillary and the cone voltages were 3000 and 30 V, respectively; MS data was acquired in the full scan mode (50.00–550.00 *m/z*).

2.3. Sample preparation

For each standard, 1 mg/mL of stock solution was prepared in methanol. A working standard mixture in methanol, including fluoroamphetamines (10 μg/mL), cathinone derivatives (5 μg/mL), synthetic cannabinoids/cannabimimetics (3–10 μg/mL), and 2C-compounds (5 μg/mL), was prepared from their stock solutions. Individual or a mixture of 2-, 3-, and 4-ring positional isomers of fluoroamphetamine (FA), fluoromethcathinone (FM), or methylethcathinone (MEC) at 5–10 μg/mL in methanol was also prepared from their stock solutions for the positional isomer study.

For plant materials, 5 mg of ground plant material was extracted with 1 mL of methanol, followed by 5 min of sonication and 30 min of soaking. The solution was filtered through a 0.45 μm RC membrane (Sun SRI, TN, USA) into a UPLC vial before injection.

For powdered materials, 5 mg of powder was dissolved in 3 mL methanol. The solution was sonicated for 5 min, and then filtered through a 0.45 μm RC membrane into an UPLC vial before injection. If necessary, the filtrate was further diluted to a suitable concentration.

3. Results and discussion

3.1. Separation and detection of emerging drugs

Although UHPLC has been utilized for the separation of emerging drugs, different mobile phase conditions have been reported depending on the class of solutes analyzed. In this vein, two separate methods were originally developed at this laboratory to screen powders suspected to contain cathinone or phenethylamine derivatives and plant materials suspected to contain synthetic cannabinoids/cannabimimetics, respectively. However, difficulties were encountered when the plant materials were laced with cathinone or phenethylamine derivatives in addition to synthetic cannabinoids/cannabimimetics. A more comprehensive separation method was needed to screen for such samples. Therefore it was advantageous to develop a comprehensive screen utilizing chromatographic conditions allowing for the separation of a wide variety of designer drugs in a single run.

Considering the structural variety among the emerging drugs, the following types of stationary phases were investigated with mobile phases containing 0.1% formic acid mixed with either methanol or acetonitrile at 30 °C: Waters BEH C18 (100 mm × 2.0 mm, 1.7 μm), Waters BEH phenyl (100 mm × 2.0 mm, 1.7 μm), and Phenomenex Kinetex C18 (100 mm × 2.0 mm, 1.8 μm). BEH phenyl column