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Rapid analysis of synthetic cannabinoids using a miniature mass spectrometer with ambient ionization capability

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ABSTRACT

Synthetic cannabinoids are an emerging class of drugs of abuse and are of a great concern for transport control and usage regulation. In this study, we have developed rapid analytical methods using a miniature mass spectrometer for the identification of synthetic cannabinoids, as the traces of bulk powders on surfaces or substances in blood and urine. Significantly simplified work flows were developed by employing two ambient ionization methods, the paper spray and extraction spray ionization. Using five synthetic cannabinoids as examples, a limit of detection of 2 ng was estimated for the detection of trace powders on a bench surface and limits of quantitation as good as 10 ng/mL were obtained for the analysis of blood and urine samples.

1. Introduction

Synthetic cannabinoids were initially developed in pharmacological research for the study of the endocannabinoid system [1]; however, recently they have emerged as a class of designer drugs. Synthetic cannabinoids for illicit use are distributed mainly in the forms of dried herbs or powdery products [2] and are deceptively marketed as herbal blends, room deodorizers, air fresheners, or incense products [3]. Although the chemical structures of the synthetic cannabinoids can differ substantially from that of delta-9-tetrahydrocannabinol (Δ^9-THC), they have a similar psychoactive effect of the primary natural cannabinoids [4]. They have a high binding affinity to the cannabinoid receptor CB_1, found primarily in the brain and central nervous system, or to CB_2 found in the peripheral nervous system, especially in cells associated with the immune system [5,6]. Easy access of the synthetic cannabinoids at low costs [7] and the lack of effective means for routine screening have contributed to the fast growth in their use [8], especially by the young and first-time drug users [9,10]. Emerging evidence has shown that the administration of synthetic cannabinoids may cause various adverse psychological and physiological effects on human health, with symptoms including anxiety, agitation, panic, paranoia, intoxication, psychosis, and seizures [11,12]. The social and medical issues associated with the use of synthetic cannabinoids have drawn a significant attention from the international community and legal actions are being undertaken to control their use [8,13]. Enforcement of the restrictions on the use of synthetic cannabinoids overall is a complicated process; however, the lack of governmental regulations in many countries on use of these compounds certainly is resulting in uncontrolled prevalence of the drug use. There are no established cutoff levels yet for regulatory detection of synthetic cannabinoids, because information about their dose-dependent effects is very limited [1].

Legal surveillance of the distribution and use of synthetic cannabinoids calls for the development of effective analytical methods for detecting and quantifying the synthetic cannabinoids from samples in a variety of forms, including the herbal blends [14–17], bulk powders [18,19], urine [20–22], whole blood [23,24], serum [25–27], hair [28–30], and saliva samples [31–33]. The analytical techniques that have been demonstrated for analyzing synthetic cannabinoids mainly include micellar electrokinetic chromatography [14,16], immunoassay [19,20], nano-liquid chromatography [17], gas chromatography mass spectrometry [15,18], and high-performance (HP) or ultra-performance (UP) liquid chromatography (LC) coupled with mass spectrometry (MS) [21–32]. These methods are typically implemented in analytical laboratories using the bench-top

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equipment and often require laborious sample preparation procedures. The development of rapid and on-site analytical methods for fast identification of synthetic cannabinoids is highly desirable for a variety of applications, such as the forensic investigation, roadside inspection, workplace drug testing, and the screening at check points.

As already demonstrated in conjunction with HPLC, MS can provide high sensitivity and high selectivity for both qualitative and quantitative analysis of the synthetic cannabinoids. Conventional laboratory-scale mass spectrometers are large and heavy, which limits their usage for in-field applications. Miniature mass spectrometers have been developed to enable the on-site chemical analysis [34]. As opposed to the traditional chemical analysis work flow, where samples are brought to the laboratory for analysis, the miniaturized instruments can now be brought to the samples [34,35]. However, sample preparation would also need to be done quickly in the field. Ambient ionization, in which the analytes in untreated samples are directly sampled and ionized for MS analysis, represents a promising solution for simplification of the sample preparation during on-site analysis.

Direct chemical analysis using MS with ambient ionization methods has advanced significantly in the past decade [36]. Since desorption electrospray ionization (DESI) [37] and direct analysis in real time (DART) [38] were reported in 2004 and 2005, respectively, more than 40 ambient ionization methods have been developed [36,39–41]. Sample pretreatment and chromatographic separation, traditionally required for MS-based analysis, can now be bypassed. Analysis of synthetic cannabinoids using DART with a time-of-flight instrument has been previously demonstrated [42,43]. Notably, miniature mass spectrometry systems with ambient ionization capability, *e.g.*, paper spray [41,44], extraction spray [45], or low temperature plasma [46], have been shown to be promising for on-site applications in food safety [47,48], pharmaceutical drug development [49], environment monitoring [50–52], and homeland security [53,54], as well as for biomedical diagnosis [55].

In this study, direct identification of synthetic cannabinoids in bulk powder or biofluid samples has been developed using a miniature ion trap mass spectrometry system with two ambient ionization methods, the paper spray and extraction spray ionization (Fig. 1). Five synthetic cannabinoids, exemplary of a class of drugs of a significant concern for regulatory control, were selected for method development and validation.

2. Experimental

2.1. Chemicals and reagents

Naphthalen-1-yl-(1-pentylindol-3-yl) methanone (JWH-018), naphthalen-1-yl-(1-pentylindol-3-yl)-1,1,2,2,3,3,4,4,5,5,5-d$_{11}$-methanone (JWH-018-d$_{11}$), 4-methoxynaphthalen-1-yl-(1-pentylindol-3-yl) methanone (JWH-081), 1-[(5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl) methanone (AM-2201), 2-(4-methoxyphenyl)-1-(1-pentylindol-3-yl) methanone (RCS-4), and [1-(5-fluoropentyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl) methanone (XLR-11) (structures shown in Fig. 2), each dissolved in methanol at concentrations of 1 mg/mL, were purchased from Lipomed AG (Arlesheim, Switzerland). All synthetic cannabinoids reference standards had purities greater than 99%. Whatman Grade 1 cellulose chromatography paper was purchased from Whatman (Piscataway, NJ, USA) and used to prepare sample substrates for paper spray and extraction spray ionization. Bovine whole blood stabilized with EDTAK$_2$ was purchased from Innovative Research (Novi, MI, USA). Synthetic urine was purchased from CST Technologies (Great Neck, NY, USA). Methanol of HPLC grade was purchased from Mallinckrodt (Hazelwood, MO, USA). Other chemicals used in the experiment were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Stock solutions of the analytes were prepared by dilution with the methanol and were subsequently spiked into the raw samples for analysis.

2.2. Instrumentation

A desktop miniature mass spectrometry system, Mini 12 [56], was utilized to perform the analysis. The integrated Mini 12 system weighed 25 kg, had outside dimensions of 19.6 × 22.1 × 16.5 in.^{3}, and consumed a power less than 100 W. The pumping system was composed of a HiPace 10 turbomolecular pump (Pfeiffer Vacuum, Nashua, NH, USA) and a two-stage diaphragm pump (MPU 1091-N84.0-8.99, KNF Neuberger, Trenton, NJ, USA). A discontinuous atmospheric pressure interface (DAPI) [57,58] was used to enable efficient transfer of ions from the ambient ionization sources to a rectilinear ion trap (RIT) [59] located within the vacuum manifold in a pulsed fashion. For each scan, the DAPI was opened briefly for about 15 ms for ion introduction and closed during the rest of the time in each scan cycle. The ions trapped in the RIT were then mass analyzed using an rf (1 MHz) amplitude scan. Resonance ejection was performed using an AC excitation (350 kHz) with its amplitude ramped with the rf scan. A scan speed of 10,000 *m/z* per second was used. The user interface for instrument control and data acquisition was developed in-house.

2.3. MS/MS analysis of synthetic cannabinoids using Mini 12

The MS/MS capability of the Mini 12 miniature mass spectrometer plays an essential role in the direct, *in situ* MS analysis of complex samples without any traditional sample pretreatment or chromatographic separation. The sensitivity can be significantly improved by the elimination of chemical noise through the MS/MS process [60]. Characteristic fragmentation patterns corresponding to the structural features of the analytes are useful, together with information of the molecular mass, for confirmation of chemical

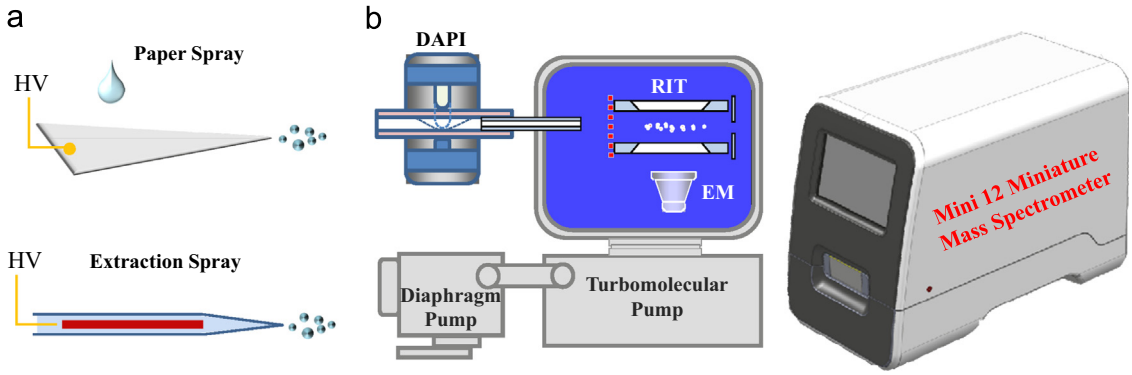

Fig. 1. (a) Paper spray ionization, extraction spray ionization, and (b) Mini 12 desktop ion trap mass spectrometer for the identification of synthetic cannabinoids.