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Extraction, identification and detection of synthetic cannabinoids found pre-ban in herbal products in Victoria, Australia

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ABSTRACT

Measures to control the expanding use of synthetic cannabinoids demand new analytical methodology to identify and determine compounds within this rapidly evolving class. Herein, we identify seven synthetic cannabinoids (**AM-2201**, **UR-144**, **XLR-11**, **A796,260**, **5F-AKB48**, **PB-22** and **5F-PB-22**) present in eleven herbal products sold in Victoria, Australia, prior to their ban in 2014, using a combination of GC-MS, HPLC, ESI-MS, and NMR. In aid of this work, we synthesised the synthetic cannabinoids **AM-2201** and **5F-AKB48**. We then explore for the first time, the chemiluminescence detection of synthetic cannabinoids using three commonly used reagents: permanganate, manganese(IV), and tris(2,2′-bipyridine)ruthenium(III). Using the permanganate reagent, no chemiluminescence signal was obtained, but the manganese(IV) and tris(2,2′-bipyridine)ruthenium(III) reagents gave analytically useful responses with all synthetic cannabinoids under investigation except **5F-AKB48**. Calibration curves for **PB-22, 5F-PB-22, AM-2201** and **5F-AKB48** prepared using HPLC with UV absorbance and/or chemiluminescence detection were used to determine the total cannabinoid content extracted with methanol (1 mL) from of six of the herbal products (10 mg), which ranged from 0.072 to 0.77 mg.

1. Introduction

Synthetic cannabinoids were initially developed as therapeutic agents [1,2], but since the early 2000s they have been marketed as herbal marijuana alternatives and become of increasing concern to law enforcement agencies and health professionals as chemicals of abuse. Synthetic cannabinoids have a similar effect in the human body as tetrahydrocannabinol (THC) as they interact with the cannabinoid receptors in the brain [1,3,4]. A series of emergency department case studies have found users suffer from anxiety, tachycardia, tachypnoea, hypertension, hallucinations, psychosis and seizures [5]. Hopkins and Gilchrist reported a link between these compounds and cannabinoid hyperemesis syndrome, which is commonly found in heavy marijuana smokers and believed to be caused by an over stimulation of the CB1 receptor [6]. By May 2014, the Victorian state government in Australia had legislated against all these compounds under Schedule 11 of the Drugs, Poisons and Controlled Substances Act 1981, banning their sale, purchase and possession.

Synthetic cannabinoids are commonly made in a powdered form that is then dissolved in a solvent such as acetone and sprayed onto mixtures of herbs [7,8]. These mixes often contain the bark, flowers, roots and leaves of different types of plants, such as damiana (*Turnera diffusa*), marshmallow leaf, mullein leaf [9,10] and various additives to provide 'flavour'. There are a large number of known synthetic cannabinoids and new analogues are constantly entering the market [11]. New cannabinoids have been produced by varying the (usually heterocyclic) core, bioisosteric replacement and/or changing or adding functional groups, often with the intention of creating derivatives that were not specifically prohibited by the law [3,12,13].

Due to the non-polar nature of the synthetic cannabinoids, organic solvents (e.g., methanol, ethanol or acetonitrile) are commonly used for their extraction, typically at a ratio of 1 mL of the solvent to 10 mg of dried plant material [9,14,15]. Generally, the samples are then either sonicated or vortexed and centrifuged

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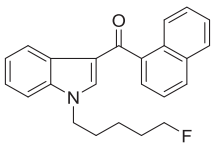
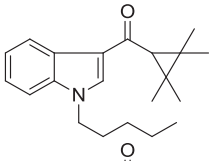
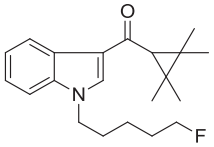
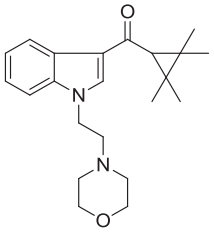
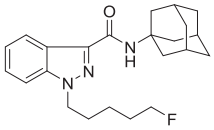
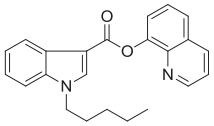
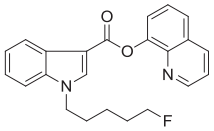
before filtering with a 0.45 μm filter [10,16,17]. Reverse-phase high performance liquid chromatography (HPLC) is commonly used for the determination of synthetic cannabinoids with the majority of published studies utilising solvent gradients of either acetonitrile or methanol in water, with modifiers such as formic acid [18].

Chemiluminescence is a sensitive and selective mode of detection, easily coupled to HPLC, which has been exploited for the determination of numerous compounds of forensic importance [19,20]. Rasanen and co-workers have used gas-phase chemiluminescence nitrogen detection for designer drugs including some synthetic cannabinoids [21], but to date, liquid-phase chemiluminescence has not been reported for the detection of these compounds. Tris(2,2′-bipyridine)ruthenium(III) ($[Ru(bipy)_3]^{3+}$) is a useful liquid-phase chemiluminescence reagent for the detection of alkaloids, biomolecules, pharmaceuticals and controlled drugs containing secondary or tertiary amines [22–25] and may be suitable for the detection of synthetic cannabinoids that contain nitrogen heterocycles (Table 1). Acidic potassium permanganate has also been used for the chemiluminescence detection of alkaloids and related compounds [26,27], and was previously applied to the HPLC determination of natural cannabinoids in hemp extracts [28]. The colloidal manganese(IV) chemiluminescence reagent has a broader selectivity than acidic potassium permanganate and has been used for analytes of forensic interest [29–32] and plant-based extracts [33,34]. Herein, we explore the complementary selectivity derived from solvent extraction, chromatographic separation and chemiluminescence detection for the determination of synthetic cannabinoids in complex herbal sample matrices. This was conducted with a view to opening new avenues to develop rapid, preliminary screening techniques based on miniaturised or portable devices in conjunction with very simple extraction and/or separation procedures.

Table 1
Synthetic cannabinoids discussed in this study.

Structure	Common name(s)	IUPAC name
	AM-2201	1-[(5-fluoropentyl)-1*H*-indol-3-yl]-(naphthalen-1-yl)methanone
	UR-144	(1-pentylindol-3-yl)-(2233-tetramethylcyclopropyl) methanone
	XLR-11	(1-(5-fluoropentyl)-1*H*-indol-3-yl) (2233-tetramethylcyclopropyl) methanone
	A796,260	[1-(2-morpholin-4-ylethyl]-1*H*-indol-3-yl]-(2233-tetramethylcyclopropyl) methanone
	5F-AKB48; 5F-APINACA	*N*-(adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
	PB-22; QUPIC	1-pentyl-1H-indole-3-carboxylic acid 8-quinolinyl ester
	5F-PB-22; 5F-QUPIC	1-pentyfluoro-1*H*-indole-3-carboxylic acid 8-quinolinyl ester

2. Materials and methods

2.1. Samples

Eleven commercially available herbal synthetic cannabinoid samples (brands: Puff, Red Dot, Bombay Blue – pineapple flavour, Cloud 9, Code Black – strawberry flavour, Northern Lights – Special K, Malibu, Atomic Bomb, Stoner, Storm and Supanova; Figure S1 in Electronic Supplementary Information) were obtained pre-ban from a range of stores in Victoria, Australia. Synthetic cannabinoids were extracted by adding 10 mg of the herbal mixture to 1 mL of solvent and sonicated for 10 min. The organic solvents trialled in this study were acetone, methanol, ethanol, ethyl acetate (Chem-Supply, SA, Australia), chloroform (Merck, Vic., Australia), dimethylsulfoxide (DMSO), dimethylformamide (DMF) (Hopkin and Williams, Essex, UK) and acetonitrile (Ajax Fine Chemicals, NSW, Australia); all solvents were of analytical grade or higher.

2.2. Synthetic cannabinoid standards

Two cannabinoid standards (**AM-2201** and **5F-AKB48**) were synthesised as described in the Electronic Supplementary Information. Two other standards (**PB-22** and **5F-PB-22**) were obtained from the National Measurement Institute (NSW, Australia). Stock solutions of these were initially prepared at 1 mM using HPLC-grade methanol (Merck) and diluted as required.

2.3. High performance liquid chromatography

An Agilent Technologies 1260 HPLC including autoinjector, solvent degasser, quaternary pump and column thermostat (20 °C) was used with a DAD (λ = 254 nm) and an in-house built chemiluminescence detector, comprising a coiled tubing flow-cell mounted against the circular-shaped window of a photomultiplier tube (Electron Tubes model 9828SB; ETP, NSW, Australia) within a light-tight housing [28]. Chemiluminescence reagents were pumped at 1 mL/min by a Gilson Minipuls peristaltic pump to the detector, where they merged with the column eluent in a T-piece shortly prior to entering the coiled flow-cell [35]. An Agilent Eclipse XDB-C18 column (4.6 mm × 15 mm, 5 μm particle diameter) was used for all separations. The sample (20 μL) was injected onto the column with a flow rate of 1 mL/min. In method A, the mobile phase comprised methanol and deionised water; the methanol component was increased from 0% to 100% over 40 min, and then held for 20 min, with 5 min re-equilibration time. In method B, both the methanol and deionised water contained 0.1% (v/v) formic acid; the methanol component was increased from 10% to 80% over 10 min, and then to 100% over 10 min, with 5 min re-equilibration time.

2.4. Chemiluminescence detection

A 1 mM solution of acidic potassium permanganate (Chem-Supply) was prepared using 1% (m/v) sodium polyphosphate