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Assessment of ultra high performance supercritical fluid chromatography as a separation technique for the analysis of seized drugs: Applicability to synthetic cannabinoids

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ABSTRACT

The recent development of modern methods for ultra high performance supercritical fluid chromatography (UHPSFC) has great potential for impacting the analysis of seized drugs. In the separation of synthetic cannabinoids the technique has the potential to produce superior resolution of positional isomers and diastereomers.

To demonstrate this potential we have examined the capability of UHPSFC for the analysis of two different groups of synthetic cannabinoids. The first group was a mixture of 22 controlled synthetic cannabinoids, and the second group included JWH018 and nine of its non-controlled positional isomers The clear superiority of UHPSFC over other separation techniques was demonstrated, in that it was capable of near baseline separation of all 10 positional isomers using a chiral column. In total we examined four achiral columns, including Acquity UPC^2 Torus 2-PIC, Acquity UPC^2 Torus Diol, Acquity UPC^2 Torus DEA and Acquity UPC^2 Torus 1-AA (1.7 μm 3.0 × 100 mm), and three chiral columns, including Acquity UPC^2 Trefoil AMY1, Acquity UPC^2 Trefoil CEL1 and Acquity UPC^2 Trefoil CEL2 (2.5 μm 3.0 × 150 mm), using mobile phase compositions that combined carbon dioxide with methanol, acetonitrile, ethanol or isopropanol modifier gradients. Detection was performed using simultaneous PDA UV detection and quadrupole mass spectrometry. The orthogonality of UHPSFC, GC and UHPLC for the analysis of these compounds was demonstrated using principal component analysis. Overall we feel that this new technique should prove useful in the analysis and detection of seized drug samples, and will be a useful addition to the compendium of methods for drug analysis.

1. Introduction

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) [1] has the responsibility for setting worldwide minimum standards for the analysis of seized drugs, allowing for the identification of a drug using a combination of various instrumental, microscopic and wet chemical techniques. These techniques are included under three categories in descending order of specificity (A, B, C), with category B encompassing separation techniques. Often, more than one separation technique is utilized in the analysis to increase specificity and to minimize sample mix-up. Acceptable SWGDRUG separation techniques include TLC, GC, LC (HPLC and UHPLC) and CE. Supercritical fluid chromatography, while similar in nature to both gas chromatography and HPLC is not currently included as an approved method.

Ultra high performance supercritical fluid chromatography (UHPSFC), is a recently developed manifestation of supercritical fluid chromatography that uses packed columns with sub 2 μm particles, or equivalent, to produce highly efficient and rapid separations. The columns are utilized on a new generation of instruments that overcome previous limitations such as poor back pressure control and excessive UV detector noise. UHPSFC is similar to liquid chromatography in that it can analyze compounds that are thermally labile, polar or non-volatile without pretreatment or derivatization (unlike gas chromatography). However, when compared to UHPLC, UHPSFC mobile phases are more diffusive with lower viscosities. This results in separations that are up to four times faster than UHPLC with the same resolving power. Furthermore, because UHPSFC operates most effectively in a normal phase

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mode, it offers excellent selectivity for structurally related compounds.

Synthetic cannabinoids are often modified to produce different analogs of the parent drug in order to circumvent laws banning their use as recreational drugs. These modifications often result in structural analogues, structural homologues, positional isomers and stereoisomers. These newly synthesized compounds are easily available over the internet and at local "head shops". Synthetic cannabinoids are typically sprayed on plant material prior to ingestion. Presently there are over 20 of these compounds under federal control in the United States [2]. It is a constant struggle for laboratories and government agencies to keep pace with the proliferation of these newly emerging drugs.

For the analysis of synthetic cannabinoids, a wide variety of separation and analysis methods have been described [3] including TLC [4,5] GC–MS [5–9], GC–FID [10], GC-NPD [7], HPLC-PDA UV [5,11], nano-HPLC-PDA UV [12], nano-HPLC Ion Trap MS [12], HPLC- Orbitrap MS [13], UHPLC-PDA UV-Single Quad (SQ) MS [14,15], UHPLC TOF MS [5,9,16], UHPLC -QTOF MS [17,18], MEKC-PDA UV [19] and MEKC Triple Quad (TQ) MS [20]. Recently SFC has been applied to the toxicological analysis of synthetic cannabinoids and their metabolites in urine [21] and UHPSFC has been proposed for the analysis of synthetic cannabinoids in drug seizures [22]. Four synthetic cannabinoids, several metabolites and the natural cannabinoids Δ9-THC, cannabinol and cannabidiol were fully resolved using a 5 μm Zorbax Rx-SIL column with a carbon dioxide/acetonitrile mobile phase and PDA UV detection [21]. In a second study, UHPSFC methods were developed for cannabidiol and nine synthetic cannabinoids using carbon dioxide with a methanol modifier. While these compounds were not completely resolved, the authors did show that with the aid of a chiral column, separation of cis and trans-CCH and the enantiomers of CP-47, 497 was possible [22]. Obviously due to the multitude of possible compounds for the synthetic cannabinoids, the separation of these analytes using a single chromatographic system is difficult, especially for positional isomers and diastereomers [9]. For example in a study of JWH-018 and nine non-controlled positional isomers only four out of ten compounds were resolved using GC, and even using a combination of both GC and UHPLC these compounds were not completely resolved. It should be noted that for legal requirements it is of upmost importance to distinguish between a controlled and non-controlled substance.

For this study the utility of UHPSFC as a SWGDRUG category B separation technique for synthetic cannabinoids was investigated. Various UHPSFC separation conditions were examined including column type, choice of modifier, gradient steepness, applied back pressure and temperature. For this investigation 31 synthetic cannabinoids including positional and stereoisomers were examined. Both UV/PDA and quadrupole mass spectrometry were investigated for detection of these compounds. Principal component analysis was utilized to investigate the degree of orthogonality of the separation technique with comparison to GC and UHPLC.

2. Experimental

2.1. Chemicals and reagents

Synthetic cannabinoid reference standards were obtained from Cayman Chemical (Ann Arbor, MI, USA). LC/MS grade water, formic acid, methanol, acetonitrile, and 2-propanol was obtained from Fischer (Fairlawn, NJ, USA). HPLC grade ethyl alcohol was obtained from Sigma-Aldrich (St. Louis, MO, USA). Certified A.C.S. ammonium hydroxide was obtained from Fischer. 99.9% liquid carbon dioxide was obtained from Roberts Oxygen (Rockville, MD, USA).

2.2. Preparation of solutions

The injection solvent consisted of heptane/ethyl acetate/methanol (50:25:25). For UHPSFC separations, individual standards or mixtures of standards of either 1.0 mg/mL or 10 mg/mL methanol stock solutions of the individual synthetic cannabinoids were diluted with injection solvent to the appropriate concentration(s).

For MS analysis the makeup solvent consisted of either 0.1% formic acid or 0.07% ammonium hydroxide in 95:5 methanol: water delivered at a flow rate of 0.45 mL/min. The latter conditions gave a more stable MS signal and thus was used for all figures of merit experiments.

2.3. Instrumentation and data analysis

The Waters UHPSFC-PDA UV-SQ MS system consisted of an Acquity UPC^2 chromatograph equipped with a binary solvent delivery pump, an autosampler with a 10 μL loop for partial injection, a PDA UV detector, and a single quadrapole Acquity QDA detector fitted with a Z-spray electrospray ionization source (Waters, Milford, MA, USA). Makeup solvent was delivered by either a Waters Model 515 pump or Waters isocratic solvent manager and mixed with effluent prior to MS detection using a splitter. Empower version 3 was used for instrument control, data acquisition and processing. For MS detection nitrogen was delivered using a Peak Scientific Model NM32LA nitrogen generator (Inchinnan, Scotland, UK). UHPSFC separations were performed using four achiral Waters columns (100 mm × 3.0 mm × 1.7 μm): Acquity UPC^2 Torus 2-PIC, Acquity UPC^2 Torus DEA, Acquity UPC^2 Torus DIOL, and Acquity UPC^2 Torus 1-AA, and three chiral Waters columns (150 mm × 3.0 mm × 2.5 μm): Acquity UPC^2 Trefoil AMY1, Acquity UPC^2 Trefoil CEL1, and Acquity UPC^2 Trefoil CEL2.

Peak resolution was determined using standard resolution curves and the ratio of the valley to the height of the peak [23]. Principal component analysis (PCA) was performed on the UHPSFC, GC and LC retention time data using IBM SPSS Statistics Version 21 (International Business Machines Corporation, Armonk, NY, USA). For PCA analysis the data were autoscaled. Each variable was adjusted so that it had zero mean and unit standard deviation or variance.

3. Results and Discussion

3.1. Separation of synthetic cannabinoids using achiral stationary phases

Two different mixtures were analyzed. The first mixture included a random collection of 22 controlled synthetic cannabinoids while the second mixture consisted of JWH-018 and nine of its non-controlled positional isomers. The separation of these two mixtures was examined using four different achiral stationary phases and a carbon dioxide mobile phase mixed with a methanol modifier. The identity of the solutes in the individual chromatographic runs was ascertained by an examination of a combination of UV spectra and MS $[M-H]^+$ data. The structures, UV spectral data and MS spectral data for the controlled synthetic cannabinoids as well as the nine positional isomers of JWH-018 are shown in Table 1. For these separations, five minute gradients were employed with usually a one minute hold at the end of the gradient. Gradient conditions were adjusted such that the chromatographic peaks occupied approximately the entire gradient time. For each column, identical separation conditions were used for both sets of compounds.

The effect of column type on the retention time of the controlled synthetic cannabinoids is shown in Fig. 1. Synthetic cannabinoids