A20 Optimized Screening of Synthetic Cannabinoids With Phenyl Reversed-Phase Liquid Chromatography

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After attending this presentation, attendees will understand the fundamental principles of Reversed-Phase Liquid Chromatography (RPLC) with UV-Vis detection as pertaining to forensic chemistry, and the necessary elements for optimized separation and identification of illegal substances, specifically synthetic cannabinoids in herbal incenses. Through the comparison of several different phenyl-functionalized columns, the optimal screening technique for synthetic cannabinoids can be determined and used in a practical setting.

This presentation will impact the forensic science community by providing an optimized and simple chromatographic separation and quantification method for synthetic cannabinoids in herbal incenses in order to improve the accuracy and throughput of "spice" evidence processing.

The recently-passed S.3187 included five classes of synthetic cannabinoids into Schedule I controlled substance list. It creates great challenges for forensic scientists to rapidly separate, identify, and quantify these "cannabimimetic agents" in numerous herbal incenses due to the similarity of isomer or analog structures. Newer compounds are being synthesized promptly to circumvent the ban, which exacerbates the analytical difficulties for forensic labs, some of which are already experiencing backlogs. In this presentation, a novel yet simple RPLC separation method with phenyl functionalized column and Diode Array Detector (DAD) was dicovered to possess several advantages over conventional C-18 LC/MS methods. When the phenyl RPLC method is coupled with a simple liquid extraction method, the identification and quantification of synthetic cannabinoids in herbal incenses can be completed in less than 30 minutes.

All cannabinoids contain aromatic functional groups such as phenyl, naphthyl, indole, and pyrrole, which readily interact with phenyl stationary phases through pi-pi interactions. Compared to conventional C-18 or C-8 columns, these phenyl columns provide greater resolution in the separation of similar cannabinoids. Six different phenyl columns were compared to determine the ideal separation conditions for synthetic cannabinoids. The injection volume of the sample, composition of the mobile phase, and temperature are optimized to achieve ideal resolution and speed. A mixture of ten synthetic cannabinoids (6-16µg/mL methanol), including JWH-122, AM-2201, RCS-4, and various isomers and analogs, were prepared to evaluate the separation efficiency. For the herbal incense samples, a simple methanol extraction with paper filtration was employed as LC sample preparation. All separations were carried out isocratically on a HPLC system with pure acetonitrile and water as mobile phases flowing at 1-1.5mL/min. The detection wavelengths were set at 214nm and 280nm to capture all synthetic cannabinoids in 1-10µL injection volumes. Elevated temperatures (50-60˚C) increased separation speed.

After the optimization of separation conditions, all six columns can separate seven out of ten cannabinoids with satisfactory resolution in under 20 minutes. Phenylhexyl columns provide better separation compared to phenylpropyl or biphenyl columns, thanks to the diverse functional groups presented on the stationary phase surface and in the analytes. Compact UPLC-type columns with porous shell provide more surface area in a shorter column, which increases speed without the sacrifice of resolution. Polymer reversed phase columns provide more uniform distribution of phenyl groups on the stationary phase, which performed better than silica-based phenyl columns. One polymer-based phenylhexyl column separated all 10 standards in under 14 minutes. With standard calibration, the synthetic cannabinoids in 30 samples were identified and quantified. Most herbal incense contain two to three cannabinoids with concentrations varying from 1-20mg/g of herb.

In conclusion, phenyl RPLC columns with DAD can be used to quickly separate, identify, and quantify synthetic cannabinoids in herbal incenses. When coupled with a simple methanol extraction method, the isocratic separation provides better resolution and rapid quantification compared to conventional C-18 LC/MS methods.

Cannabinoids, Chromatography, Drug Analysis

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