B207 High-Performance Thin-Layer Chromatography (HPTLC) Densitometric Analysis of Cannabinoids in *Cannabis sativa* L.

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Learning Overview: The goal of this presentation is to report the results of the evaluation of different mobile phase systems for the analysis of cannabinoids in *Cannabis sativa* L. using HPTLC, as well as a method to differentiate marijuana samples from hemp samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by reporting the results of the evaluation of ten mobile phase systems used in the HPTLC analysis of cannabis products. The most useful HPTLC systems for the analysis of cannabinoids in *Cannabis sativa* L. in forensic casework will be presented.

TLC is a planar chromatographic method that has been used as a routine, quick-and-easy tool for screening seized drugs in forensic casework. However, historically, traditional TLC has resulted in poor resolution due to systematic errors from hand-spotting, temperature/humidity control, and measurement of the Retardation factor (R_f). HPTLC has been applied in the pharmaceutical field for years, yet it is not widely used in forensic science field. HPTLC has the potential to provide better resolution of forensic samples and to generate reports for more convenient documentation for peer review of casework.

There has been a variety of methods used to analyze cannabinoids by TLC, but the literature is void of a critical review of the different mobile phase systems. This presentation will report the results of the evaluation of ten different mobile phase systems for the analysis of cannabinoids in *Cannabis sativa* L. using HPTLC. All systems were run in triplicate. R_f were recorded and the resolution was calculated for 11 different cannabinoid certified standards. A CAMAG® HPTLC instrument setup was used for all analyses. The setup included an Automatic TLC Sampler 4, an Automatic Development Chamber 2, a TLC visualizer, and a TLC Scanner 3. HPTLC silica gel 60 F_{254} 20 x10cm plates were used for nine systems and an RP-18 WF 10 x 10cm plate was used for one additional system. Parameters for plate development included 30 seconds pre-drying, 20 minutes of humidity control and tank saturation with mobile phase, 70mm migration distance, and 5 minutes of drying after development. Samples were visualized on the plate under white light and at 254nm and 366nm wavelengths. A CAMAG® Chromatogram Immersion Device III was used to dip the developed plate into 0.5% Fast Blue B salt solution for five seconds. visionCATS CAMAG® HPTLC software (version 2.5) was used to control, document, and analyze all results from experiments. Two systems, xylene-hexane-diethylamine (25:10:1) and 6% diethylamine in toluene gave the best results in separating the three major cannabinoids, Δ9-*trans*-tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), and Cannabinol (CBN). The results of the analysis of various cannabis products from casework will be presented.

The xylene:hexane:diethylamine (25:10:1) system was used to develop a calibration method for Δ9-THC. Regression analysis showed a linear relationship (R^2 >0.99) with a range of quantitation between 50–500ng. Marijuana samples and suspected hemp samples were able to be differentiated based on color intensity of sample when compared to standard calibrators of Δ9-THC and CBD.

HPTLC is a superior method to lower-resolution traditional TLC systems for qualitative identification of the common cannabinoids in cannabis products submitted to crime laboratories. It can also be used for quantitative analysis of THC in cannabis products when necessary. Using the proper mobile phase system with HPTLC can eliminate systematic errors from lower-resolution TLC, increase resolution, and make documentation easier. It is possible to differentiate hemp from marijuana, which could be very useful in cannabis casework, given recent legislative changes.

HPTLC, Cannabinoids, Mobile Phase

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