A84 Validation of Thin Layer Chromatography With AccuTOF-DART™ Detection for Forensic Drug Analysis

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After attending this presentation, attendees will be familiar with the results of validation for the identification of several pharmaceutical preparations on Thin Layer Chromatography (TLC) plates using the Direct Analysis in Real Time (DART™) ion source and an exact mass, time-of-flight mass spectrometer in conjunction with physical examination.

This presentation will impact the forensic science community by offering the potential benefits of this identification method relative to current pharmaceutical identification methods using TLC and Gas Chromatography-Mass Spectrometry (GC-MS).

At the conclusion of this presentation, attendees will be familiar with the results of validation for the identification of several pharmaceutical preparations on Thin Layer Chromatography (TLC) plates using the Direct Analysis in Real Time (DART™) ion source and an exact mass, time-of-flight mass spectrometer in conjunction with physical examination. The potential benefits of this identification method relative to current pharmaceutical identification methods using TLC and Gas Chromatography-Mass Spectrometry (GC-MS) will also be offered.

Thin Layer Chromatography (TLC) is a technique that is commonly employed in forensic drug analysis. Detection is typically accomplished using various spray reagents – forming visible chromophores indicative of the compounds analyzed. Direct Analysis in Real Time (DART™) is an ionization source, coupled to an accurate mass time-of-flight, mass spectrometer that has the capability to ionize materials in ambient conditions. The AccuTOF-DART™ system is currently used at the Virginia Department of Forensic Science to screen drug samples with identification being made only after the use of other confirmatory techniques.

Analysis of pharmaceutical preparations in Virginia's Department of Forensic Science laboratories begins with the comparison of physical identifiers of the tablet based on the size, color, shape and markings compared with the expected characteristics detailed in the published pharmaceutical libraries. TLC is then employed to separate the components of the preparation and compare the relative retention factor of the sample against the relative retention factor of the standards, with spray reagents used for detection. Once TLC is successfully completed, the sample is then analyzed with GC-MS against the expected standards. Three common pharmaceutical preparations, tablets of codeine, hydrocodone, and oxycodone mixed with acetaminophen, were chosen for this study.

This study consisted of four main steps: (1) determination of the lower limit of detection (LLOD) of codeine, hydrocodone, and oxycodone standards spotted on TLC plates with detection by DART™; (1) determination of the selectivity of TLC-DART™; (3) DART™ detection of codeine, hydrocodone and oxycodone when dissolved from tablets containing acetaminophen after TLC;, and, (4) reproducibility of the results. In the LLOD portion of the experiment, serial dilutions were made of each standard and spotted onto TLC plates. The plates were then vertically incised, sprayed with 1:25 glycerol in methanol (to enhance detection) and analyzed with the DART™ to determine the best gas heater temperature. The ideal temperature was determined to be 325° C for all three preparations. Additional TLC plates were spotted, incised, sprayed with the glycerol solution and analyzed to determine the LLOD. The LLOD was determined to be 0.3 mg/mL for codeine and 0.5 mg/mL for hydrocodone and oxycodone. For the selectivity determination, standards were obtained for drugs with similar empirical formulae to determine the ability to differentiate them using the TLC-DART™ method. Orifice 1 voltages of 30 V and 90 V were used to give both molecular weight and fragmentation data. While there was some overlap in retention factors for TLC and peaks seen with the DART™, there were enough differences in both the chromatography and the DART™ mass spectra that the combination allowed for specific identification. The detection of codeine, hydrocodone and oxycodone tablets containing acetaminophen was determined by crushing a portion of the tablet and dissolving in an appropriate solvent. TLC plates were spotted, chromatographed, incised, sprayed with the glycerol solution and analyzed to determine if the separation achieved by the TLC baths allowed for the identification of the components of the preparations. Ten replicates were run to test reproducibility. The reproducibility study was repeated twice more on separate days.

The combination of TLC with DART™, after physical examination, streamlines the analytical scheme used to screen and identify pharmaceutical preparations while still meeting the requirements of SWGDRUG guidelines for drug identification. This study validates the use of TLC-DART™ in the forensic identification of the components of several pharmaceutical preparations.

Thin Layer Chromatography, Direct Analysis in Real Time, Mass Spectrometry

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