K19 Extraction and Analysis of Warfarin From Whole Blood Using a Long Chain SPE Sorbent

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The goal of this presentation is to present information on a solid phase extraction method that will improve on existing procedures for the analysis of warfarin in postmortem blood samples.

This presentation will impact the forensic community and/or humanity by improving the analysis of this drug in post mortem samples by utilizing a more efficient extraction system i.e., a long chain SPE sorbent in conjunction with both liquid and gas chromatographic systems.

Warfarin (Coumadin) is a popular pharmaceutical used as a blood- thinning agent. In therapeutic use, blood levels range from 1000 ng to

3100 ng per mL has been reported.[1] Several methods have been used for the analysis of this drug using liquid-liquid extraction.[2,3] This project was developed in order to study this drug at low levels in post mortem samples using a novel (C_{30}) solid phase sorbent.

In this method, Warfarin and the internal standard (p- chlorowarfarin (100 ng)) were spiked into whole blood samples (1mL) over a concentration range 0 through to 200 ng per mL. The samples were treated with an aqueous phosphate buffer (9 mL) and the drugs extracted onto a C_{30} SPE columns (200 mg). The columns were washed with the phosphate buffer and hexane (1x 3 mL each) and eluted with 14% methanol acid in ethyl acetate (2x 3mL). The eluents were collected and evaporated for further chromatographic analysis. Using GC-MS, the samples were derivatized prior to analysis using BSTFA, for analysis with LC-PDA the samples were reconstituted in DI water.

GC-MS separation was carried out using an Agilent Technologies 6890 GC coupled to a 5975 MSD for SIM analysis. HPLC analysis was carried isocratically out using both PDA and Fluorescence detection.

From this method LOQ's of 25 ng per mL of sample is easily achievable by either chromatographic system. By using GC-MS (SIM) in EI mode, 10 ng per mL of sample can be detected.

Examples of chromatograms and calibration curves are presented to show the simplicity and efficiency of this methodology.

References:

1 C.Winek *et al*, Forensic Sci. Int'l 122 (2001) 107-123

2 Locatelli *et al*, J.Chrom.B., 818 (2005) 191-8

3 Naidong *et al*., J.Pharm.Biomed. Anal. 25 (2001) 219-29

Warfarin, SPE, Toxicology

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