K10 Distribution of Methadone and Its Metabolites in Plasma and Blood Cell

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After attending this presentation, attendees will learn that data derived from the analysis of plasma do not represent the concentration of methadone in whole blood.

This presentation will impact the forensic science community by illustrating that methadone, perhaps most other drugs and their metabolites, is distributed between plasma and blood cell. Therefore, whether plasma or whole blood should selected as the test specimen depends on the test objective.

Methadone has long been adopted as the primary substitution drug for "treating" heroin addicts elsewhere and recently in Taiwan. Accurate determination of this compound and its metabolites in patients' blood provides valuable information helpful to safe and effective implementation of the substitution therapy policy.

Simulated samples (drug-free whole blood spiked with the analytes of interest) were first separated into plasma and blood cells portions. Resulting whole blood, plasma, and blood cell samples were used for developing effective sample preparation approaches to determine the distribution characteristics of the analytes of interest in plasma and blood cell portions. Clinical samples collected from patients under treatment were then analyzed to: (a) validate the findings derived from simulated samples; and, (b) study factors — e.g., treatment dosage, time lapse between drug intake and sample collection, and genetic variations, such as ABCB1 (C1236T, C3435T, and G2677T/A) and CYP2C19 (G681A and C990T) — that may affect the distributions. Typical sample preparation steps included: (a) addition of internal standards (MTD-d9 and EDDP-d3); (b) deproteinization twice by acetonitrile; and, (c) extraction with isopropanol/hexane (1:8, v/v) at pH 10.2 (carbonate buffer). Extracts were dried, then reconstituted with ethyl acetate for analysis. GC-MS was used as the primary analytical method, while a significant number of samples were also analyzed by LC/MS/MS (triple quadrupole configuration) to confirm the accuracy of data derived from GC-MS analysis. Since the concentrations of the commonly monitored metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP), in most clinical specimens were below the GC-MS quantification limits, MTD data were more fully evaluated.

The validity of a set of analytical data was examined by comparing the compatibilities of: (a) finding of the whole blood sample and the sum of the findings of the plasma and the blood cell samples; and, (b) the analytical findings derived from the GC-MS and LC-MSMS for those clinical specimens that have been analyzed by both methods. Data derived from simulated samples indicated: (a) 80% (standard deviation = 0.52%; n = 5) of MTD and 77% (standard deviation = 0.95%; n = 5) of EDDP in whole blood were found in plasma; and, (b) portions of MTD and EDDP (especially MTD) on the blood cells can be removed by washing the blood cell with phosphate buffer (pH 7.4). Data derived from GC-MS and LC-MSMS methods for the analysis of clinical specimens were found compatible. Among the 26 clinical specimens studied, the amount of MTD found in the plasma portion ranged from 70 to 86%. Specimens were re-collected from a small group of patients (n = 5). Data derived from the follow-up analysis of these specimens appeared to indicate the observed inter-patient variations (in MTD distribution) was unlikely caused by differences in treatment dosage or the time lapse between drug intake and specimen collection. It is currently being examined.

Methadone, Distribution, Blood

 ** Presenting Author*