K15 Identification and Quantification of Tapentadol and N-Desmethyltapentadol in Human Urine Using Gas Chromatography-Mass Spectrometry

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After attending this presentation, attendees will learn about a Gas Chromatography-Mass Spectrometry (GC/MS) method developed to identify and quantify tapentadol and its main metabolite N-desmethyltapentadol (NDT) in human urine. Attendees will also understand that preliminary investigations demonstrated that the NDT metabolite does not have the same extraction characteristics and chemical derivatization properties as the parent drug. Therefore, special considerations were necessary when developing a method for simultaneous identification and quantification of tapentadol and NDT in human urine.

This presentation will impact the forensic science community by describing to forensic toxicologists and chemists the first GC/MS method developed and validated to identify and quantify tapentadol and its main metabolite NDT in human urine.

Chronic pain is one of the most persistent health care problems in the United States. When physicians fail to properly address pain in patients, it can lead to additional health problems or decrease the patient's quality of life. In many health care settings, opiate and opioid drugs have become the treatment of choice for pain management because of their effective analgesic properties. Tapentadol (Nucynta®) is a relatively new drug that is approved for treatment of both immediate and chronic pain. Tapentadol causes analgesia by acting as an agonist at the brain's mu receptors and as a norepinephrine reuptake inhibitor. Combining these two mechanisms of action makes tapentadol different from "traditional" opiate and opioid drugs, which do not act as norepinephrine reuptake inhibitors. As an analgesic drug, it is likely that incorporation of tapentadol into pain management and pain-monitoring programs will become more widespread, and it will be necessary for toxicology laboratories to be able to identify and quantify the drug and its metabolite in human urine specimens.

The development and validation of the first GC/MS assay developed for the identification and quantification of tapentadol and its major metabolite NDT in human urine samples will be described. Method development studies were initially performed to design an assay that was optimized for the extraction, identification, and quantification of tapentadol and NDT. The optimized procedure involved sample alkalinization with saturated borate buffer (pH 9.5) and extraction into chloroform: isopropanol (9:1) solvent. Samples were evaporated to dryness under a stream of nitrogen and derivatized with 25µl MTBSTFA + 1% TBDMCS: acetonitrile (1:2) at 55°C for >2h.

Quantification of tapentadol and NDT required two internal standards. Deuterated tapentadol-d3 was used for tapentadol quantification and 4-(2-methylamino)propyl)phenol was used for NDT because deuterated NDT was not commercially available. Two internal standards were needed because of the extraction differences between tapentadol and NDT, arising from the metabolite's secondary amine structure. The secondary amine structure of NDT also limited the compound's solubility in MTBSTFA + 1% TBDMCS derivatizing reagent, making it necessary to solubilize the metabolite with acetonitrile to maximize silation derivatization.

Glucuronide conjugates are the primary route of tapentadol and NDT elimination. Hydrolysis studies were performed to liberate the glucuronide conjugates from urine samples. Tapentadol-β-D-glucuronide and NDT were analyzed using the developed GC/MS program and no free tapentadol was detected. Overnight hydrolysis with helix pomatia H-2 was found to be the optimal hydrolysis method, with approximately 50% tapentadol liberated at concentrations of 150ng/ml, and 47% at concentrations of 600ng/ml.

The assay met all laboratory validation criteria with respect to linearity, sensitivity, accuracy, inter-assay precision, intra-assay precision, selectivity, matrix effects, process efficiency, recovery, bench-top stability, and instrument stability. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for tapentadol and NDT were administratively set at 10ng/ml and 50ng/ml, respectively.

Five quality-control samples were run in triplicate over an eight-day validation period with tapentadol and NDT at concentrations of 50, 150, 600, 1500, and 2500ng/ml. The accuracy of the quality-control samples were within ±16% of the target value and the precision %CV values (inter and intra) were <16%. Matrix effect, process efficiency, and recovery were assessed by analyzing six replicates of tapentadol and NDT at concentrations of 150 and 600ng/ml. At both concentrations, tapentadol recovery was 100% and NDT recovery was 83% at 150ng/ml and 96% at 600ng/ml. The validated method allows for the identification and quantification of tapentadol and NDT in human urine.

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