Clinical and Forensic Monitoring of Zopiclone Through the Use of a Degradation Product

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Overview

Purpose: To determine if the unstable analytes zopiclone, its stereoisomer eszopiclone (Lunesta®, Sunovion Pharmaceuticals Inc.), and their metabolites can be accurately quantified via a degradation compound, 2-amino-5-chloropyridine (ACP) used as a surrogate analyte.

Methods: Sample analysis initiates with a rapid LC-MS/MS analysis monitoring for a panel of "Z-Drug" non-benzodiazepine sedative hypnotic compounds consisting of zolpidem, zaleplon, and zopiclone and their primary metabolites zolpidem phenyl-4-carboxylic acid, 5-oxo-zaleplon, zopiclone-N-oxide, and N-desmethylozopiclone. In addition, ACP is semi-quantitatively monitored as an indicator of zopiclone or zopiclone metabolites. When zopiclone, zopiclone metabolites, or ACP are detected, a second analysis is performed under alkaline conditions forcing the degradation of all remaining zopiclone and zopiclone metabolites to ACP prior to the quantitative analysis of ACP.

Results: The production of ACP was characterized to ensure ACP levels accurately reflect the load of zopiclone within a sample.

Background

Zopiclone is present in urine samples in low concentrations of parent drug and is primarily found as the two metabolites; zopiclone-N-oxide and N-desmethylozopiclone. However, in alkaline samples zopiclone and metabolites rapidly degrade into ACP (see Figures 1 and 2). The uncontrolled loss of zopiclone and metabolites in these samples complicates the ability to confidently measure concentrations of these analytes and interpret the findings.

Clinical analysis of urine specimens were prepared by organic dilution with acetonitrile, vortex mixing, and centrifugation. Supernatant aliquots were analyzed via LC-MS/MS (see Table 1) monitoring for a panel of non-benzodiazepine hypnotic compounds consisting of zolpidem, zaleplon, and zopiclone and their primary metabolites. Samples with detectable levels of zopiclone, zopiclone metabolites, or ACP were then analyzed using a subsequent ACP analysis following a forced degradation of all zopiclone compounds into ACP.

Zopiclone and metabolites were fully converted into ACP using an alkaline degradation procedure including potassium hydroxide and incubation at 65°C. Complete degradation can be achieved in under 15 minutes and is not impacted by the analyte concentration or variations in the initial pH of the specimen. Samples are then processed by an acetonitrile dilution, vortex mixing, and centrifugation prior to LC-MS/MS analysis.

Results

60 randomly selected patient samples which initially screened positive for zopiclone were selected for further evaluation. Samples were analyzed for zopiclone, zopiclone-N-oxide, N-desmethylozopiclone, and ACP to determine initial concentrations.

90% of the samples were acidic thereby explaining the existence of detectible zopiclone levels in these samples. ACP was additionally detected in 87% of the samples prior to forced degradation. The relative ratio of zopiclone to ACP in untreated samples varied from sample to sample. See color coding in Figure 5.

Following the forced degradation procedure, measured ACP levels directly correlated to the summed molar concentrations of zopiclone, zopiclone metabolites and initial ACP (presented as relative concentration, Target ACP). See Figure 5.

Conclusions

A two part procedure has been developed monitoring for non-benzodiazepine hypnotics. Zolpidem, zaleplon and their primary metabolites are directly monitored and quantified.

Zopiclone and metabolites are unstable in alkaline samples reducing the ability to directly measure these compounds.

Zopiclone can be indirectly measured through a quantitative, forced degradation procedure. The resulting degradation product, ACP, can be measured via LC-MS/MS and the levels directly correlate to the concentration of zopiclone and metabolites.

References