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

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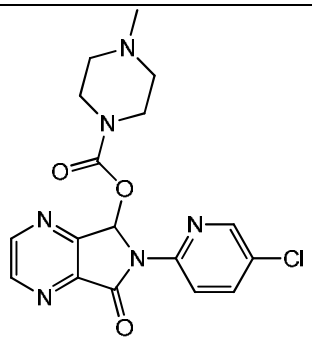
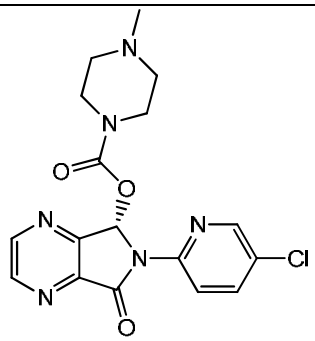
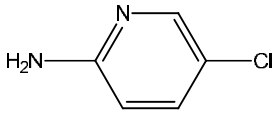
Sleep aids are widely used today; the CDC reports 4% of the US population has used prescription sleep aids within the last month[1]. Identifying these agents within biological samples is of interest from both a clinical and forensic toxicology perspective to identify compliance, abuse, and impairment. However, monitoring for the presence of zopiclone or the optically pure isomer eszopiclone is severely limited by poor *ex vivo* stability of both parent drugs and their primary metabolites zopiclone-N-oxide and N-desmethylzopiclone. All degrade to 2-amino-5-chloropyridine (ACP)[2,3] under ambient and refrigerated conditions, with limited degradation also occurring over long term storage of frozen samples.

While zopiclone and its primary metabolites can be successfully stabilized by acidifying the samples, this is an unattractive strategy due to the logistical difficultly of ensuring the addition of stabilizer at the time of sample collection. An initial rapid LC-MS/MS screening procedure is performed detecting any trace levels of zopiclone, zopiclone N-oxide, and ACP in conjunction with an analysis detecting other non-benzodiazepine hypnotics (Z-drugs), zolpidem and zaleplon and their respective metabolites. If the initial screen is positive for zoplicone, zopiclone N-oxide, or ACP,an alternative strategy is implemented to chemically convert all zopiclone and primary metabolites into ACP through an alkaline incubation with ammonium hydroxide. ACP is then extracted through a simple solvent dilution procedure followed by UPLC-MS/MS analysis on an AB Sciex 5500. Measured molar quantities of ACP formed in the alkaline samples reflect the total combined number of moles of zopiclone and the major metabolites present in the original sample.

Additionally, we found ACP to be a specific indicator of zopiclone compounds, as ACP was not produced when a battery of 200 common pharmaceutical agents and drugs of abuse were subjected to the degradation protocol. Specifically, the other non-benzodiazapines, zolpidem and zaleplon, did not produce ACP.

In summary, a previously described strategy of using ACP levels as a surrogate analyte reflecting zopiclone concentrations has been successfully incorporated into a UPLC-MS/MS procedure for

monitoring non-benzodiazepine hypnotics (Z-drugs) and successfully applied to urine matrices; the procedure is currently being evaluated for other biological matrices.

		
Zopiclone	Eszopiclone	ACP

1. Chong Y, Fryar CD, and Q Gu, Prescription Sleep Aid Use Among Adults: United States, 2005–2010; NCHS Data Brief Number 127, Hyattsville, MD: National Center for Health Statistics. 2013.
2. Mannaert E, Tytgat J, and P Daenens, Detection of 2-Amino-5-Chloropyridine in Urine as a Parameter of Zopiclone (Imovane®) Intake using HPLC with Diode Array Detection, *J of Anal Toxicol,* 21 (1997) 208-212.
3. Nilsson GH, Kugelberg FC, Ahlner J, and R Kronstrand, Quantitative Analysis of Zopiclone, N-desmethylzopiclone, Zopiclone-n-oxide, and 2-Amino-5-chloropyridine in Urine Using LC-MS-MS, *J Anal Toxicol,* 38 (2014) 327-334.