HPLC-MS-MS Methods for the Detection of Zopiclone and its Alkaline Hydrolysis Product 2-amino-5-chloropyridine (ACP) in Post-Mortem Blood

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BACKGROUND

Zopiclone (Zimovane™) is a cyclopyrrolone hypnotic used for the short-term treatment of insomnia. It also possesses anxiolytic, sedative, anti-convulsant and muscle-relaxant properties. Despite sharing the pharmacological actions of benzodiazepines, zopiclone acts on different receptors. Zopiclone has an oral bioavailability of approximately 80%, and is absorbed rapidly, producing peak blood concentrations within 1.5 to 2 hours. It is extensively metabolised in the liver to zopiclone-N-oxide (active) and N-desmethylzopiclone (inactive). The elimination half-life is approximately 5 hours and no accumulation has been seen after repeated dosing.

Recently there has been an increase in the number of reports of zopiclone abuse and misuse.[1] Drug abusers are now thought to be using zopiclone as its affects are comparable to those of benzodiazepines, yet routine drug screening programmes are often unable to detect its presence. Zopiclone's rapid onset of action, coupled with its short half-life also makes it a potential candidate for drug-facilitated crime.[2] For these reasons it is no surprise that the number of zopiclone cases presenting to forensic toxicology laboratories is on the increase.

The detection and subsequent quantification of zopiclone is plagued with problems. It is unstable in nucleophilic solvents such as methanol or ethanol, it has a high molecular weight (388.8) and therapeutic concentrations are low (30-60ng/mL peak).[3] While fluoride preservatives may assist in preserving zopiclone concentrations in post-mortem samples, prior exposure to unusual conditions, such as putrefaction may have already resulted in significant degradation.[4] Unfortunately due to difficulties associated with zopiclone stability and analysis, routine screening procedures often overlook its presence.

Whilst the instability of zopiclone can hinder its detection, this phenomenon has the potential to be exploited. Under alkaline conditions zopiclone rapidly hydrolyses to 2-amino-5-chloropyridine (ACP). This lower molecular weight (128.6) compound is far easier to detect through routine screening. We describe HPLC-MS-MS methods for the detection of zopiclone and its main alkaline hydrolysis product, ACP in post-mortem blood and evaluate the potential value of ACP quantification as a means of establishing zopiclone overdose.

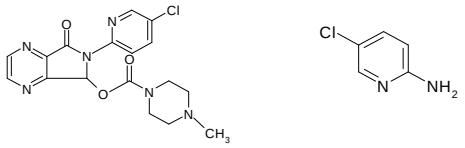

Figure 1. The Chemical structures of zopiclone and its alkaline hydrolysis product 2-amino-5-chloropyridine (ACP).

EXPERIMENTAL

Materials

Zopiclone was obtained from Aventis (Nanterre, France) and ACP was obtained from Fluka (Seelze, Germany). HPLC grade methanol, acetonitrile and methyl-tert-butyl-ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Analar grade formic acid and sodium hydroxide (40% solution) were obtained from BDH (Poole, Dorset, England). Trizma Base was obtained from Sigma (St Louis, USA) De-ionised water was prepared on site (ELGA Limited).

Extraction

Zopiclone and ACP methods involved liquid-liquid extraction into MTBE. Both analytes were analysed in duplicate due to the absence of suitable internal standards. Zopiclone was extracted using 2M tris (250µL), while ACP was extracted using 1M sodium hydroxide (250µL), each using a 200µL sample of post-mortem blood. Zopiclone and ACP were then back-extracted into 0.1% formic acid (250µL). The extracts were transferred to autosampler vials and left to stand for 5 minutes to allow any residual MTBE to evaporate, prior to analysis.

HPLC Conditions and MS Parameters

The HPLC equipment consisted of a Perkin Elmer PE200 series autosampler and pump, and a Schimadzu CTO-10A column oven. Detection was by tandem mass spectrometry (HPLC-MS-MS), using a Sciex API2000 triple quadrupole mass spectrometer equipped with a turbo-ion spray interface (Applied Biosystems).

ACP: The analytical column used for ACP was a Supelcosil LC-18-DB (15cm x 4.6cm, 5µm) and the mobile phase consisted of methanol:de-ionised water (85:15, v/v), supplemented with ammonium acetate to achieve a final concentration of 2mmol/L. The method was run in positive ionisation mode and set to measure precursor to product ion transitions of m/z 129.0/112.1 and 131.0/114.1 for ACP, which eluted after 2.3 minutes.

Zopiclone: The analytical column used for zopiclone was a Supelcosil LC-Si (10cm x 4.6, 5µm) and the mobile phase used was acetonitrile:de-ionised water:formic acid (50:50:0.1, v/v/v). The method was run in positive ionisation mode and set to detect the precursor and product ion transitions of m/z 389.1/245.1 and 391.2/247.0 for zopiclone, which eluted after 6.8 minutes. Concentrations of both analytes could be detected at 5ng/mL.

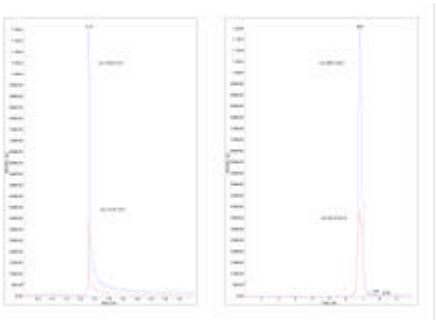

Figure 2. Chromatograms illustrating the retention times and the precursor to product ion transitions of ACP (left) and zopiclone (right).

RESULTS

Table 1. Shows the ACP and zopiclone concentrations observed in 12 post-mortem samples referred to the Forensic Toxicology Service by HM Coroner. The presence of zopiclone was indicated in the history of all cases presented. A very strong correlation was observed between the concentration of zopiclone and ACP ($r = 0.994$, $p < 0.001$).

Case Number	ACP (mg/L)	Zopiclone (mg/L)
1	0.437	0.71
2	0.106	0.88
3	0.474	2.63
4	0.005	0.03
5	0.021	0.23
6	0.007	0.01
7	1.281	5.26
8	0.028	0.02
9	0.136	0.21
10	0.010	0.00
11	<0.005	0.01
12	4.080	14.62

Table 1. Post-mortem concentrations of ACP and zopiclone in 12 femoral blood samples submitted to the Forensic Toxicology Service.

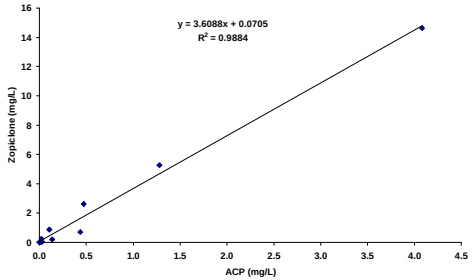

Figure 3. A graph showing the correlation between ACP and zopiclone concentrations in the samples studied.

CONCLUSION

The data show that ACP was detectable in all cases of zopiclone overdose and in all except one case of therapeutic use. The authors believe that ACP can be used reliably for routine zopiclone screening and that its use for this purpose will lead to an increase in the detection of zopiclone positive cases. The data obtained also suggest the potential quantitative use of ACP in the estimation of zopiclone concentrations. However, further research into this area is required.

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