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Case Report

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Case Report

Potential 2-Aminoindane Fatality Invalidated by Careful Mass Spectrometric Analysis

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Abstract

We describe herewith the case of a patient presenting to the emergency department for worsening ear–nose–throat symptoms. As chemsex was evocated by the family, patient's serum was submitted to a new psychoactive substances screening. After a simple liquid–liquid extraction, serum was injected on a high-resolution mass spectrometer using quite usual conditions (C18 column, gradient mode with acidic buffer, methanol and acetonitrile). An almost perfect match with 2-aminoindane (2-AI) was observed considering that the precursor ion was present in the sample but absent in the commercial library. Literature concerning 2-AI is sparse, and further investigations were undertaken. After injection of the reference standard, a small retention time shift has been observed (0.3 min) between the standard and the sample. The case was only closed while spiking the sample with the standard, giving rise to two distinct peaks. As a result, 2-AI was then considered as absent from the sample and death was attributed only to infection. Moreover, a rapid liquid chromatography–tandem mass spectrometry method dedicated to 2-AI was developed. It generated the same false-positive result highlighted by significant differences observed in ion ratios (2.37 for the sample versus 6.62 for the neat standard).

Introduction

With only four compounds reported to the European Monitoring Center for Drugs and Drug Addiction between 2005 and 2017, aminoindanes represent the smallest category of new psychoactive substances (NPS) detected in Europe (1). The simplest compound of this class is 2-aminoindane (2-AI), which is structurally related to amphetamine, as indicated in Figure 1.

Other derivatives potentially used as NPS are, for example, 5,6-methylenedioxy-2-aminoindane, 5-iodo-2-aminoindane and *N*-methyl-2-aminoindane; however, these will not be discussed herein (2).

2-AI's mechanism of action includes a selective inhibition of the norepinephrine transporter (dopamine and serotonin transporters are not affected) and a release of norepinephrine and dopamine in the synapse (3).

Scientific information about the clinical effects of 2-AI is sparse. According to user reports, 2-AI consumption sometimes leads to very mild effects, while some consumers report physical and mental stimulating effects as potent as those of 3,4-methylenedioxymethylamphetamine (MDMA). Pain relief, wakefulness and euphoria are also described (4–6). 2-AI can be consumed by oral ingestion or nasal insufflation even if snorting is frequently associated with a sensation of pain or burning of the nostrils (4, 5).

Only a few cases are available in the literature in which 2-AI was found in biological samples. In their 2014 publication, Elliott et al. list the NPS that they found in casework during a 3-year period and 2-AI was detected once in 2011; yet, no details were given about the case (7). A fatality mainly attributed to methadone (blood concentration = 0.807 mg/L) and 2-AI (0.101 mg/L) but also including other medications (zopiclone and sertraline at a supratherapeutic level and aripiprazole at an infratherapeutic level) and ethylphenidate (blood concentration = 0.11 mg/L) was reported by Maskell et al. (8). This paper is the only one indicating a 2-AI blood concentration. Eventually, 2-AI was also detected but not quantified in an MT-45 overdose (9) and in pooled urine collected from a London nightclub urinal (10).

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Figure 1. Chemical structure of amphetamine (a) compared to 2-AI (b).

In this paper, we report an analytical investigation concerning a dying patient with serum sample potentially positive for 2-AI.

Case History

A 24-year-old man came for the third time at the emergency room. He was suffering from worsening ear–nose–throat symptoms for a week, unsuccessfully treated with paracetamol, ibuprofen and oxymetazoline. Medical history was irrelevant. He presented with a Glasgow Coma Score of 15/15, profuse sweating and generalized erythema. A light edema was observed on the right eyelid, left elbow and wrist combined with a painful left leg. Sinusal tachycardia was also observed. He was rapidly admitted to the intensive care unit where he was sedated and intubated. Antibiotherapy (ceftriaxone and clindamycine) and iterative filling were initiated. Nevertheless, he rapidly developed a violent septic shock leading to disseminated intravascular coagulation associated with cardiac, renal, hepatic and respiratory failure. Necrotic tissue combined with blisters appeared on the left elbow, consequently requiring a surgery. Despite close monitoring and supportive treatment, he finally died of a virulent group A *Streptococcus pyogenes* the day after his admission. Chemsex was mentioned by the family; hence, a screening for NPS was undertaken.

Materials and Methods

Standard and reagents

Analytical standard of 2-AI was purchased from LGC standards (Teddington, UK). Prazepam, used as internal standard (IS), was purchased from Certa (Braine-l'Alleud, Belgium). All solvents were liquid chromatography–mass spectrometry or high-performance liquid chromatography grade and supplied by J.T. Baker (Phillipsburg, USA). For mobile phase preparation, ammonium formate was supplied by Fisher Chemical (Merelbeke, Belgium), whereas formic acid was purchased from Biosolve (Dieuze, France).

Sample preparation

After addition of an IS (prazepam 1 mg/L) and basification (0.5 mL of Na_2CO_3, 1M), 5 mL of a mix of diethyl ether/dichloromethane/*n*-hexane/*n*-amylic alcohol (50/30/20/0.5: V/V) was added to 1 mL of the sample. After mixing for 15 min and centrifugation, the organic layer was evaporated to dryness and reconstituted in 100 µL of the mobile phase.

Instrumentation and chromatographic conditions

The ultra-high-performance liquid chromatograph coupled to a time-of-flight mass spectrometer (UHPLC-TOF-MS) apparatus was an Eksigent® LC 100 XL combined with a TripleTOF® 4600 from Sciex (Framingham, USA). The screening method was developed by Sciex (11). Injections of 10 µL of the samples were done on a Kinetex C18 column, 2.6 µm, 100 Å, 50 × 3.00 mm (Phenomenex, Torrance, USA). A gradient was applied at 30°C, with mobile phase A consisting of 10 mM ammonium formate and mobile phase B made of a mix of acetonitrile and methanol with 0.1% formic acid (50/50). A constant flow of 0.4 mL/min was applied using the following gradient: the initial condition of 98% of mobile phase A was held for 1 min. Then, the gradient linearly decreased to 0% of A in 10 min, held for 3 min. Finally, the gradient returned to initial conditions and was maintained for 2.5 min prior to the next injection. The mass spectrometer was equipped with a DuoSpray ion source working in positive electrospray ionization mode. Source conditions were as follows: ion source gas 1 (GS1) 40 psi, ion source gas 2 (GS2) 60 psi, curtain gas (CUR) 30 psi, source temperature 500°C and ion spray voltage floating 5500 V. The mass acquisition method was a TOF survey scan from 50 to 1,100 Da (cycle time 1 s and accumulation time 0.150 s). It was combined with a second experiment of product ion scan using an information-dependent acquisition method on a maximum of 20 candidates per cycle. Data were acquired with Analyst 1.7.1 software and finally processed with the PeakView software 2.2, including a comparison to a regularly updated in-house library purchased by Sciex.

Another method dedicated to 2-AI was also developed on an Acquity UPLC® coupled with a Quattro Premier XE® mass spectrometer from Waters (Milford, MA, USA). Injection volume was 5 µL. We used a BEH C18 column (1.7 µm 2.1 × 50 mm) maintained at 40°C and a mix of respectively 0.1% formic acid in water and 0.1% formic acid in methanol defined as mobile phases A and B. A flow rate of 0.4 mL/min was applied with a gradient mode as follows: 95% of mobile phase A was initially applied and decreased to 70% of A in 6 min and then rapidly to 0% in 0.5 min. Initial conditions were reapplied in the next minute, maintained for 1 min before the next injection. Source was operating in positive electrospray ionization, with a capillary voltage of 1.5 kV. Temperatures were set at 120°C for the source and 350°C for the desolvation. Nitrogen was used as cone and desolvatation gas at a flow rate of 50 and 800 L/h. The instrument was working in multiple reaction monitoring (MRM) mode and the collision gas was argon settled at a flow rate of 0.20 mL/min. The cone voltage was set at 20 V and transitions monitored were 133.9 > 116.9 (collision energy: 15 eV) and 133.9 > 115.1 (collision energy: 20 eV) for 2-AI, whereas it was 325.3 > 271.1 (collision energy: 24 eV) for the IS.

Method validation

Both methods considered in this paper are qualitative methods. Therefore, the validation procedure was restricted to the following parameters: specificity, limit of detection (LOD) and matrix effect (ME). Each experiment has been carried out on both instruments.

Specificity

Ten supposed negative serum samples were submitted to the sample preparation as described before, with no addition of the IS. They were injected into both instruments in order to check for the absence of endogenous compound generating a peak corresponding to 2-AI.

Simultaneously, blank matrix was also spiked with 500 µg/L of the most commonly prescribed drugs to determine wheter they would lead to a signal that could be confused with 2-AI. Paracetamol, benzodiazepines and Z-drugs (alprazolam, hydroxy-alprazolam, bromazepam, clonazepam, desalkylflurazepam, diazepam, lora-zepam, lormetazepam, midazolam, nordiazepam, oxazepam and zolpidem), antidepressants (citalopram, fluoxetine, mirtazapine, trazadone, venlafaxine and desmethylvenlafaxine), neuroleptics (clozapine, haloperidol, olanzapine, pipamperone, prothipendyl, quetiapine and risperidone) and opiates (codeine, methadone, morphine, morphine-3-glucuronide and tramadol) were tested. Usual drugs of abuse (amphetamine, cocaine,

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benzoylecgonine, MDMA and tetrahydrocannabinol (THC)) were also tried out in order to screen for interfering compounds.

Limit of detection

LOD was evaluated by injecting decreasing amount of 2-AI spiked in blank serum and submitted to the extraction procedure. Three different sources were tested, and each source was processed on a different day while each level was prepared in duplicate. The LOD was defined as the smallest level generating a signal-to-noise ratio >3. This was combined with fulfilled identification criteria on the TOF instrument, i.e., a mass error <5 ppm, a retention time error <5.1%, an isotope ratio difference <20% and a library score >70. 2-AI concentrations in the tested levels were 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5 and 10 µg/L.

Matrix effect

ME was evaluated on 12 different sources of blank serum using the post-extraction addition procedure described by Matuszewski et al. (12). Six of them were spiked post-extraction with 5 µg/L of 2 AI, whereas the six other matrices were spiked post-extraction with 100 µg/L of 2-AI. The mean peak area of each level was compared with the mean peak area of six neat standards at the same concentration. Ionization suppression or enhancement was calculated with the following formula: $\left(\frac{Mean\ area\ spiked\ sample}{Mean\ area\ neat\ standard} - 1\right) \times 100$.

Results and Discussion

Method validation

Specificity

Based only on the recommended tests, both methods demonstrated a sufficient specificity. Indeed, no interfering compound was noticed neither in the 10 matrices tested nor among the 36 spiked compounds.

Limit of detection

A LOD of 0.5 µg/L was defined on the liquid chromatography–tandem mass spectrometry (LC–MS-MS) method, whereas a concentration of 2.5 µg/L was the smallest that could be detected in TOF-MS with fulfilled identification criteria. Indeed, a concentration of 1 µg/L was detectable with a chromatographic peak showing a signal-to-noise ratio >3, but the compound was not always fragmented or mass spectrum/isotopic patterns were inconclusive.

It is in accordance with usual practice that tends to use tandem mass spectrometry rather than TOF-MS in order to increase the sensitivity, even if this lack of sensitivity comes from an unfair comparison between an untargeted acquisition method and a dedicated quantitative triple quadrupole method (13). However, NPS are potent compounds that can sometimes be present in biological samples at low concentration while exerting significant effect, making this relatively high LOD a potential limitation of our TOF-MS method. It has to be noticed that LOD is compound dependent, and the LOD that was measured for 2-AI is not necessarily representative of all NPS. As reviewed elsewhere (14), screening methods dedicated to NPS are mainly targeted LC–MS-MS methods, but publications using untargeted high-resolution mass spectrometry are also available. The choice between both methods is sometimes cost dependent, as TOF instruments are expensive and cannot be bought by each laboratory.

Matrix effect

Calculated ionization suppression/enhancement is represented in Table I: little to no significant ME was observed.

Table I. Matrix Effects Calculated on Both Instruments

	LC–MS-MS		TOF-MS	
	Without IS (%)	With IS (%)	Without IS (%)	With IS (%)
5 µg/L	−0.29	41.9	−1.91	70.9
100 µg/L	4.29	43.4	−2.39	48.8

Moreover, the relative standard deviation calculated on peak area ranged from 2.56% to 8.99%, depending on the level and the instrument. It confirmed that no matrix led to a significant ionization suppression or enhancement.

Area ratios calculated with the IS led to significantly increased results. This illustrates that the use of an IS, which is not the labeled analogue of the compound of interest, can be misleading. Indeed, by definition, MEs are due to compounds present in the matrix that coelute with the compound of interest and are entering the source at the same time, leading to potential interference with its ionization. The choice of a totally different IS that does not share the retention time of the compound of interest is a mistake. Even if sometimes it is inevitable in case labeled ISs are not available.

Patient sample

Initial analysis of the sample by UHPLC-TOF-MS led to a match with 2-AI, as indicated by the exact mass (134.0958; mass error = 1.2 ppm) and the three major fragments (91.0536, 115.0535, 117.0694). However, retention time of this compound was not known, and slight differences were observed in the mass spectrum (e.g., precursor ion was present in the sample but absent in the commercial library), see Figure 2.

2-AI reference standard was then ordered to check its retention time with the method and make a decision. Upon arrival (2.5 months later), reference standard was injected on UHPLC-TOF-MS and its mass spectrum and retention time were compared with the sample. A small difference of 0.3 min between the neat standard and the extracted serum was observed, which could potentially be explained by MEs. Moreover, precursor ion (134.0958) was not observed for the neat standard while it was in the sample.

Then, as several milliliters of sample were luckily available, we decided to spike the patient serum with 2-AI. Two distinct peaks were observed, closing the case (Figure 3).

Finally, an even smaller retention time shift (0.1 min) was also observed in the LC–MS-MS method while analyzing the standard together with the patient sample. Indeed, this method was developed to be as fast as possible. If we used solely the LC–MS-MS method, a false-positive result would probably be unsuspected, unless ions

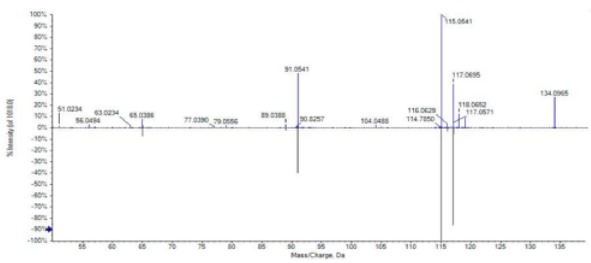

Figure 2. 2-AI mass spectrum in the sample (top) compared to library (bottom).

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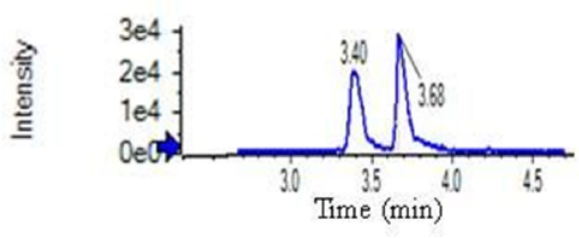

Figure 3. Chromatogram of the sample spiked with 2-AI.

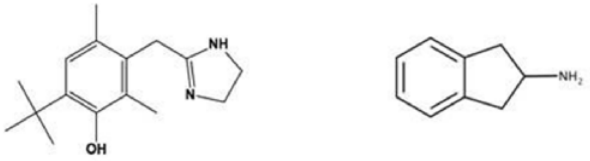

Figure 4. Chemical structure of oxymetazoline (a) compared to 2-AI (b).

ratios were calculated as a significant difference was observed for the sample compared to the neat standard (2.37 vs 6.62, respectively).

Ion ratios are not systematically assessed during the analytical validation process. Maybe, because it can be tricky due to inter-batch variation as well as concentration variation (15). However, it was demonstrated in other areas that this criterion was important to prevent false-positive result (16, 17). In the present case, an erroneous identification of 2-AI would not have had a huge impact. However, this is transposable to all compounds analyzed with LC–MS-MS methods. In other cases, misidentification can have serious consequences in a forensic context (driving license restitution, child custody, drug facilitated sexual assault, etc.).

Stability issue

In our case, delivery time of the reference standard was so long (2.5 months) that it gave rise to a stability issue if we had to quantify the compound in the patient sample. Indeed, stability in biological matrices have not been studied for all NPS. Concerning 2-AI, no degradation was demonstrated when plasma samples were stored at room temperature during 28 days (18). We can reasonably assume that a patient sample containing 2-AI would remain stable when stored in a freezer during 2.5 months.

Origin of the interference

The patient was treated with oxymetazoline, which is a nasal vasoconstrictor that shows some structural analogy with 2-AI, i.e., two carbon atoms creating a link between a nitrogen atom and a phenyl ring (Figure 4).

In order to see if an oxymetazoline metabolite wasn't responsible for this false-positive result, a volunteer inhaled the recommended dose of oxymetazoline the 3 days before his blood was screened for NPS. No interference was observed, which is similar to an *in vitro* study (19).

Finally, ~900 other samples were tested with the TOF method, and the same interference was observed in 6 of them, representing 0.66% of the tested samples. This low frequency of occurrence allows us to exclude an endogen compound. Unfortunately, neither patient files analysis nor compounds found in the samples allowed us to identify the nature of this interfering compound.

Conclusion

One aspect of the analytical challenge represented by NPS is once again depicted in this case report. Even if MRM mode is often preferred in order to increase the sensitivity, identification criteria—including retention time and two transitions—are sometimes insufficient to be 100% sure of the compound identification, especially if the runtime is short. Calculating ion ratios is mandatory if identification is to be based on MRM data. Finally, this case will not expand the number of 2-AI-related fatalities.

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