



First detection of ethylphenidate in human fatalities after ethylphenidate intake[☆]



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ABSTRACT

Methylphenidate, a psychostimulant drug from the group of amphetamines is, among others, established in the treatment of attention deficit hyperactivity disorder and narcolepsy. It is also known to have a certain potential of abuse. In combination with alcohol, the metabolite ethylphenidate was detected in human plasma in small amounts. However, ethylphenidate is sold as “research chemical” via the Internet. It was put under German narcotics law in July 2013. In a recent case, where a deceased person was found in his apartment, the police seized a plastic bag with the inscription “ethylphenidate”. An autopsy of the 32-year-old man yielded a mitral valve endocarditis, which must have persisted a while before death, in combination with a pneumonia.

At the Forensic Toxicological Centre (FTC) in Munich femoral blood, liver, pericardium fluid, urine, stomach content and hair of the deceased were analyzed for ethylphenidate after sample preparation by an LC-Triple TOF 5600. Calibration curves were spiked with a methanolic 1 mg/mL solution of ethylphenidate (substance provided by the State Office of Criminal Investigation in Munich) in whole blood in comparison to liver and femoral blood, in serum in comparison to pericardium fluid and in urine in comparison to urine and stomach content, respectively.

Ethylphenidate was detected in all analyzed matrices. The spectrums of the human specimen were compared to those obtained from the calibration curves and identified as ethylphenidate. The measured concentrations were for femoral blood 110 ng/mL, for liver 180 ng/g, for pericardium fluid 131 ng/mL, for urine 987 ng/mL and for stomach content 20.7 ng/mL, respectively. The stomach contained 200 mL of a brownish-coloured liquid, resulting in a total amount of 4000 ng ethylphenidate. The lowest calibrator for whole blood and serum was 1 ng/mL and for urine 10 ng/mL.

As far as it is known to the authors, these are the first ethylphenidate levels measured in a case of ethylphenidate intake. Therefore these results can only be compared to methylphenidate concentrations with therapeutic levels ranging from 5 to 60 ng/mL in serum. As the toxic levels for methylphenidate start from approximately 500 ng/mL serum, we estimate that ethylphenidate in the concentrations mentioned above is not in a directly lethal range. But it has to be considered, that amphetamine-like drugs as methylphenidate are known for their cardiovascular side effects (like tachycardia and arrhythmia) and might therefore have contributed to death, which was attributed to endocarditis in combination with pneumonia.

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1. Introduction

Methylphenidate, a psychostimulant drug from the group of amphetamines is, among others, established in the treatment of attention deficit hyperactivity disorder and narcolepsy. It inhibits the reuptake of noradrenaline and dopamine and increases the amount of these neurotransmitters in the synaptic cleft. Methylphenidate is also known to have a certain potential of abuse [1]. In

Internet forums users state that ethylphenidate produces the same effects as methylphenidate but is less potent and more pleasant. The typical method of administration is nasal and oral, but with both methods the mucosa is severely irritated. Its main application, similar to methylphenidate when used illegally, is to enhance concentration, but with fewer side effects [2]. In combination with alcohol the metabolite ethylphenidate (Fig. 1) is formed in vivo similar to the forming of cocaethylene and was detected in human plasma in small amounts [3]. For the manufacturing of ethylphenidate, methylphenidate has to be hydrolyzed with hydrochloric acid and afterwards esterified with ethanolic hydrochloric acid [4]. As methylphenidate is known to be unstable in human plasma at room temperature, ethylphenidate is assumed to behave in the same way. In Germany ethylphenidate was sold as a “research chemical” via the Internet [5], which means buying and selling was not illegal as long as it was declared as “not for human consumption”. However, in July 2013 it was subjected to German narcotics law because of the rising potential of abuse, so the selling of ethylphenidate is now illegal. In Sweden and Austria it is also considered to be a controlled substance. In the USA and Australia it is considered by the schedules as an analogue of methylphenidate. It is not covered by the laws of other countries yet. The State Office of Criminal Investigation (LKA) in Munich reported seized ethylphenidate in 5 cases in 2012, three times in a mixture combining other substances and twice as a pure substance (verified via infrared spectroscopy at the LKA). In 2013 two seizures of ethylphenidate were reported. It is also reported that a small amount of 0.19 g ethylphenidate was confiscated at Frankfurt customs. An assumed ethylphenidate intake of a deceased person has led to the presented investigations.

2. Case reports

A 32-year-old man was found dead in his apartment, where the police seized a plastic bag labelled “ethylphenidate”. The deceased’s mother was last in contact with him three weeks prior to his death, after this, there was no contact. The corpse was decomposed, when found. An autopsy of the man was carried out 2 days later and revealed a mitral valve endocarditis, which must have persisted for a while before death. Beyond this, pneumonia was also verified. Toxicological analysis was carried out and yielded no methylphenidate but ritalinic acid in a large amount. Blood alcohol concentration was also measured. This case is referred to as case 1.

Retrospectively, cases from 2013 and the second half of 2012, in which toxicological analysis yielded ritalinic acid in high concentrations and no or little methylphenidate were prepared and analyzed for ethylphenidate. The only other positive result, which is referred to as case 2 in this article, was a 38-year-old man

who was a known drug addict. When he was found, several recent puncture marks were visible on his body and a syringe, fentanyl patches and Lyrica® pills containing pregabalin were found in the apartment. An autopsy was carried out the day after his death and revealed aspiration of stomach content to be the cause of death. Additional centrally acting substances were found in routine analysis.

3. Materials and methods

3.1. Histological examination

For histological examination, aortic valve tissue and representative lung tissue of the 32-year-old man were taken, cut for microscopy and stained with haematoxylin–eosin–stain.

3.2. Toxicological analyses

3.2.1. Routine screening

A qualitative routine screening for central nervous acting substances as well as selected drugs was carried out on urine and stomach content by means of an LC–MS/MS device according to a procedure previously described [6]. For quantification, femoral blood was analyzed with the same device. An additional general unknown analysis by means of GC–MS was carried out for the urine samples. Sample preparation is analogical to that mentioned below for ethylphenidate.

In the routine alcohol analysis blood alcohol was not detected in both cases

3.2.2. Additional analysis for ethylphenidate

3.2.2.1. Preparation and analysis of the samples. At the Forensic Toxicological Centre (FTC) in Munich, femoral blood, liver, pericardium fluid, urine and stomach content of the deceased in case 1, and femoral blood in case 2, were prepared as follows: 100 µL of femoral blood and pericardium fluid, respectively, were treated with acetonitrile for protein precipitation, the supernatant was vaporized to dryness under nitrogen flow and reconstituted in a methanolic ammoniumformate solution. Of liver tissue 1 g was homogenized with 2 mL of isotonic sodium chloride solution in an ultra-turrax, following an analogical preparation to that of femoral blood and pericardium fluid by protein precipitation of 100 µL of the fluids. 100 µL of urine was treated with β-glucuronidase and, like the stomach content, diluted 1:10 before analysis. The internal standard 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) was added to all samples before preparation. The extracts were analyzed for ethylphenidate by an LC–Triple TOF 5600 device with electrospray ionization operated in positive mode. For the calibration curves, different matrices as follows were spiked with a methanolic solution of ethylphenidate in a concentration of 1 mg/mL with seized ethylphenidate, provided by the LKA in Munich (the substance was verified as ethylphenidate in a purity of more than 90% by means of Infrared-Spectrometry). A calibration curve including five different concentrations, covering a range of 1–250 ng/mL was spiked in whole blood, provided by a volunteer without noteworthy medication, as a comparison for femoral blood and liver tissue. In urine, four different concentrations of ethylphenidate, covering a range of 10–1000 ng/mL were spiked in urine provided by a volunteer without noteworthy medication. This curve served as calibration for urine and stomach content, as the stomach contained a predominantly liquid content. To range the concentration in pericardium fluid five different concentrations in a range from 1 ng/mL to 250 ng/mL were spiked in bovine serum and used for calibration.

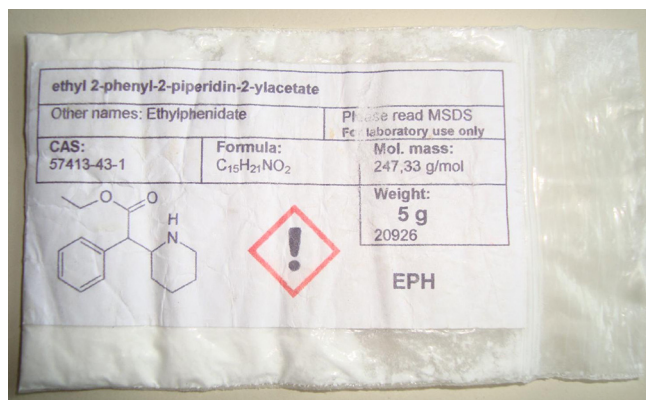


Fig. 1. Seized plastic bag with ethylphenidate.

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