3,4-Methylenedioxypyrovalerone (MDPV) is a cathinone derivative. It has recently been classified as a controlled substance in many countries. This substance is a stimulant that can be snorted, smoked or taken orally. MDPV has been determined in biological material from four cases sent to the Institute of Forensic Research in 2011. In the first case, a passenger car crashed into a truck; the driver of the vehicle suffered severe injuries, resulting in his death. In the second case, biological material was obtained from the decedent male individual, who did not wake up after a party. In the two cases, the material was secured on suspicion of the possession of narcotic drugs or psychotropic substances, in which the suspects admitted to using “legal highs.” The MDPV blood concentrations of the deceased driver and deceased man were 38 and 17 ng/mL, respectively. In the two other cases, the determined concentrations were 306 and 124 ng/mL. However, MDPV was not the sole substance detected in these cases: in each, other drugs were also determined. Analyses of blood were conducted using liquid chromatography–tandem mass spectrometry.

Introduction

3,4-Methylenedioxypyrovalerone (MDPV) is a designer drug derived from cathinone, which is structurally similar to 3,4-methylenedioxy-N-methamphetamine (MDMA) (Figure 1), with potent stimulant properties and the potential for abuse and drug dependency. The abuse of psychoactive “bath salts,” “plant food/fertilizers” or “research chemicals” containing MDPV has increasingly grown since 2005; in the last few years, this substance has seemed to be one of the most popular of the synthesized cathinone stimulants (1–5). MDPV was a major phenomenon in Finland (4) and has also been sold in Sweden, Ireland and the United Kingdom under many names, including MDPK, MTV, Magic, Maddie, Black Rob, Super Coke, PV and Peeve. This substance appeared in 2007 on the market of “legal highs” in Poland, and between 2008 and 2011, it was identified in many products, e.g., Coca Life, Coco Classic, Coco Speed, Charge + , Ivory, Ivory Wave, Ivory Speed, Speedway, Doves Red, Star Dust and Crack Inside. MDPV was the sole component in only 39% of products. When it was sold in mixtures, other psychoactive substances primarily included lidocaine, 4-methylcathinone (4-MEC), butylone and 3,4-methylenedioxypyrrolidin-1-ybutio phenone (MDPBP) (5). Over the last few years, MDPV has been reportedly linked with some fatalities and severe intoxications in users of “legal highs” in several countries (6, 7). As a result, MDPV is to be classified as a controlled substance in some countries, including the United States. Since 2011, MDPV has also been controlled under the Polish Drug Addiction Counteraction Act of July 29, 2005, as amended.

MDPV is a light brown or white hygroscopic, crystalline powder, which is usually supplied in the form of tablets, capsules or powders. The purity of “legal highs” with MDPV varies from 10–100%. The dosages contained in a single package range widely from 10 to 200 mg (5). It may be administered via oral ingestion, nasal insufflation, smoking, and more rarely, intravenous or intramuscular injection or via the rectum. Oral and non-oral routes have the same overall effect, but non-oral routes produce faster and shorter effects (1). The dosages vary according to the route of administration and the dependence of an individual. The starting dosages are 1 to 5 mg and typical dosages range between 5 and 20 mg (2, 8). Redosing is very common; the amount consumed over a single session can be higher than 20 mg and dosages as high as 200 mg have been reported. MDPV users may quickly develop tolerance and consequently tend to consume higher dosages (9). MDPV, like other cathinones, is often used in conjunction with alcohol or other controlled substances.

MDPV acts as a central nervous system stimulant and has been reported to produce effects similar to those of methamphetamine, methylphenidate and amphetamine. MDPV does not have an empathogen action like MDMA, but some effects can be compared to cocaine (10). Sometimes the names of products containing MDPV even suggest its connection with cocaine (Coca, Coco or Crack). The mechanism of action of MDPV is primarily to inhibit the reuptake of noradrenaline and dopamine (11). The primary psychoactive effects last 3 to 4 h, with the after-effects lasting from 6 to 8 h.

Little is known about the pharmacology, human or animal toxicity, addiction or acute overdose potential or the long-term effects of MDPV. The desired psychoactive effects include euphoria, increased energy and motivation, mental stimulation, increased concentration, increased sociability and sexual stimulation. Overdoses can lead to tachycardia, hypertension, arrhythmia, hyperthermia, sweating, insomnia, nausea, dizziness, rhabdomyolysis, seizure, stroke, cerebral edema, cardiorespiratory collapse, myocardial infarction and death. Behavioral effects include panic attacks, anxiety, agitation, confusion, severe paranoia, auditory and visual hallucinations, psychosis, suicidal ideation, self-mutilation and behavior that is aggressive, violent and self-destructive (12–14). MDPV can produce hallucinatory delirium, a condition also described with phencyclidine (PCP) (15).

This paper describes the methodology developed for the determination of MDPV and its application to four cases sent to the Institute of Forensic Research (IFR) in 2011, in which MDPV was detected and quantitated in blood. In addition, the paper also discusses the concentrations and interpretations of MDPV levels.

Case Histories

**Case 1**

A passenger car crashed into a truck and the driver of the car suffered severe injuries, resulting in his death. During the external inspection of the deceased, the police revealed packages of white powders, which had the names Ivory Speed and Exclusive...
Dust and the inscription “collector’s product for field stone rinsing.”

Case 2
A man with a history of drug addiction was found unresponsive after a night of partying. A concubine testified that he had taken a product called Speedway while at the party. The autopsy showed emacitation, external hydrocephalus and atherosclerosis. The man also suffered from human immunodeficiency virus (HIV) infection.

Case 3
A 25-year-old woman was arrested by police on suspicion of the possession of narcotics. She admitted that on the same day she took “legal highs” (mosquito repellent) approximately 8 h earlier. During the blood sample collection, she showed emacitation, external hydrocephalus and atherosclerosis. The woman was submitted for toxicological analysis for the presence of drugs of abuse.

Case 4
A 19-year-old driver was stopped for a routine traffic control. During the blood sample collection, he admitted to the use of “legal highs” (mosquito repellent) approximately 8 h earlier. Poor reactions to light and slurred speech were observed in the driver.

Experimental
Reagents and materials
MDPV and mephedrone-\(d_3\) were purchased from LGC Standards (Dziekanow Lesny, Poland). High-performance liquid chromatography (HPLC)-grade acetonitrile (MeCN), methanol and formic acid, 98–100%, were purchased from Merck (Warsaw, Poland). Orthophosphoric acid, 85%, was obtained from POCH (Gliwice, Poland). Drug-free whole blood samples used for the development and validation of the method and for preparing controls were obtained from a regional blood donation center. Blank blood was screened for common drugs of abuse (including MDPV) and alcohol, with negative results. All biological materials were stored at +4°C before the analysis.

Standard, calibrators and control preparation
A stock solution of MDPV (1 ng/mL in methanol, stored at −22°C), was spiked into 0.2 mL of drug-free blood samples to prepare calibrators at the following concentrations: 5, 10, 20, 50, 100, 200 and 500 ng/mL. Control samples at concentrations of 10 and 100 ng/mL in addition to negative controls were analyzed with each analytical run. Mephedrone-\(d_3\) spiking solution (1 µg/mL) was prepared for use as the internal standard (IS) to obtain a concentration of 100 ng/mL.

Analysis
Extraction
To the blood samples (0.2 mL) placed in Eppendorf vials, 20 µL of 1 µg/mL methanolic solution of mephedrone-\(d_3\) (IS) was added to obtain a final concentration of 100 ng/mL. The analytes were isolated by precipitation with MeCN. Six hundred microliters of MeCN were added in 50 µL portions, and after each addition, the samples were vortex-mixed for 10 s. The samples were mixed for 5 min and centrifuged at 13,000 rpm for 3 min. The organic solvent was transferred to a 2 mL glass vial. MeCN was evaporated to dryness under nitrogen at 37°C. The dry residues were dissolved in 100 µL of 0.1% formic acid in water (v/v) and transferred to inserts for autosampler vials. The injection volume was 10 µL.

Chromatographic and spectrometric conditions
The analysis of blood samples was performed on an Agilent Technologies 1200 series liquid chromatograph coupled to a 6460 Triple Quad mass spectrometer operating in positive electrospray ionization mode (+ESI) and controlled by MassHunter software (version B.02.01). Separation was achieved on a Zorbax SB-C18 column (2.1 x 50 mm, 1.8 µm; Agilent Technologies, Palo Alto, CA) maintained at 25°C. The mobile phase consisted of a mixture of 0.1% formic acid in MeCN (v/v) and 0.1% formic acid in water (v/v) and was eluted under the following gradient conditions (shown in relation to MeCN content): 0 min, 10%; 6 min, 100%; 7 min, 10%; 14 min, 10%. The mobile phase was delivered at a flow rate of 0.3 mL/min and the total analytical run time was 14 min. Under these conditions, the retention time of MDPV was 4.97 min [relative retention time (RRT): 1.61]. Multiple reaction monitoring (MRM) was applied with positive ion detection. The following precursor ions and three fragment ions were monitored for each compound: 276.2 \(\rightarrow\) 126.1 (quantifiers), 276.2 \(\rightarrow\) 175.1, 276.2 \(\rightarrow\) 135.0 for MDPV, and 181.1 \(\rightarrow\) 163.1 (quantifiers), 181.1 \(\rightarrow\) 148.1, 181.1 \(\rightarrow\) 91.1 for mephedrone-\(d_3\). The optimized MS conditions were as follows: gas flow (nitrogen), 10 L/min; gas temperature, 280°C; sheath gas flow, 10 L/min; sheath gas temperature, 400°C; nebulizer pressure, 40 psi; capillary voltage, 4,000 V; dwell time, 25 ms. The fragmentor voltages were 124 V for MDPV and 87 V for mephedrone-\(d_3\). Collision energies (V) for MDPV and mephedrone-\(d_3\) transitions were 16, 20, 24 and 8, 20, 36, respectively.

Method validation
The liquid chromatography–tandem mass spectrometry (LC–MS-MS) method for the quantification of MDPV was validated with the data validation summarized in Table I. A seven-point MDPV blood calibration curve (number of replicates for each level: 5) was linear in the range from 5 to 500 ng/mL. The coefficient of determination (R²) value for the curve was 0.994. The limit of detection (LOD), based on a signal-to-noise ratio

Figure 1. Chemical structures: cathinone (A); MDPV (B); MDMA (C).
(S/N = 3) of the least abundant transition, was 0.5 ng/mL; the limit of quantification (LOQ) was defined as the lowest concentration of the calibration curve (5 ng/mL). The specificity (matrix) of the assay was determined by analyzing MDPV-free blood samples taken from eight persons. The total extraction recovery was determined to be 110% (100 ng/mL, n = 5) by comparing the responses (analyte area/IS area) of MDPV extracted from blood to those of blank blood spiked with the IS and MDPV added after the extraction. LC–MS-MS matrix effect (ME) was calculated by comparing the responses of known amounts (100 ng/mL) of unextracted MDPV (Set A, n = 5) with those measured in blank blood spiked after extraction with the same amount of the analyte (Set B, n = 5). The following formula was used: $ME(\%) = \frac{B}{A} \times 100$. The ME for MDPV was 89.5% and showed signal suppression.

### Results

The results of the analysis showed that MDPV was found in each of discussed cases; however, it was not the sole substance present in the materials. In the blood taken from the decedent driver (Case 1), MDPV and buphedrone were determined at concentrations of 38 and 127 ng/mL, respectively. In the blood sample of the deceased man (Case 2), MDPV was found at a concentration of 17 ng/mL. Clonazepam and its metabolite 7-aminoclonazepam were also detected at concentrations of 1.2 and 96 ng/mL. The woman suspected of possession and drug abuse (Case 3) had, in blood, MDPV at a concentration of 306 ng/mL and diazepam at a concentration of 147 ng/mL. In the fourth case, the concentration of MDPV in blood was 124 ng/mL. Traces of tetrahydrocannabinol (THC) at a concentration below 1 ng/mL, its metabolite 11-nor-9-carboxy-THC (THCCOOH) at a concentration of 7.9 ng/mL and the synthetic cannabinoid metabolite JWH-018 5-hydroxyindole at a concentration of 24 ng/mL were also detected in this case. MRM chromatograms of MDPV and mephedrone-$d_3$ isolated from the blood sample (Case 3) are presented in Figure 2.

### Discussion

The developed method for the detection and determination of MDPV has been successfully applied to the routine analysis of blood samples collected from people suspected of ingesting this substance. As presented in the following, parameters of the method such as the LOD and LOQ demonstrate that the method is well suited for the analysis of blood samples for the presence of MDPV and covers the range of typical concentrations that may be expected in blood, from the so-called normal to toxic levels.

The first case concerned a man who died in a car crash. He was driving a car after using MDPV and buphedrone; blood concentrations were 38 and 127 ng/mL (16). Kriikku et al. analyzed

---

**Table 1**

<table>
<thead>
<tr>
<th>Validation Data for Quantification of MDPV in Blood by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) Method</th>
<th>Mean concentration (± SD) (ng/mL)</th>
<th>RSD (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraday (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>10.61 ± 1.02</td>
<td>9.63</td>
<td>106.1</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>101.90 ± 5.14</td>
<td>5.04</td>
<td>101.9</td>
</tr>
<tr>
<td><strong>Interday (n = 10)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>11.72 ± 1.97</td>
<td>15.96</td>
<td>117.2</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>108.53 ± 12.45</td>
<td>11.47</td>
<td>108.5</td>
</tr>
<tr>
<td>LOD</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>5.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD – standard deviation.
RSD – relative standard deviation.
n – number of replicates.
LOD – limit of detection.
LOQ – limit of quantification.

---

**Figure 2.** MRM chromatograms of blood sample from Case 3 (LC–MS-MS).
blood samples from 219 impaired automobile drivers and determined MDPV at concentrations of 16–8,400 ng/mL with a median of 60 ng/mL (17, 18). These authors showed that MDPV impaired driving performance in 84% cases; however, in only 7%, the impairment was rated as moderate or greater. The observed aberrations include difficulty in speech, defining the current time, walking in a straight line and turning around. Data from such a large number of cases suggests that MDPV is responsible for at least a portion of behavioral abnormalities and driving difficulties. However, it has remained unclear whether the observed psychophysical achievement deficiency is caused only by MDPV, because the concentrations of other substances present in blood, primarily stimulants, were often high. Other studies performed in rodents also showed the substantial influence of MDPV on locomotor activity (19, 20). In the authors’ opinion, the driver in this case was under the influence of buphedrone and MDPV and these synthetic cathinones impaired his driving ability, leading to a fatal vehicular collision (16).

In the second case, the MDPV concentration was determined to be relatively low, at 17 ng/mL; clonazepam metabolite and traces of clonazepam were also found. Concentrations determined in blood in fatal cases were mostly in the range of 440–1,090 ng/mL (8, 21, 22). In a single fatality, MDPV was detected in blood at 170 ng/mL (23). Murray et al. describe a fatal case after the recreational use of MDPV with a serum concentration at 82 ng/mL (6). Kriikku et al. found MDPV in 13 deceased individuals, but this compound was not the sole cause of death in any of these cases. In eight of these cases, the concentrations of MDPV in postmortem blood were in the range of 20–4,800 ng/mL (median: 130 ng/mL) (17, 18). The MDPV concentration in blood in this case was lower than that reported in other fatal cases and other substances were also present. MDPV, in addition to benzodiazepines, was unlikely to be the cause of death of the deceased, but rather many diseases and emaciation. Generally, cathinones were not the primary cause of death in most described fatalities with these compounds. In most cases, the death was attributed to multiple drug toxicity associated with MDPV and/or other drug use, including alcohol.

On the other hand, in the third case of the arrested woman, the concentration of MDPV was 20 times higher (306 ng/mL) than in the aforementioned fatal case. Shown in this case, MDPV blood concentrations were also higher than typical concentrations described in the literature in the cases of the abuse of this compound. Blood or plasma MDPV concentrations in individuals using the drug recreationally are estimated to range from 10 to 50 (150) ng/mL. Blood or plasma MDPV concentrations in overdosed patients ranged from 24 to 330 ng/mL (23, 24). Thornton et al. described a patient with psychosis who had MDPV and flephedrone in serum at concentrations of 186 and 136 ng/mL, respectively (25). Alexy et al. presented the case of a male who was admitted to the hospital with hallucinations and severe paranoia. He had been abusing MDPV for approximately six months prior to this event and had blood MDPV and THCCOOH concentrations of 750 and 17 ng/mL, respectively (26). The concentrations determined in these presented case studies confirm that the patients’ overdose effects were consistent with the observed symptoms in the woman in Case 3. Presumably, this woman had also developed a tolerance to MDPV, which may explain the higher than normal concentrations of this compound.

In the fourth case, the concentration (124 ng/mL) was typical for a person using the drug recreationally, which indicates that the driver was under the influence of this drug, as explained previously. The presence of cannabinoids intensified the negative influence on psychomotor performance.

MDPV is often combined with other substances, such as alcohol, cannabinoids, amphetamines and other stimulants (benzylpiperazine, methylene and mephedrone), γ-hydroxybutyric acid (GHB), γ-butyrolactone (GBL), opiates and benzodiazepines. The latter are most often used during the comedown period. Kriikku et al. (18) detected MDPV in 219 cases, in which amphetamine and benzodiazepines were also present in 63% of these cases. The levels of benzodiazepines were often low; however, the levels of stimulants found together with MDPV were high in most cases (17). When analyzing the aforementioned data, similar situations with low concentrations of benzodiazepines were observed in the current cases (Cases 2 and 3). Benzodiazepines are mostly used by addicts to reduce the adverse effects caused by MDPV.

The concentrations of MDPV determined in blood and literature case studies are summarized in Table II.

---

<table>
<thead>
<tr>
<th>MDPV Concentration (ng/mL)</th>
<th>Type of Case</th>
<th>Other Detected Substances (concentration in ng/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>fatal car accident</td>
<td>buphedrone – 127</td>
<td>our case 1</td>
</tr>
<tr>
<td>17</td>
<td>fatal</td>
<td>clonazepam – 1,2, 7-aminoconazepam – 96</td>
<td>our case 2</td>
</tr>
<tr>
<td>306</td>
<td>possession and drug abuse</td>
<td>diazepam – 147</td>
<td>our case 3</td>
</tr>
<tr>
<td>124</td>
<td>driving under the influence</td>
<td>tetrahydrocannabinol (THC) &lt; 1, 11-nor-9-carboxy-THC (THCCOOH) – 7.9, JWH-018 S-hydroxyindoled – 24, flephedrone – 346</td>
<td>our case 4</td>
</tr>
<tr>
<td>186 (serum)</td>
<td>non-fatal intoxication</td>
<td>-</td>
<td>(17)</td>
</tr>
<tr>
<td>24–241</td>
<td>13 patients of regional poisoning center</td>
<td>-</td>
<td>(23)</td>
</tr>
<tr>
<td>16–8000</td>
<td>259 cases of driving under the influence</td>
<td>-</td>
<td>(18)</td>
</tr>
<tr>
<td>20–4800</td>
<td>8 fatal cases</td>
<td>-</td>
<td>(21)</td>
</tr>
<tr>
<td>330.950</td>
<td>non-fatal intoxication</td>
<td>-</td>
<td>(17)</td>
</tr>
<tr>
<td>750</td>
<td>non-fatal intoxication</td>
<td>-</td>
<td>(24)</td>
</tr>
<tr>
<td>82 (serum)</td>
<td>fatal intoxication</td>
<td>-</td>
<td>(26)</td>
</tr>
<tr>
<td>1090</td>
<td>abuse, fatal</td>
<td>-</td>
<td>(6)</td>
</tr>
<tr>
<td>440 (femoral) 500 (heart)</td>
<td>fatal</td>
<td>caffeine, fluoxetine – 290, lamotrigine – 410, JWH-18 – 0.48, JWH-250 – 4.6</td>
<td>(22)</td>
</tr>
</tbody>
</table>

---

References:
(6), (17), (18), (21), (23), (24), (25), (26), (19), (20), (22).
Conclusions
There are several reports of the analysis of MDPV in biological material. This paper describes four cases in which this compound was detected. MDPV blood concentrations in the presented case studies were found to be within the range of typical concentrations, as described in previous literature regarding cases in which this compound was abused. The presence of other substances in the blood, particularly benzodiazepines, suggests that these substances reduce the adverse effects caused by MDPV.

Additionally, an LC–MS–MS method was successfully developed and applied in this study.

References
9. Watterson, L.R., Kufahl, P.R., Nemirovsky, N.E., Sewalia, K., Grabenauer, M., Thomas, B.F. et al.; Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV); Addiction Biology, July 11, 2012: 10.1111/j.1569-1600.2012.00074.x.