# Improving Wastewater-Based Epidemiology for New Psychoactive Substance Surveillance by Combining a High-Throughput *In Vitro* Metabolism Assay and LC−HRMS Metabolite Identification

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# Abstract:

One of the primary criteria for a suitable drug biomarker for wastewater-based epidemiology (WBE) is having a unique source representing human metabolism. For WBE studies, this means it is important to identify and monitor metabolites rather than parent drugs, to capture consumption of drugs and not fractions that could be directly disposed. In this study, a high-throughput workflow based on a human liver S9 fraction *in vitro* metabolism assay was developed to identify human transformation products of new chemicals, using α-pyrrolidino-2-phenylacetophenone (α-D2PV) as a case study. Analysis by liquid chromatography coupled to high resolution mass spectrometry identified four metabolites. Subsequently, a targeted liquid chromatography – tandem mass spectrometry method was developed for their analysis in wastewater samples collected from a music festival in Australia. The successful application of this workflow opens the door for future work to better understand the metabolism of chemicals and their detection and application for wastewater-based epidemiology.



**Keywords:** synthetic cathinones; music festivals; α-D2PV; α-Pyrrolidino-2-phenylacetophenone; wastewater analysis; metabolite discovery

# 1. Introduction:

Wastewater-based epidemiology (WBE) is currently gaining attention to monitor the presence of a wide variety of chemicals and viruses (Choi et al., 2018). The method relies on the detection of biomarkers indicative of chemical or biological exposure in wastewater influent, which can then be extrapolated to estimates of community-wide exposure or use. In general for WBE studies, there are four criteria each biomarker needs to meet for it to be considered suitable, including for it to be 1) excreted via urine in consistent amounts; 2) detectable in wastewater; 3) stable in wastewater (both in the collected sample and in the sewer); and 4) having a unique source representing human metabolism (Gracia-Lor et al., 2017). From this first aspect, without having reference materials to conduct pharmacokinetic studies, excretion of compounds remains unknown. For example, most new psychoactive substances (NPS) are not fulfilling these criteria due to insufficient knowledge on the metabolism, leading to most studies using the parent drug as the target biomarker (Bijlsma et al., 2021; Gent and Paul, 2021; Reid et al., 2014). As such, the interpretation or biomarker selection of these NPS detections can be problematic, as the drug can be either directly discarded (contributing to biomarker signal without human consumption) or the parent drug could be a sub-optimal target if it is largely or entirely metabolised when consumed (leading to poor detection rates). As NPS are intended to have psychoactive properties, conducting studies to determine their metabolism in humans is ethically challenging (Meyer, 2016).

Due to the infrequent consumption and therefore low expected levels of NPS in wastewater, highly sensitive and selective methods are required for the analysis of even the most common NPS with most methods utilising targeted, quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), with most targeting the parent compound, as metabolism information is not readily known (Bade et al., 2020, 2017; Borova et al., 2015; González-Mariño et al., 2016; Kinyua et al., 2015; Reid et al., 2014).

*In vitro* metabolism bioassays have been proposed as a potential means to expand the capabilities of wastewater-based epidemiology (Lai et al., 2015; Lopardo et al., 2017; Reid et al., 2014). For example, external metabolization systems like the liver S9 fraction *in vitro* bioassay have been developed (Shao et al., 2020) and refined for high-throughput application (Escher et al., 2020; Huber et al., 2021; Villeneuve et al., 2019). Metabolism is also an important determinant of toxicity since chemicals can be activated or deactivated (detoxified) by, for example, Phase 1 cytochrome P450 monooxygenase (CYP) enzymes (Yu, 2020) and

Phase II conjugation enzymes (e.g., glucuronosyltransferases) (Lepri et al., 2017). These *in vitro* experiments subject parent compounds to metabolism, generating potential human metabolites which could be further investigated as WBE biomarkers. The metabolism products can be then qualitatively screened using non-targeted (less sensitive than targeted) techniques using LC coupled to high resolution mass spectrometry (HRMS) to allow the acquisition of accurate-mass full-spectrum data (Kaufmann et al., 2010); but requires analytical reference standards for confirmation of spectral matches (Hernández et al., 2018). For qualitative identification purposes matching retention time and spectral matching to analytical reference standards of the target analyte is required when analysing real samples such as urine or wastewater (Bade et al., 2019; Bijlsma et al., 2021).

The combination of an S9 fraction *in vitro* metabolism bioassay with LC-HRMS was recently demonstrated as a high-throughput workflow for production of pesticide metabolites to generate a LC-HRMS database consisting of retention time, precursor ion and fragments (Huber et al., 2021). By combining the *in vitro* assay on parent NPS drugs with HRMS screening of the metabolism products, potential metabolites of NPS can be identified, and subsequently tested for use in WBE. The primary aim for this work was to develop a workflow for the identification of NPS metabolites using α-D2PV as a case study. A second aim was to show applicability of the method through the analysis of α-D2PV and its metabolites in wastewater samples collected from a music festival.

# 2. Materials and Methods:

## 2.1 Chemicals

α-Pyrrolidino-2-phenylacetophenone (α-D2PV) (1 mg/mL) was kindly donated for this work by Forensic Sciences Queensland. The stock solution was stored in the dark at -20 °C until analysis. Phenacetin (CAS: 62-44-2), acetaminophen (CAS: 103-90-2), (2S,3S)-hydroxybupropion hydrochloride (CAS: 106083-71-0), midazolam (CAS: 59467-70-8) and midazolam-D4 maleat (CAS: 1435938-30-9) were purchased from Sigma-Aldrich, bupropion hydrochloride (CAS: 31677-93-7) was purchased from TCI Chemicals, 1-hydroxymidazolam (CAS: 59468-90-5) was purchased from Cayman Chemical Company and bupropion-D9 hydrochloride (CAS: 1189724-26-5) was purchased from Expert Synthesis Solution.

## 2.2 Materials

Pooled-donor human liver S9 fraction (protein content 20 g/L) was purchased from Thermo Fisher (Article No. HMS9PL) and Aroclor 1254-induced rat liver S9 fraction from Molecular Toxicology (Article No.11-101). The S9 was stored at -80 °C and thawed on ice directly before use. NADPH tetrasodium salt (CAS: 2646-71-1) was purchased from Roth and Trizma® hydrochloride solution (1 M) from Sigma-Aldrich (Article No. T2194-100ML). Strata™-X Polymeric Reversed Phase 96-well microelution plates (Article No. 8M-S100-4GA) were purchased from Phenomenex and 96 glass-coated microtiter plates and deep-well glass-coated microtiter plates (Article Nos. 60180-P334 and 60180-P336) from Thermo Fisher. LC-MS grade methanol was purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) was purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Fisher Chemical (Australia). Ultrapure water was obtained from a Milli-Q system (Merck Millipore, USA, 0.22 µm filter, 18.2 $m\Omega \cdot cm^{-1}$).

## 2.3 Wastewater Samples

Wastewater samples were collected from a music festival that was held in May 2022. The festival site had fixed on-site ablution facilities which drained into a site-specific on-site wastewater treatment plant. Twenty-four-hour composite wastewater influent samples were collected on each day of the festival, from 3pm to 3pm the following day using an Avalanche portable refrigerated sampler (TELEDYNE ISCO). Samples were collected time-proportionally at 20-minute intervals to provide a composite sample over 24 hours. Once collected, the samples were preserved by acidification to pH 2 with 2 M HCl (approximately 1

mL per 100 mL sample), and stored on ice until delivery at the laboratory, where they were frozen immediately at -20°C and stored until analysis.

A direct injection method was used for this work. Briefly, thawed samples (3 mL) were filtered through a 0.2 µm RC filter into a glass tube, with 1 mL of the filtered wastewater transferred to a glass vial for LC-MS analysis.

### 2.4 S9 workflow optimization

The metabolization of three pharmaceuticals with known metabolites (phenacetin, bupropion and midazolam) was tested with five concentrations of rat liver S9. Aliquots of the chemicals were added to 200 µL phosphate buffered saline (PBS, pH 7.4), leading to a final chemical concentration of 1 mg/L. After 5 min incubation at room temperature, rat liver S9 was added to all samples leading to final S9 concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 mg/mL. 9 µL of a 20 mM NADPH solution in PBS was added to the vials (final NADPH concentration of 0.8 mM). Negative controls without S9 extracts were prepared for all chemicals. The vials were incubated at 37 °C and 800 rpm (BioShake iQ, QInstruments) for 3 h. After 1.5 h, 4.5 µL NADPH solution were added to all vials. The reaction was stopped with 1 mL cold MeOH containing 0.05 mg/L internal standard (bupropion-D9 or midazolam-D4, no internal standard was available for phenacetin) and the vials were incubated at -20 °C for 20 min. The vials were centrifuged at 4000 rpm (1789 RCF) for 10 min (Heraeus Multifuge X1R, Thermo Scientific) and 200 µL of the supernatant were measured with LC-MS/MS. The method details are shown in the Supplementary Material, Section S1 and Table S1. Standard solutions (0.001-10 mg/L) of the chemicals in methanol with 0.05 mg/L internal standard were measured together with the samples. The chemicals were quantified via the ratio of analyte to internal standard.

### 2.5 Final S9 Metabolism Procedure

For the *in vitro* metabolization, the Trizma® hydrochloride solution (1 M) was diluted to a final concentration of 0.05 M and the pH was adjusted to pH 7.4. 200 µL of Trizma® buffer was transferred into clear HPLC vials. Aliquots (2 µL) of the α-D2PV stock solutions were transferred into two vials leading to a final chemical concentration of 9 mg/L. 5 µL of the human liver S9 were added to all vials leading to a final S9 content of 0.45 mg/mL. The vials were incubated at room temperature for 5 min and a 20 mM NADPH solution in Trizma® buffer (9 µL) was added to the vials (final NADPH concentration of 0.8 mM). All samples were prepared in duplicates. Additionally, negative controls without S9 were prepared (5 µL of

Trizma® buffer was added instead of S9). Three blank samples were prepared containing all components except for α-D2PV.

The vials were covered with aluminum foil and incubated at 37 °C and 40 rpm for 3 h (Thermoline, orbital shaker incubator TU-454). After 1.5 h, 4.5 µL of the NADPH solution was added to all vials. After the incubation, 200 µL cold methanol was added to all vials to stop the enzymatic activity and the vials were stored at -20 °C overnight. The methanol was removed under a nitrogen stream (Ratek, nitrogen blow-down system DBH30D) until approx. 200 µL of the samples were left. The vials were centrifuged at 3000 × g for 10 min (Microsolv Vial Centrifuge) and 130 µL of the supernatant was transferred to a glass coated 96-well plate.

Sample clean-up was performed using a Strata™-X Polymeric Reversed Phase 96-well microelution plate (2 mg sorbent per well). For the solid-phase extraction, a 96-well positive pressure manifold (UCT, VMF96PPM) was used. The Strata™-X plate was conditioned with 200 µL methanol per well and equilibrated with 200 µL HPLC water per well. 100 µL of the samples were loaded and washed with 200 µL 95:5 HPLC water:methanol per well. After washing the Strata™-X sorbent was dried for approx. 1 min. Samples were eluted with 100 µL methanol into a glass-coated deep well plate. After the clean-up, all samples were diluted with 100 µL HPLC water and transferred to HPLC vials with inserts for LC-MS analysis.

## 2.6 Instrumental analysis

### *2.6.1 SCIEX TripleTOF 5600+*

The incubated extracts were analysed using a Shimadzu UHPLC system (Sciex ExionLC) coupled to a SCIEX TripleTOF 5600+ system, using positive electrospray ionisation as previously described (Rousis et al., 2023). Briefly, chromatographic separation was achieved using a Phenomenex Kinetex Biphenyl (50 × 2.1 mm × 2.6 µm) column fitted with a Security Guard ULTRA Cartridges UHPLC Biphenyl 2.1 mm ID columns, at a flow rate of 0.3 mL/min and an injection volume of 10µL. A delay column was installed between the mobile phase mixer and the autosampler (Phenomenex Kinetex 5 µm EVO C18 100Å 20 × 2.1 mm). A mobile phase of 95:5 (v/v) MilliQ water: methanol (solvent A) or methanol: MilliQ water (solvent B), both with 0.1% formic acid was used. The initial percentage of B was 5 %, which was kept steady for the first 1.5 minutes. The concentration of B was linearly increased to 95% over 12.5 minutes and held for 3 minutes before being brought back to the starting conditions over 0.1 minutes and kept steady for the final 2.9 minutes to equilibrate the system. The total run time was 20 minutes. Mass spectroscopic data were collected over a m/z range of 50–650.

Data were acquired in Sequential Window Acquisition of all THeoretical fragment-ion spectra (SWATH) mode, utilising one full scan MS (collision energy of 10 V) and 8 subsequent SWATH windows (experiments), each of which had a collision energy of 25 V with a collision energy spread of 15 V. The first experiment gave information relating to the parent mass, while the others gave information relating to the fragment ions.

The data was processed manually using Peakview (Version 2.2.0.11391) to identify potential metabolites.

*2.6.2 SCIEX X500R*

Data-dependent analysis (DDA) was conducted using a high-performance liquid chromatography system (ExionLC AD, AB Sciex, Ontario, Canada) coupled with a SCIEX X500R Quadrupole Time-of-Flight (QTOF) mass spectrometer (AB Sciex, Ontario, Canada), equipped with electrospray ionization (ESI). A sample volume of 10 µl was injected onto a Kinetex Biphenyl (50 × 2.1 mm × 2.6 µm) column, (Phenomenex, Lane Cove, Australia), with an accompanying guard cartridge (SecurityGuard™, Biphenyl 2.1 mm ID, Phenomenex, Lancove, Australia), using the autosampler. A delay column was installed between the mobile phase mixer and the autosampler (Phenomenex Kinetex 5 µm EVO C18 100Å 20 × 2.1 mm). The mobile phases employed, consisting of ultra-pure water (A) and methanol (B), were both acidified with 0.1% formic acid. Initially, the concentration of B was 5% and after one minute, it was linearly increased to 50% over 3 minutes. Over the next 13 minutes, the concentration of B was linearly increased to 99%, where it was held for 3 minutes. It was then returned to starting conditions over 0.1 minutes and held for 2.9 minutes to equilibrate the system. The total run time was 23 minutes. A constant flow rate of 0.4 mL/min was maintained, as detailed in Table S3. Full-scan data spanning 100−600 m/z for MS1 and 50−600 m/z for MS/MS were acquired. High-purity nitrogen served as the nebulizer, curtain, and collision gases. The curtain gas flow was set at 35 L/min, while ion source gases 1 and 2 were maintained at 70 psi. The ion source temperature was stabilized at 600 °C, with an ion spray voltage of 5500 V. Collision energy settings were 10 V for MS1 and 25 V for MS/MS, along with a declustering potential of 50 V. For MS/MS experiments, the ten most intense ions exceeding 1000 cps were chosen with active dynamic background subtraction.

### *2.6.3 SCIEX 7500*

The incubated extracts and wastewater samples were analysed using a Shimadzu Nexera LC-40 coupled to a SCIEX Triple Quad 7500 system, following a previously published method (Rousis et al., 2023). Briefly, chromatographic separation was achieved using a Phenomenex Kinetex Biphenyl (50 × 2.1 mm × 2.6 µm) column fitted with a SecurityGuard ULTRA Cartridges UHPLC Biphenyl 2.1 mm ID columns, at a flow rate of 0.45 mL/min and an injection volume of 2 µL. A delay column was installed between the mobile phase mixer and the autosampler (Phenomenex Kinetex 5 µm EVO C18 100Å 20 × 2.1 mm). The mobile phase consisted of 95:5 (v/v) MilliQ water: methanol with 0.1% formic acid (solvent A) and 95:5 (v/v) methanol: MilliQ water with 0.1% formic acid (solvent B). The initial percentage of B was 5%, which was kept steady for the first 1 minute. The concentration of B was linearly increased to 100% over 6 minutes and held for 2 minutes before being brought back to the starting conditions over 0.1 minutes and kept steady for the final 1.9 minutes to equilibrate the system. The total chromatographic run time was 11 minutes. The mass spectrometer was run in scheduled multiple reaction monitoring (sMRM) mode in positive ion mode, with at least two transitions per analyte and a 30 or 60 s retention time window around each analyte.

As reference standards were not available for the metabolites, the S9 incubated extract was used to estimate the retention time of the metabolites. Due to the limited volume of the metabolite extracts, the MS parameters of the metabolites could not be optimised, so the entrance potential, collision energy and exit potential were the same as for the parent compound. The ion source gas 1 and 2 were set at 60 psi, curtain gas at 40 psi, ion source temperature at 450 °C and ion spray voltage at 2600 V.

# 3. Results and Discussion

α-D2PV is a synthetic cathinone, similar in structure to α-PVP ("flakka"), which was a popular NPS in the mid-2010s and had overdoses and deaths linked to its consumption. α-PVP was first identified in early 2021 in Slovenia (European Monitoring Centre for Drugs and Drug Addiction, 2022) and has previously been reported in Australia through wastewater analysis (Bade et al., 2019; Tscharke et al., 2016). Research into the online trawling of the social media platform Reddit and its associated drug-oriented subreddits showed that interest in α-D2PV peaked in late 2021/early 2022. It has been shown that mentions of NPS on Reddit correlate with toxicological findings in the United States (Barenholtz et al., 2021; National Drug Early Warning System (NDEWS), 2022). With similar trends observed in wastewater for the NPS use in the United States and Australia (Bade et al., 2023b, 2022), it is thus important to also monitor the consumption of α-D2PV in Australia.

## 3.1 S9 Workflow Validation

We adapted a high-throughput *in vitro* metabolization method by Huber et al (Huber et al., 2021) to produce α-D2PV metabolites. Since the method by Huber et al. was mainly focused on pesticides, we selected three pharmaceuticals with known metabolites (phenacetin, bupropion and midazolam) for the method optimization. Figure 1 shows the amount of the parent chemicals and the respective metabolites after incubation with different concentrations of S9. At the lowest S9 concentration (0.05 mg/mL) only a small decrease in the concentration of the parent chemical was seen for phenacetin and midazolam. The metabolites acetaminophen and hydroxymidazolam could be detected but showed lowest abundance for this low enzyme concentration. The strongest transformation of all three chemicals was achieved with the highest S9 concentration (5 mg/mL). However, the amount of metabolite formed was highest only for hydroxymidazolam. The use of such a high concentration is not advised as it can lead to more matrix interferences, which can affect the interpretation of the LC-MS spectra. The largest amount of acetaminophen was formed at 0.5 mg/mL S9 and the largest amount of hydroxybupropion was formed at 0.1 mg/mL S9. Based on the results of this pretest, an S9 content of 0.45 mg/mL was selected for the metabolic degradation of α-D2PV. Although a higher S9 concentration could metabolise a higher amount of chemical, relevant metabolites formed could also be further transformed and thus this concentration compromise was reached.

### 3.2 Metabolite Identification

To identify prospective metabolites of α-D2PV, a small database was developed based on predicted metabolites from similar work performed in literature (Krotulski et al., 2018; Tyrkkö et al., 2013). The predominant metabolic pathways in literature for related pyrrolidinyl substituted cathinones are hydroxylation and dehydrogenation (Zaitsu, 2018), with the proposed metabolites shown in Figure 2. The S9 metabolite extracts were analysed on the SCIEX 5600+ Triple TOF instrument, operating in SWATH mode and on the SCIEX X500R, operating in DDA mode (Figure 3 and Table 1).

All the proposed metabolites were found in the incubated extract in both the SWATH and DDA analyses. In the SWATH analysis, the signal of the metabolite M3 was also found in the negative control (Figure S1). Metabolite M3 is the hydroxylated form of α-D2PV. As such, its presence in the negative control could also be due to the $^{13}C$ isotopic peak of the parent compound, which was present at a high concentration. To better distinguish M3 from the parent drug, the DDA experiments clearly showed a difference from the parent drug, with a loss of water (*m/z* 250.1606) and pyrrolidine ring (*m/z* 197.0970) the most intense fragment ions, together with the pyrorolidinium (*m/z* 72.0813), tropylium (*m/z* 91.0547) and benzylium (*m/z* 105.034) ions (Figure 3D). All metabolites showed similar fragmentation, with the m/z 195.0804 ($C_{14}H_{11}O^+$) and m/z 167.0862 ($C_{13}H_{11}^+$) in all metabolites except M3 (Table 1). This indicates that the hydroxylation (M1, M2) was on the pyrrolidine ring, followed by dehydrogenation to the corresponding lactam (M4).

### 3.3 Application to Wastewater Samples

To cater for the potential low levels of α-D2PV and its metabolites in wastewater, a targeted LC-MS/MS method was utilised for the analysis of wastewater. The method was based on a previously validated method (Bade et al., 2023a; Rousis et al., 2023) including the transitions of the most sensitive fragment ions from the HRMS analysis. All mass spectrometric parameters are included in the supporting information (Table S2).

Wastewater samples from a music festival were analysed for these metabolites. α-D2PV, the two hydroxylated metabolites (M1 and M2) and a dehydrogenated metabolite (M4) were identified (Figure 3). Although we could not quantify these metabolites as no reference standards were available, the hydroxylated metabolites M1 and M2 were the most abundant, followed by M4.

From both the SWATH and DDA analyses of the incubated extracts, these were the three most prevalent metabolites and thus were expected to have a greater chance of detection in the wastewater samples. The identification was confirmed based on retention time similarity to the enzyme extract and the ion ratio between the two monitored transitions.

# Conclusion

This work presents an optimised workflow for chemical biomarkers identification for WBE, using α-D2PV as a case study. We successfully identified three metabolites in a wastewater sample collected from a music festival. This work shows the importance of including metabolites in WBE methods, to confirm consumption, which cannot be ascertained analysing solely the parent drug. The success of this work opens the door for future studies into the metabolism of NPS and other substances following this high throughput S9 workflow, and their incorporation into analytical methods for wastewater-based epidemiology as well as forensic and health applications, thereby expanding the already diverse suite of compounds being monitored.

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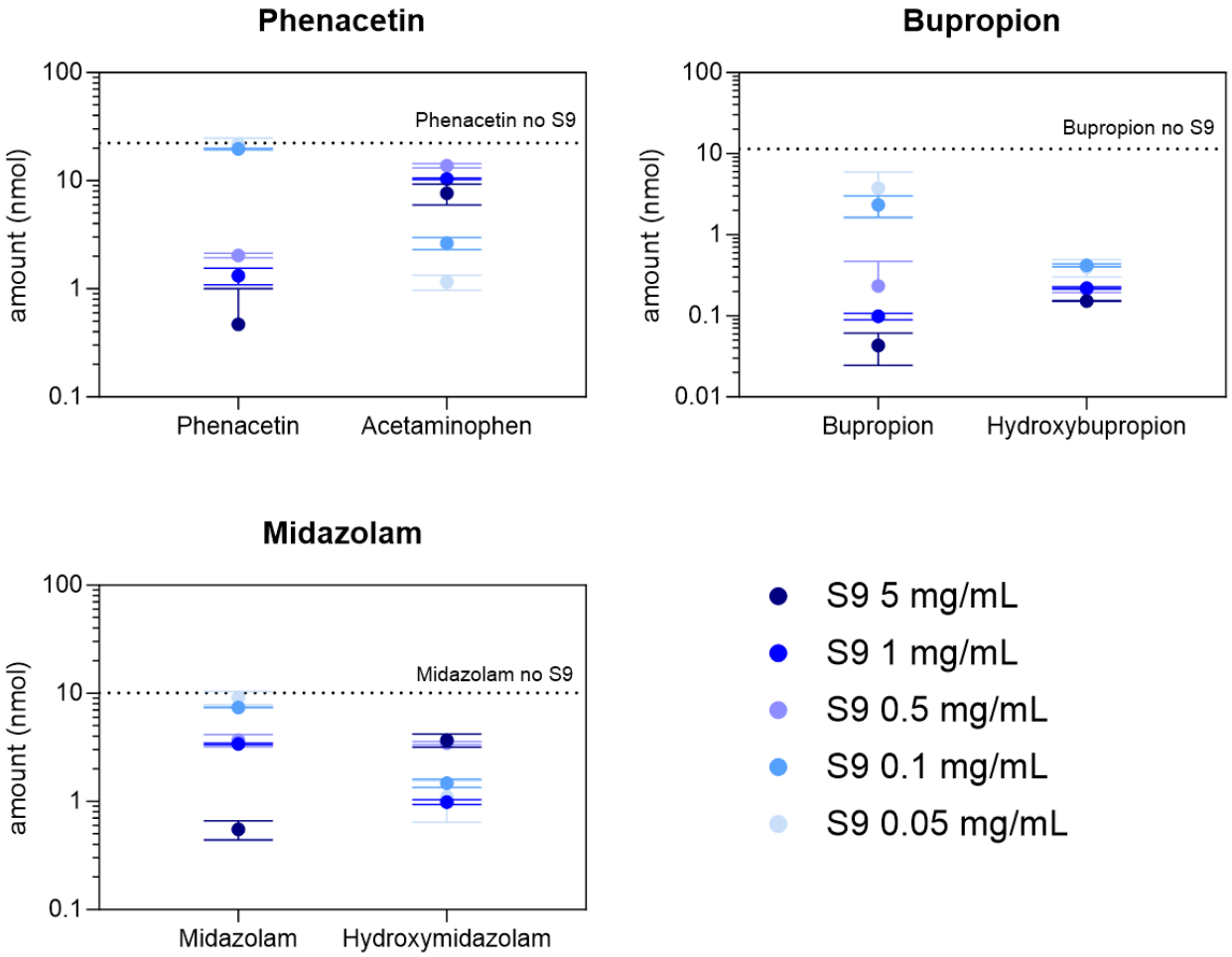


*Figure 1:* S9 method optimisation of phenacetin, bupropion and midazolam.

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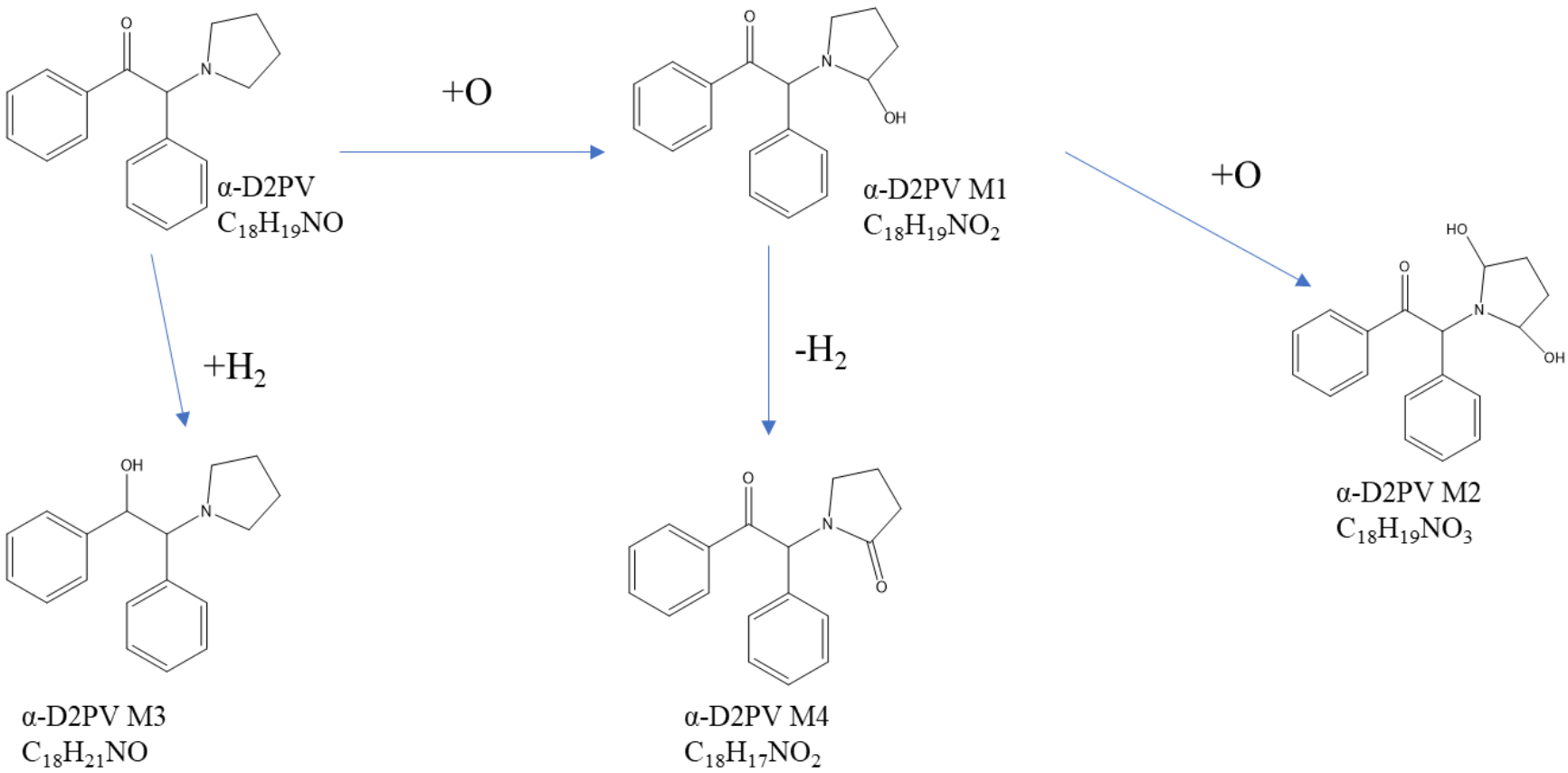


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491 *Figure 2:* Proposed metabolites of α-D2PV based on reported metabolism of similar compounds (Zaitsu, 2018).

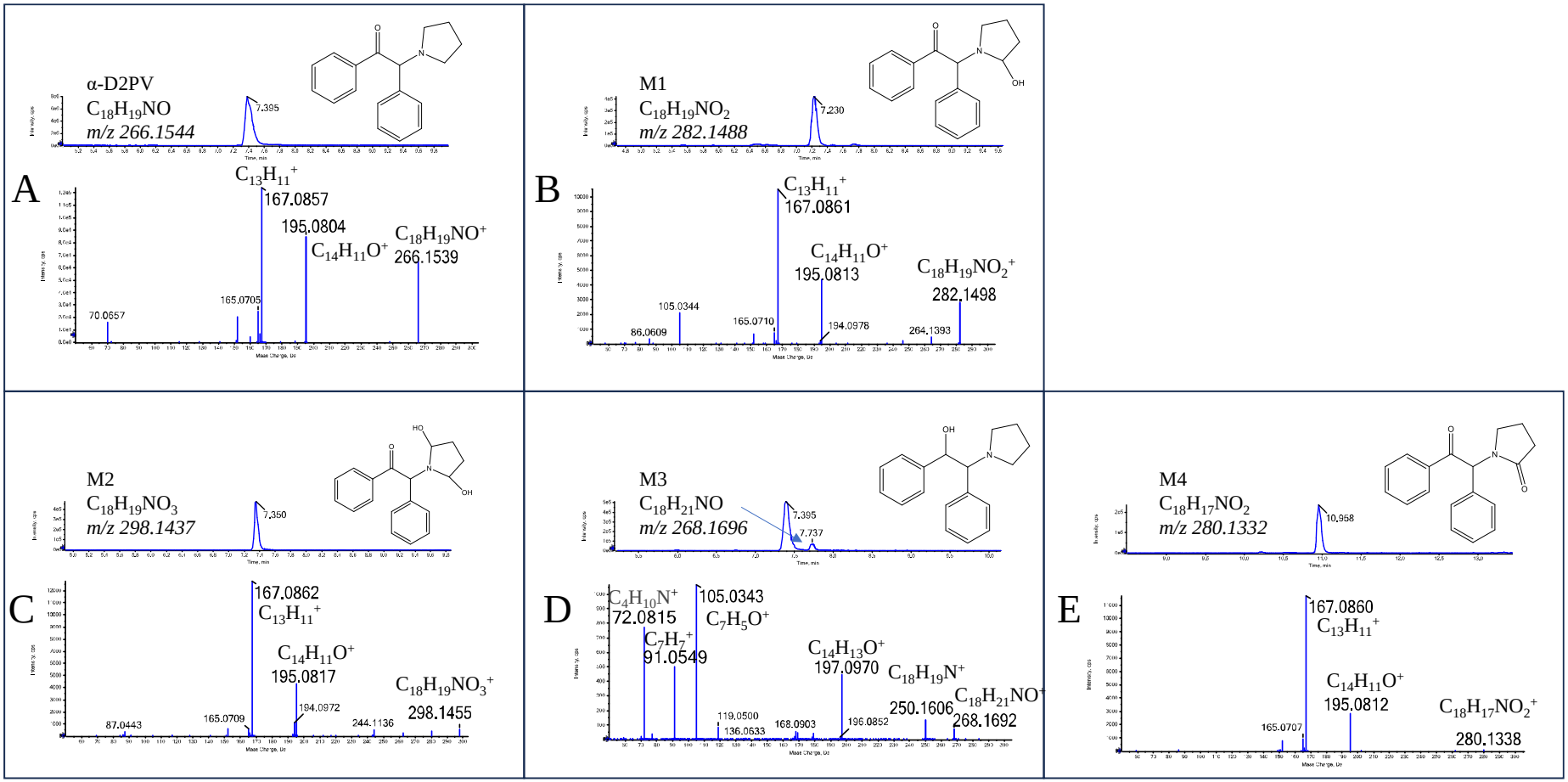


*Figure 3:* Data Dependent Acquisition (DDA) of the parent drug (A) and all proposed metabolites (B-E), including the extracted ion chromatograms measured in the S9 extract. Main fragment ions are annotated as in Table 1.

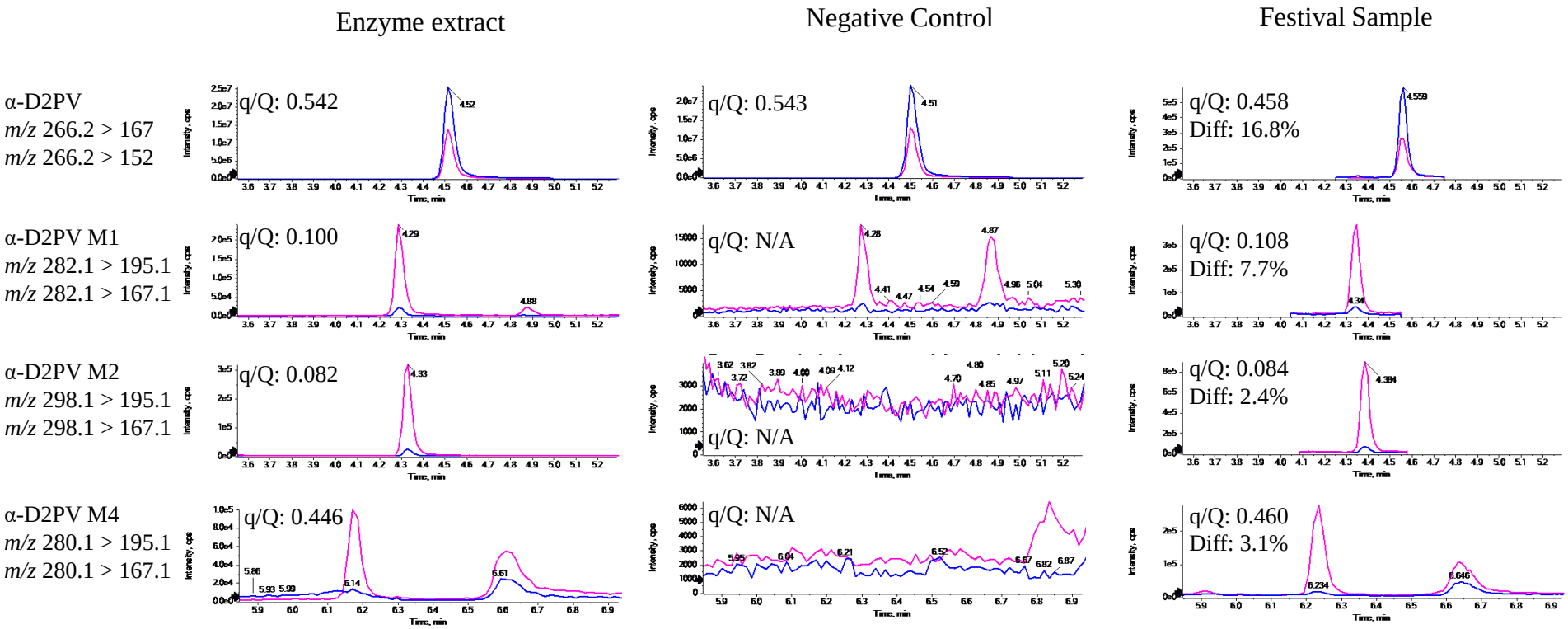


*Figure 4:* Three metabolites of α-D2PV found in wastewater samples from music festival. Ion ratios are also shown.

497 *Table 1:* Mass Spectrometric properties of α-D2PV and its four tentative metabolites in both 5600+ and X500R

| | Retention Time (5600+/X500R) | | Chemical Formula | Exact Mass | Measured Mass (5600+) | Measured Mass (X500R) | Mass Error (5600+/X500R; ppm) |
|---|---|---|---|---|---|---|---|
| α-D2PV | 8.66/7.39 | | $C_{13}H_{11}^+$ | 167.0855 | 167.0846 | 167.0857 | 5.39/1.20 |
| | | | $C_{14}H_{11}O^+$ | 195.0804 | 195.0789 | 195.0804 | 7.69/0.00 |
| | | $[M+H]^+$ | $C_{18}H_{19}NO^+$ | 266.1544 | 266.1526 | 266.1539 | 6.76/1.88 |
| α-D2PV M1 | 8.23/7.23 | | $C_{13}H_{11}^+$ | 167.0855 | 167.0862 | 167.0861 | 4.19/3.59 |
| | | | $C_{14}H_{11}O^+$ | 195.0804 | 195.0819 | 195.0813 | 7.69/4.61 |
| | | $[M+H]^+$ | $C_{18}H_{19}NO_2^+$ | 282.1494 | 282.1501 | 282.1498 | 2.48/1.42 |
| α-D2PV M2 | 8.38/7.35 | | $C_{13}H_{11}^+$ | 167.0855 | 167.0856 | 167.0862 | 0.6/4.19 |
| | | | $C_{14}H_{11}O^+$ | 195.0804 | 195.0809 | 195.0817 | 2.56/6.66 |
| | | | $C_{18}H_{17}NO_2^+$ | 280.1332 | 280.1340 | NA | 2.86/NA |
| | | $[M+H]^+$ | $C_{18}H_{19}NO_3^+$ | 298.1442 | 298.1438 | 298.1455 | 1.34/4.36 |
| α-D2PV M3 | 9.01/7.74 | | $C_4H_{10}N^+$ | 72.0813 | NA | 72.0815 | NA/2.77 |
| | | | $C_7H_7^+$ | 91.0547 | NA | 91.0549 | NA/2.20 |
| | | | $C_7H_5O^+$ | 105.034 | NA | 105.0343 | NA/2.86 |
| | | | $C_{14}H_{13}O^+$ | 197.0961 | 197.0970 | 197.0970 | 4.56/4.56 |
| | | | $C_{18}H_{19}N^+$ | 250.159 | 250.1606 | 250.1606 | 6.40/6.40 |
| | | $[M+H]^+$ | $C_{18}H_{21}NO^+$ | 268.169 | 268.1701 | 268.1692 | 4.10/0.75 |
| α-D2PV M4 | 12.67/10.96 | | $C_{13}H_{11}^+$ | 167.0855 | 167.0862 | 167.086 | 4.19/2.99 |
| | | | $C_{14}H_{11}O^+$ | 195.0804 | 195.0812 | 195.0812 | 4.10/4.10 |
| | | $[M+H]^+$ | $C_{18}H_{17}NO_2^+$ | 280.1332 | 280.1338 | 280.1338 | 2.14/2.14 |

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