University of Staffordshire

Development of an HPLC Method for the Identification & Quantification of MDPHP

by

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Abstract

The synthetic cathinone 3,4-methylenedioxy-α-pyrrolidinohexanophenone (MDPHP), colloquially known as 'monkey dust', has increasingly appeared in forensic casework in recent years, but a quantitative method for seized samples has yet to be published. This study aimed to develop and validate an accurate and reproducible HPLC-UV/Vis method to detect and quantify MDPHP in confiscated samples. Stemming from the UNODC 2015 HPLC method for the quantification of synthetic cathinones, multiple HILIC and reversed-phase stationary phases have been systematically evaluated. The final method employs an ACE UltraCore Super 5 C18 column (150 x 4.6 mm i.d.), with a gradient mobile phase comprised of a 10 mM Ammonium Formate (AF), pH 3, and Acetonitrile (ACN) (95:5, v/v), delivered at 0.6 mL/min and 30°C, with detection at 238 nm (bandwidth ±10 nm). Validation demonstrates a limit-of-detection (LOD) of approximately 0.0003 mg/mL, a limit-of-quantification (LOQ) of 0.001 mg/mL, good linearity (R^2 = 0.993), and intra- and inter-day relative standard deviation (%RSD) of <5% for peak area. This method delivers accurate and precise results across multiple injections and days, demonstrating potential suitability for routine forensic laboratory analysis.

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"We used to be complete wholes in our original nature, and now 'love' is the name for our pursuit of wholeness, for our desire to be complete. […] Thus, from such ancient times, the desire for one another is innate in human beings, seeking to restore the unity of our original nature, to make one out of two, and to heal the condition of humanity. Each of us, therefore, is but a fragment of the original whole human being; and for each person there exists another who is his or her complement, since that single being was split in two, like flatfish. For this reason, each of us is constantly searching for the other half that completes us." (*Symposium*, Plato, 191a–193d)

Table of Contents

1. List of abbreviations (in alphabetical order)

ACMD: Advisory Council on the Misuse of Drugs

ACN: Acetonitrile

AF: Ammonium Formate

ATR-FTIR: Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy

BAC: Blood Alcohol Content

CV: Column Volume

EMCDDA: European Monitoring Centre for Drugs and Drug Addiction

EUDA: European Union Drugs Agency

GC-MS: Gas Chromatography-Mass Spectrometry

HILIC: Hydrophilic Interaction Liquid Chromatography

HPLC: High-Performance Liquid Chromatography

HPLC-UV/Vis: High-Performance Liquid Chromatography-Ultraviolet/Visible Spectroscopy

HRMS: High-Resolution Mass Spectrometry

HS-GC: Headspace Gas Chromatography

LC-HRMS: Liquid Chromatography-High Resolution Mass Spectrometry

LC-MS/MS: Liquid Chromatography-Tandem Mass Spectroscopy

LC-QTOF: Liquid Chromatography-Quadrupole Time Of Flight Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantification

MeOH: Methanol

MDPHP: 3,4-methylenedioxy-α-pyrrodilinohexanophenone

MDPV: Methylenedioxypyrovalerone

MPA: Mean Peak Area

NMR: Nuclear Magnetic Resonance

NPS: New Psychoactive Substances

RP: Reversed-phase

SD: Standard Deviation

S/N: Signal-to-Noise Ratio

UHPLC-MS/MS: Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry

UNODC: United Nations Office on Drugs and Crime

UNODC EWA: United Nations Office on Drugs and Crime Early Warning Advisory

%RSD: Relative Standard Deviation

2. List of tables

3. List of figures

4. Introduction

4.1 New Psychoactive Substances (NPS)

New Psychoactive Substances (NPS) is the term used to refer to drugs specifically created to mimic the effects of other illegal substances, including cannabinoids, hallucinogens and psychostimulants (Home Office, 2015). The United Nations Office on Drugs and Crime (UNODC) defines NPS as 'substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat' (UNODC EWA, 2025). The term 'new' does not necessarily refer to new inventions, "but to substances that have recently become available on the market" (UNODC EWA, 2025). Despite being commonly referred to as "legal highs", the United Kingdom has put NPS under the control of the *Misuse of Drugs Act 1971* and the *Psychoactive Substances Act 2016*.

New psychoactive substances can be classified based on their origin and legal situation (Zapata *et al*, 2021), though the most common approach in literature categorises them by pharmacological effects. The latter divides NPS into three main classes, (i) hallucinogens, (ii) depressants and (iii) stimulants (UNODC, 2018; Global Commission of Drug Policy, 2019):

i. Hallucinogens: substances that alter human sensory perceptions, leading to a distorted view of reality where time, space, shapes and colours are contorted. Examples include LSD and ibogaine.
ii. Depressants: substances that, by slowing down the central nervous system's activity, provide a sense of relaxation, drowsiness and pain relief. Morphine and diazepam fall into this category.
iii. Stimulants: substances that make the users more alert, euphoric and energetic by speeding up the central nervous system's activity. Synthetic cathinones are classified within this category.

4.1.1. NPS Global Emergence

The UNODC Early Warning Advisory (EWA) has reported the emergence of NPS in 153 countries globally, with a prevalence of consumption in the northern hemisphere up to July 2024 (UNODC EWA, 2025). As of 2026, the total number of NPS identified has reached 1,444. Stimulants and synthetic cannabinoid receptor agonists account for 30% and 28.8% of

the total, respectively, followed by classic hallucinogens (12.97%), synthetic opioids (11.89%), sedatives/hypnotics (4.66%) and dissociatives (3%). The remaining 8.31% of the total NPS reported are not yet assigned to a specific pharmacological category (UNODC EWA, 2025).

In the United States of America alone, the Center for Forensic Science Research & Education (CFSRE) has reported 201 newly discovered NPS between 2018 and 2025, with NPS opioids being the largest subclass (Krotulski *et al*, 2026). The EU Early Warning System was monitoring 1000 NPS by the end of 2024, 47 of which had first been reported in the last year (EUDA, 2025). In the United Kingdom, NPS have been circulating on the drug market for over a decade, with the first recorded occurrences dating back to 2008/2009 (Home Office, 2015).

4.2 Synthetic Cathinones

In recent years, among the various NPS groups, synthetic cathinones have gained particular attention due to their rapid proliferation in global drug markets. Synthetic cathinones are a class of NPS derived from the naturally occurring drug 'cathinone' found in the khat plant (*Catha Edulis*) (Riley *et al*, 2020). This flowering evergreen shrub, native to Eastern and Southeastern Africa, gives stimulant-like effects (United States Department of Justice/Drug Enforcement Administration, 2024). Synthetic cathinones are often referred to as 'bath salts', since they mainly circulate as white or brown powders or crystals. Despite being less common, capsules, tablets and cathinone vapes can also be found on the market. Synthetic cathinones are often mixed with adulterants such as benzocaine, lidocaine, caffeine, piperazines and paracetamol (EMCDDA, 2009).

Synthetic cathinones are the β-keto analogues of amphetamines (Coppola and Mondola, 2012), as they are structurally related to the latter but present a carbonyl (keto) group on the side chain (a benzylic group) in the β-position (Advisory Council on the Misuse of Drugs, 2025). As such, synthetic cathinones usually exhibit amphetamine-like effects (Simmler *et al*, 2012), stimulating the central nervous system by increasing synaptic concentrations of dopamine, norepinephrine and, to a lesser extent, serotonin, which amplify their neurotransmitter activity (Baumann *et al*, 2018). Potential effects include paranoia, hallucinations, increased sociability, as well as panic, agitation and diminished appetite (Drug Policy Alliance, 2016). Their consumption can induce tachycardia, hypertension, chest pain and rhabdomyolysis (Arillotta *et al,* 2024), additionally to involuntary body movements, anxiety and depression (Pieprzyca *et al*, 2021).

Synthetic cathinones are currently controlled by the UK Government as 'Class B' drugs under the *Misuse of Drugs Act 1971*: the maximum penalty for possession is up to 5 years in prison and/or unlimited fine, while the supply and production can lead to up to 14 years in prison and/or an unlimited fine. However, as stated in the 'Cover letter from ACMD with advice on synthetic cathinones' (2025a), the Advisory Council on the Misuse of Drugs (ACMD) has recently started to consider changing the classification of some selected synthetic cathinones to Class A, given "their involvement in 'monkey dust' preparations and the effects of these substances on local communities, as well as their association with suspected drug-related deaths in the UK".

The UNODC's *World Drug Report 2025* highlighted the major role played in recent years by synthetic cathinones in both Eastern Europe and Central Asia, as well as the global dominance of amphetamine-type synthetic stimulants in drug seizures. Even though the European Union Drugs Agency (EUDA) has reported a decrease in new synthetic cathinones detected annually, from 30 in 2014-2015 to 7 in 2024, more than 60 previously known synthetic cathinones were still detected and seized on the European drug market in 2023 (EUDA, 2025). As the *European Drug Report 2025* states, seizure quantities have reached 37 tonnes in 2023, with early data showcasing continued high levels in 2024. The data shows that synthetic cathinones' confiscations in Europe in 2023 were three times greater than the total amphetamine and methamphetamine seizure in the same year (EUDA, 2025).

The recreational use of 'first-generation' synthetic cathinones in the United Kingdom has become an increasing public health concern since the early 2000s, particularly following the widespread proliferation of methylone, mephedrone and MDPV (ACDM, 2025b). Recent studies have demonstrated the decline in use of mephedrone and first-generation synthetic cathinones in the past decade, followed by the appearance of a 'second' and 'third generation' (ACDM, 2025b).

Despite the new generations, the use of synthetic cathinones and mephedrone-related deaths have been declining since 2010 and 2015 respectively (ACMD, 2025a). Between 2019 and 2023, a total of 75 deaths involving one or more out of 34 synthetic cathinones were reported (ACMD, 2025c). The mean age was 40 years, with 93% aged over 25 years. Out of the 75 cases, 33% of individuals lived in the two lowest socioeconomic deciles, and 9% were of no fixed abode. Over half of the victims were unemployed, and 84% were previously known drug users (ACMD, 2025c). The Staffordshire County accounted for 52% of total cases.

In 2024, the ACMD reported an increased consumption of synthetic cathinones, especially in Staffordshire, Shropshire and Cheshire, with market concentrated in Stoke-on-Trent, Telford,

Shrewsbury, Newcastle under Lyme, Stone, Stafford and Uttoxeter (ACMD, 2025b). Concerns were raised in North Staffordshire and the surrounding areas about the use of 'monkey dust' and the antisocial behaviour associated with its use (ACMD, 2025b).

4.3 'Monkey dust': 3,4-methylenedioxy-α-pyrrolidinohexanophenone

The UK slang 'monkey dust' refers to the synthetic cathinone 3,4-methylenedioxy-α-pyrrolidino hexanophenone ('MDPHP', $C_{17}H_{23}NO_3$), an hexanone homologue of pyrovalerone that differs from MDPV by one additional carbon in the alkyl chain (Bassi *et al,* 2024). The compound was originally developed and patented in the United States during the 1960s (Köppe *et al*, 1969), but it was first reported as a drug of abuse by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in the *Europol 2014 Annual Report*. The first reports of 'monkey dust' in the UK appeared in 2013 on *The Sentinel*, a local newspaper covering the city of Stoke-on-Trent (Atkinson and Sumnall, 2020). The coverage followed a murder case in which the perpetrator was reported to be 'high on drugs during [a] savage attack' after consuming 'the drug "monkey dust"'. Between 2019-2023, ACDM (2025c) reported 15 MDPHP-related deaths, all but one (93%) recorded in the Staffordshire County. Of these cases, 73.3% involved males, and all individuals were aged between 25 and 64 years.

MDPHP has not yet been pharmacologically characterised (Pisanu *et al*, 2022), as most case studies have only observed poly-intoxications; however, Beck *et al* (2018) identified agitation, delirium, hallucinations, excessive motor activity, seizures, tachycardia, hypertension and hyperthermia as clinical indicators of NPS intoxication, with MDPHP being the most frequently detected substance through the cases examined. As general MDPHP intoxication symptoms, Grapp *et al*, (2020) reported loss of consciousness, aggressive behaviour, delayed physical response, impaired balance and mental confusion. As MDPHP was consumed concomitantly with other substances in all cases observed, such as THC and diazepam, the reported symptoms cannot be attributed solely to 'monkey dust' consumption.

Estimating the number of global fatalities as a consequence of 'monkey dust' ingestion has been difficult: most of the reported deaths are the result of polydrug fatal intoxication involving MDPHP (Adamowicz and Hydzik, 2018; Casati *et al*, 2025; Croce *et al*, 2025), and, therefore, can't directly be attributed solely to the synthetic cathinone. The first fatality by MDPHP with no other substances co-ingested was reported in Italy in 2022 (Di Candia *et al*, 2022): the patient presented high MDPHP concentrations in femoral blood (399 ng/mL) and in urine (222 ng/mL).

The symptoms reported included tachycardia, psychosis, and neurological problems, which are commonly associated with cases of synthetic cathinones' poisonings.

4.3.1 Qualitative analysis techniques

Qualitative analysis methods of MDPHP samples usually include Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS) (Casati *et al*, 2025), and Liquid Chromatography-Quadrupole Time Of Flight Mass Spectrometry (LC-QTOF) (Krotulski and Logan, 2018). Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) may also be employed for qualitative MDPHP analysis: Di Candia *et al* (2022) applied a Q-Exactive Orbitrap High-Resolution Mass Spectrometry (HRMS) alongside LC/MS-MS in their studies for confirmation of 'monkey dust' poisoning. Multi-analytical approaches have also been applied: Gonçalves *et al* (2021) screened multiple cathinone derivatives by combining Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR), GC-MS and Nuclear Magnetic Resonance (NMR) analysis, while Croce *et al* (2025) utilised Headspace Gas Chromatography (HS-GC) for Blood Alcohol Content (BAC) alongside LC-MS/MS.

4.3.2 Quantitative analysis techniques

The analytical techniques employed for the quantitative determination of MDPHP and synthetic cathinones depend largely on the matrix (e.g., urine, blood, plasma) and the required level of sensitivity. For example, Hong *et al* (2016) employed GC-MS to quantify synthetic cathinones in urine samples from poisoning cases, whereas Aldubayyan *et al* (2022) utilised LC-MS/MS for the same purpose. MDPHP quantitation from plasma samples can be carried out by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) (Pelletier *et al*, 2024). To the author's knowledge, no quantitative methods for seized MDPHP samples have been published to date. The lack of quantitative analysis techniques for seized MDPHP, rather than toxicological samples, highlights the need for this project.

4.3.3 HPLC-UV/Vis methods for synthetic cathinones

Despite the increasing prevalence of MDPHP in forensic casework, especially in the Staffordshire County, the quantitative analysis of 'monkey dust' samples still present significant analytical challenges, mostly related to the complexity, cost and speed of the techniques. Most laboratories rely on mass spectrometric methods, such as LC-HRMS, which are often not available to smaller forensic or research laboratories, creating the need for more efficient and effective quantitative methods. Additionally, multiple studies have shown that SC often require chemical derivatisation

to improve mass spectral quality by GC-MS (Kranenburg *et al*, 2020; Kerrigan, 2015) and can degrade during thermal analysis (Kerrigan and Savage, 2016), which results in longer analysis time, supplementary costs and less reliable results.

In 2015, the UNODC released a HPLC-UV/Vis method for analysis of synthetic cathinones in seized materials (UNODC, 2015) that was initially developed for the identification and quantification of mephedrone and methylone by Santali *et al* (2011). High-Performance Liquid Chromatography (HPLC) is regarded as a fast and straightforward separation technique that allows for highly precise results and efficient recovery of analytes, particularly in forensic drug analysis (Ohtsuki *et* al, 2024). Additionally, HPLCs coupled with UV/Vis detectors are usually cheaper and more straightforward than MS-based systems (Leghissa *et al*, 2017).

To the author's knowledge, there is no MDPHP-specific quantification method published that uses HPLC-UV/Vis. Nevertheless, the method developed by Santali *et al* (2011) could address the limitations of current MS-approaches and provide a valuable technique for 'monkey dust' samples' quantification, simultaneously supporting both forensic caseworks and public health monitoring.

5. Aims and Objectives

The aim of this project is to develop an accurate, reproducible and validated HPLC-UV/Vis method to detect and quantify MDPHP in seized street samples. The samples (ST24026) will be provided by the Staffordshire and West Mercia constabularies. The chromatographic conditions explored in J. Robinson's student project (2025), based on the UNODC method (2015) for HPLC-UV/Vis quantification of synthetic cathinones, will initially be evaluated. Depending on the results obtained, alternative chromatographic conditions will be investigated, including variations in mobile phase composition and pH, as well as buffer type and concentration. Different stationary phases, including C18 and HILIC columns, will also be assessed to determine their suitability for retention and separation of MDPHP. Following the development of a working method, further optimisation of chromatographic parameters, such as column temperature, will be carried out, and the method's analytical performance will be assessed via limit-of-detection/limit-of-quantification, intra-day repeatability, and inter-day reproducibility evaluation.

6. Materials and methods

6.1 Instrumentation

- pH regulation:
    - Thermo Scientific ORION STAR A211 Benchtop pH Meter
    - SLS Ltd. Select Electrode pH Epoxy PHE1000
- GC-MS:
    - PerkinElmer Clarus 690 GC
    - PerkinElmer Clarus SQ 8T MS
- UV detector:
    - Thermo Scientific EVOLUTION 201 UV-Visible Spectrophotometer
- HPLC instrumentation:
    - PerkinElmer Series 200 HPLC
    - PerkinElmer UV/Vis UHPLC Detector
    - ACE UltraCore 5 SuperC18 HPLC Column, CORE-5A-1546U, 150 x 4.6 mm i.d.

6.2 Reagents and materials

- Seized sample ST24026 (confirmed to contain only MDPHP, see *7.1.1.3*)
- Fisher Scientific HPLC grade Methanol
- Sigma-Aldrich Ammonium Formate (eluent additive for LC-MS)
- Deionised water
- 2 M Hydrochloric Acid (HCl)
- Fisher Scientific HPLC grade Acetonitrile
- Eluent filtering:
    - Sigma-Aldrich 1000 mL volumetric Flask
    - Sigma-Aldrich Sinter Funnel 40/35
    - Sigma-Aldrich Vacuum and Reservoir Kit
    - Sigma-Aldrich 0.20 µm Nylon Membrane 47 mm

6.3 Preparation of stock and working standard solutions (MDPHP)

A stock solution was prepared by accurately weighing 10.0 mg of sample ST24026 into a 10 mL volumetric flask and dissolving it completely in methanol (MeOH) to volume, yielding a concentration of 1.0 mg/mL. In a second 10 mL volumetric flask, 1 mL of the stock solution was transferred and made up to volume using methanol to obtain a 1:10 dilution. 1 mL of the solution

was transferred into an autosampler vial with a red PTFE/silicone septum. All stock solutions and samples were stored in the freezer at -20°C to prevent degradation and evaporation.

6.4 Preparation of blank solutions

A methanol blank was prepared and stored under identical conditions to the stock solutions and samples.

6.5 Preparation of HPLC eluents

6.5.1. 5 mM Ammonium Formate buffer

In a 500 mL solvent reservoir, 0.1574 g of Ammonium Formate (NH_4HCO_2) was dissolved in 500 mL of deionised water. Using the Thermo Scientific ORION STAR A211 pH meter (average calibration slope: 97.8%) and the pHE100 SLS electrode, the pH was adjusted from 5.53 to 3.07 using 2M HCl added dropwise. The eluent was then filtered using the Sigma-Aldrich© filtering kit. The buffer was then connected to channel A of the HPLC system.

6.5.2. Acetonitrile eluent

The Acetonitrile (ACN) HPLC grade eluent was transferred in a 500 mL solvent reservoir and connected to the HPLC system on channel B.

6.6 HPLC analytical conditions

- Column:
  - ACE UltraCore 5 SuperC18 HPLC Column, CORE-5A-1546U, 150 x 4.6 mm i.d.
- Pump Program:

Table 1: Initial Method Pump Program

Step	Time (min)	Flow (mL/min)	Channel A (%)	Channel B (%)	Curve
0	3.0	0.40	90	10	0
1	3.0	0.40	90	10	0
2	10.0	0.40	10	90	1.0
3	5.0	0.40	10	90	0

- Flow rate: 0.4 mL/min
- Temperature: 25°C
- Pressure: approximately 710 psi under initial conditions
- Injection volume: 10.0 µL
- Sampling rate: 10.00 pts/sec

- Detection wavelength: 238 nm

6.7 Safety and waste disposal

A Probabilistic Risk Assessment (PRA) for the handling and disposal of all chemicals involved in the method development process was completed in accordance with the University of Staffordshire's H&S policy and the Control of Substances Hazardous to Health (COSHH) Regulations (Health and Safety Executive, 2002). The PRA was reviewed and signed off by the supervising staff before any laboratory activity started. A full copy of the document can be found in *APPENDIX A*. An Ethics Form, required under the University of Staffordshire's Ethical Review Policy (2024), was completed alongside the PRA, and can be found in *APPENDIX B*.

6.8 Statistical analysis

Statistical analysis of replicated injections was performed using Microsoft Excel (Microsoft Corp., USA). The standard deviation (SD) was calculated using the sample standard deviation function (STDEV.S), and the relative standard deviation (%RSD) was determined according to equation (1):

$$\%RSD = \frac{SD}{mean} \times 100 \quad (1)$$

7. Results and Discussion

7.1. Column Chemistry Investigation

7.1.1. Reversed-Phase Screening

The initial phase of the method development process was carried out using the ACE UltraCore 5 Super C18 (150 mm x 4.6 mm i.d.), a reversed-phase (RP) HPLC column with a stationary phase that features octadecyl carbon chains (18-C) bonded to a silica surface via siloxane (Si-O-Si) links. It provides hydrophobic interaction-based separation, good efficiency, and is particularly suitable for moderately non-polar organic compounds (Ganesh *et al*, 2023). Given the structural characteristics of MDPHP (see *APPENDIX C*), a C18 stationary phase was selected as an appropriate starting point to develop an efficient retention and separation method.

7.1.1.1. Gradient Elution and Retention Behaviour

Mobile phase composition was systematically varied over the duration of the separation process through gradient elution. This technique is generally adopted to improve resolution and separation of analytes in complex mixtures (Ornaf and Dong, *2005*). Contrary to isocratic elution for RP chromatography, gradient HPLC typically provides better separation for hydrophobic compounds, such as MDPHP, in a reasonable time frame (LCGC, 2013). At the beginning of the elution gradient, a low organic modifier fraction (Φ) was selected to maximise analyte retention and improve on-column focusing (Ganesh *et al*, 2023). Under these weak elution conditions, the retention factor (*k*) is high, resulting in slow analyte migration and strong partitioning into the stationary phase (Jouyban *et al*, 2009). As Φ increases during the gradient, mobile-phase strength increases and *k* decreases continuously, leading to progressive desorption and acceleration of the analyte through the column (Molnar, 1981).

7.1.1.2. Mobile Phase Composition

Ammonium Formate (AF), dissolved in distilled water, was selected as the aqueous mobile phase component. AF is highly polar and fully water soluble by dissociating into NH_4^+ and $HCOO^-$ in solution. In RP HPLC, AF mainly acts as a buffering agent to control mobile phase pH and to minimise undesirable interactions between basic analytes and potential residual silanol groups on the silica surface (Maiellaro *et al*, 2024), thereby improving peak symmetry

and reproducibility. As an ionic and highly polar species, AF is not retained under RP conditions and elutes near the solvent front, as reported in published studies, such as Roemer *et al* (2014).

Acetonitrile (ACN) is a polar aprotic organic solvent and was used as the organic modifier. ACN does not ionise or buffer pH, but plays a critical role in modulating mobile phase polarity and elution strength (Subirats *et al*, 2023). Increasing ACN concentration reduces hydrophobic interactions between the analyte and the C18 stationary phase (Steinhoff *et al*, 2025). The retention time decreases as a result, and controlled gradient elution is enabled. Therefore, the mobile phase gradient was initially set from 90:10 (AF:ACN) to 10:90 (AF:ACN) over the course of each run.

7.1.1.3. Analyte

The analyte was obtained from the sample ST24026. The previously conducted GC-MS analysis (full chromatogram and library report provided in *APPENDIX D*) confirmed the presence of MDPHP as the principal component of the sample (RT 18.996 min, match factor 837), as shown in Figure 1. A second peak at RT 21.807 min was identified as a phthalate ester, a synthetic plasticiser or additive commonly used in the cosmetic and pharmaceutical industries (Aldegunde-Louzao *et al*, 2024). Phthalates are frequently detected during GC-MS analysis as background contamination, often deposited on injector surfaces, which leads to persistent peaks that are not directly derived from the sample (Marega *et al*, 2013). Therefore, the peak was tentatively attributed to laboratory or packaging contamination rather than manufacturing impurity.

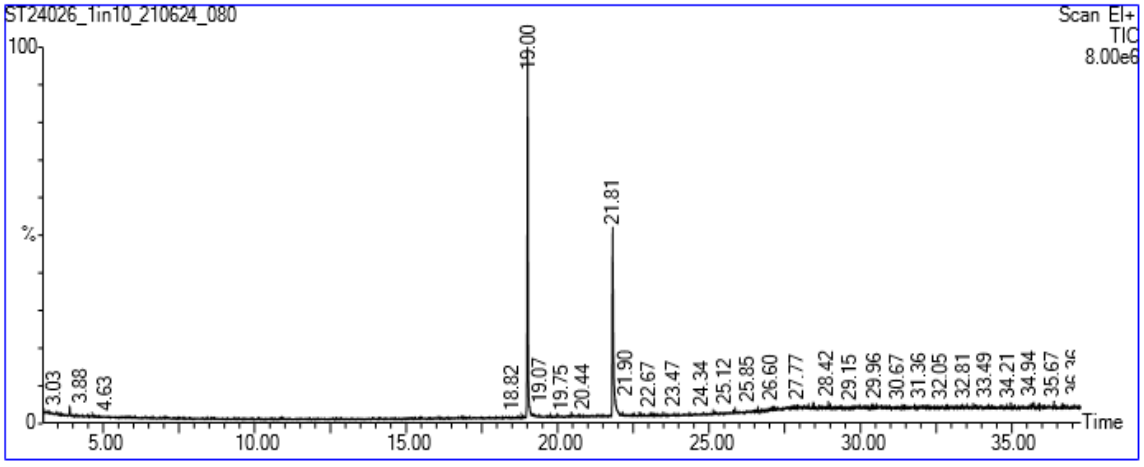

Figure 1: ST24026 GC-MS Qualitative Report

The UV-Vis spectra derived from three ST24026 dilutions (1:10, 1:100, and 1:1000; see *APPENDIX E*) showed the characteristic aromatic absorption bands that are consistent with methylenedioxy-substituted β-ketophenethylamine structures. As shown in Figure 2, no other significant UV-active components were detected, which supported the conclusion that MDPHP (observed λ_{max} = 231 nm, ±1 nm) was the predominant organic component in the sample.

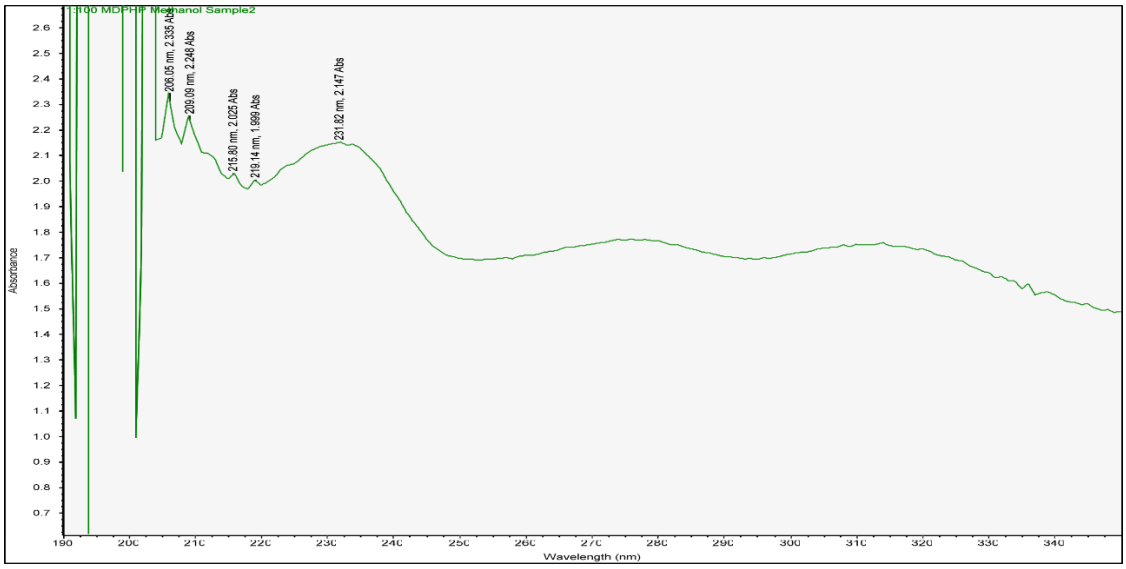

Figure 2: 1:100 dilution ST24026 UV-Vis Spectrum

7.1.1.4. Pump Program and Analytical Conditions

The initial pump program, based on a previous student project (Robinson, 2025), is shown in Table 2, while the analytical conditions are registered in Table 2. The gradient (Step 3) was followed by a high-organic isocratic hold (Step 4) to ensure complete elution of strongly retained or hydrophobic compounds, preventing carryover and improving reproducibility between injections (Ampe *et al*, 2021).

Step 0 acts as the column re-equilibration phase at initial mobile phase conditions, to allow k to return to its initial value (Schellinger *et al*, 2008). The re-equilibration time was estimated from the column void volume (V_M) for a 150 x 4.6 mm i.d. column, and was calculated using:

$$V_M \approx 0.5 \times L \times d_c^2 \times 10^{-3} \quad (2)$$

where L is the column length (mm) and d_c is the internal diameter (mm), giving $V_M \approx 1.6$ mL (Dolan, 2015). Traditional methods suggest that adequate re-equilibration of a RP

column following solvent gradient elution may require flushing the column for a minimum of 10 column volumes (CV). However, prior studies using the same stationary phase reported adequate re-equilibration at approximately 2 CV (Stoll and Seidl, 2019; Schellinger *et al*, 2008). Between Step 0–1, the total time at initial isocratic mobile phase conditions is 6.0 minutes, equal to 1.6 CV, and it was considered sufficient based on the results obtained by the previous student project (Robinson, 2025). Since no data is recorded during Step 0, the total analytical run time amounted to 18.0 minutes. The dotted blue line in Table 2 indicates when injection occurred. The visual representation of the elution gradient can be found in *APPENDIX T*.

Table 2: Pump Program ACE UltraCore 5 SuperC18 HPLC Column

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	3.0	0.40	90	10	0
1	3.0	0.40	90	10	0
2	10.0	0.40	10	90	1.0
3	5.0	0.40	10	90	0

Table 3: Initial Methodologies Analytical Conditions

Wavelength (λ)	Injection Volume	Temperature	Column	Sampling Rate
238 nm	10.0 µL	25°C	RP C18	10.0 pts/sec

Although the UV-Vis spectrophotometric analysis indicated a λ_{max} for MDPHP at 231 nm (±1 nm), the detection wavelength on the PerkinElmer UV/Vis UHPLC Detector was set on 238 nm (Table 3), as instructed by the method developed in a previous student project (Robinson, 2025). Given the instrument bandwidth of ±10 nm, this setting effectively encompassed the observed absorption maximum, ensuring adequate analytical sensitivity.

Column temperature was initially set at 25°C during method development (Table 3), as it is a neutral and widely adopted starting condition in RP HPLC (Bharathi Tejas and Bhadre Gowda, 2021). As highlighted by D. Bolliet (1998), column temperature is less influential on retention changes compared to mobile phase composition. Therefore, method development was primarily focused on solvent strength and buffer conditions before optimising the thermal parameters.

7.1.1.5. Buffer Trialled

7.1.1.5.1. Ammonium Formate

A 1:10 dilution of MDPHP in MeOH was used as the analyte, after a MeOH blank run was performed to ensure that the column was clean. Under the initial conditions described in the previous student project (Robinson, 2025), 5 mM AF, pH 3, pump program and analytical condition shown in Table 2 and Table 3 respectively, the sample produced a sharp, early-eluting peak at approximately 2.9 minutes (Figure 3) (full chromatograms in *APPENDIX F*). The peak showed reasonable symmetry and a narrow width, which initially suggested acceptable chromatographic performance.

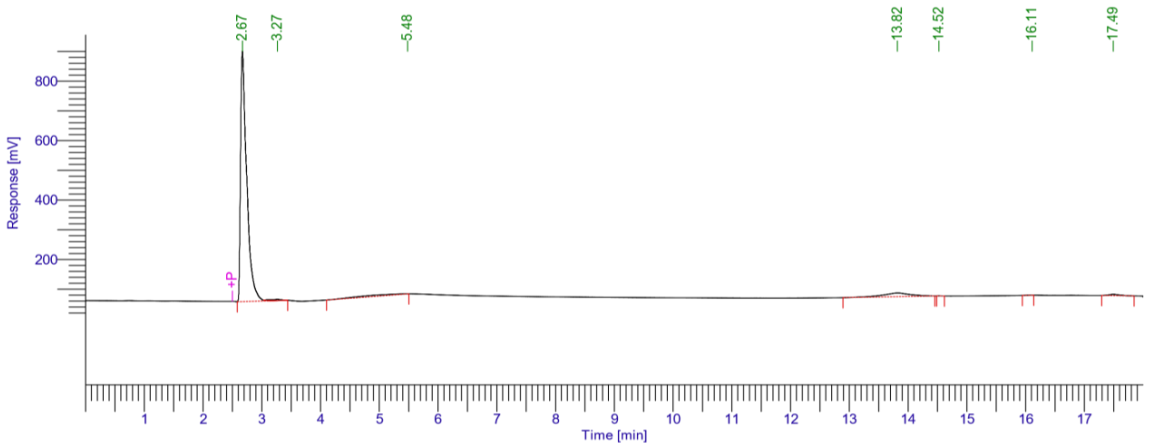

Figure 3: 1:10 MDPHP in MeOH 5 mM AF, pH 3

However, the peak's retention time appeared to be earlier than the expected column void time (t_0) under the applied flow rate, calculated as follows:

$$t_0 = \frac{V_M}{flow} = \frac{\sim 1.6\ mL}{0.4\ mL/min} \approx 4\ min \quad (3)$$

Therefore, this suggested that there was very limited hydrophobic interaction with the C18 stationary phase (Ligor *et al*, 2014). The limited retention indicated that the analyte was eluting at or near the solvent front and was not undergoing true RP partitioning. This was unexpected under strongly aqueous initial conditions (90% aqueous phase) for a moderately hydrophobic compound such as MDPHP. The results prompted further investigation into both mobile phase pH and buffer composition to determine whether the analyte was co-eluting with the injection peak.

In order to reach MDPHP's pKa (approximately 8.0–10.0), adjustments to the mobile phase pH were done to modulate the analyte's ionisation state and increase the hydrophobic interactions with the stationary phase (Ganesh *et al*, 2023). At pH ≈ pKa, a

greater fraction of the neutral species is present, which usually results in increased retention (Kumar *et al*, 2013). Therefore, the buffer pH was increased to 7 and then 9 to try to push the analyte elution after the solvent front. In both cases, the peak did shift to 3.432 min and 3.295 min respectively (see *APPENDIX G* and *APPENDIX H*), but the solvent retention time still appeared to be shorter than t_0.

Changes to the buffer concentration were investigated next, initially lowering it to 1 mM AF (0.0314 g AF in 500 mL of deionised water) and then raising it to 20 mM, whilst keeping the pH at 9. Under the first conditions (full HPLC report in *APPENDIX I*), MDPHP eluted at approximately 3.3 min. The peak showed significant asymmetry and tailing (Figure 4), with noticeable distortion after the apex. The baseline stability was moderately affected in the post-elution region, suggesting analyte–stationary phase secondary interactions (Ganesh *et al*, 2023). At 20 mM AF (full HPLC report in *APPENDIX J*), MDPHP eluted at a similar time (approximately 3 min) as a sharp peak, while a pronounced baseline elevation was registered following elution during the high-organic phase of the gradient, potentially due to the mobile phase composition changes (Figure 5) (Karu, 2012).

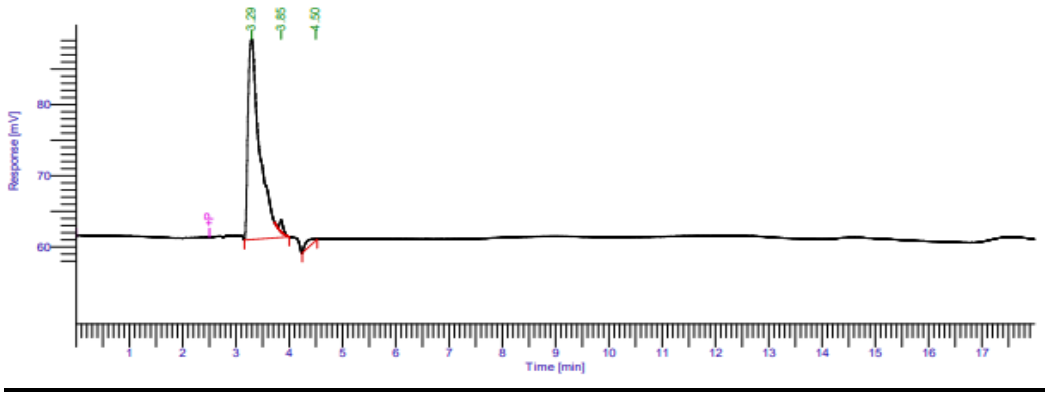

Figure 4: MDPHP 1mM AF, pH 9 - ACE Super C18

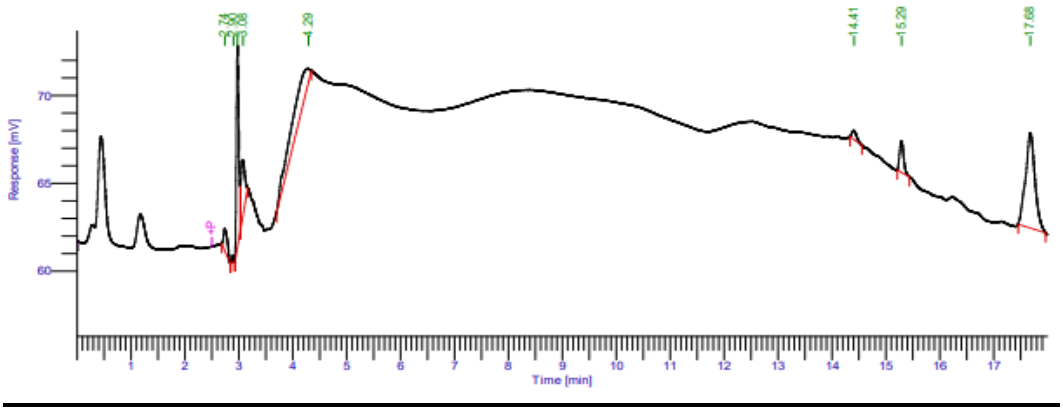

Figure 5: MDPHP 20 mM AF, pH 9 - ACE Super C18

7.1.1.5.2. Sodium Phosphate

As the AF buffer did not produce the expected results, a 50 mM Sodium Phosphate buffer (pH 3) was connected on channel A of the HPLC system, while maintaining the same analytical conditions. The eluent was pre-made for a different student project; therefore, no additional preparation had to be done. The resulting chromatogram (see *APPENDIX K*) showed significant negative absorbance, potentially due to intrinsic buffer absorbance (Dong and Wvsocki, 2019). At 50 mM concentration, increased background absorbance is likely to enhance the difference between the mobile phase and the injected solvent, which may have been the cause for the transient negative deflections (Nevado *et al*, 2013).

Consequently, the buffer was diluted to 5 mM concentration, while maintaining pH 3. The chromatograms showed no negative absorbance (full HPLC reports in *APPENDIX L*), but the peak detected displayed noticeable asymmetry and tailing (Figure 6). Additionally, the analyte retention time (approximately 2.70 min) was still shorter than the expected column void time.

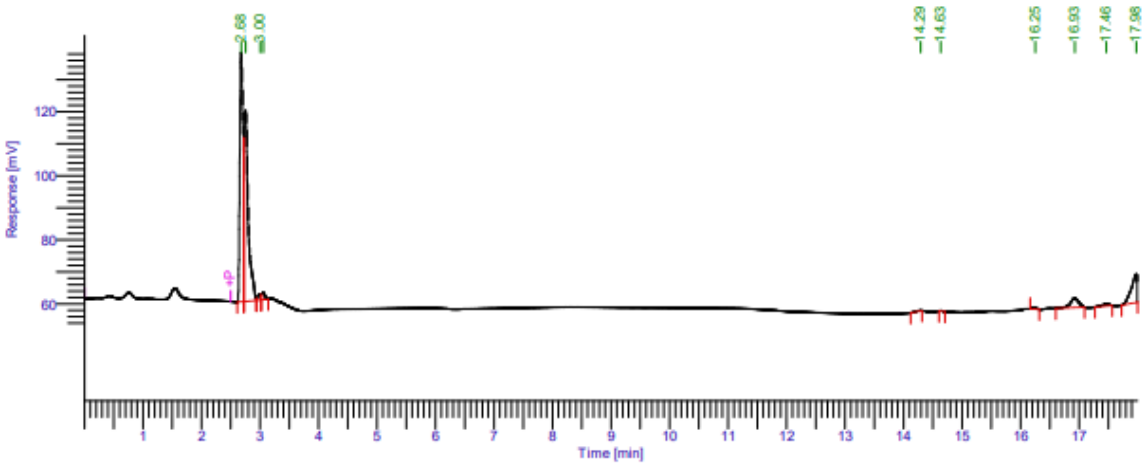

Figure 6: MDPHP 5 mM Sodium Phosphate, pH 3 - ACE Super C18

Given that neither changing the buffer concentration nor the pH appeared to significantly delay analyte elution time, the next step was to try switching to a different column.

7.1.2. HILIC Column Screening

Hydrophilic Interaction Liquid Chromatography (HILIC) is a leading technique for the separation of polar molecules (Cavazzini *et al*, 2023). It employs polar stationary phases that are involved in hydrophilic interactions with the analytes in high organic content mobile phases (such as ACN) to retain polar solutes (Aristoy and Toldrá, 2016). As HILIC had already been used for the quantification of synthetic cathinones (Peters *et al*, 2016), three different columns were selected for the method development: ACE 3 µm HILIC-A, HILIC-B, and HILIC-N. The first two columns have stationary acidic and basic phases with an ionisable negative and positive surface charge, respectively, which depends on the mobile phase pH (Burgos-Gil *et al*, 2019). Conversely, HILIC-N has a neutral polyhydroxy stationary phase (Burgos-Gil *et al*, 2019).

HILIC-B was first tested using a 20 mM AF buffer, pH 3, and ACN gradient elution, following the pump program and analytical conditions in Table 2 and Table 3 respectively. The AF buffer strength and low pH cause the ionisation of the HILIC silanol groups to reduce, minimising electrostatic interactions, and the ammonium ions compete with basic analytes for interaction sites, influencing retention (Al-Tannak *et al*, 2019). MDPHP at pH 3 is fully protonated, and more likely to undergo partition into the HILIC water-rich layer. Therefore, MDPHP retention relies on partitioning, not electrostatics.

A new MDPHP working solution was prepared by transferring 10 µL of the 1.0 mg/mL stock solution (in MeOH) into a 10 mL volumetric flask and diluting to volume with a 1:9 (v/v) mixture of ACN and 20 mM AF, yielding a final concentration of 1 µg/mL. A blank was prepared under identical conditions. Since the retention time and the *k* factor are inversely proportional to solvent strength in HILIC separation (Travedi *et al*, 2012), the analyte composition was changed. Therefore, compared to MeOH, ACN tends to increase retention due to its weaker organic solvent nature, which promotes partitioning into the water-rich layer at the stationary phase surface (Travedi *et al*, 2012).

A sharp, high-intensity peak was observed in the resulting chromatograms (Figure 7) at approximately 2 minutes, followed by a pronounced negative deflection around 3 minutes and an unstable baseline region. The results still clearly showed an early-eluting peak (full HPLC report in *APPENDIX*), which led to a change in column.

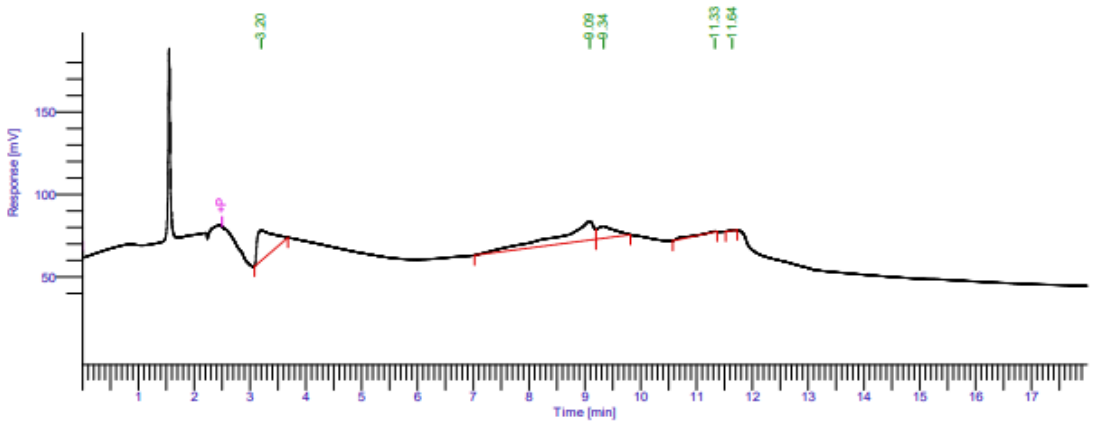

Figure 7: ACE HILIC-B Results

Both HILIC-A (*APPENDIX N*) and HILIC-N (*APPENDIX O*) generated very sharp peaks between 2.0–2.6 minutes and noticeable baseline disturbance. The consistent early elution across all three HILIC stationary phases indicated that the selected mobile phase and operational conditions were insufficient for effective HILIC retention of MDPHP. Consequently, the method development trials were carried out on the ACE Super C18 column again.

7.2. Mobile Phase Optimisation on Selected Column

After reverting to the ACE Super C18 column, a 10 mM AF concentration, pH 3, was trialled on Channel A of the HPLC system, while ACN was kept on Channel B. A 1:10 dilution of MDPHP in MeOH previously produced was used as analyte, alongside a MeOH blank. The HPLC pump

program was slightly modified, as shown in Table 4, whilst maintaining the operational conditions unchanged:

Table 4: Second Pump Program - ACE Super C18 HPLC Column

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	1.5	0.40	90	10	0
1	1.5	0.40	90	10	0
2	7.0	0.40	10	90	1.5
3	3.0	0.40	10	90	0

However, results showed once again that the analyte co-eluted with the injection peak, yielding broadened and split peaks in the estimated column dead time (see *APPENDIX P*).

7.3. Final Method

Since the prior method (Table 4) did not yield the desired results, the pump program was modified to further optimise the chromatographic conditions, as registered in Table 5. Furthermore, the column temperature was changed to 30°C (Table 6).

Table 5: Final Method Pump Program

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	1.5	0.60	95	5	0
1	1.5	0.60	95	5	0
2	7.0	0.60	60	40	1.5
3	3.0	0.60	60	40	0

Table 6: Final Method Analytical Conditions

Wavelength (λ)	Injection Volume	Temperature	Column	Sampling Rate
238 nm	10.0 µL	30°C	RP C18	10.00 pts/sec

The MeOH blank reported a shift in the injection peak (height: 91 mAU) approximately between 2.0–2.8 minutes, due to the change in the pump program flow to 0.6 mL/min (Figure 8). No

additional peaks were observed throughout the run, confirming the absence of system-derived contaminants.

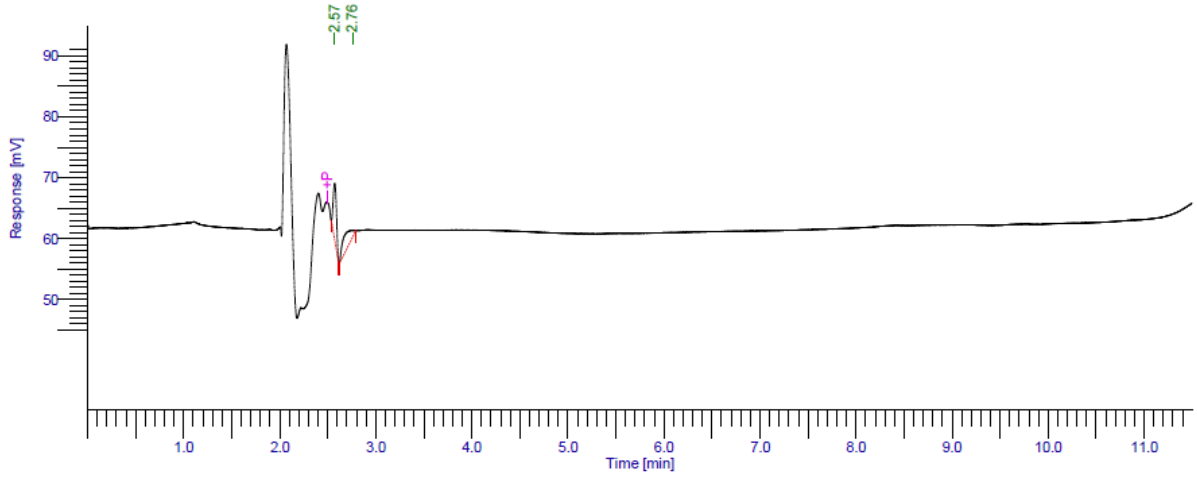

Figure 8: 95:5 10 mM AF (pH 3):ACN - MeOH blank

The following 1:10 dilution MDPHP in MeOH samples ('November 1:10 MDPHP in MeOH', Figure 9; 'January 1:10 MDPHP in MeOH', Figure 10) both showed a sharp, symmetric peak after the injection peak region, eluted at 2.99 min and 3.01 min respectively, that accounted for approximately 99.5% of the total peak area (full report in *APPENDIX Q*). The peaks' separation from the injection disturbance clearly demonstrated that effective partitioning was occurring under the selected conditions. Retention time was consistent across the injections (Δt_R = 0.03 min), demonstrating good chromatographic repeatability. However, since the sample size was limited to two injections, repeatability and reproducibility were calculated more reliably after the method optimisation trials in 7.3.2.2.

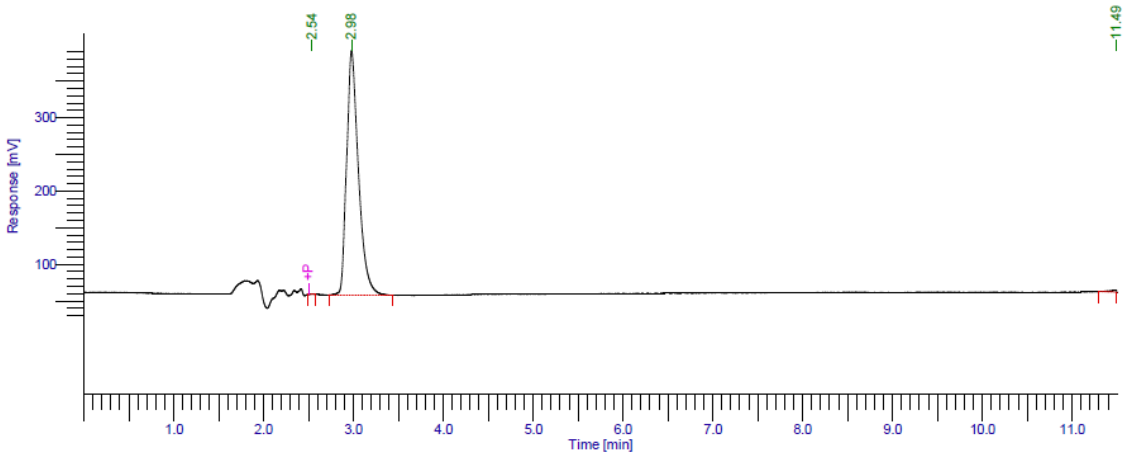

Figure 9: November 1:10 MDPHP in MeOH - Working Method

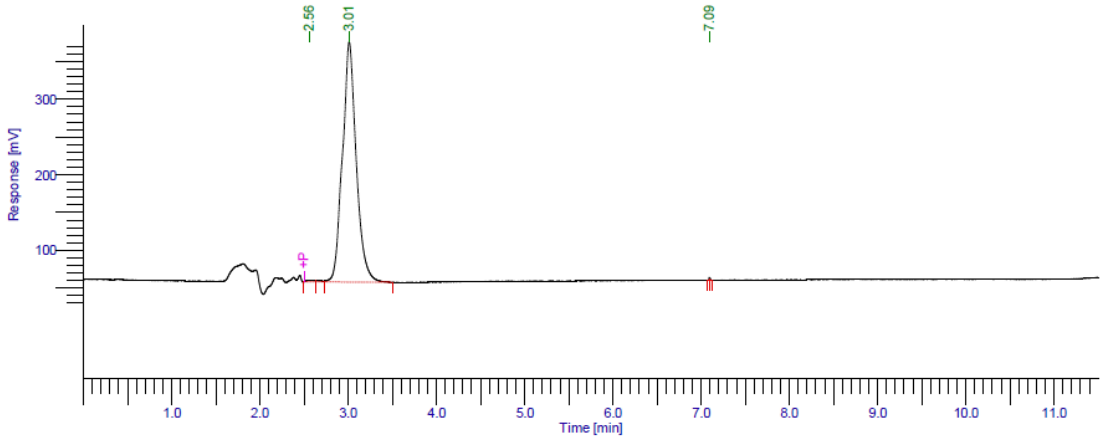

Figure 10: January 1:10 MDPHP in MeOH - Working Method

7.3.1. Optimisation Trial

Given that elution began at approximately 1.7 minutes following the start of Step 2, this indicated insufficient retention under initial analytical conditions. To shift MDPHP elution into an analytically preferable window (5–8 minutes from run start), systematic modifications were applied to certain chromatographic parameters, including column temperature, mobile phase composition, and the duration of the initial hold (Step 0–1).

The peak shape was not one of the criteria considered to determine a positive result of the optimisation trials, since most chromatograms presented asymmetric, split or distorted peaks. The observed peak morphology was likely influenced by the age of the aqueous buffer, as most mobile phase buffers have a limited usable lifespan of approximately one week (Losch, 2026), and the eluent had been created two weeks prior to the beginning of the optimisation trials. All of the optimisation trials chromatograms are present in *APPENDIX R.*

7.3.1.1. Column Temperature

It has long been recognised that retention in reversed-phase liquid chromatography follows the van't Hoff equation (3), by which the natural logarithm of the retention factor is described as linearly related to the reciprocal of temperature (4) (Asnin and Stepanova, 2024).

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \Phi \qquad (3)$$

where:

$$\ln k \propto \frac{1}{T} \qquad (4)$$

Consequently, retention generally decreases with increasing column temperature, due to enthalpic and entropic factors that regulate solute partitioning between the stationary and mobile phases (Bidlingmeyer and Henderson, 2004). Increasing column temperature typically weakens analyte–stationary phase interactions and reduces retention, which translates to shorter elution times (Guillarme *et al*, 2004). Therefore, the temperature of the RP C18 column was reduced from 30°C to 25°C, while maintaining the pump program and all other analytical conditions unchanged, as shown in Table 7 and Table 8.

Table 7: Column Temperature Optimisation HPLC Pump Program

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	1.5	0.60	95	5	0
1	1.5	0.60	95	5	0
2	7.0	0.60	60	40	1.5
3	3.0	0.60	60	40	0

Table 8: Analytical Conditions of Column Temperature Optimisation

Wavelength (λ)	Injection Volume	Temperature	Column	Sampling Rate
238 nm	10.0 µL	25°C	RP C18	10.00 pts/sec

After running a 1:10 dilution of MDPHP in MeOH with the new analytical conditions, the results showed that the injection peak had slightly shifted to the right of the chromatogram, and that the peak shape had deteriorated and split into two separate peaks. Based on the obtained chromatograms, it was decided to change the operating column temperature back to 30°C, and then try changing the mobile phase composition.

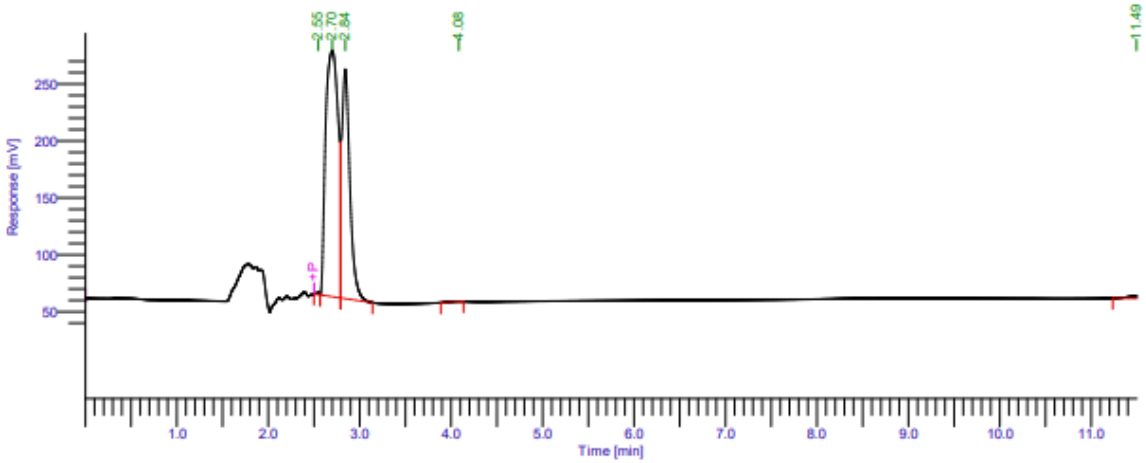

Figure 11: Column Temperature Optimisation Trial Chromatogram

7.3.1.2. Mobile Phase Composition

The pKa values refer to the negative log of the acid dissociation constant (Ka), which represents the degree of acid ionisation.

$$pKa = -\log_{10} Ka \quad (5)$$

According to the Henderson-Hasselbalch equation (6) for a weak base, used to determine the pH of a buffer solution composed of a weak base (B) and its conjugate acid (BH^+), the ratio of neutral (B) to protonated (BH^+) species depends on the difference between pH and pKa.

$$pH = pKa + \log(\frac{[B]}{[BH^+]}) \quad (6)$$

Given that MDPHP is a weak base, with an estimated pKa of approximately 8.0–10.0, and that the mobile phase pH was maintained at 3 using AF, the compound exists almost entirely in its protonated (BH^+) form, under the applied analytical conditions. Therefore, the changes in mobile phase that impact solvent strength and hydrophobic partitioning influence retention rather than altering the ionisation degree.

The organic modifier fraction (Φ) influences the retention factor in RP chromatography. The apparent pKa under chromatographic conditions ($pK_{a,chrom}$), which is the pH at which half of the analytes are protonated, depends on the composition of the mobile phase (Heinisch and Rocca, 2004). The van't Hoff equation (1) states that the polarity of the mobile phase relative to the RP C18 stationary phase increases as Φ decreases. This then leads to the hydrophobic analytes (such as MDPHP) interacting more strongly with the stationary phase (David and Moldoveanu, 2024).

Lowering Φ makes the mobile phase environment more similar to aqueous conditions, which shifts the ionisation equilibrium of weak bases toward a protonated state at a certain mobile phase pH (Heinisch and Rocca, 2004). This makes the apparent $pK_{a,chrom}$ closer to the aqueous value ($pK_{a,aq}$) and the predominance of the protonated (BH^+) form is reinforced under the acidic AF conditions used (pH 3). These effects, along with the increased hydrophobic interactions resulting from the lower Φ factor, cause a higher retention factor (*k*), a longer analyte elution time, and a greater initial focusing on the column head before the gradient increases. Therefore, the ACN% was reduced from 5% to 2% in Step 0–1, while maintaining identical analytical conditions, as shown in Table 9 and Table 10.

Table 9: Mobile Phase Composition Optimisation HPLC Pump Program

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	1.5	0.60	98	2	0
1	1.5	0.60	98	2	0
2	7.0	0.60	60	40	1.5
3	3.0	0.60	60	40	0

Table 10: Analytical Conditions of Mobile Phase Composition Optimisation

Wavelength (λ)	Injection Volume	Temperature	Column	Sampling Rate
238 nm	10.0 µL	30°C	RP C18	10.00 pts/sec

The same 1:10 dilution of MDPHP in MeOH, used for the column temperature optimisation trial, was run with the new chromatographic conditions. However, the peak was significantly smaller than the one registered the previous week, possibly due to sample degradation caused by erroneous storage conditions, as the sample had not been deposited in the freezer. More importantly, the peak did not shift, therefore the mobile phase composition was changed back to 95:5 AF:ACN.

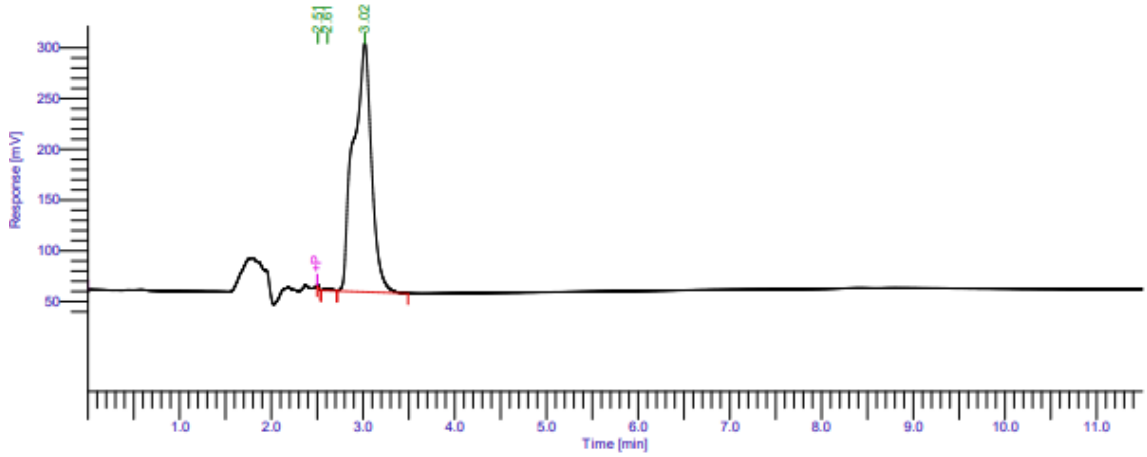

Figure 12: Mobile Phase Composition Optimisation Trial Chromatogram

7.3.1.3. Initial Hold Duration

In gradient RP HPLC, extending the initial isocratic hold at a low Φ maintains a high *k* for a longer duration before the gradient ramp begins (Grushka *et al*, 2004). Under isocratic conditions, retention is described by:

$$t_R = t_0(1 + k) \quad (7)$$

$$k = \frac{t_R - t_0}{t_0} \quad (8)$$

where t_R is the retention time, and t_0 is the dead time. For RP systems, the retention factor depends on the organic modifier fraction Φ according to the linear solvent strength model (9), as reported by Snyder *et al* (2010):

$$\ln k = \ln k_w - S\phi \quad (9)$$

where k_w is the retention factor in pure water, and *S* is a solvent strength constant. As Φ decreases, *k* increases exponentially, resulting in stronger retention. In gradient elution, Φ increases with time:

$$\phi(t) = \phi_i + \beta t \quad (10)$$

where Φ_i is the initial organic fraction, and β is the gradient slope. Analyte elution occurs when *k* decreases to approximately 1–2 as solvent strength increases, where, for *k*=1, the analyte spends the same amount of time in the stationary phase as the mobile phase, and for *k*=2, the analyte spends twice as much time in the stationary phase (Ismail, 2024).

Extending the initial hold time maintains the mobile phase at a low Φ_i for a longer period, keeping k high before the gradient ramp begins (Baeza-Baeza and García-Álvarez-Coque, 2014). Although the thermodynamic retention factor at a given Φ does not change, the analyte remains strongly retained for longer, consequently increasing the observed retention time, given that:

$$t_R \approx t_h + t_{gradient} \quad (11)$$

where t_h is the initial hold time. Additionally, prolonged exposure to weak mobile phase enhances analyte focusing on the column head, improving both peak shape and effective retention (Buckenmaier *et al*, 2025). Thus, increasing the initial hold time delays elution by postponing the analyte's exposure to stronger solvent conditions (Kaliszan *et al*, 2003).

Therefore, the initial hold time (Step 0–1) was extended from 1.5 minutes to 3 minutes, for a total run time of 13 minutes, while every other parameter was maintained unchanged (see Table 11 and Table 12).

Table 11: Initial Hold Duration Optimisation HPLC Pump Program

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	3.0	0.60	95	5	0
1	3.0	0.60	95	5	0
2	7.0	0.60	60	40	1.5
3	3.0	0.60	60	40	0

Table 12: Analytical Conditions of Initial Hold Duration Optimisation

Wavelength (λ)	Injection Volume	Temperature	Column	Sampling Rate
238 nm	10.0 µL	30°C	RP C18	10.00 pts/sec

However, the previously observed MDPHP peak was no longer detected under the modified chromatographic conditions (Figure 13), suggesting instability in the retention behaviour.

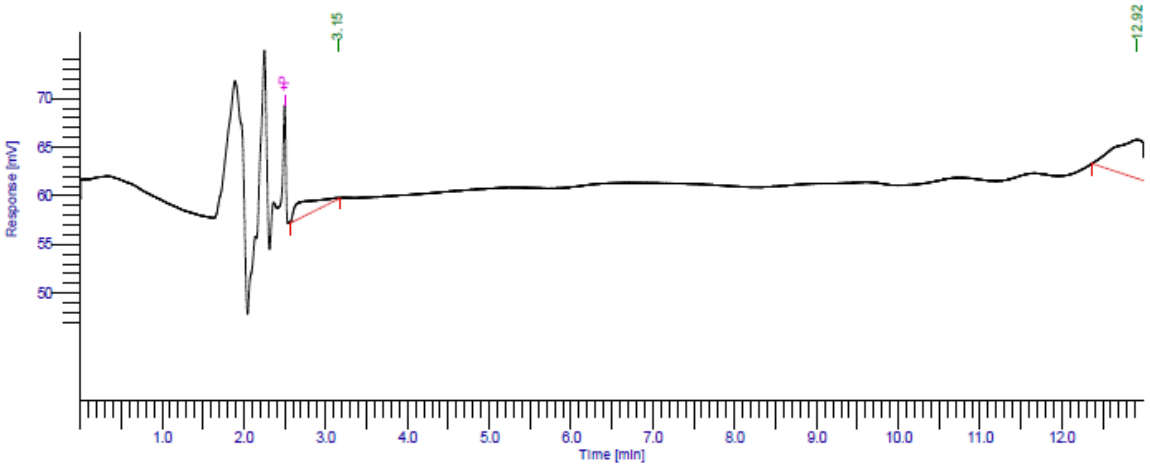

Figure 13: 3 Minutes Hold Optimisation Trial Chromatogram

Consequently, the hold duration was modified again to two minutes, to verify whether a time change in Step 1 could still benefit overall retention. However, the results were consistent with those of the initial hold duration optimisation trial, with no MDPHP being detected (Figure 14). Therefore, the hold duration was reduced to 1.5 minutes once again.

Table 13: Second Hold Duration Optimisation Trial Pump Program

Step	Time (min)	Flow (mL/min)	A(%)	B(%)	Curve
0	2.0	0.60	95	5	0
1	2.0	0.60	95	5	0
2	7.0	0.60	60	40	1.5
3	3.0	0.60	60	40	0

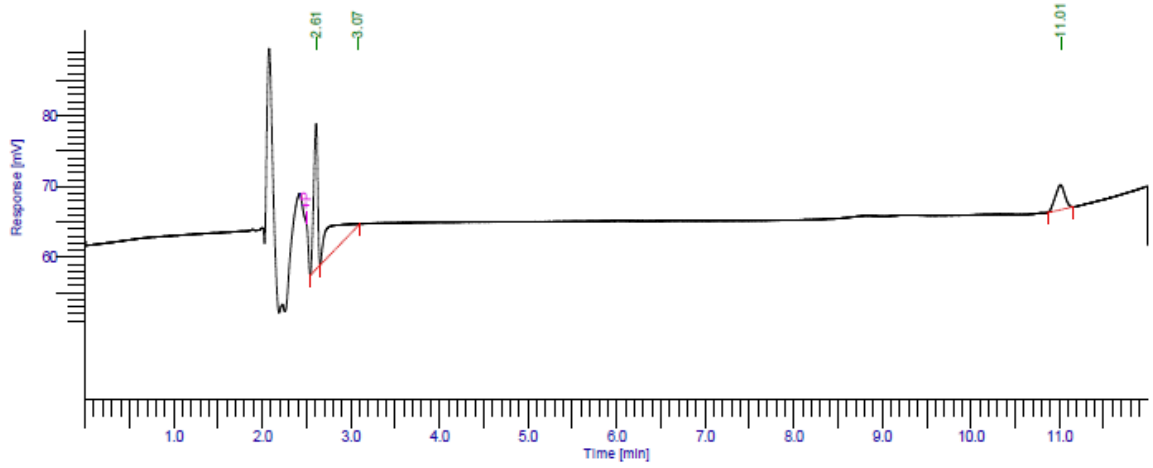

Figure 14: 2 Minutes Hold Optimisation Trial Chromatogram

7.3.2. Validation Parameters

7.3.2.1. LOD/LOQ

A series of serial dilutions (1:10–1:1000) were run using the final method. Their respective chromatograms (see *APPENDIX S*) showed a progressive reduction in peak area and peak height with decreasing concentration (Table 14). The analyte is clearly detectable all throughout the serial dilutions, but its signal approaches the baseline noise at the 1:1000 dilution, indicating that it is close to the Limit of Detection (LOD). The LOD represents 'the lowest concentration that can be measured (detected) with statistical significance by means of a given analytical procedure' (Konieczka, 2012).

Table 14: LOD Serial Dilutions Peak Area and Height

MDPHP in MeOH dilution	Peak Area (UV*sec)	Height (UV)
1:10	3,825,032.82	272,630.79
1:100	449,544.71	38,596.76
1:1000	52,889.26	3,961.53

A signal-to-noise (S/N) ratio of 3:1 was used to determine the method's LOD. The 1:1000 dilution of MDPHP in MeOH had resulted in a signal of 3.0 mAU and a baseline noise level of 0.3 mAU, yielding a S/N ratio of 10:1. Assuming that the detector response within the studied concentration range was linear, the LOD for a S/N ratio of 3:1 was calculated proportionally (ThermoFisher Scientific, 2022). This corresponds to an estimated MDPHP in MeOH dilution of approximately 1:3333 MDPHP in MeOH, or 0.0003 mg/mL.

The Limit of Quantification (LOQ), 'the smallest amount or the lowest concentration of a substance that is possible to be determined by means of a given analytical procedure with the established accuracy, precision, and uncertainty' (Konieczka, 2012), was set using a 10:1 S/N ratio (ThermoFisher Scientific, 2022). Under the chromatographic conditions applied for the method, the 1:1000 dilution (0.001 mg/mL) meets this criterion and, therefore, represents the LOQ.

7.3.2.2. Intra-day Repeatability and Inter-day Reproducibility

Intra-day repeatability was evaluated by assessing method precision through six replicated injections (n=6) of a 1:10 dilution of MDPHP in MeOH under identical analytical conditions on the same day. The peak areas (UV*sec) obtained for each injection are summarised in Table 15 (full chromatograms in *APPENDIX U*).

Inter-day reproducibility was assessed using a new sample of the same concentration, analysed on a separate day under equivalent chromatographic conditions. The corresponding peak areas are also shown in Table 15 (full chromatograms in *APPENDIX V*).

Table 15: Intra-day Repeatability and Inter-day Reproducibility Peak Areas

Run Number	Intra-day Repeatability Peak Area (UV*sec)	Inter-day Reproducibility Peak Area (UV*sec)
R1	3,646,260.01	3,339,100.05
R2	3,560,098.56	3,445,570.82
R3	3,314,334.29	3,505,037.35
R4	3,532,843.39	3,672,168.75
R5	3,699,219.88	3,666,914.79
R6	3,593,441.39	3,589,146.00

The mean peak area (MPA), standard deviation (SD), and relative standard deviation (%RSD) were calculated to evaluate method precision. The SD and %RSD for both intra-day repeatability and inter-day reproducibility were initially calculated using all six injections (R1–R6) to evaluate overall method precision, as shown in Table 16. However, visual inspection of the data showed that the final three injections (R4–R6) demonstrated improved consistency in the peak area response.

To assess whether early injections were influenced by system equilibration effects (e.g., flushing the column for only 15 minutes instead of the recommended 30 minutes), the SD and %RSD for intra-day repeatability and inter-day reproducibility were also calculated separately for injections R4–R6. This allowed to assess the method's accuracy once chromatographic conditions appeared stabilised. The results are registered in Table 16.

Table 16: Intra-day Repeatability and Inter-day Reproducibility MPA, SD, and %RSD

	Intra-day Repeatability			Inter-day Reproducibility		
Injection Range	MPA (UV*sec)	SD (UV*sec)	%RSD	MPA (UV*sec)	SD (UV*sec)	%RSD
R1–R6	3,557,699.59	133,415.65	± 3.75	3,536,322.96	131,461.17	± 3.72
R4–R6	3,608,501.55	84,204.46	± 2.33	3,642,743.18	46,490.80	± 1.28

The intra-day repeatability for injections R1–R6 showed a MPA of 3,557,699.59 UV*sec with a %RSD of ±3.75%, which corresponds to a calculated variability interval of 3,424,285.86–3,691,113.32 UV*sec based on MPA ± (%RSD × MPA). The inter-day reproducibility MPA (R1–R6) was 3,536,322.96 UV*sec, which lies within this interval, indicating comparable analytical performance between days.

For injections R4–R6, the intra-day MPA was 3,608,501.55 UV*sec with a %RSD of ±2.33%, corresponding to a calculated interval of 3,524,422.91–3,692,579.09 UV*sec. The inter-day MPA (R4–R6) was 3,642,743.18 UV*sec, which also falls within this calculated dispersion range, further supporting method consistency once system stability was achieved.

Even though some regulatory pharmaceutical methods aim for %RSD values under ±2%, this method's %RSD values are consistently under ±5%, which is generally considered acceptable for quantitative chromatography coupled with UV detectors (Green, 2011). UV detection is acknowledged to be less sensitive and more frequently subject to baseline noise compared to MS detectors. Thereby, the strict <2% precision threshold is not always applicable for HPLC methods %RSD values, unless analytical conditions are highly optimised (AlRabiah *et al.*, 2020). Additionally, the decrease of the %RSD to ±2.33% and ±1.28% after system stabilisation further indicated that the higher variability observed initially was likely related to equilibration effects rather than the method's imprecision itself.

7.3.2.3. System Equilibration Time

At the beginning of the method development, the initial system equilibration time was set at around 10 minutes, as suggested by existing literature (Seidl *et al*, 2019), where short equilibration times are considered acceptable during early optimisation. However, during the method validation process, the need for a longer initial system equilibration time became evident: the improvement in %RSD values between injections R1–R6 and R4–R6 (Table 16) highlighted the incomplete equilibration in the initial results. Given that the final method employs an ACE Super C18 column, 150 x 4.6 mm i.d., the column volume (CV) was calculated using the following equation:

$$CV = \pi r^2 L \approx 2.5\, mL \tag{12}$$

where r was 0.23 cm, and L was 15 cm. The optimal equilibration volume was calculated to be between 10–20 CV, 25 mL and 50 mL respectively. Based on the final method flow rate of 0.6 mL/min, the equilibration time was estimated between 42 minutes (10 CV) and 83 minutes (20 CV). Experimentally optimal results were achieved after the initial 10-minute

system equilibration time combined with three injections (R1–R3, see 7.3.2.2), corresponding to a total equilibration time of approximately 50 minutes (~12 CV). This was, therefore, considered sufficient to ensure adequate initial column stabilisation.

7.3.2.4. Pure Standards

Four pure MDPHP standards in MeOH at increasing concentrations were used to construct a calibration curve for the developed method. The solutions were prepared from a Chron Chemicals' powder reference standard and had been previously prepared and stored as stock solutions. The concentrations of the standards were 47.7 mg L^{-1}, 95.4 mg L^{-1}, 190.8 mg L^{-1}, and 286.2 mg L^{-1} respectively. Each standard was analysed using the working method, and the resulting peak areas were recorded (Table 17). The 47.7 mg L^{-1} sample did not produce a detectable MDPHP peak. However, since both the LOD and LOQ were estimated to be of lower concentrations compared to 47.7 mg L^{-1} (see 7.3.2.1), the result was attributed either to potential degradation of the sample, as they were made in 2024, or due to a previous error during standard preparation. Each resulting chromatogram can be found in *APPENDIX W*.

Table 17: Pure MDPHP Standards Peak Area (Human Error)

Concentration Standard	Peak Area (UV*sec)
47.7 mg L^{-1}	/
95.4 mg L^{-1}	3,263,484
190.8 mg L^{-1}	11,143,699
286.2 mg L^{-1}	17,064,295

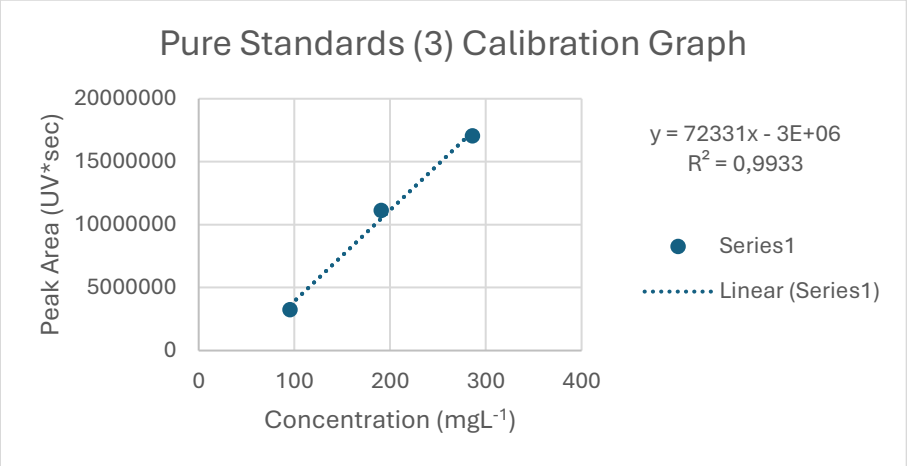

Figure 15: Pure Standards Calibration Graph

The expected peak area for the 47.7 mg L^{-1} MDPHP in MeOH standard was imputed using the trendline equation (y = 72331x – 3E+06) in Figure 15. All expected peak areas are registered in Table 18.

Table 18: Expected Pure MDPHP Standards Peak Areas

Concentration Standard	Peak Area (UV*sec)
47.7 mg L^{-1}	450,189
95.4 mg L^{-1}	3,263,484
190.8 mg L^{-1}	11,143,699
286.2 mg L^{-1}	17,064,295

The accuracy of the regression model (Figure 15) was assessed by back-calculation of the calibration standards (Sonawane *et al*, 2019). Concentrations were calculated from the calibration equation (y = 72331x – 3E+06) and compared to their nominal value (c_{nom}) with the following equation:

$$\%Error = \frac{C_{calc} - C_{nom}}{C_{nom}} \times 100 \quad (13)$$

Table 19: %Error of the Regression Model

Back-calculated Concentration	Nominal Concentration	%Error
≈ 86.6 mg L^{-1}	95.4 mg L^{-1}	≈ –9.2%
≈195.5 mg L^{-1}	190.8 mg L^{-1}	≈ 2.5%
≈ 277.3 mg L^{-1}	286.2 mg L^{-1}	≈ –3.1%

The %Error values (Table 19) remained within acceptable analytical limits (<±15%) (Raposo, 2016; ICH, 2022), which demonstrated that the model provides reliable concentration estimates within the investigated range.

7.3.3. Uncertainty Analysis

Using the repeatability and calibration linearity values as primary contributors, an approximate analysis of measurement uncertainty was conducted. Other potential sources of uncertainty, such as LOD and detector wavelength variation, were considered negligible when compared to these dominant factors and, therefore, were not included in the calculations (Ellison and Williams, 2012). As stated in Section *7.3.2.2*, the responses for injections R1–R3 showed higher

variability compared to the last three repeats, likely due to chromatographic system equilibration. Consequently, the repeatability uncertainty calculations were performed using the stabilised injections (R4–R6), as to better represent the method's precision under steady-state conditions.

The stabilised intra-day repeatability (%RSD = 2.33%) corresponded to a relative standard uncertainty (u_{rep}) of 0.0233. Calibration uncertainty (u_{cal}) was estimated from the deviation from the perfect linearity of the calibration model (R^2 = 0.993) (Figure 15) and resulted in approximately 0.007. The combined uncertainty (u_c) was calculated using the following equation (14):

$$u_c = \sqrt{u_{rep}^2 + u_{cal}^2} \tag{14}$$

with $u_c \approx 0.024$, approximately 2.4%. Using a coverage factor of k = 2, corresponding to approximately 95% confidence level, the expanded uncertainty (U) of the method was estimated to be ± 4.9%, calculated as (15):

$$U = k \times u_c \tag{15}$$

This approach is consistent with the EURACHEM Guide recommendations for quantifying uncertainty in analytical measurements (Ellison and Williams, 2012). This estimated uncertainty was based solely on the dominant sources of variability present in the method, as suggested in the EURACHEM Guide (Ellison and Williams, 2012), which states that "where an effect is detected and is statistically significant, but remains sufficiently small to neglect in practice", it may reasonably be excluded from the uncertainty budget.

The U = ± 4.9% indicates that the measured concentrations of MDPHP samples analysed with this method are expected to fall within ± 4.9% of the reported value with a certainty level of ~95%. The predefined acceptance limit for measurement uncertainty in HPLC-UV/Vis methods generally fall below ± 5%–10% of the true value (El-Ghaly et al, 2025; Al-Farhan and Eldin, 2025); therefore, the ± 4.9% value confirms that this method provides measurements that are sufficiently reliable to be used for forensic screenings and quantitative analysis. MDPHP concentrations (C) will be expected to fall within the following range (16):

$$C = C_{measured} \pm U \tag{16}$$

This uncertainty level is consistent with the calibration accuracy observed in 7.3.2.4, where the higher concentration levels (190.8 mg L^{-1} and 286.2 mg L^{-1}) showed deviations of only 2.5%

and –3.1% respectively . The larger deviation showed by the lower concentration standard, 95.4 mg L^{-1}, is potentially attributable to the non-zero intercept of the regression equation, which has greater influence at lower detector responses. The intercept represents a significant part of the 95.4 mg L^{-1} standard's total peak area, which inevitably leads to higher error margins in back-calculated concentrations.

7.3.4. Statistical Framing

The performance of the method was compared against typical acceptance criteria from validation guidelines, such as ICH Q2(R1) Guideline (2005), in Table 20. The acceptance limit for linearity typically is $R^2 \geq 0.99$–0.995, while for repeatability is %RSD ≤ 5% in UV methods (ResolveMass Laboratories, 2025). LOD and LOQ values of approximately 0.3 µg/mL and 1.0 µg/mL respectively are standardly validated for HPLC-UV methods (ResolveMass Laboratories, 2025). As stated before (see 7.3.3), the method's extended uncertainty lies below the expected parameters for HPLC-UV/Vis methods. Therefore, the verdict for all validation parameters for this HPLC-UV/Vis method was a pass, according to the guidelines.

Table 20: Method Validation Summary

Parameter	Obtained Value	Acceptance limit	Verdict
Linearity (R^2)	0.993	$R^2 \geq 0.99$	Pass
Repeatability (%RSD)	2.33%	%RSD ≤ 5%	Pass
LOD	0.0003 mg/mL	LOD ≤ 0.001 mg/mL	Pass
LOQ	0.001 mg/mL	LOQ ≤ 0.01 mg/mL	Pass
Expanded Uncertainty (U)	± 4.9%	< ± 5–10%	Pass

7.4. Method Limitations

The validation of this method was significantly limited by the availability of only four calibration standards, one of which did not yield a detectable peak for reasons that have already been discussed in Section 7.3.2.4. Consequently, the linear regression (Figure 15) was determined with only three working calibration points. While this was sufficient to establish that the relationship between concentration and detector response was approximately linear, it reduces the statistical confidence in the slope and intercept estimates (Cheng *et al*, 2022).

From a statistical perspective, many statistical indicators used to assess linearity, such as residual plots, ANOVA and lack-of-fit tests, would have become unreliable or impossible to perform meaningfully with only three points (Miller and Miller, 2018). Therefore, linearity was only

approximated using the correlation coefficient, which is usually considered to be a weaker indicator with small data sets (Xinbo *et al*, 2022). Moreover, the non-zero intercept strongly influenced the lowest available standard. Calibration uncertainty was estimated mainly using repeatability data, as a comprehensive regression-based uncertainty model could not be created due to unstable statistical parameters, such as standard error of the slope (Linnet, 1999).

In addition to the weakness in the method validation, several other limitations relating to HPLC-UV/Vis as an analytical technique remain: UV detection generally shows low sensitivity compared to MS detectors (Mokh *et al*, 2022), resulting in high LOD/LOQ; thus, trace levels of impurities or co-eluting compounds may not be detected using UV-based techniques. MS detection provides greater selectivity due to its ability to distinguish compounds based on mass-to-charge ratio (m/z), which makes it less susceptible to interference caused by co-eluting compounds in complex matrices (Tuzimski and Petruczvnik, 2020). A full comparison between the developed HPLC-UV/Vis method and typical LC-MS/MS based ones can be found in Table 21.

Table 21: Developed HPLC-UV/Vis Method Compared with Typical LC-MS/MS Methods

Parameter	Developed HPLC-UV/Vis method	Typical LC-MS/MS Methods
Sensitivity (LOD)	~0.0003 mg/mL	Typically ng/mL range
Selectivity	Moderate	Very high
Run time	Short analytical run	Often short analytical run
Instrument cost	Low; widely available	High purchase and maintenance cost
Operational complexity	Simple operation and method development	Requires specialised training and optimisation
Suitability for routine analysis	High	Often limited to specialised laboratories

However, despite these limitations, HPLC-UV/Vis remains a widely accessible and cost-effective analytical technique and is suitable for laboratories that lack access to advanced MS instrumentation.

7.5. Further Work

Future work should focus on verifying the statistical validity of the developed method by creating a linear regression model using more calibration standards, possibly more than 6, and perform the test discussed in Section 7.4. The method should also be tested on samples that contain additional organic components, such as common adulterants (e.g. caffeine) and/or other synthetic cathinones. This would allow the assessment of the method's selectivity in the presence of potential co-eluting

compounds. Additionally, further evaluation of the method's robustness should be conducted by investigating the effects of variations in chromatographic parameters, including column temperature and mobile phase pH.

8. Conclusion

The aim of this project was to develop and validate an accurate and reproducible HPLC-UV/Vis method for the quantification of seized MDPHP samples. The final method utilises the reversed-phase ACE UltraCore 5 Super C18 column, coupled with a gradient mobile phase comprised of 10 mM AF (pH 3) and ACN, progressing from a 95:5 to a 60:40 ratio (v/v) over an 11.5-minute run. The mobile phase is delivered at 0.6 mL/min, with the column temperature set at 30°C. The UV/Vis detector is set at 238 nm (bandwidth ±10 nm). Based on experimental observations, an initial column equilibration time of a minimum of 50 minutes (~12 CV) is required to achieve consistent chromatographic performance and acceptable repeatability.

Method validation produced positive results: LOD and LOQ resulted in approximately 0.0003 mg/mL and 0.001 mg/mL, while intra-day repeatability and inter-day reproducibility showed %RSD values below ± 5%. Analysis of pure MDPHP standards demonstrated good linearity based peak area (R^2 = 0.993). The regression model's linearity was determined by back-calculation of the standards, and results showed overall %Error lower than ±10%, which is within the acceptable analytical limit of ±15%. The expanded uncertainty was estimated at U = ±4.9%, with a certainty level of approximately 95%. All parameters assessed to determine the method performance (linearity, repeatability, LOD/LOQ, expanded uncertainty) were within the respective acceptance criteria established by forensic validation guidelines, such as the ICH Q2(R1) Guideline. Collectively, the results indicate that a reliable and reproducible HPLC-UV/Vis method for 'monkey dust' quantification was successfully developed using accessible instrumentation.

To the author's knowledge, there are no other published HPLC-UV/Vis quantitative methods specific to MDPHP. This method will be able to respond to forensic laboratories' need for effective and streamlined methods of 'monkey dust' quantification that do not require supplementary analytical cost, such as MS-based techniques. Additionally, the method's relatively short run time allows for efficient analysis of multiple samples, which is advantageous in high-performance forensic laboratory environments. Future research should revolve around the complete validation of method robustness and application to complex samples containing multiple organic

components, such as common adulterants or other synthetic cathinones, prior to potential standardisation across forensic laboratories.

9. References

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10. Appendices

10.1. List of Appendices

University of Staffordshire

APPENDIX A

Probabilistic Risk Assessment (PRA)

Procedure:

- Academics or session lead to complete Risk Assessment for all practical classes/activities, Technical team for all support aspects this is then reviewed as required
- Researchers/Experimenters are to complete a Risk Assessment in consultation with their project advisor and technical staff as appropriate.
- No laboratory work is to commence without a suitable and comprehensive risk assessment being signed off by a competent person detailed in the laboratory handbook.
- Researchers/Experimenters to keep copies of Risk Assessments when working in the laboratories.

Notes:

- The risk assessment must be reviewed when any changes are made to the equipment, materials, procedure, personnel or if there is a near miss or accident
- Any staff member can stop experimental work if no risk assessment is in place, or if, in their opinion, there is a risk to safety. If anybody else has concerns, they must raise it immediately to a member of staff.
- Add rows as necessary
- If substances are used, then you must fill out the COSHH section 3-6. The COSHH regulations link is available here: - Control of substances hazardous to health (COSHH). The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (hse.gov.uk)

Review Date	Reviewed by	Amended – Yes/No	Approval

Risk assessment Reference **(Technical Services Only)**	

Name	Camilla Elettra Pesce	Supervisor name	Jodie Dunnett

Email address	p018804n@student.staffs.ac.uk	Supervisor email	J.C.Dunnett@staffs.ac.uk
level of study	Level 6	Course title	Forensic Science BSc (Hons)
Module number	FORE60369	Module title	Forensic Research Project
Session/project title	Development of an HPLC Method for the Identification & Quantification of MDPHP		
Ethics approved (use BABAO for skeletal remains)	Yes ☒ No☐ Not applicable☐		

Description of experimental procedure/practical session (500 words max)

This project aims at developing an accurate, reproducible and validated HPLC method to quantify 3,4-methylenedioxy-alpha-pyrrolidinohexaphenone (MDPHP, "monkey dust") in seized street samples. Heroin and caffeine might be present in the samples. The project requires constant use of personal protective equipment (PPE), as a preventive measure to minimise the risk of exposure to chemical substances or cathinones' derivatives. The handling of MDPHP samples throughout the entire process, including unpackaging, inspection, weighting and sample preparation, will be performed in a fume hood.

The UNODC HPLC method for quantification of synthetic cathinones will be trialled first. Sample solutions will be prepared by dissolving the powdered MDPHP samples in HPLC mobile phase, 28:72 (v/v) methanol : 10 mM ammonium formate buffer (pH 3.5, adjusted with formic acid), at a concentration of 10 µg/mL. To adjust the buffers pH, 2M NaOH and HCl might be used. Samples will first be screened by GC-MS to identify constituent compounds and impurities, followed by HPLC-UV/Vis analysis. Based on the outcomes of the UNODC HPLC method, alternative published methods may be adapted as necessary.

Risk Assessment

Risk assessment score

		Consequence				
		Negligible (minimal first aid only) 1	Minor (minor injuries) 2	Moderate (major injury) 3	Major (life changing injury) 4	Catastrophic (Danger of death) 5

Likelihood						
	Almost certain 5	5	10	15	20	25
	Likely 4	4	8	12	16	20
	Possible 3	3	6	9	12	15
	Unlikely 2	2	4	6	8	10
	Rare 1	1	2	3	4	5

Hazard list

Hazards inherent in the work, record details and possible injury: (e.g., Equipment, procedures, general chemical hazards, invertebrate work, body fluid sampling etc.)	Risk score	Record precautions which will be taken: (*e.g.,* Include any standard operating procedures, codes of practice, faculty policies you will be following) **Use Hierarchy of Control Measures to reduce risks.**	New risk score
Chemical exposure to toxic and flammable solvent used as mobile phase (e.g. Methanol etc.) and samples.	9	Individual chemicals must be risk assessed in specific PRAs written for the procedure being used. Always correctly wear PPE (safety glasses, gloves, lab coat and mask). Prepare the solvent and samples by carrying out vacuum filtration in the fume hood. Do not breath the fumes of the solvent. Ensure solvent reservoir and waste are gas tight. Ensure correct ventilation.	2
High-pressure flowing liquids in HPLC.	4	Always correctly wear PPE (safety glasses, gloves, lab coat and mask). Leave the purge valve open while turning the HPLC on. Depending on the system, pressure can reach 3000 psi. Ensure the pump pressure is well below maximum limit. Set pump with low flow rate and slowly increase. Keep power and electric connections away from HPLC pump.	2
Use of needles and syringes	4	Always correctly wear PPE (safety glasses, gloves, lab coat and mask). Experienced staff will demonstrate the standard operating procedures for the instrument and equipment. Refer to AN 12 HPLC.	1

Hazards inherent in the work, record details and possible injury: (e.g., Equipment, procedures, general chemical hazards, invertebrate work, body fluid sampling etc.)	Risk score	Record precautions which will be taken: (*e.g.,* Include any standard operating procedures, codes of practice, faculty policies you will be following) **Use Hierarchy of Control Measures to reduce risks.**	New risk score
		Syringes must only be changed by technical staff.	
Solvent and samples spills.	9	Always correctly wear PPE (safety glasses, gloves, lab coat and mask). Use the spill kit to clean the area. Do not wash solvents down the drain. Dispose of all contaminated materials in the designed hazardous waste container.	1
Analysing samples.	2	Always correctly wear PPE (safety glasses, gloves, lab coat and mask). Handle samples carefully and ensure the correct placement in the auto injector. Dispose of sample vials in sharp containers. Use HPLC waste containers for the samples injected with mobile phase. Do not dispose of samples down the drain.	1
Analytical laboratory hazards (e.g. hot instruments, sharps, chemicals). Asphyxiant gases, compressed air. Trip and noise hazards.	8	Restricted areas are marked with "authorised personnel only" signage. Warning labels indicate specific hazards. Instruments have to be switched off where possible to allow cooling after use. Oxygen monitoring system is in place. See Safe Working Procedure R318 for further guidance.	2
Eluents kept on top of HPLC.	4	Individual chemicals must be risk assessed in specific PRAs written for the procedure being used. COSHH information for eluent used is provided below. Eluents may be kept in sealed bottles on top or next to the instrument.	1

Who may be at risk?

Staff – Day shift	Staff – Out of hours	Postgraduate students	Undergraduate students	New or expectant mothers	Contractors	Public	Other, please state below
☒	☐	☐	☒	☐	☐	☐	

What level of risk do you assign to this work?

Low	Medium Low	Medium	High
☒	☐	☐	☐

If the risk assessment is classified as high, then **no work** is to be undertaken. First, follow hierarchy of controls to reduce risks.

If no COSHH assessment is required, then please click here.

Control of Substances Hazardous to Health (COSHH)

COSHH assessment

Control of substances hazardous to health (COSHH). The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (hse.gov.uk)

Links and table below to aid completing the COSHH assessment.

Minimum PPE are lab coat/safety glasses

Addition PPE:		
Fume cupboard (FC)	Laminar flow cabinets (LF)	Microbiological Safety cabinet (Cab)
Nitrile gloves (NG)	Vinyl gloves (VG)	Cryogenic gloves (CG)
Face shield (FS)	Face Mask FFP1	Face Mask FFP2
Face Mask FFP3	Respirator (R)	Other (provide details)
All INFORMATION CAN BE FOUND WITHIN MSDS (MATERIAL SAFETY DATA SHEETS) ON THE INTERNET, SCIENCES CHEMICAL DATABASE ON-LINE OR WITHIN EACH OF THE LABORATORIES		

Use safety data sheets where possible.

Hazard and Precaution statements list, should also be stated on the MSDS - GHS Classification (nih.gov)

Work Exposure Limits (WEL) if not stated on (M)SDS - EH40/2005 Workplace exposure limits (hse.gov.uk)

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
Ammonium Formate	Sigma Aldrich	H, I	NG	S -> L	<5g 1mM	H319 - Causes serious eye irritation.	P264 - Wash skin thoroughly after handling; P280: Wear eye protection/face protection; P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 - If eye irritation persists: Get	n/a

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							medical advice/attention.	
Methanol	Sigma Aldrich	F, T+, M	NG, FC	L	100% 1 L	H225 – Highly flammable liquid and vapour. H301 + H311 + H331 – Toxic if swallowed, in contact with skin or if inhaled. H370 – Causes damage to organs (Eyes, Central nervous system).	P210 – Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 – Keep container tightly closed. P280 – Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P310 – IF SWALLOWED: Immediately call a	STEL 250 ppm 333 mg/m3

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							POISON CENTER/ doctor. P303 + P361 + P353 – IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P311 – IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor.	
Formic Acid	Sigma Aldrich	F, C, T+	FC, NG	L	10 mL	H226 - Flammable	P210 - Keep away from heat, hot	TWA: 5 ppm

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
						liquid and vapour. H302 -Harmful if swallowed. H314 - Causes severe skin burns and eye damage. H331 - Toxic if inhaled.	surfaces, sparks, open flames and other ignition sources. No smoking. P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P312 - IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all	9.6 mg/m^3

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							contaminated clothing. Rinse skin with water. P304 + P340 + P310 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy	

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							to do. Continue rinsing.	
2M Hydrochloric Acid	Fisher Scientific	C	FC, NG, R	L	10 mL	H290 - May be corrosive to metals.	P234 - Keep only in original packaging; P390 - Absorb spillage to prevent material damage.	STEL: 5 ppm 15 min STEL: 8 mg/m^3 15 min
2M Sodium Hydroxide	Fisher Scientific	C	FC, NG	L	10 mL	H290 - May be corrosive to metals; H314 - Causes severe skin burns and eye damage; H318 – Causes serious eye damage.	P280 - Wear protective gloves/protective clothing/eye protection/face protection; P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting;	2 mg/m^3 STEL

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P310 - Immediately call a POISON CENTER or doctor/physician; P234 - Keep only in original packaging.	
Ammonium acetate	Fisher Scientific	n/a	NG	S	<5 g 5 mM	n/a	n/a	n/a

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
3,4-methylenedioxy-alpha-pyrrolidinohexaphenone (MDPHP)	Cayman Chemical	H, I	LF, NG, FC	S	10 mg	H319 - Causes serious eye irritation; H335 + H336 - May cause respiratory irritation, drowsiness or dizziness.	P261 - Avoid breathing dust/fume/gas/mist/vapours/spray; P264 - Wash thoroughly after handling; P271 - Use only outdoors or in a well-ventilated area; P280 - Wear eye protection / face protection; P304 + P340 - If inhaled: Remove person to fresh air and keep comfortable for breathing; P305 + P351 + P338 - If in	STEL: 130 mg/m^3

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P312 - Call a poison centre/doctor if you feel unwell; P337 + P313 - If eye irritation persists: Get medical advice/attention; P403 + P233 - Store in a well-ventilated place. Keep container tightly closed;	

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							P405 - Store locked up; P501 - Dispose of contents/container in accordance with local/regional/national/international regulations.	
Caffeine	Sigma-Aldrich	H, I	PPE	S	<10 mg	H302 – Harmful if swallowed	P264 - Wash skin thoroughly after handling; P270 - Do not eat, drink or smoke when using this product; P301 + P312 - IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell;	n/a

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							P501 - Dispose of contents/ container to an approved waste disposal plant.	
Diacetylmorphine (Heroin)	Cayman Chemical	T+, F, M	LF, G, FC	S	<10 mg	H300 + H310 + H330 - Fatal if swallowed, in contact with skin or if inhaled.	P260 - Do not breathe dust/fume/gas/mist/vapours/spray; P262 - Do not get in eyes, on skin, or on clothing; P264 - Wash thoroughly after handling; P270 - Do not eat, drink or smoke when using this product; P271 - Use only outdoors or in a	n/a

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							well-ventilated area; P280 - Wear protective gloves / protective clothing; P284 - [In case of inadequate ventilation] wear respiratory protection; P301 + P310 - If swallowed: Immediately call a poison center/doctor; P330 - Rinse mouth; P302 + P352 - If on skin: Wash	

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							with plenty of water; P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing; P320 - Specific treatment is urgent (see on this label); P361 + P364 - Take off immediately all contaminated clothing and wash it before reuse; P403 + P233 - Store in a well-ventilated place.	

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							Keep container tightly closed; P405 - Store locked up; P501 - Dispose of contents/container in accordance with local/regional/national/international regulations.	
Industrial Methylated Spirit	Fisher Scientific	F, H, I, M	NG, FC	L	<10 mL	H225 - Highly flammable liquid and vapor; H302 + H332 - Harmful if swallowed or if inhaled; H319 - Causes serious eye irritation;	P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking; P280 - Wear eye protection/ face protection;	250 ppm

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
						H371 - May cause damage to organs.	P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting; P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower; P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing;	

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							P312 - Call a POISON CENTER or doctor if you feel unwell.	
Triethylammonium phosphate	Sigma-Aldrich	H, I, C	NG	L	10 mM	H302 – Harmful if swallowed; H319 – Causes serious eye damage.	P264 – Wash thoroughly after handling; P270 – Do not eat, drink or smoke when using this product; P280 – Wear protective gloves/clothing/eye protection/ face protection; P301 + P312 – IF SWALLOWED: Call a POISON	n/a

Substance involved (e.g., chemicals including reagents, intermediates, products, and by-products):	SDS source (e.g. Fisher Scientific/Sigma Aldrich)	Hazard Class (see below abbreviation letter e.g., C, F):	Handling precautions as above (abbreviation letter e.g., FC):	Physical State (solid (S), liquid (L), gas (G), mist (M), fume (F), dust (D)	Quantity and concentration to be used: ml/g, % solution/M	Hazard statement Code (H code)	Precautionary Statements (P-Code) Prevention	Workplace exposure limit (STEL/WEL)
							CENTRE/doctor if you feel unwell; P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P337 + P313 – If eye irritation persists: Get medical advice/attention.	

Hazard pictograms

Select all hazard pictograms that apply to **all** the work activity								
 Corrosive (C)	 Caution (H, I)	 Flammable (F)	 Longer Term Health Hazards (M)	 Acute Toxicity (T+)	 Oxidising (O)	 Dangerous to the environmen t (W)	 Explosive (E)	 Gases Under Pressure (G)
☒	☒	☒	☒	☒	☐	☐	☐	☐

Special hazards

Are there any special hazards associated with work / procedure?	Yes ☒ No ☐
If yes, then state:	
1. Is the activity/substances risk of thermal runaway or explosion?	Yes ☐ No ☒
2. Does the activity involve handling or storing pyrophoric or unstable substances such as peroxides?	Yes ☐ No ☒
3. Are any substances capable of forming an explosive atmosphere?	Yes ☒ No ☐
If the answer is yes to Q1, a Dangerous substance, and Explosives Atmosphere (DSEAR) assessment must be completed. If it is yes to Q2 and Q3 then a DSEAR procedure should be followed (i.e. check LELs using calculator and have ignition sources been considered along with emergency plans. Has the above statements and procedures been followed? Yes ☒ No ☐	

Biological hazards

In addition to COSHH, Biosafety must also be considered. Please ensure that the following biological hazards are filled out appropriately, refer to Health and Safety Executive England, Advisory Committee on Dangerous Pathogens Approved List of biological agents (hse.gov.uk) for info.

Microbiology

Microorganism	Strain designation number (If known)	Source (supplier, depositor, sample location)	Classification (Hazard group, Classification group)	Growth media	Hazard group definition (Also include any known hazards such as specific infections and/or diseases)	Precautions to be implemented in work to mitigate infection risk (includes additional PPE requirements or method of working, refer to additional PPE in COSHH section above)

Tissue Culture

Cell Line	Source	Classification	Growth media	Hazard	Precaution

Genetic modification (GM)

Are genetic modification procedures required?	Yes ☐ No☒
If yes, then follow GM protocols	
Have you submitted a GM risk assessment?	Yes ☐ No☒
Has the GM risk assessment been approved?	Yes ☐ No☒

Human Tissue Act and other Policies

Are there any specific conditions to be adhered to e.g., Human Tissue Act, body fluid policy? (If yes give details below)	Yes ☐ No☒
Enter details here	
If working with body fluids, have you completed the body fluids declaration?	Yes ☐ No☒

Other biological material

Material (e.g. fluids, bone, meat etc.)	Hazard	Precaution	Quantity to be used

Storage and Disposal

Storage requirements – How are the materials to be stored?	
Keep substances' containers tightly closed in a dry, cool and well-ventilated place. 10mM Ammonium Formate in Methanol + 0.05% Formic Acid - Keep away from open flames, hot surfaces and sources of ignition. Flammables area.	
Disposal information – How will the waste be disposed?	Waste is classified as hazardous. Dispose of in accordance with the European Directives on waste and hazardous waste. Dispose of in accordance with local regulations. Drug waste must be disposed in a labelled beaker and left in fume hood for specialist disposal.
Are there any special disposal requirements?	Yes ☐ No☒

If yes, please state requirements: -	

Emergency Plans

Do procedures require further emergency plans other than stated in codes of practice or standard procedure risk assessments? If yes, then state below	Yes ☐ No☒
Spills	
Fire	
First Aid	
Other	

Approval

Risk assessment completed by	Camilla Elettra Pesce
Date submitted	03/11/2025
Supervisor (or session lead) approval signed	
Date of supervisor approval	
H&S approval signed	
Date of H&S approval	
Review date	
Any other comments	

APPENDIX B

Ethics Form

RESEARCH ETHICS

Disclaimer Form

The following declaration should be made in cases where the researcher and the supervisor (where applicable) conclude that it is not necessary to apply for ethical approval for a specific research project.

PART A: TO BE COMPLETED BY THE RESEARCHER

Name of Researcher:	Camilla Elettra Pesce
School:	School of Health, Education, Policing and Sciences

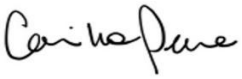
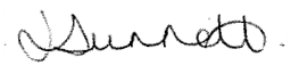
Student/Course Details (If Applicable)		
Student ID Number:		23018804
Name of Supervisor(s)/Module Tutor:		Jodie Dunnett
PhD/MPhil project: ☐		
Taught Postgraduate ☐ Project/Assignment:	Award Title:	
Undergraduate ☒ Project/Assignment	Module Title:	Forensic Research Project

Project Title:	Quantitative analysis of non-judicial drug samples of “monkey dust” for Staffordshire and West Mercia Constabularies		
Project Outline:	This project aims at developing an accurate, reproducible and validated HPLC method to quantify 3,4-methylenedioxy-alpha-pyrrolidinohexaphenone (MDPHP, “monkey dust”) in seized street samples. Samples will be screened by GC-MS to identify constituent compounds and impurities. The UNODC HPLC method for quantification of synthetic cathinones will be trialled first; If unsuitable, alternative published methods will be adapted.		
Give a brief description of research procedure (methods, tests etc.)	The project includes the use of GC-MS for qualitative screening of MDPHP, followed by HPLC analysis according to the UNODC method of quantification of synthetic cathinones. This may vary based on the method’s results, which may lead to a change in methodology.		
Expected Start Date:	06.10.2025	Expected Date:	06.10.2026

Declaration

I/We confirm that the University's Ethical Review Policy has been consulted and that all ethical issues and implications in relation to the above project have been considered. I/We confirm that ethical approval need not be sought. I/We confirm that:

The research does not involve human or animal participants	☒
The research does not present an indirect risk to non-participants (human or animal).	☒
The research does not raise ethical issues due to the potential social or environmental implications of the study	☒
The research does not re-use previously collected personal data which is sensitive in nature, or enables the identification of individuals.	☒
Has a risk assessment been completed for this project?	☒ Yes ☐ N/A

Signature of Researcher:		Date:	21/10/2025
Signature(s) of Project Supervisor(s) (if student) OR Signature of Head of Department/ Senior Researcher (if staff)		Date:	23-10-2025

NB: If the research departs from the protocol which provides the basis for this disclaimer then ethical review may be required and the applicant and supervisor (where applicable) should consider whether or not the disclaimer declaration remains appropriate. If it is no longer appropriate an application for ethical review **MUST** be submitted.

APPENDIX C

Chemical Structures of Synthetic Cathinones

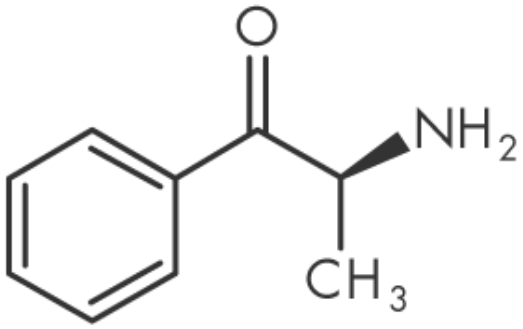


Figure 16: Cathinone Chemical Structure

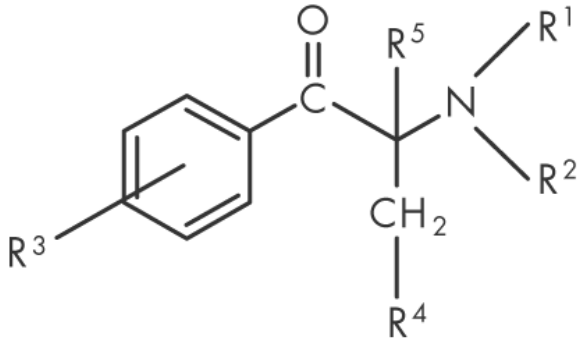


Figure 17: General Structure of a Cathinone Derivative Showing Substitution Patterns

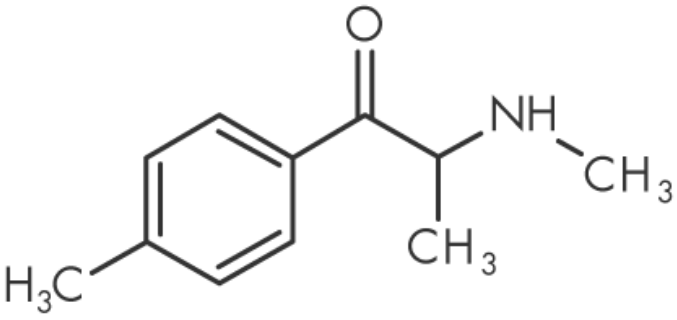


Figure 18: Mephedrone Chemical Structure

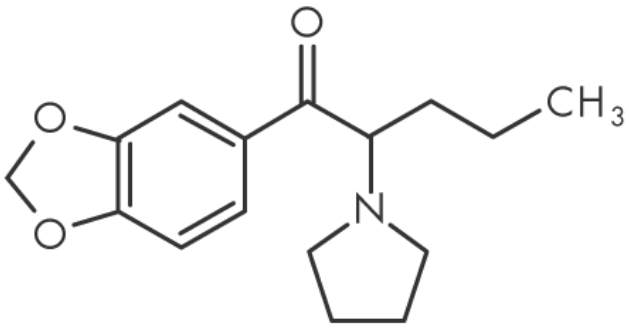


Figure 19: MDPV Chemical Structure

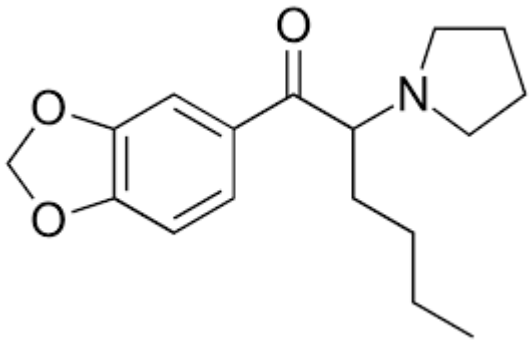


Figure 20: MDPHP Chemical Structure

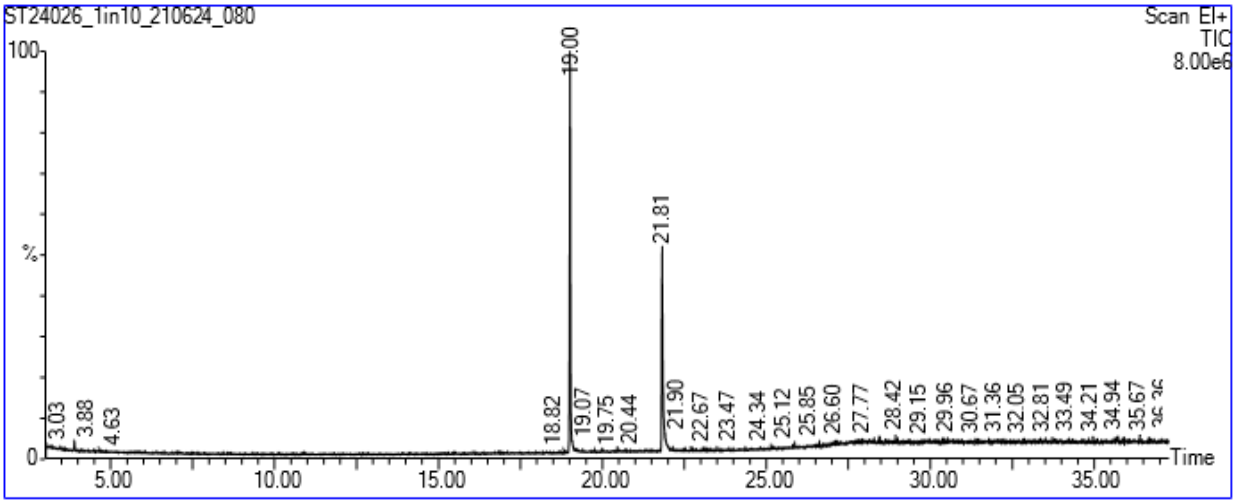
University of Staffordshire

APPENDIX D

ST24026: GC-MS Analysis

Qualitative Report

File:	C:\TURBOMASS\RESEARCH ETC.PRO\Data\ST24026_1in10_210624_080.raw		
Acquired:	22-Jun-24 12:51:07 AM	Printed:	26-Feb-26 11:42 AM
Description:			
GC/MS Method:	GC: Monkey dust method 1 - split 20 (HS on B).mth		MS: MonkePage 1 of 1
Sample ID:	delay.EXP ST24026_1in10_210624_080	Vial Number:	68



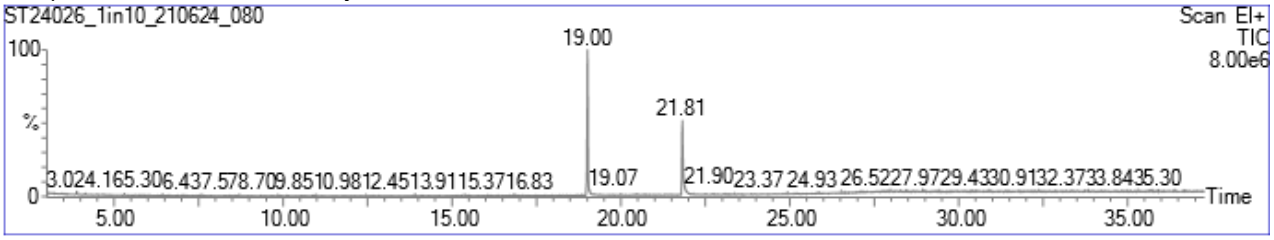
#	RT	Scan	Height	Area	Area %	Norm %
1	18.996	3198	7,818,225	227,702.7	58.563	100.00
2	21.807	3760	3,979,392	161,116.4	41.437	70.76

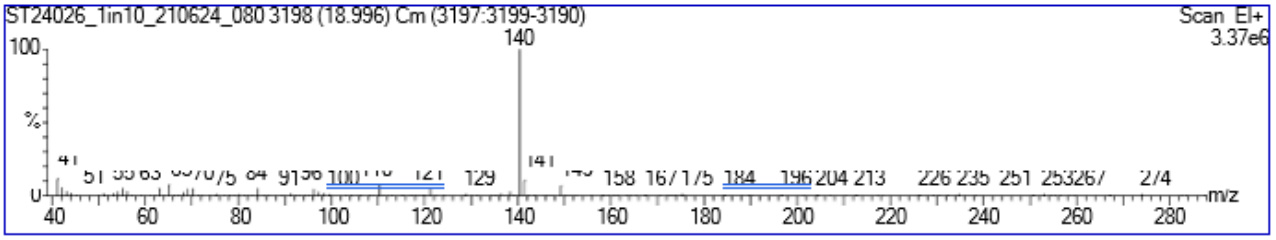
Inst () ACQUISITION PARAMETERS

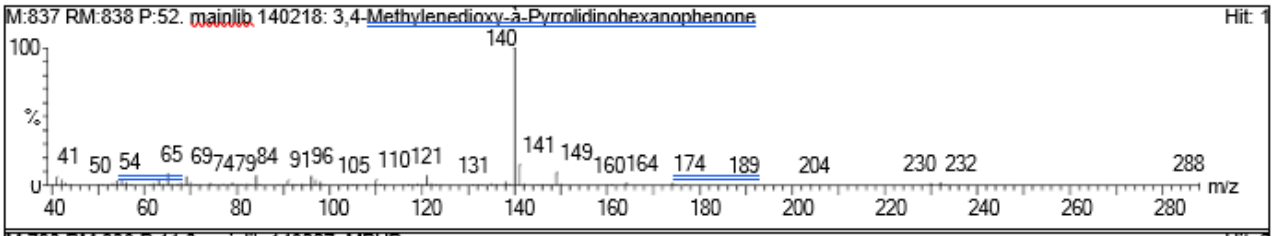
Oven: Initial temp 90°C for 1 min, ramp 8°C/min to 300°C, hold 10 min, InjBauto=1°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=3.00 min, Transfer Temp=200°C, Source Temp=180°C, Scan: 40 to 400Da, Column 30.0m x 250µm

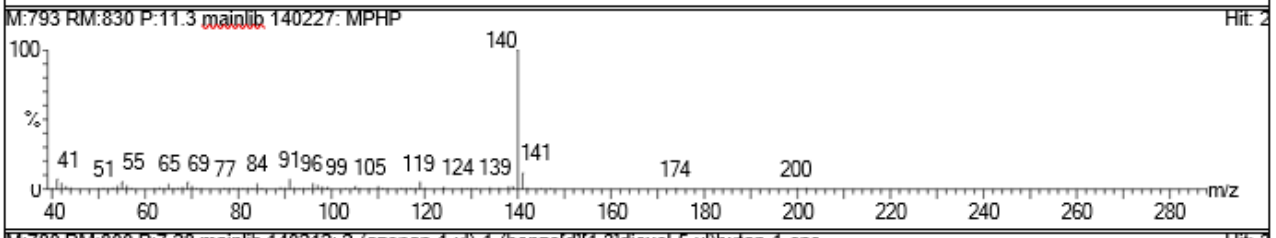
Library Search Chromatogram/Spectrum Peak Report

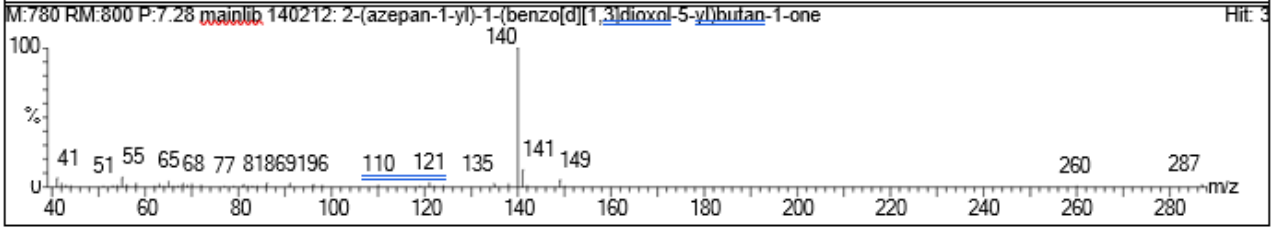
File: C:\TURBOMASS\RESEARCH ETC.PRO\Data\ST24026_1in10_210624_080.raw
Acquired: 22-Jun-24 12:51:07 AM Printed: 26-Feb-26 11:42 AM
Description:
GC/MS Method: GC: Monkey dust method 1 - split 20 (HS on B).mth MS: MonkePage 1 of 2
Sample ID: delay.EXP Vial Number: 68











Lib	Match	R.Match	Name	CAS#
mainlib	837	838	3,4-Methylenedioxy-à-Pyrrolidinohexanophenone	24622-61-5
mainlib	793	830	MPHP	
mainlib	780	800	2-(azepan-1-yl)-1-(benzo[d][1,3]dioxol-5-yl)butan-1-one	
mainlib	779	801	1-(benzo[d][1,3]dioxol-5-yl)-2-(piperidin-1-yl)pentan-1-one	
mainlib	774	792	1-(benzo[d][1,3]dioxol-5-yl)-2-(4-methylpiperidin-1-yl)butan-1-one	

Inst () ACQUISITION PARAMETERS

Oven: Initial temp 90°C for 1 min, ramp 8°C/min to 300°C, hold 10 min, InjBauto=1°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=3.00 min, Transfer Temp=200°C, Source Temp=180°C, Scan: 40 to 400Da, Column 30.0m x 250µm

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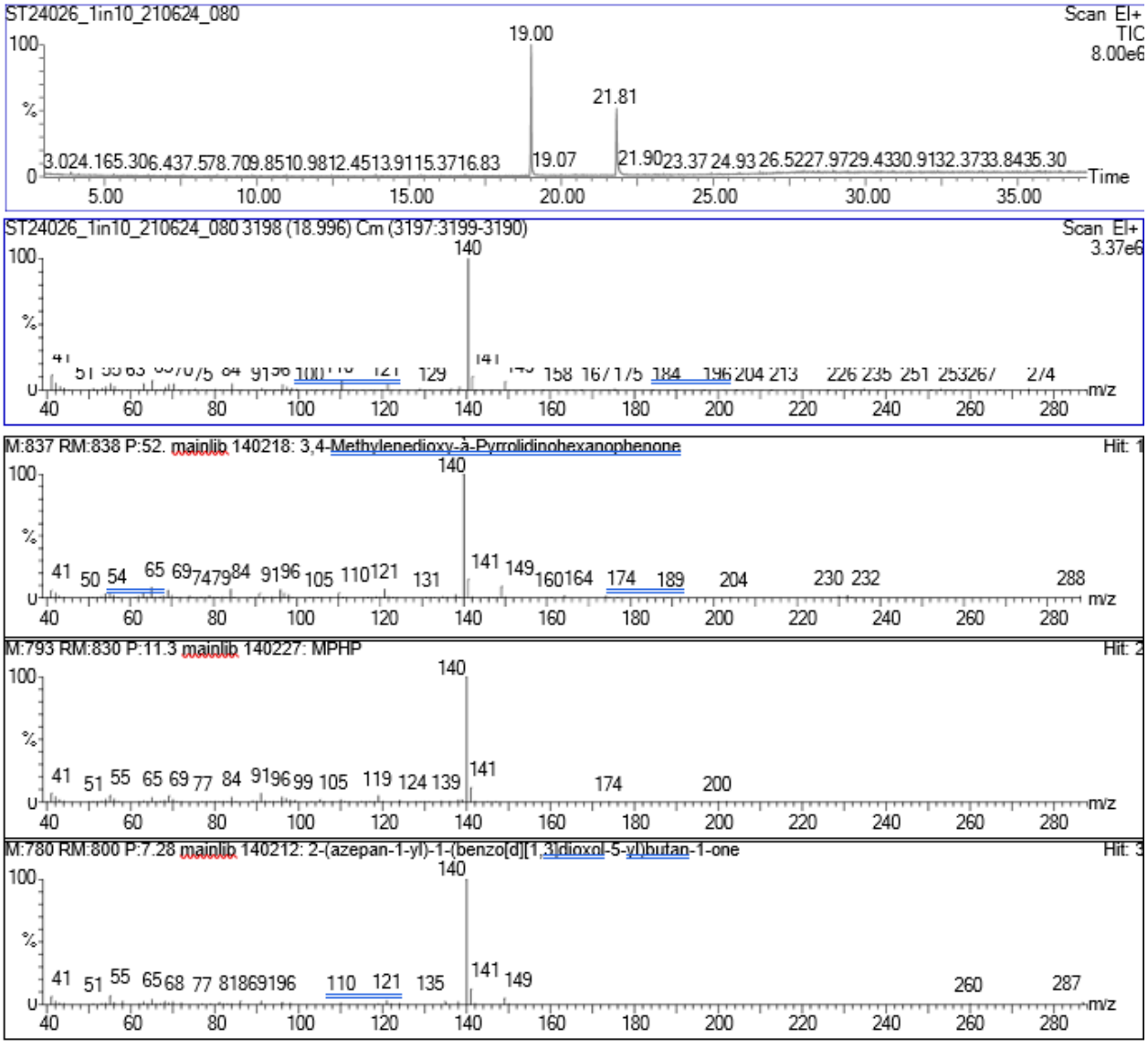
Library Search Chromatogram/Spectrum Peak Report

File: C:\TURBOMASS\RESEARCH ETC.PRO\Data\ST24026_1in10_210624_080.raw
Acquired: 22-Jun-24 12:51:07 AM Printed: 26-Feb-26 11:42 AM
Description:
GC/MS Method: GC: Monkey dust method 1 - split 20 (HS on B).mth MS: MonkePage 2 of 2
Sample ID: delay.EXP Vial Number: 68



Lib	Match	R.Match	Name	CAS#
mainlib	837	838	3,4-Methylenedioxy-à-Pyrrolidinohexanophenone	24622-61-5
mainlib	793	830	MPHP	
mainlib	780	800	2-(azepan-1-yl)-1-(benzo[d][1,3]dioxol-5-yl)butan-1-one	
mainlib	779	801	1-(benzo[d][1,3]dioxol-5-yl)-2-(piperidin-1-yl)pentan-1-one	
mainlib	774	792	1-(benzo[d][1,3]dioxol-5-yl)-2-(4-methylpiperidin-1-yl)butan-1-one	

Inst () ACQUISITION PARAMETERS

Oven: Initial temp 90°C for 1 min, ramp 8°C/min to 300°C, hold 10 min, InjBauto=1°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=3.00 min, Transfer Temp=200°C, Source Temp=180°C, Scan: 40 to 400Da, Column 30.0m x 250µm

APPENDIX E

ST24026: UV-Vis Analysis

	Sample ID	User Name	Date and Time
	1:10 MDPHP Methanol Sample	lab	02/12/2025 12:48:57

Peaks:

nm	Abs
227.182	4.977
310.933	4.945
305.930	4.880
309.190	4.525
314.015	3.880

	Sample ID	User Name	Date and Time
	1:100 MDPHP Methanol Sample2	lab	02/12/2025 12:55:55

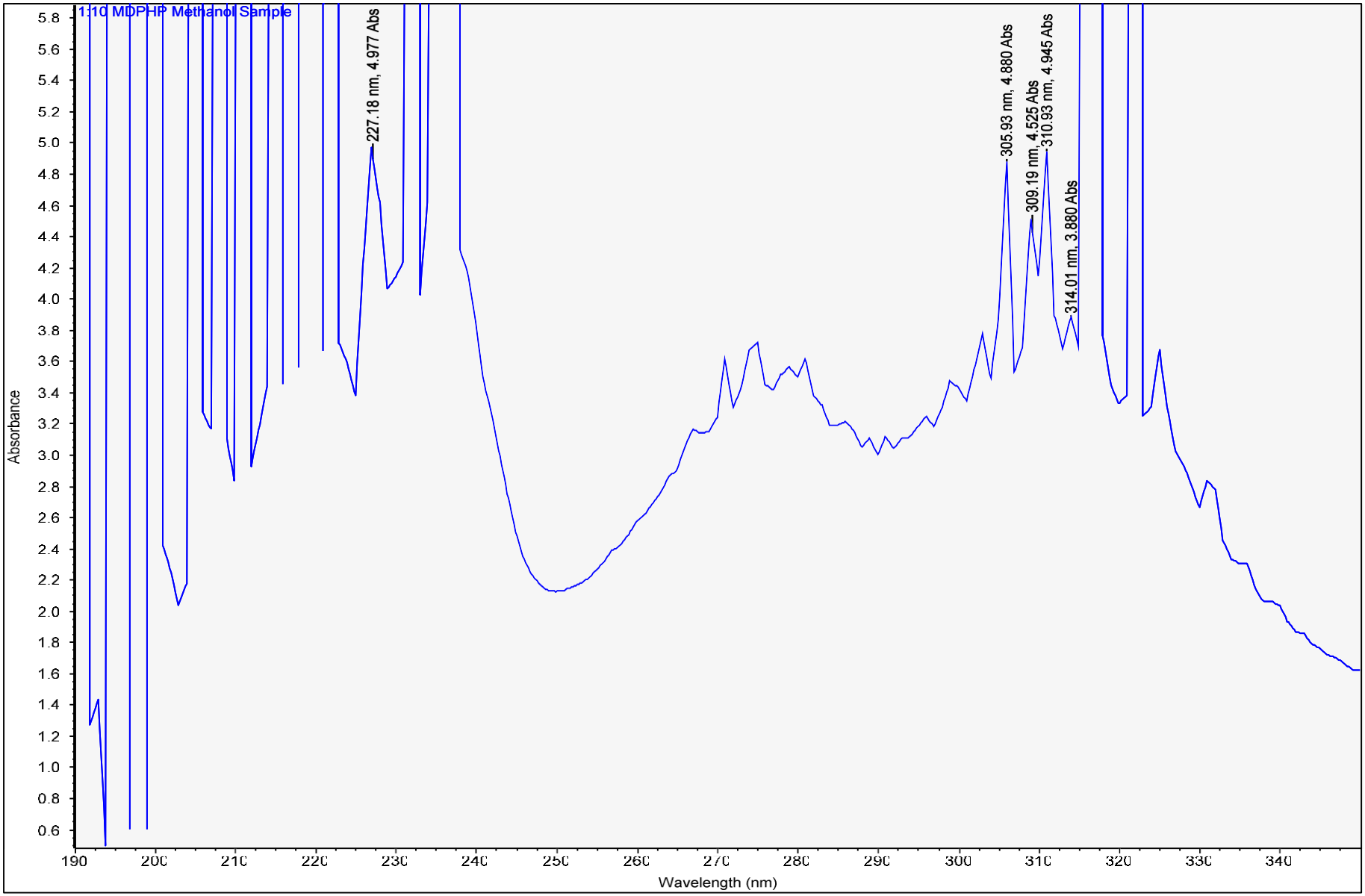
Peaks:

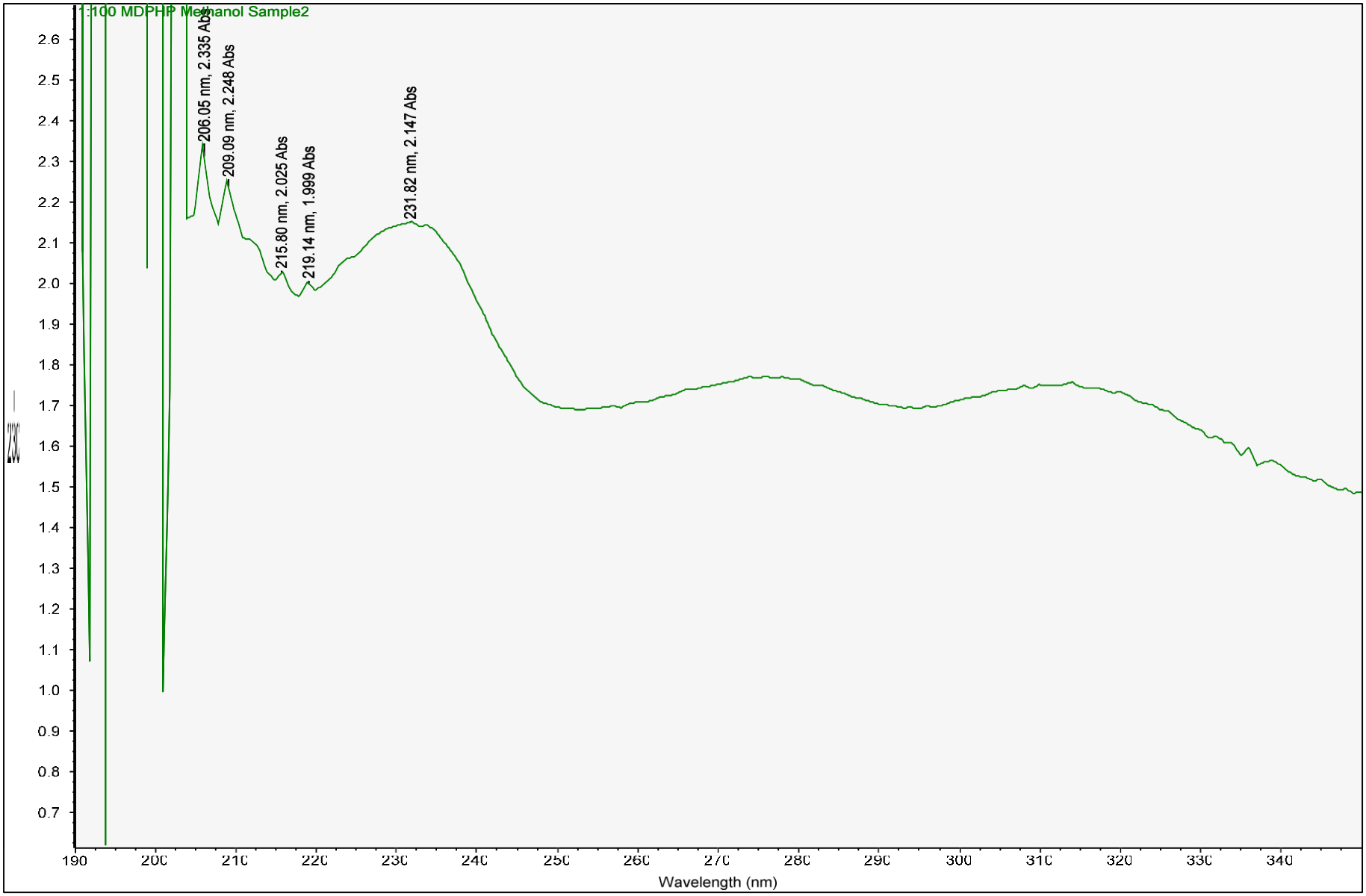
nm	Abs
206.050	2.335
209.094	2.248
231.818	2.147
215.801	2.025
219.139	1.999

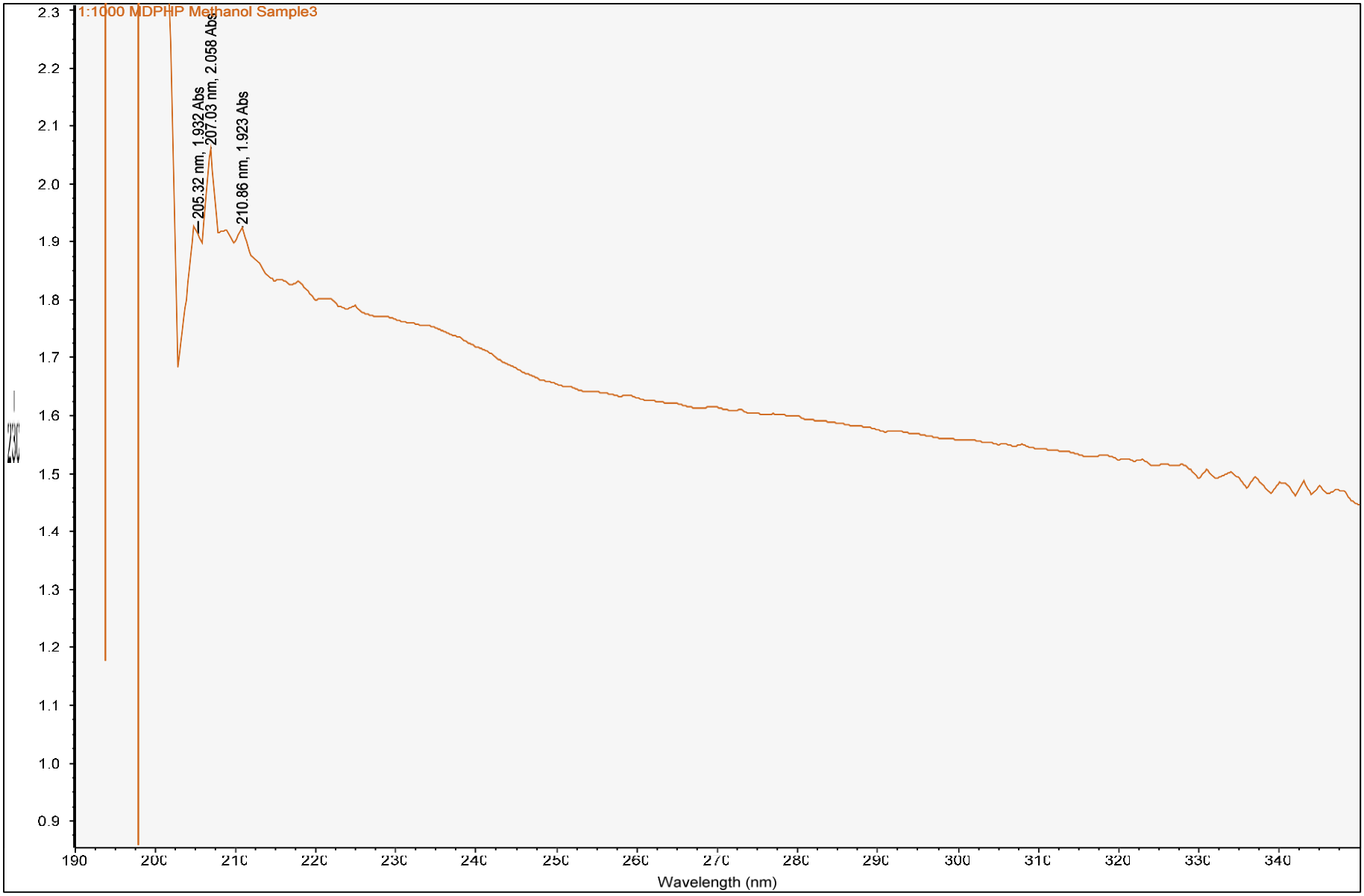
	Sample ID	User Name	Date and Time
	1:1000 MDPHP Methanol Sample3	lab	02/12/2025 13:02:50

Peaks:

nm	Abs
207.028	2.058
205.319	1.932
210.865	1.923


1:10 MDPHP Methanol Sample
227.18 nm, 4.977 Abs
305.93 nm, 4.880 Abs
309.19 nm, 4.525 Abs
310.93 nm, 4.945 Abs
314.01 nm, 3.880 Abs
Absorbance
Wavelength (nm)


1:100 MDPHP Methanol Sample2
206.05 nm, 2.335 Abs
209.09 nm, 2.248 Abs
215.80 nm, 2.025 Abs
219.14 nm, 1.999 Abs
231.82 nm, 2.147 Abs
Wavelength (nm)


1:1000 MDPHP Methanol Sample3
205.32 nm, 1.932 Abs
207.03 nm, 2.058 Abs
210.86 nm, 1.923 Abs
Wavelength (nm)

APPENDIX F

ACE Super C18, 5 mM Ammonium Formate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:01
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	02/12/2025 13:00:26
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	4		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

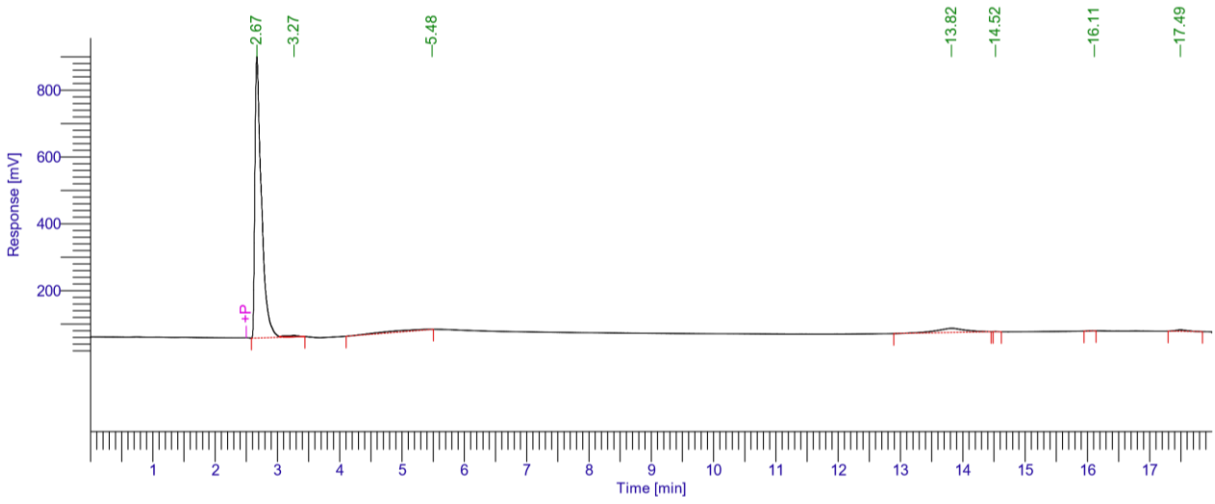


Figure 21: 1:10 MDPHP in MeOH 5 mM AF pH 3 – 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.672	6174535.47	842150.57	89.71	89.71	BE

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:11
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	02/12/2025 14:17:13
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	6		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

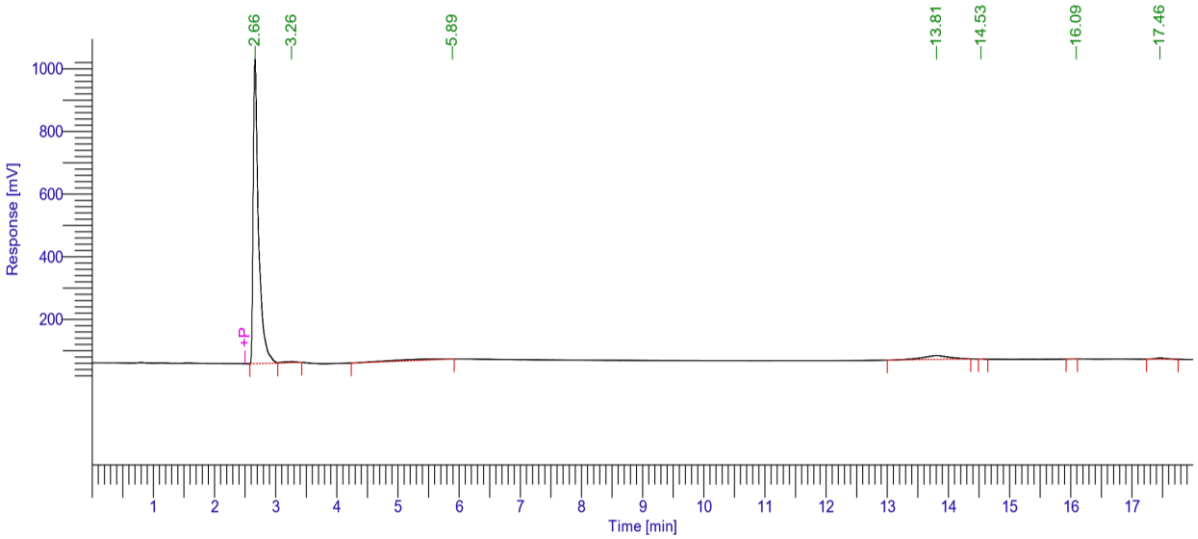


Figure 22: 1:10 MDPHP in MeOH 5 mM AF pH 3 - 2

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.664	5975466.76	972775.17	89.89	89.89	BV

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:22
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	02/12/2025 15:51:00
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	8		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

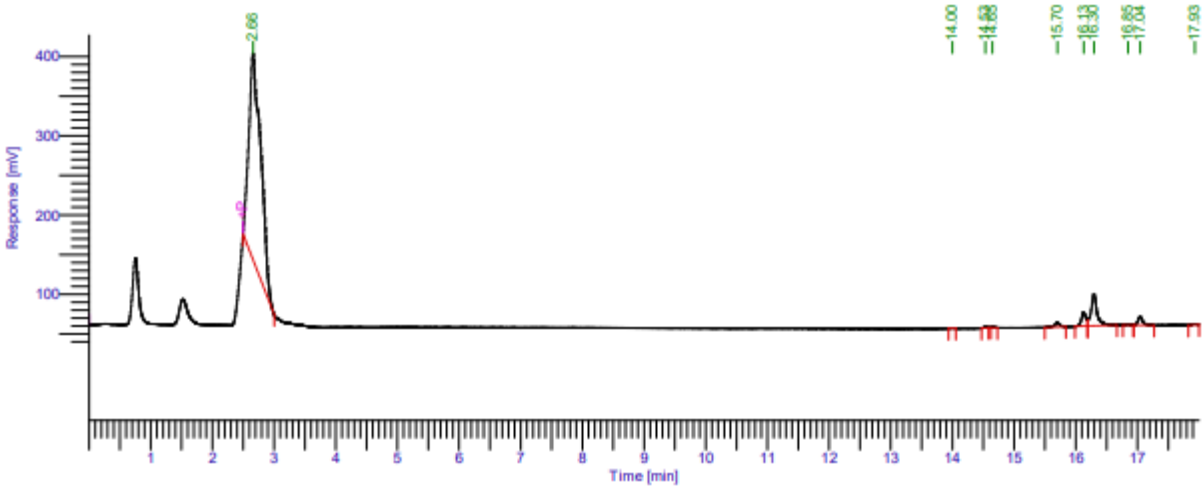


Figure 23: 1:10 MDPHP in MeOH 5 mM AF pH 3 - 3

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.660	3563083.56	261073.14	88.46	88.46	*BB

APPENDIX G

ACE Super C18, 5 mM Ammonium Formate, pH 7 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:32
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	15/12/2025 13:26:56
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	11		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5mM Ammonium Formate pH 6.96 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

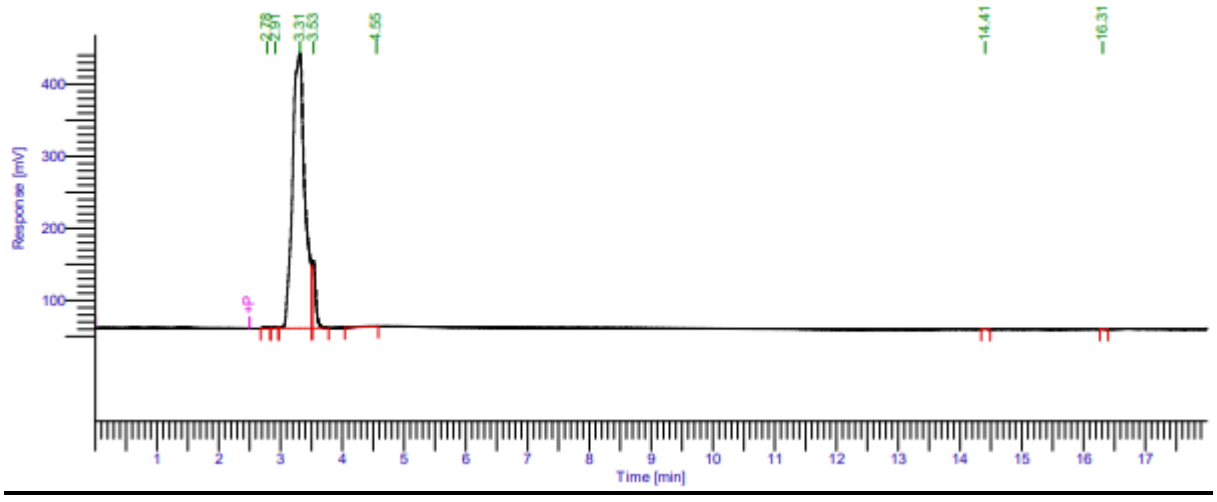


Figure 24: 1:10 MDPHP in MeOH 5 mM AF pH 7 - 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
3	3.314	5265149.80	381579.21	92.45	92.45	VV

APPENDIX H

ACE Super C18, 5 mM Ammonium Formate, pH 9 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:41
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	15/12/2025 15:51:04
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	14		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5mM Ammonium Formate pH 9.03 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

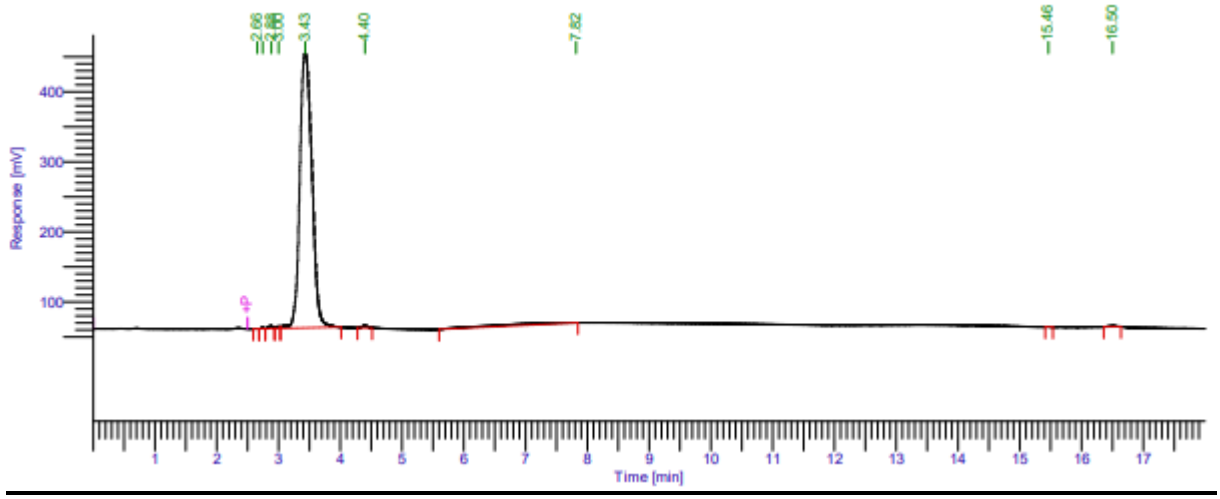


Figure 25: 1:10 MDPHP in MeOH 5 mM AF pH 9 - 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
5	3.432	5353429.50	391990.61	95.69	95.69	VB

APPENDIX I

ACE Super C18, 1 mM Ammonium Formate, pH 9 Chromatograms

Software Version:	6.3.1.0504	Date:	6/02/2026 15:17:51
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	20/01/2026 13:14:59
Sample Name:	1:100 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	17		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
1mM Ammonium Formate pH 9.14 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

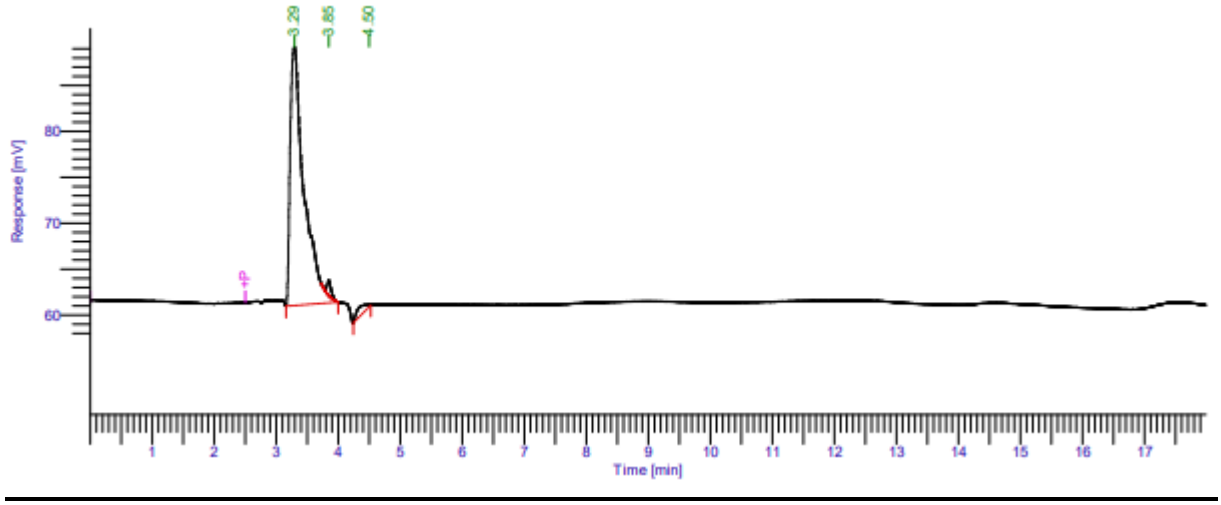


Figure 26: 1:10 MDPHP in MeOH 1 mM AF pH 9 - 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	3.295	434389.85	28176.94	95.93	95.93	BE

Software Version:	6.3.1.0504	Date:	16/02/2026 15:17:55
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	20/01/2026 13:36:39
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	18		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
1mM Ammonium Formate pH 9.14 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

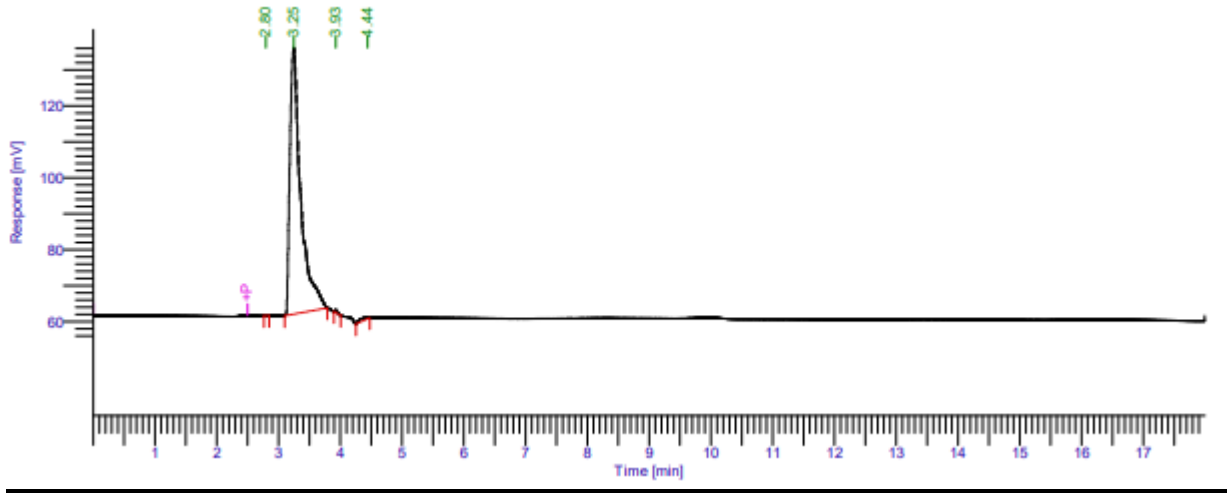


Figure 27:1:100 MDPHP in MeOH 1 mM AF pH 9 – 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.248	897220.36	74086.15	98.98	98.98	BB

APPENDIX J

ACE Super C18, 20 mM Ammonium Formate, pH 9 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:18:37
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	23/01/2026 13:05:43
Sample Name:	1:100 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	30		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
20 mM Ammonium Formate pH 9.01 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

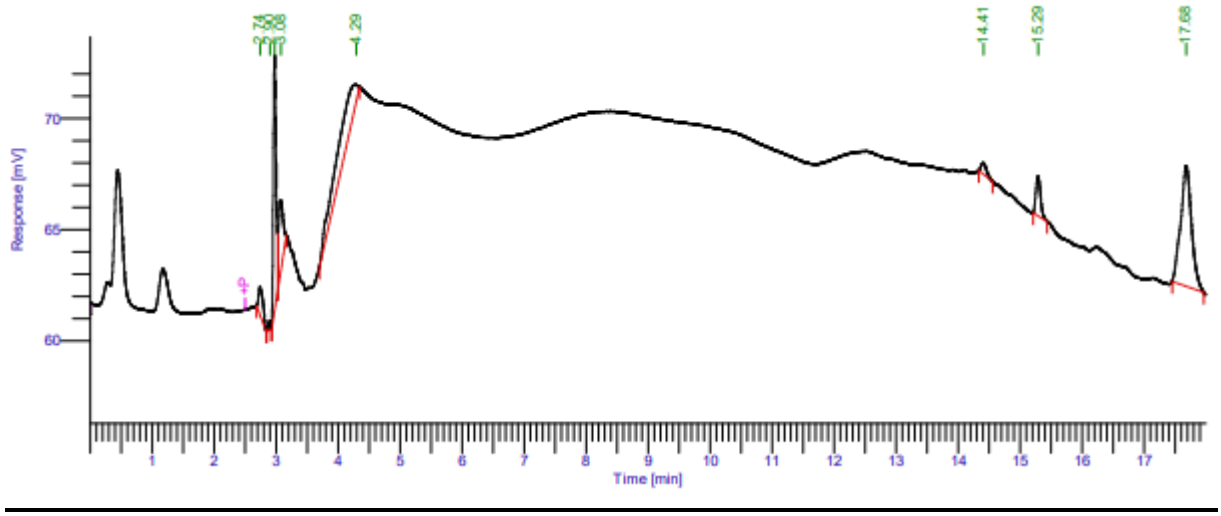


Figure 28:1:100 MDPHP in MeOH 20 mM AF pH 9 – 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
3	2.976	35940.18	11442.86	19.83	19.83	BV
4	3.078	15789.20	3102.29	8.71	8.71	VB

APPENDIX K

ACE Super C18, 50 mM Sodium Phosphate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:18:07
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	20/01/2026 16:06:38
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	22		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
50 mM Sodium Phosphate pH 3.10 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

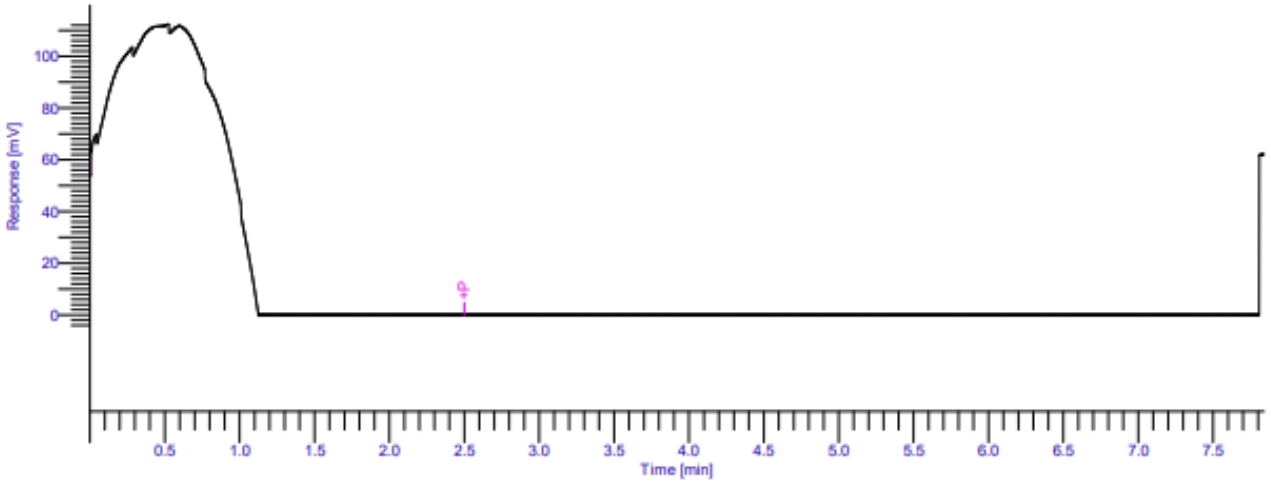


Figure 29: 1:100 MDPHP in MeOH 50 mM Sodium Phosphate pH 3 – 1

APPENDIX L

ACE Super C18, 5 mM Sodium Phosphate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:18:26
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	21/01/2026 09:25:57
Sample Name:	1:100 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	26		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5 mM Sodium Phosphate pH 3.10 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

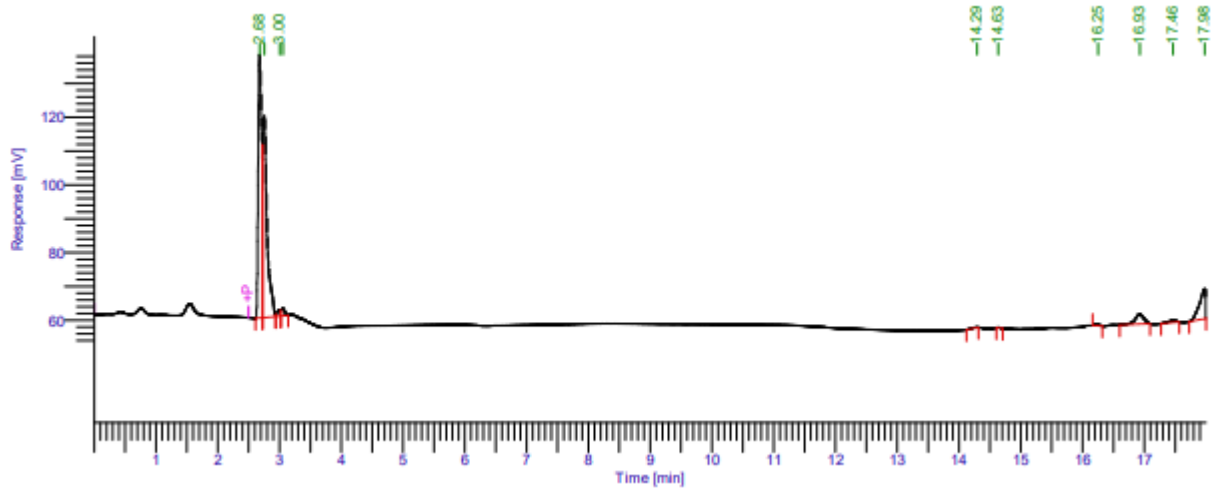


Figure 30: 1:100 MDPHP in MeOH 5 mM Sodium Phosphate pH 3 – 1

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.679	302045.79	77853.09	42.78	42.78	BV
2	2.756	288589.86	59535.89	40.88	40.88	VV

Software Version:	6.3.1.0504	Date:	16/02/2026 15:18:29
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	21/01/2026 09:47:37
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	27		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150x4.6
5 mM Sodium Phosphate pH 3.10 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

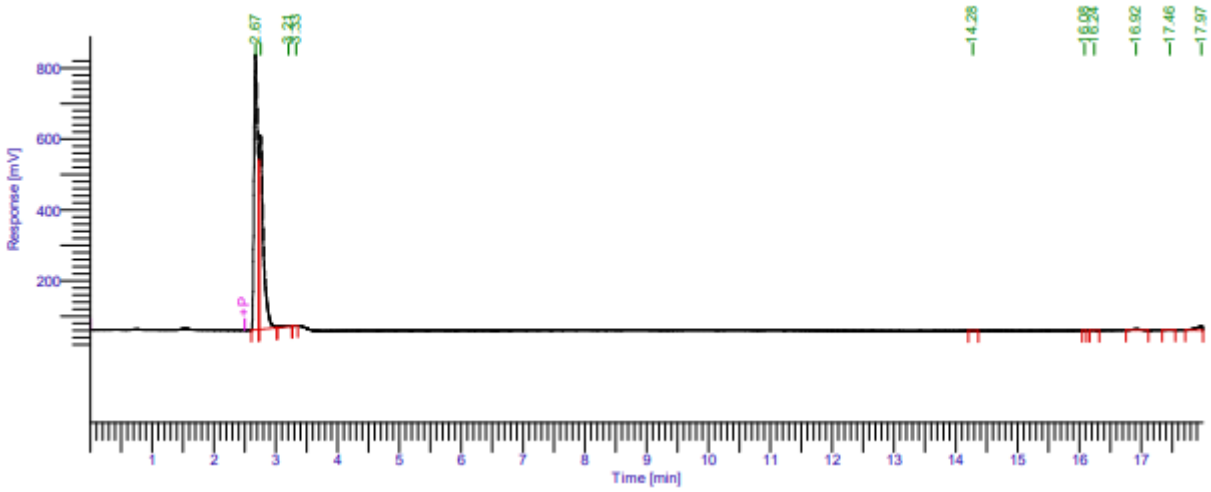


Figure 31: 1:10 MDPHP in MeOH 5 mM Sodium Phosphate pH 3 – 2

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.675	3278238.25	776160.88	53.90	53.90	BV
2	2.757	2646396.46	547878.62	43.51	43.51	VV

APPENDIX M

ACE© HILIC-B, 20 mM Ammonium Formate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:19:07
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	28/01/2026 11:12:33
Sample Name:	MDPHP 90:10 AF:ACN	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	41		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE HILIC-B column. 150 x 4.6
20 mM Ammonium Formate pH 3.48 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

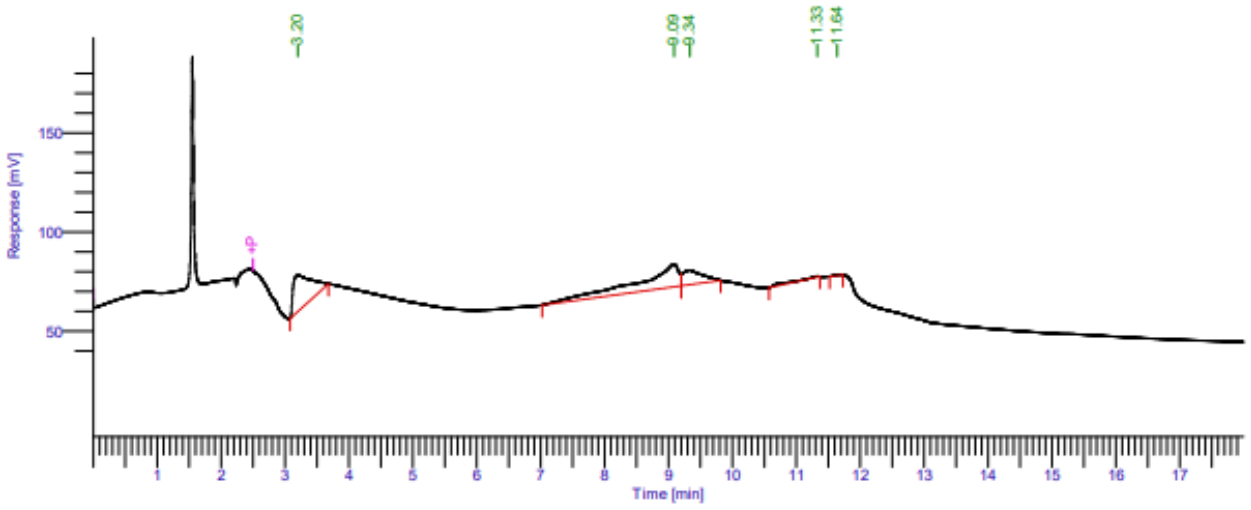


Figure 32: MDPHP in 90:10 AF:ACN, 20 mM Ammonium Formate, pH 9 - HILIC B

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	7.484	595645.71	16531.49	70.66	70.66	BV

Software Version:	6.3.1.0504	Date:	16/02/2026 15:19:16
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	28/01/2026 12:17:40
Sample Name:	MDPHP in MeOH	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/4	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	44		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE HILIC-B column. 150 x 4.6
20 mM Ammonium Formate pH 3.48 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

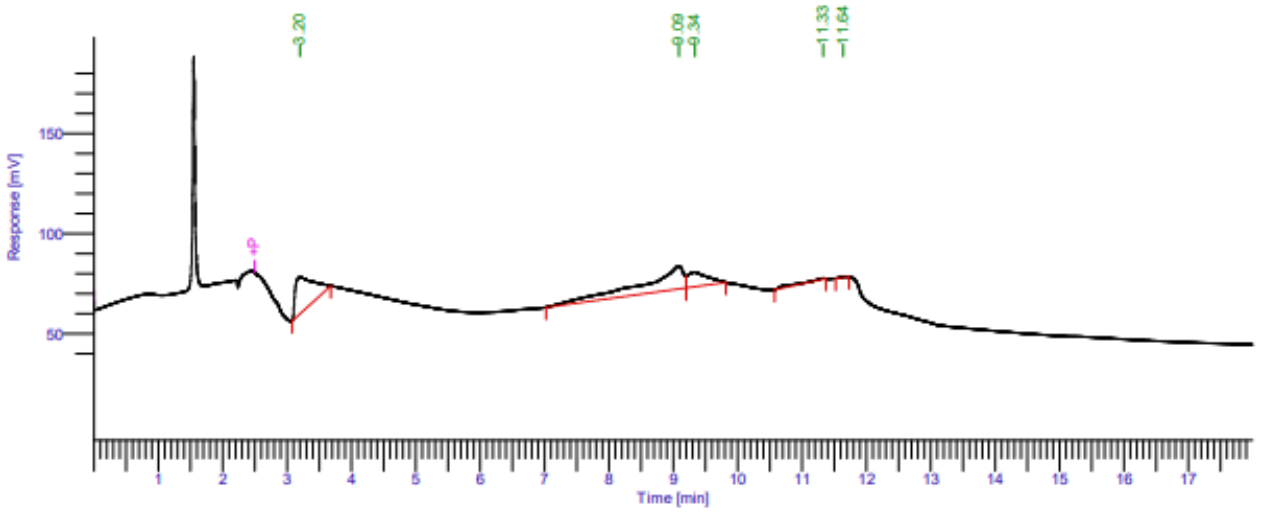


Figure 33: MDPHP in MeOH, 20 mM Ammonium Formate, pH 9 - HILIC B

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	3.200	346776.13	18238.23	33.46	33.46	BB

APPENDIX N

ACE© HILIC-A, 20 mM Ammonium Formate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:19:28
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	28/01/2026 15:20:12
Sample Name:	MDPHP in 90:10 AF:ACN	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	47		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE HILIC-A column. 150 x 4.6
20 mM Ammonium Formate pH 3.48 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

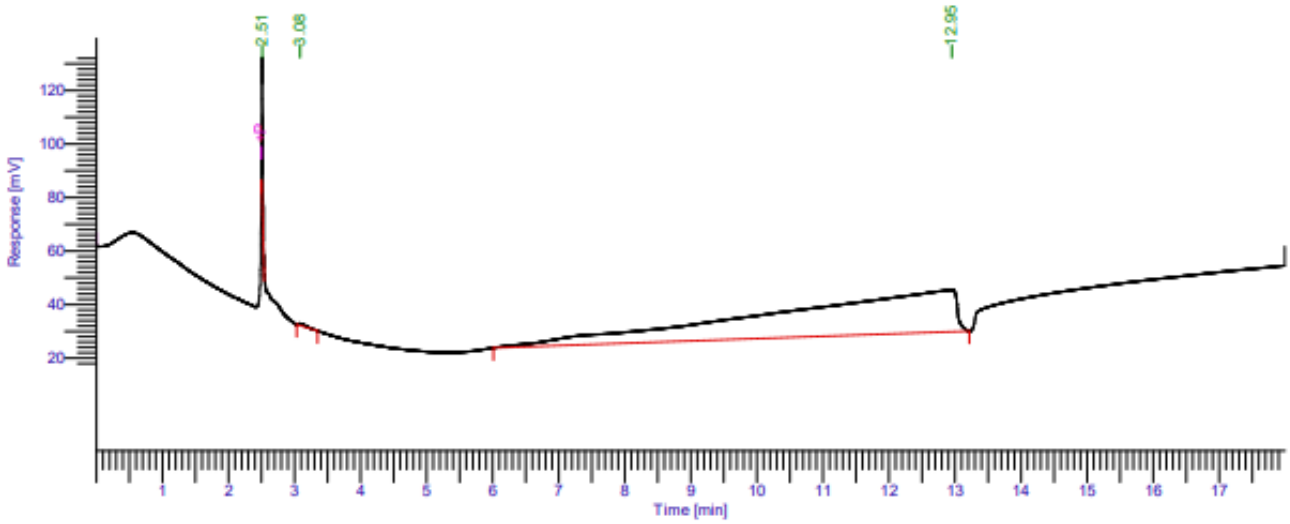


Figure 34: MDPHP in 90:10 AF:ACN, 20 mM Ammonium Formate, pH 9 - HILIC A

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.511	68313.47	55827.08	2.06	2.06	*BB

APPENDIX O

ACE© HILIC-N, 20 mM Ammonium Formate, pH 3 Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:19:33
Reprocess Number:	sci-42d4ee68db4: 4844	Data Acquisition Time:	28/01/2026 16:22:04
Sample Name:	MDPHP in 90:10 AF:ACN	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	49		

Result File: c:\projects\camilla pesce 2025\results\MDPHP_021225_002-20260216-151655.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE HILIC-A column. 150 x 4.6
20 mM Ammonium Formate pH 3.48 on A
Acetonitrile on B
Flow 0.4
25 degrees Celsius
UV 238 nm

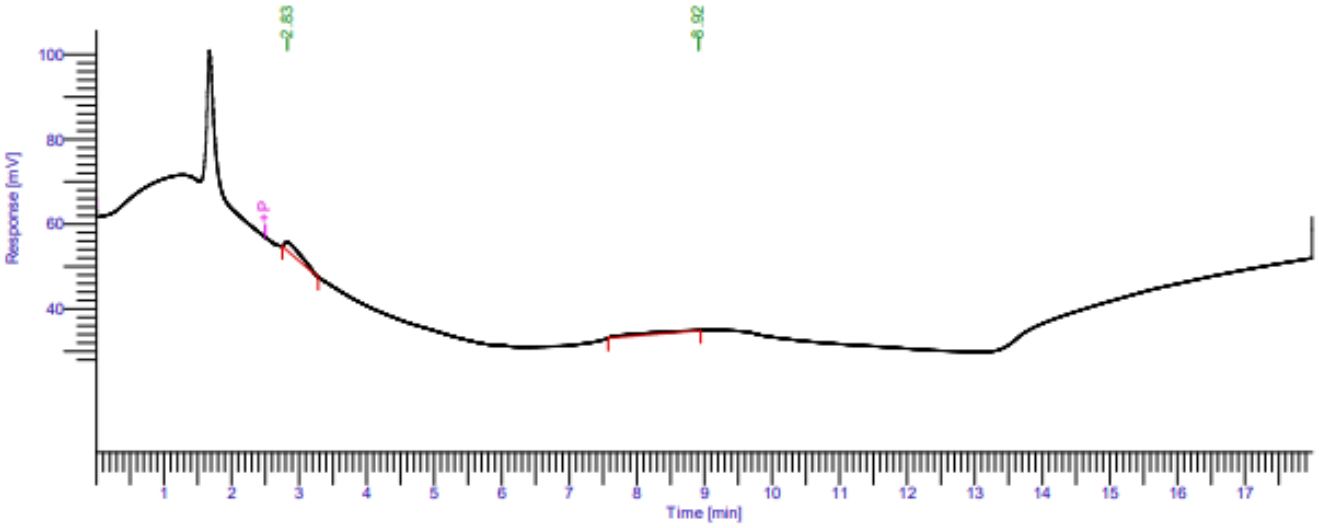


Figure 35: MDPHP in 90:10 AF:ACN, 20 mM Ammonium Formate, pH 9 - HILIC N

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.827	41228.66	2069.28	61.53	61.53	BB

APPENDIX P

ACE Super C18, 90:10 10 mM AF (pH 3):ACN Chromatograms

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:29
Reprocess Number:	sci-42d4ee68db4: 5014	Data Acquisition Time:	03/02/2026 12:49:20
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	28		

Result File C:\Projects\Camilla Pesce 2025\Results\10mMAmF-ACN_Jan1_10MDPHP_03022026_003-20260226-114227.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.4
30 degrees Celsius
UV 238 nm

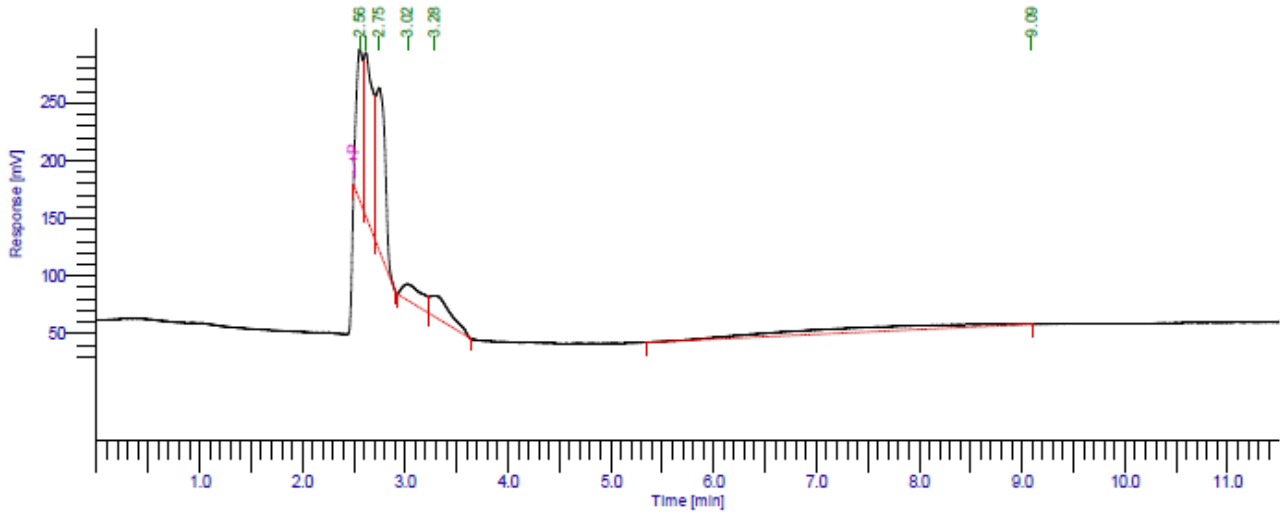


Figure 36: January MDPHP 90:10 10 mM AF (pH3):ACN - ACE Super C18

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.558	539863.79	131682.81	15.95	15.95	*BV
2	2.617	950669.66	141967.06	28.08	28.08	VV
3	2.747	866453.29	140930.88	25.60	25.60	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:32
Reprocess Number:	sci-42d4ee68db4: 5014	Data Acquisition Time:	03/02/2026 12:35:35
Sample Name:	1:10 MDPHP (28.11.25)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	29		

Result File C:\Projects\Camilla Pesce 2025\Results\10mMAmF-ACN_Nov1_10MDPHP_03022026_002-20260226-114230.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.4
30 degrees Celsius
UV 238 nm

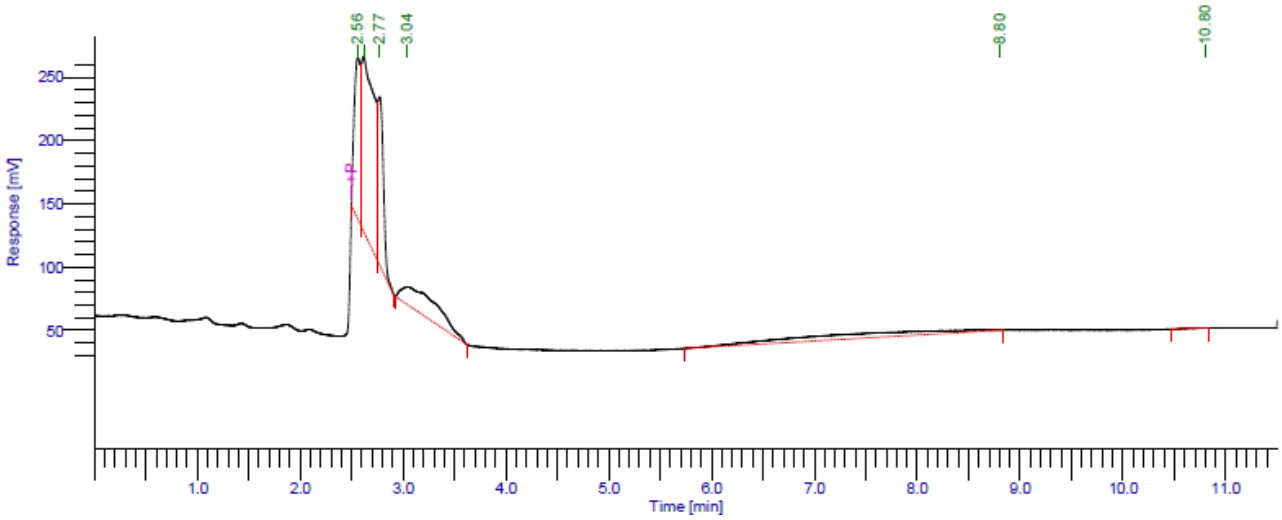


Figure 37: November MDPHP 90:10 10 mM AF (pH3):ACN - ACE Super C18

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.560	509169.24	127647.89	16.11	16.11	*BV
2	2.617	1244096.05	138054.06	39.36	39.36	VV
3	2.773	527807.23	132989.70	16.70	16.70	VB

APPENDIX Q

ACE Super C18, 95:5 10 mM AF (pH 3):ACN Chromatograms

Software Version:	6.3.1.0504	Date:	16/02/2026 15:20:10
Reprocess Number:	sci-42d4ee68db4: 5014	Data Acquisition Time:	03/02/2026 14:21:30
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	60		

Result File c:\projects\camilla pesce 2025\results\95%10mMAmF-ACN_JanMDPHP_03022026_002-20260216-152009.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

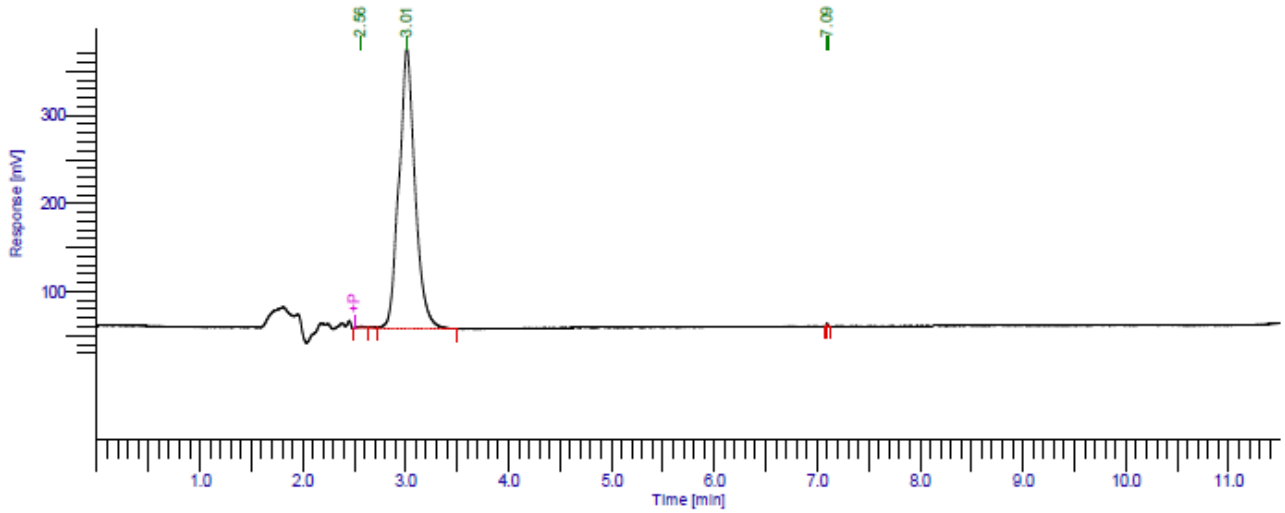


Figure 38: January MDPHP 95:5 10 mM AF (pH 3):ACN - ACE Supe C18

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.011	3449571.87	317693.80	99.55	99.55	VB

Software Version:	6.3.1.0504	Date:	16/02/2026 15:20:20
Reprocess Number:	sci-42d4ee68db4: 5014	Data Acquisition Time:	03/02/2026 15:10:15
Sample Name:	1:10 MDPHP (28.11.25)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	63		

Result File c:\projects\camilla pesce 2025\results\95%10mMAmF-ACN_NovMDPHP_03022026_002-20260216-152019.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

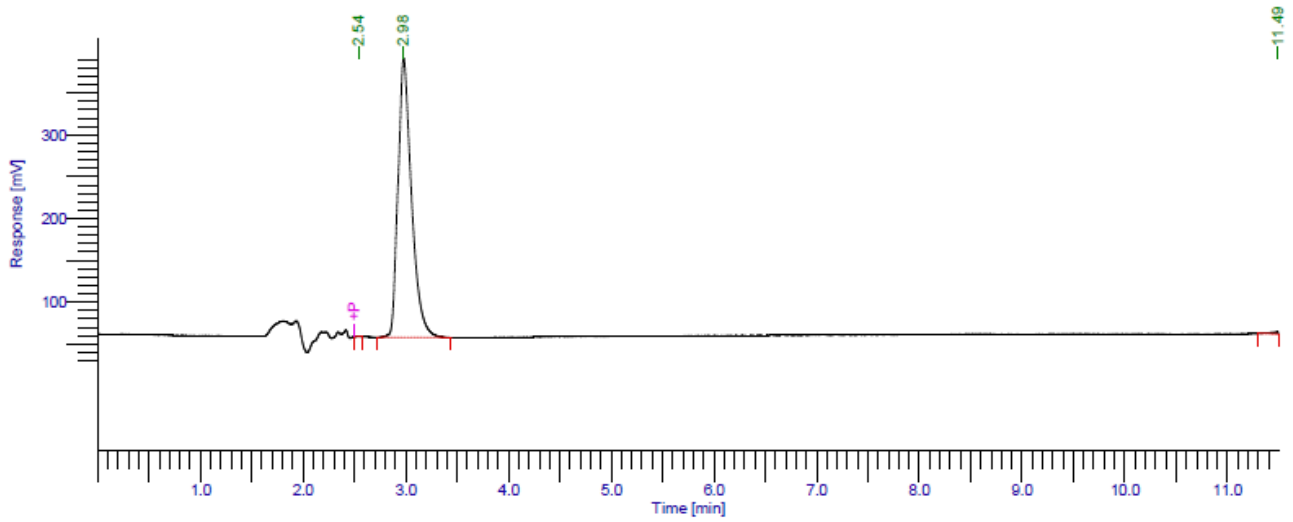


Figure 39: November MDPHP 95:5 10 mM AF (pH 3):ACN - ACE Supe C18

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.979	3050344.72	333428.21	99.51	99.51	BB

APPENDIX R

ACE Super C18, 95:5 10 mM AF (pH 3):ACN Optimisation Trials

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:09
Reprocess Number:	sci-42d4ee68db4: 5010	Data Acquisition Time:	09/02/2026 10:51:20
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	24		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF-ACN(25Celsius)_JanMDPHP_09022026_002-20260226-114208.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
25 degrees Celsius
UV 238 nm

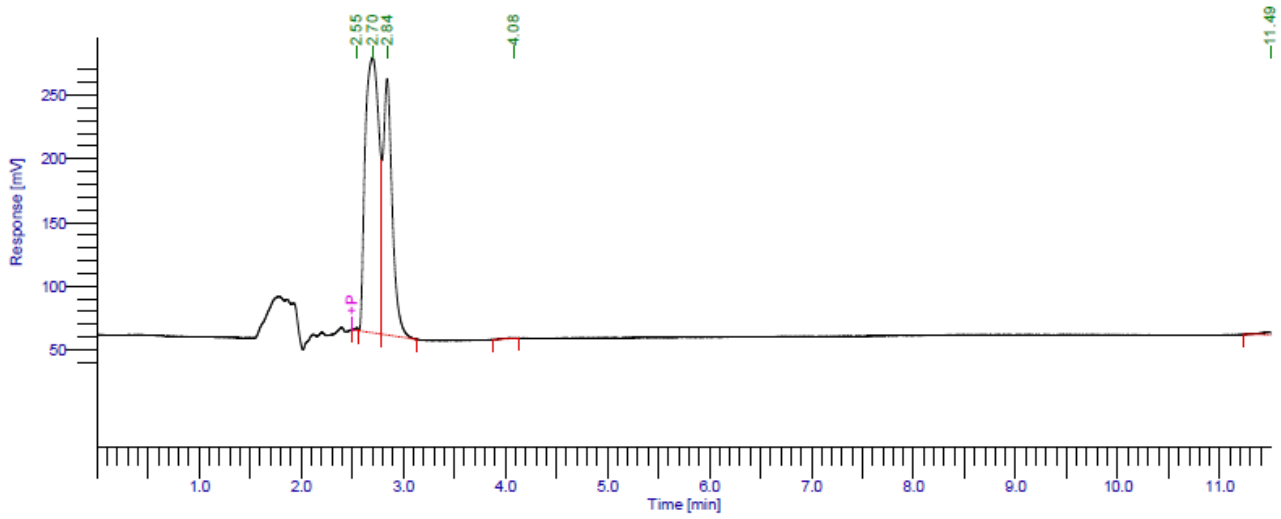


Figure 40: 1:10 MDPHP 25°C Optimisation Trial

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.699	2039158.69	216235.39	61.65	61.65	BV
3	2.842	1246674.72	201322.46	37.69	37.69	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:07
Reprocess Number:	sci-42d4ee68db4: 5009	Data Acquisition Time:	09/02/2026 11:35:49
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	23		

Result File C:\Projects\Camilla Pesce 2025\Results\98%10mMAmF-ACN_JanMDPHP_09022026_002-20260226-114205.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

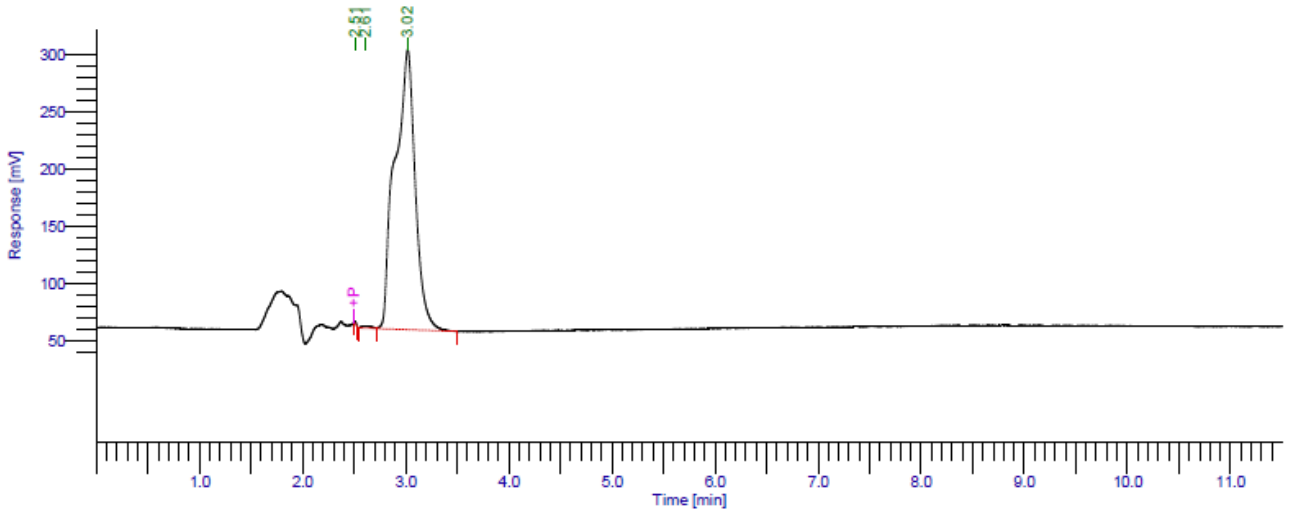


Figure 41: 1:10 MDPHP 98:2 AF:ACN Optimisation Trial

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
3	3.020	3351560.72	245023.12	99.54	99.54	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:02
Reprocess Number:	sci-42d4ee68db4: 5007	Data Acquisition Time:	10/02/2026 12:14:44
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	21		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF-ACN_3.0Step1_JanMDPHP_09022026_002-20260226-114201.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

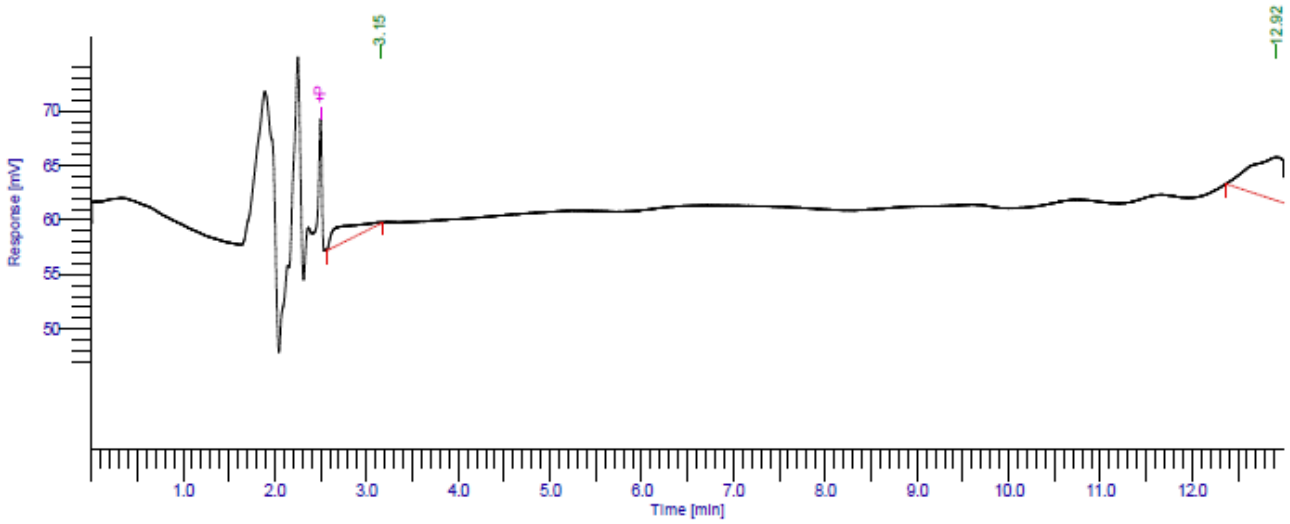


Figure 42: 1:10 MDPHP 3 min Hold Optimisation Trial

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	3.146	33551.73	164.71	26.90	26.90	BB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:42:00
Reprocess Number:	sci-42d4ee68db4: 5006	Data Acquisition Time:	10/02/2026 15:13:40
Sample Name:	1:10 MDPHP (20.01.26)	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	20		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF-ACN_2minJanMDPHP_09022026_001-20260226-114159.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

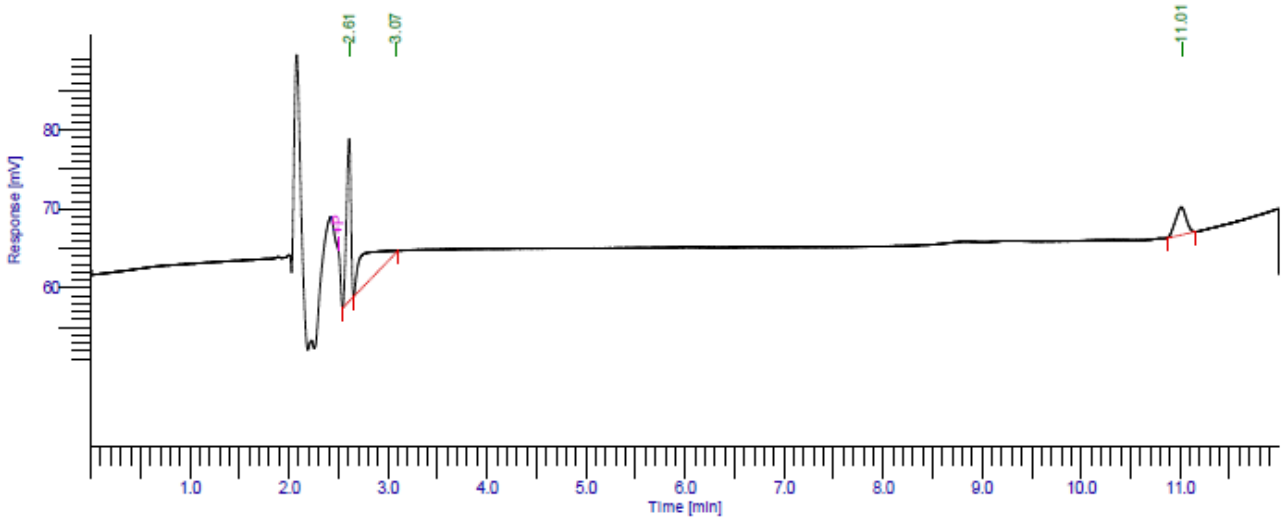


Figure 43: 1:10 MDPHP 2 min Hold Optimisation Trial

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.073	377.53	164.71	41.26	41.26	VB

APPENDIX S

ACE Super C18, 95:5 10 mM AF (pH 3):ACN Limit of Detection (LOD)

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:54
Reprocess Number:	sci-42d4ee68db4: 5003	Data Acquisition Time:	16/02/2026 13:11:49
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/4	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	17		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_LOD_1in10MDPHP_16022026_004-20260226-114153.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

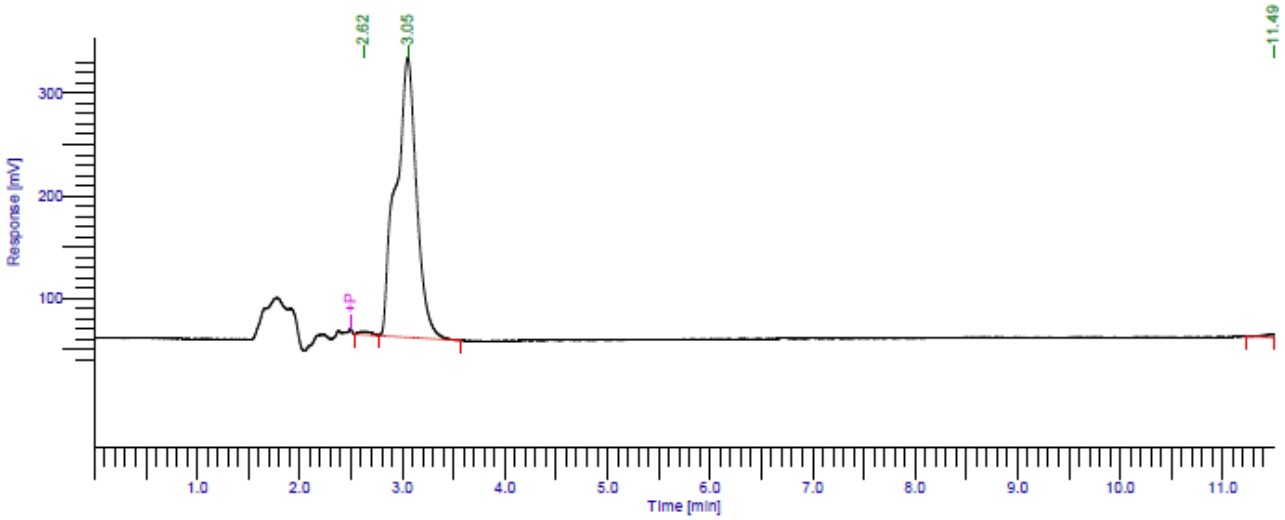


Figure 44: LOD 1:10 MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.054	3825032.82	272630.79	98.66	98.66	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:56
Reprocess Number:	sci-42d4ee68db4: 5004	Data Acquisition Time:	16/02/2026 12:58:05
Sample Name:	1:100 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	18		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_LOD_1in100MDPHP_16022026_003-20260226-114155.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

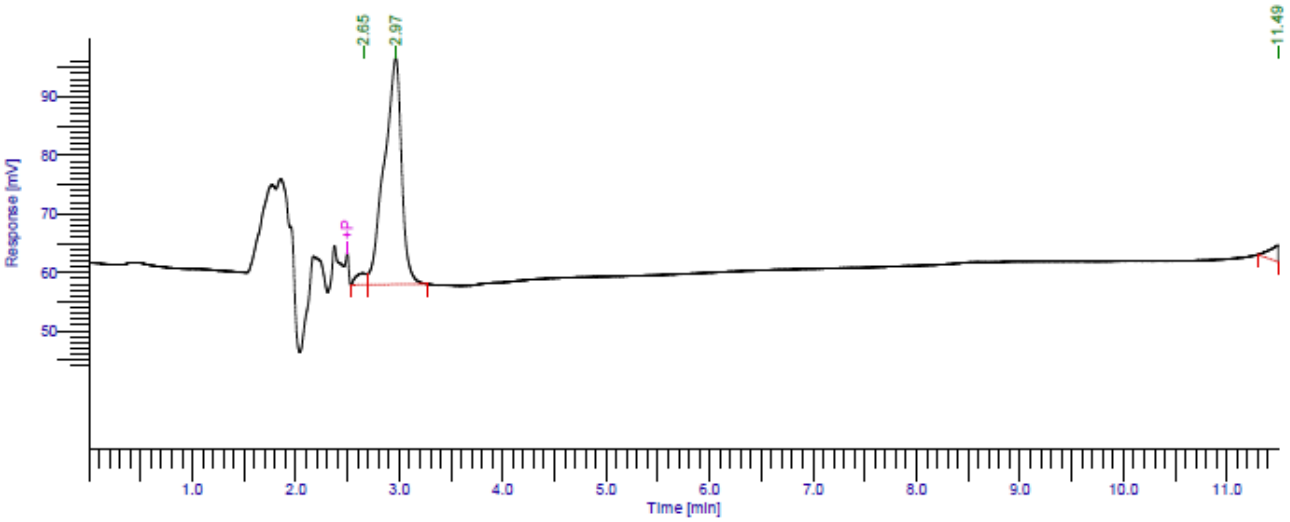


Figure 45: LOD 1:100 MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.966	449544.71	38596.76	93.94	93.94	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:58
Reprocess Number:	sci-42d4ee68db4: 5004	Data Acquisition Time:	16/02/2026 12:44:21
Sample Name:	1:1000 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	19		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_LOD_1in1000MDPHP_16022026_002-20260226-114157.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

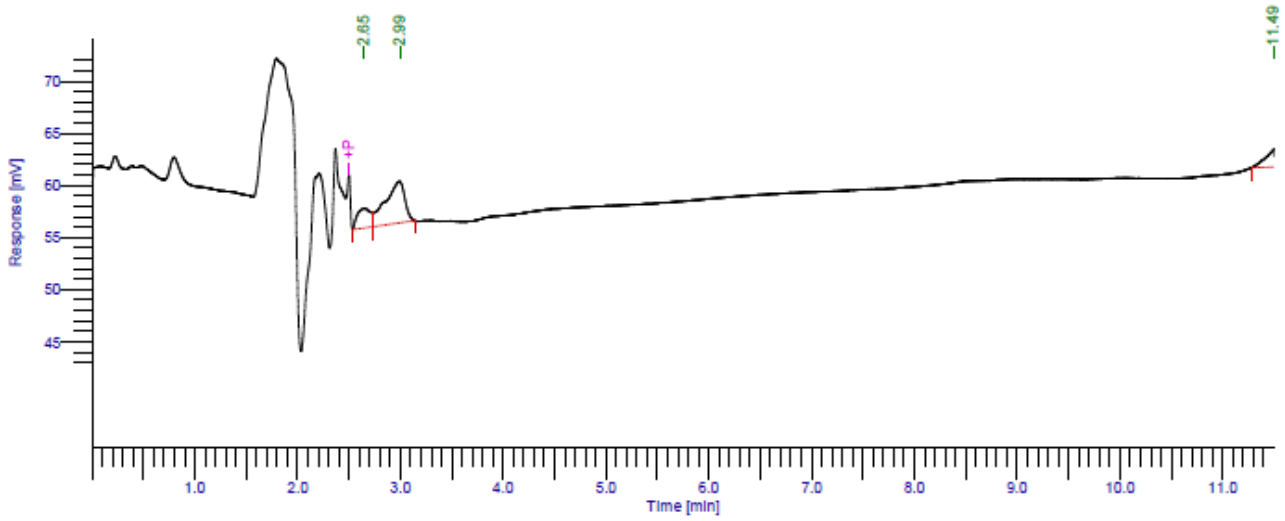


Figure 46: LOD 1:1000 MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.993	52889.26	3961.53	66.46	66.46	VB

APPENDIX T

ACE Super C18, Pump Programs Elution Gradient Visual Representations

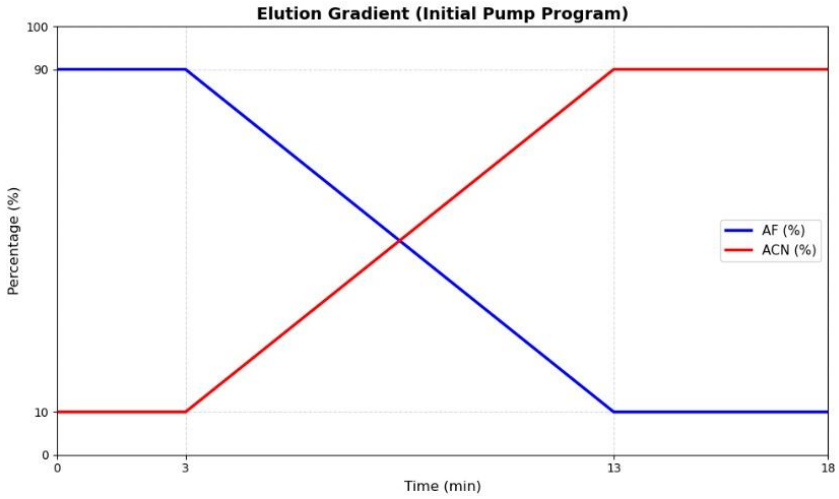


Figure 47: Initial Method Eluent Gradient Curve

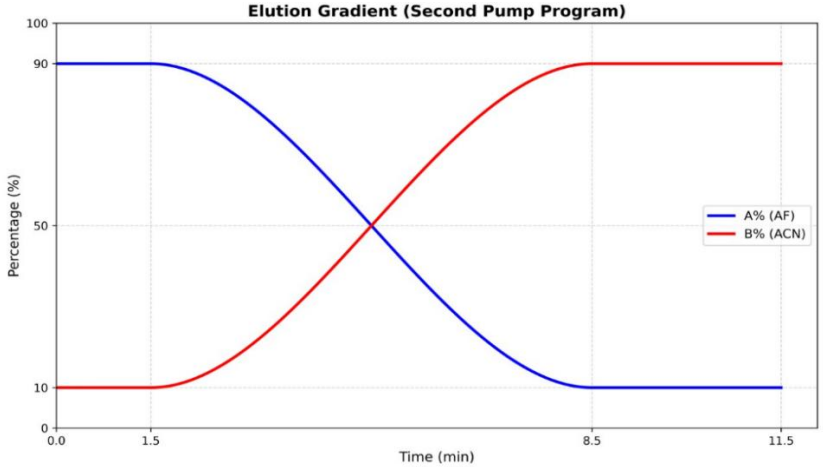


Figure 48: Second Method Eluent Gradient Curve

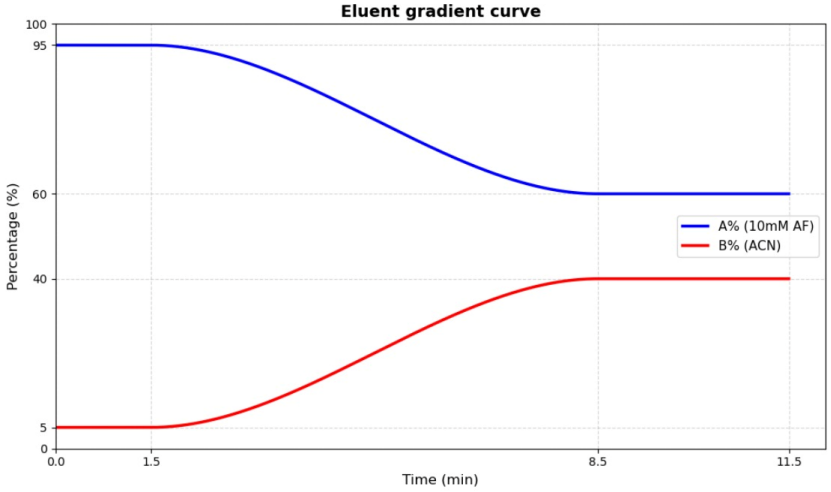


Figure 49: Final Method Eluent Gradient Curve

APPENDIX U

Intra-day Repeatability Chromatograms

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:43
Reprocess Number:	sci-42d4ee68db4: 4998	Data Acquisition Time:	19/02/2026 12:01:58
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	12		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat1_1in10MDPHP_19022026_002-20260226-114142.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

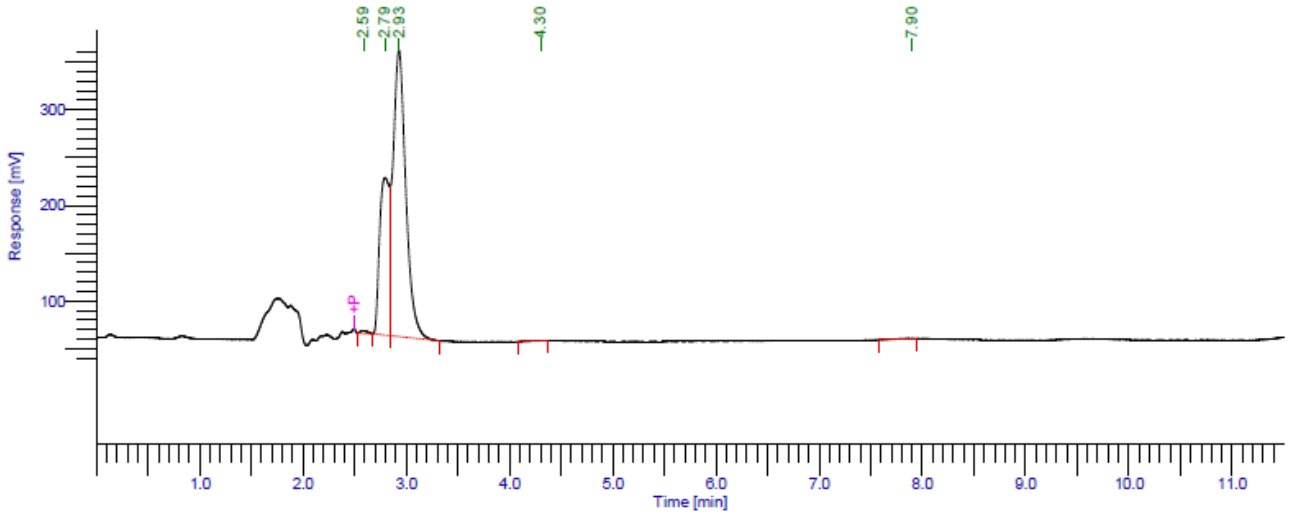


Figure 50: Intra-day Repeat 1 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.795	1043379.93	164697.91	28.44	28.44	BV
3	2.930	2602880.08	299599.32	70.95	70.95	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:41
Reprocess Number:	sci-42d4ee68db4: 4997	Data Acquisition Time:	19/02/2026 12:15:41
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	11		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat2_1in10MDPHP_19022026_003-20260226-114140.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

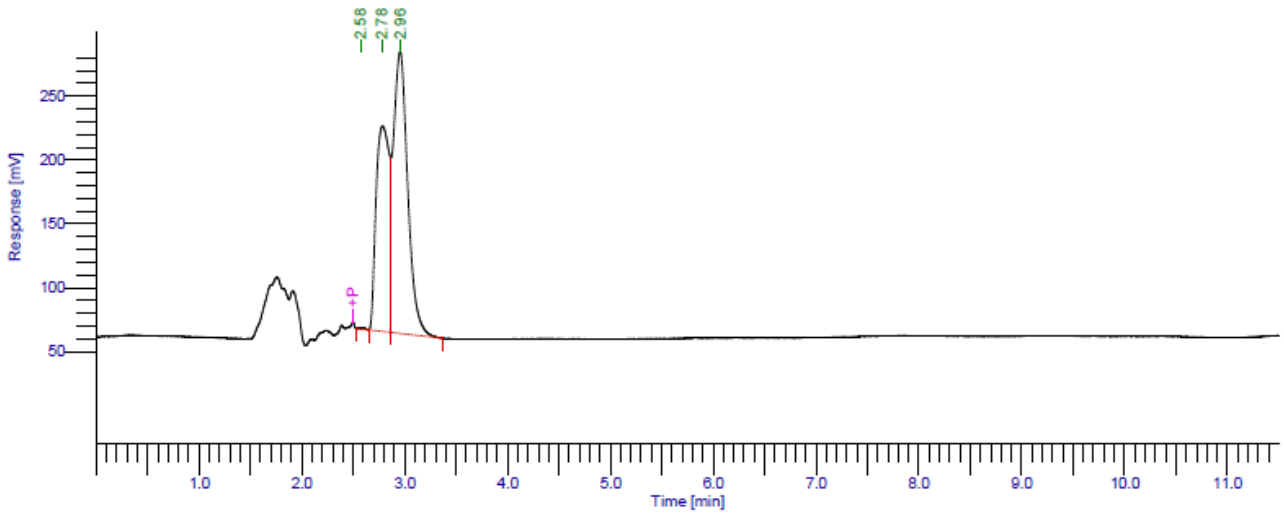


Figure 51: Intra-day Repeat 2 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.785	1427858.10	160740.92	40.05	40.05	BV
3	2.956	2132240.46	220651.94	59.81	59.81	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:39
Reprocess Number:	sci-42d4ee68db4: 4996	Data Acquisition Time:	19/02/2026 12:29:26
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	10		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat3_1in10MDPHP_19022026_004-20260226-114138.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

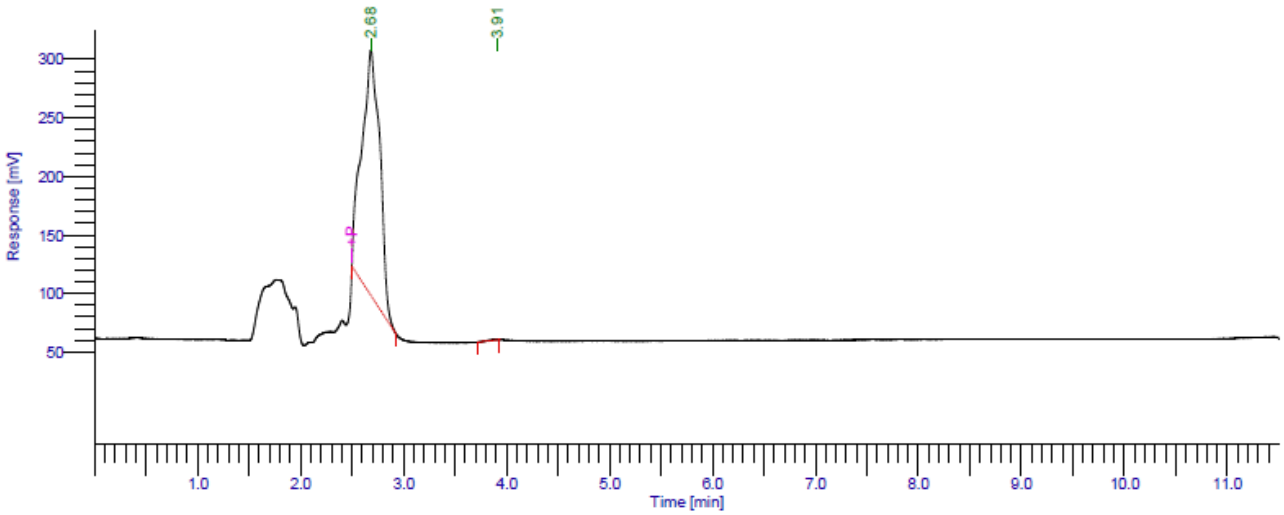


Figure 52: Intra-day Repeat 3 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.683	2559201.84	209220.67	99.85	99.85	*BB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:37
Reprocess Number:	sci-42d4ee68db4: 4995	Data Acquisition Time:	19/02/2026 12:43:10
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	9		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat4_1in10MDPHP_19022026_005-20260226-114136.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

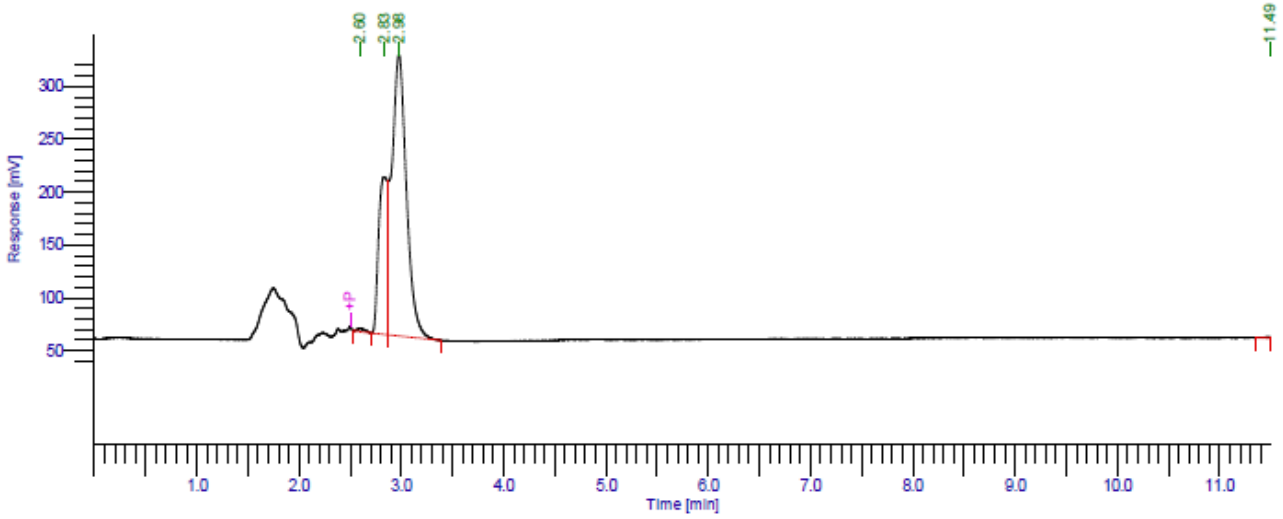


Figure 53: Intra-day Repeat 4 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.831	919584.16	149093.56	25.86	25.86	BV
3	2.977	2613259.23	265938.27	73.49	73.49	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:35
Reprocess Number:	sci-42d4ee68db4: 4994	Data Acquisition Time:	19/02/2026 12:56:54
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	8		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat5_1in10MDPHP_19022026_006-20260226-114134.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

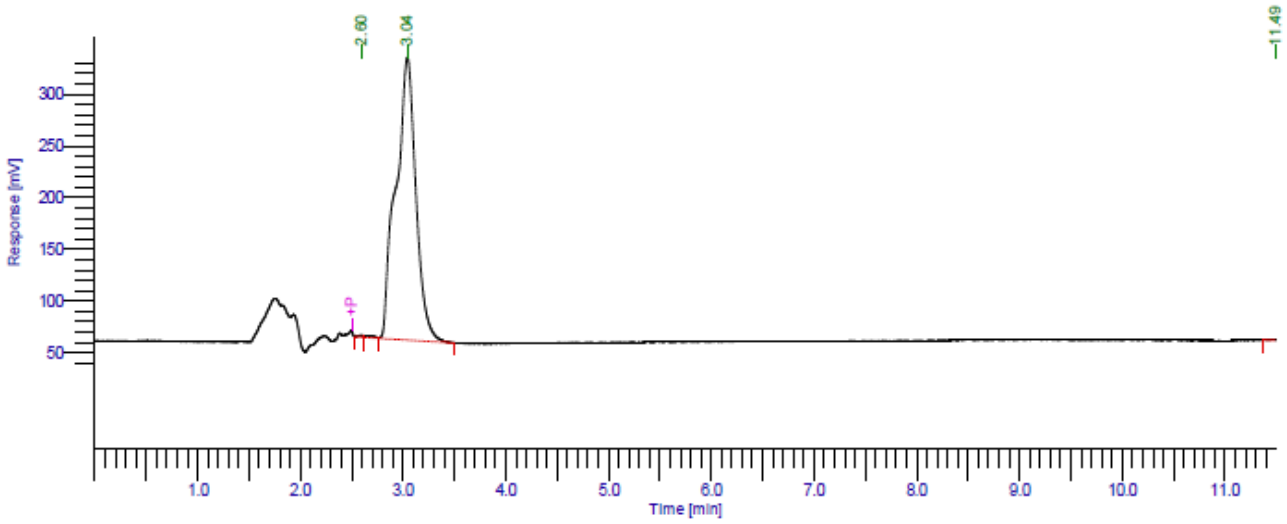


Figure 54: Intra-day Repeat 5 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.042	3699219.88	273345.50	99.69	99.69	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:33
Reprocess Number:	sci-42d4ee68db4: 4993	Data Acquisition Time:	19/02/2026 13:10:38
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	7		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFrepeat6_1in10MDPHP_19022026_007-20260226-114132.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

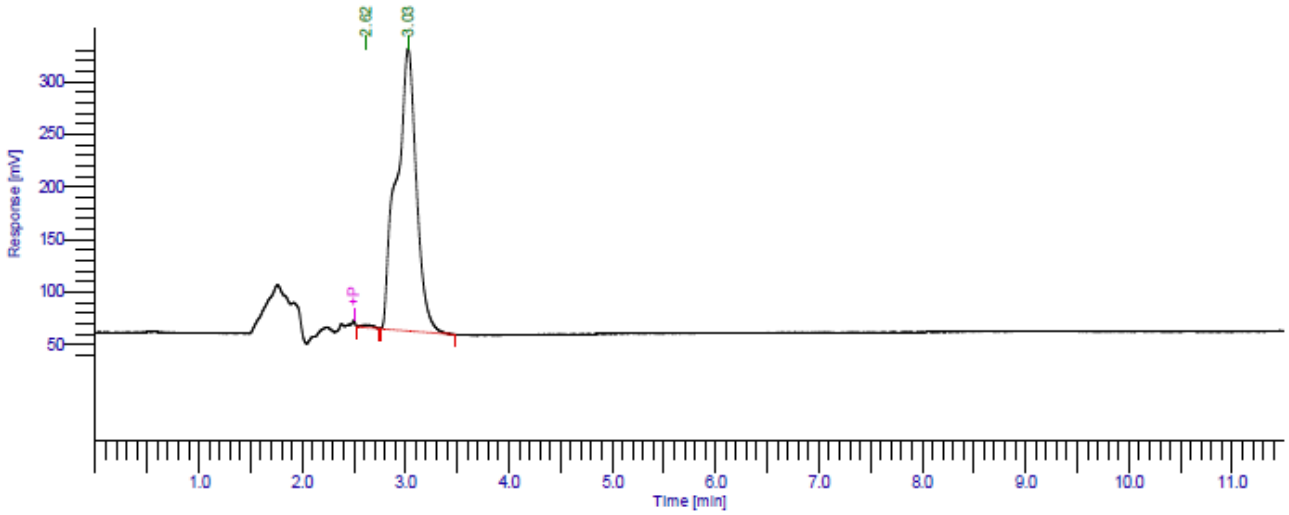


Figure 55: Intra-day Repeat 6 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.026	3593441.39	268683.25	99.43	99.43	BB

APPENDIX V

Inter-day Reproducibility Chromatograms

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:31
Reprocess Number:	sci-42d4ee68db4: 4992	Data Acquisition Time:	20/02/2026 12:32:06
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	6		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod1_1in10MDPHP_20022026_002-20260226-114129.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

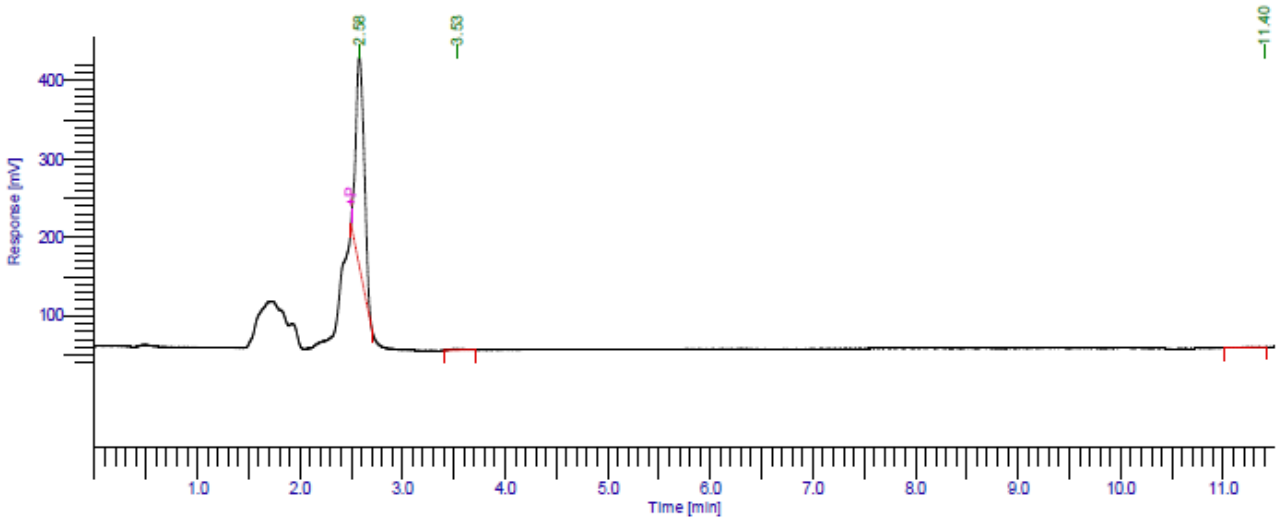


Figure 56: Inter-day Reproduction 1 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.579	1533637.06	266423.40	98.64	98.64	*BB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:28
Reprocess Number:	sci-42d4ee68db4: 4991	Data Acquisition Time:	20/02/2026 12:45:50
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	5		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod2_1in10MDPHP_20022026_003-20260226-114125.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

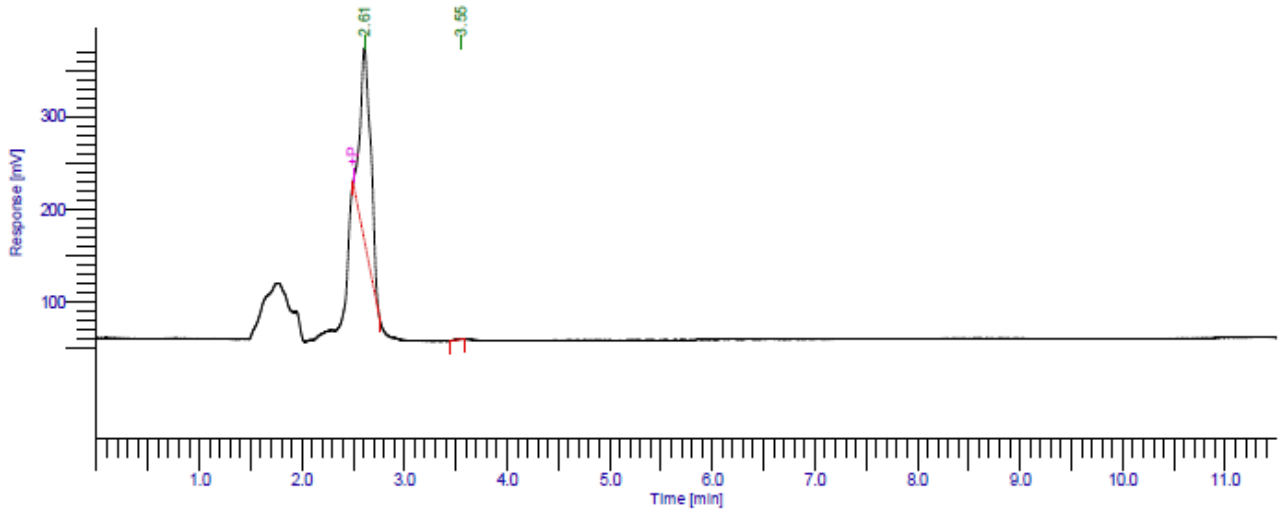


Figure 57: Inter-day Reproduction 2 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.609	1491626.83	207549.69	99.77	99.77	*BB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:24
Reprocess Number:	sci-42d4ee68db4: 4990	Data Acquisition Time:	20/02/2026 12:59:34
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	4		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod3_1in10MDPHP_20022026_004-20260226-114123.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

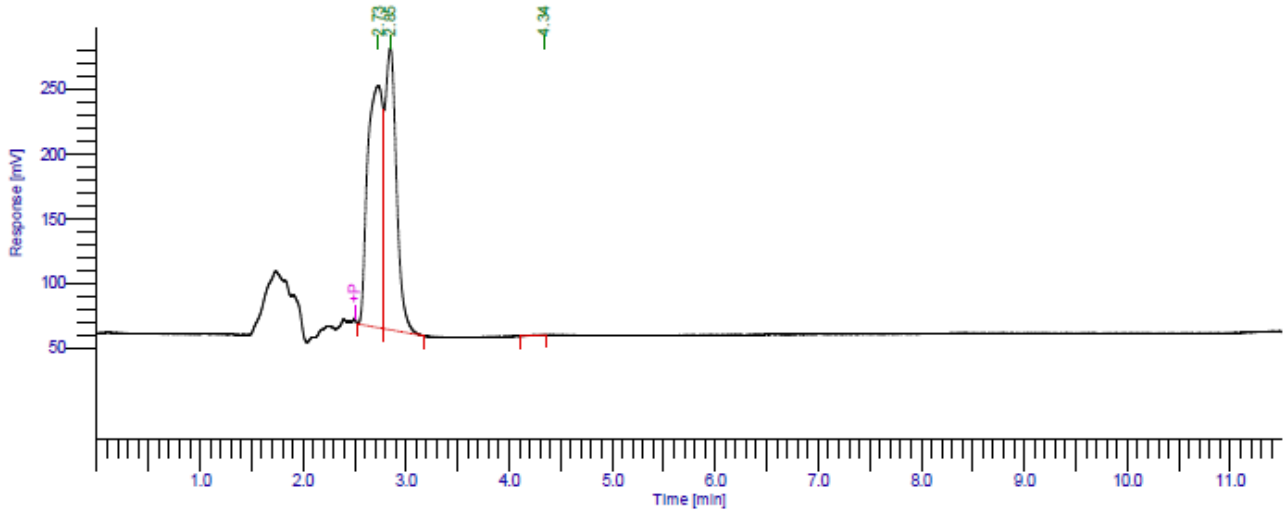


Figure 58: Inter-day Reproduction 3 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
1	2.729	1896964.51	186954.26	53.27	53.27	BV
2	2.848	1661952.99	217806.77	46.67	46.67	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:22
Reprocess Number:	sci-42d4ee68db4: 4989	Data Acquisition Time:	20/02/2026 13:13:18
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	3		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod4_1in10MDPHP_20022026_005-20260226-114120.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

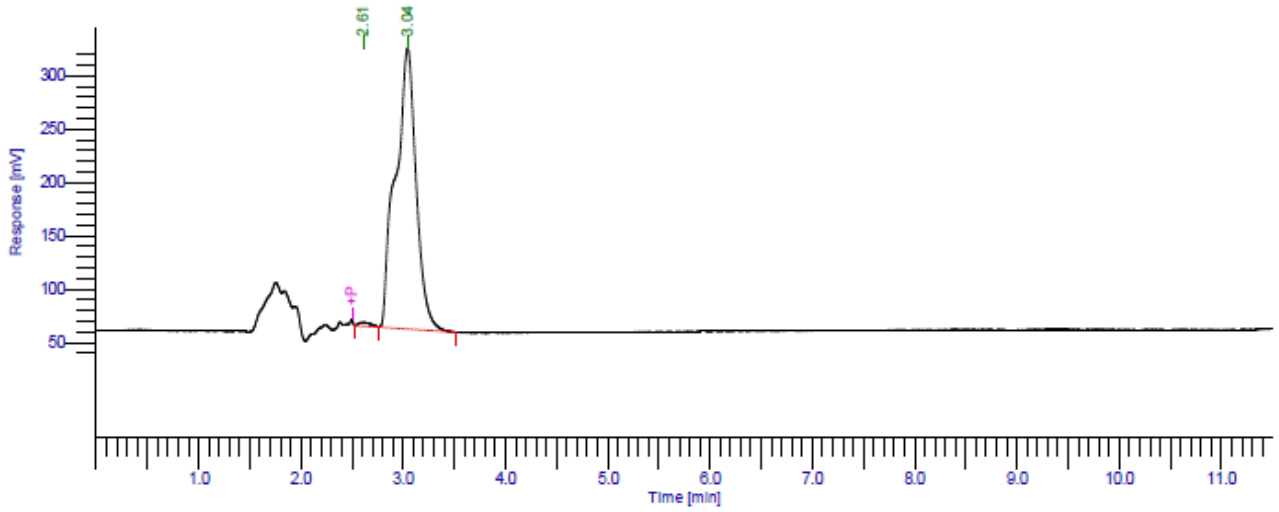


Figure 59: Inter-day Reproduction 4 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.043	3672168.75	263226.28	99.20	99.20	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:19
Reprocess Number:	sci-42d4ee68db4: 4988	Data Acquisition Time:	20/02/2026 13:27:01
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	2		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod5_1in10MDPHP_20022026_006-20260226-114116.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

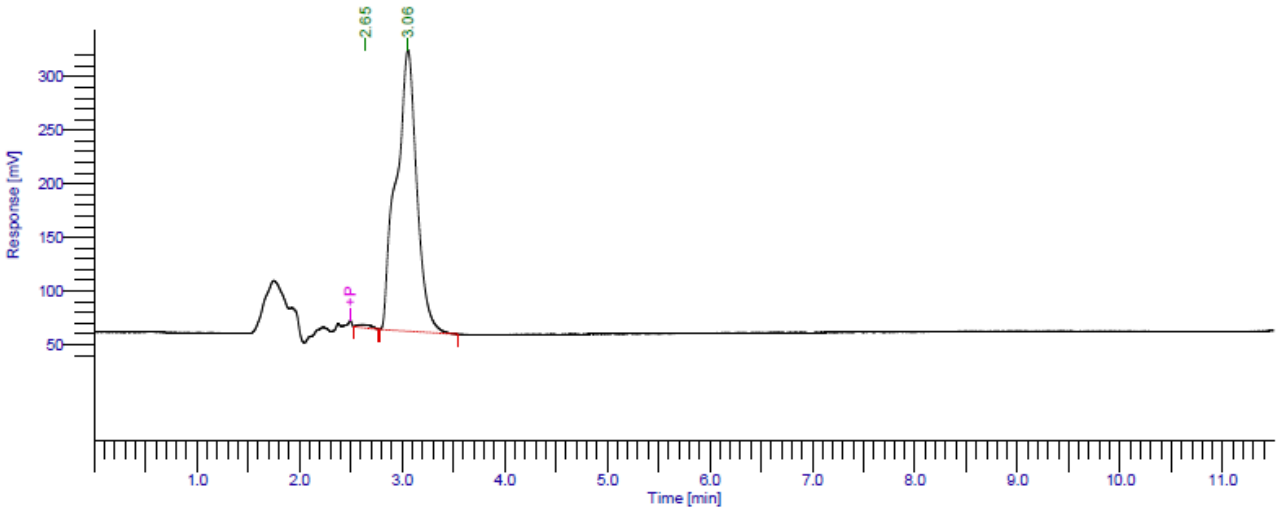


Figure 60: Inter-day Reproduction 5 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.058	3666914.79	262420.23	99.26	99.26	BB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:14
Reprocess Number:	sci-42d4ee68db4: 4987	Data Acquisition Time:	20/02/2026 13:40:46
Sample Name:	1:10 MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	1		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmFreprod6_1in10MDPHP_20022026_007-20260226-114056.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

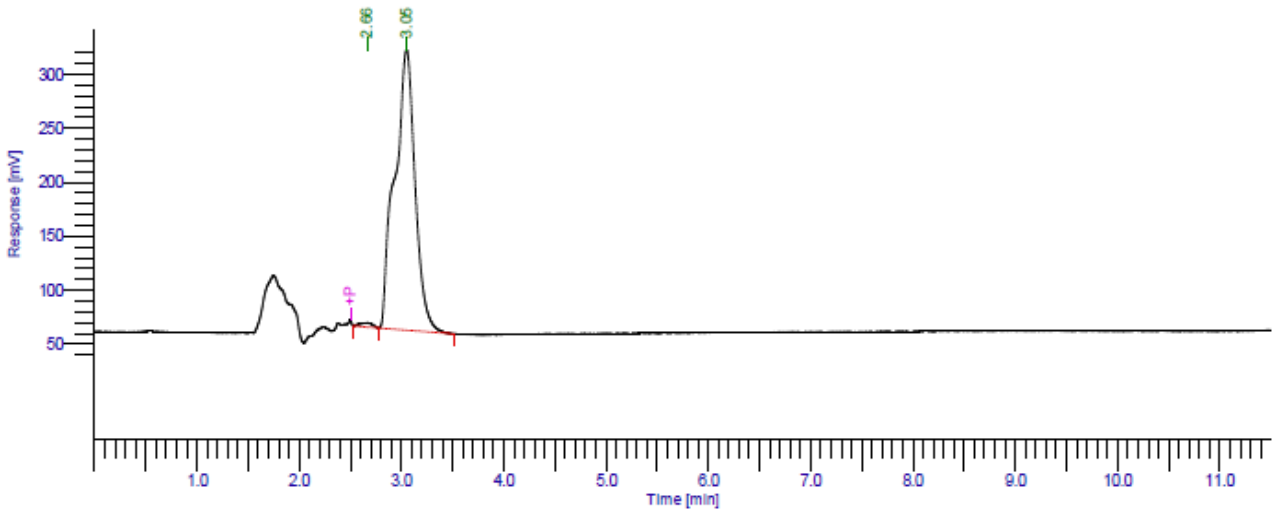


Figure 61: Inter-day Reproduction 6 Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.049	3589146.00	259737.80	99.05	99.05	BB

APPENDIX W

Pure MDPHP in MeOH Chromatograms

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:51
Reprocess Number:	sci-42d4ee68db4: 5002	Data Acquisition Time:	17/02/2026 12:39:51
Sample Name:	47.7 mgL^{-1} MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/1	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	16		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_JD47.7MDPHP_17022026_001-20260226-114151.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

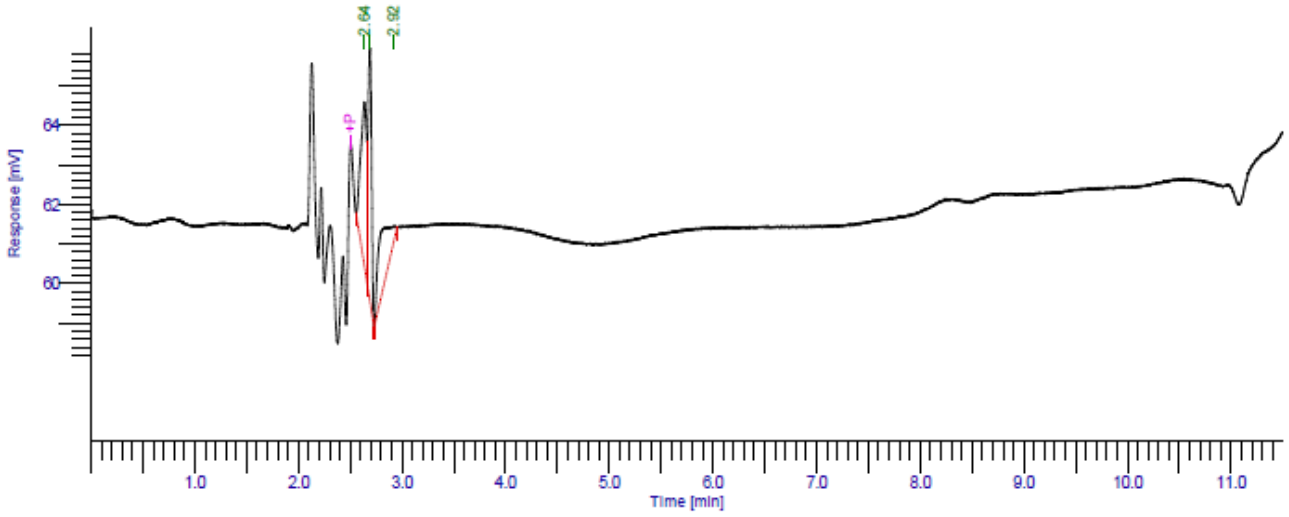


Figure 62: 47.7 mgL^{-1} MDPHP in MeOH Chromatogram

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:49
Reprocess Number:	sci-42d4ee68db4: 5001	Data Acquisition Time:	17/02/2026 12:53:35
Sample Name:	95.4 mgL^{-1} MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/2	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	15		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_JD95.4MDPHP_17022026_002-20260226-114149.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

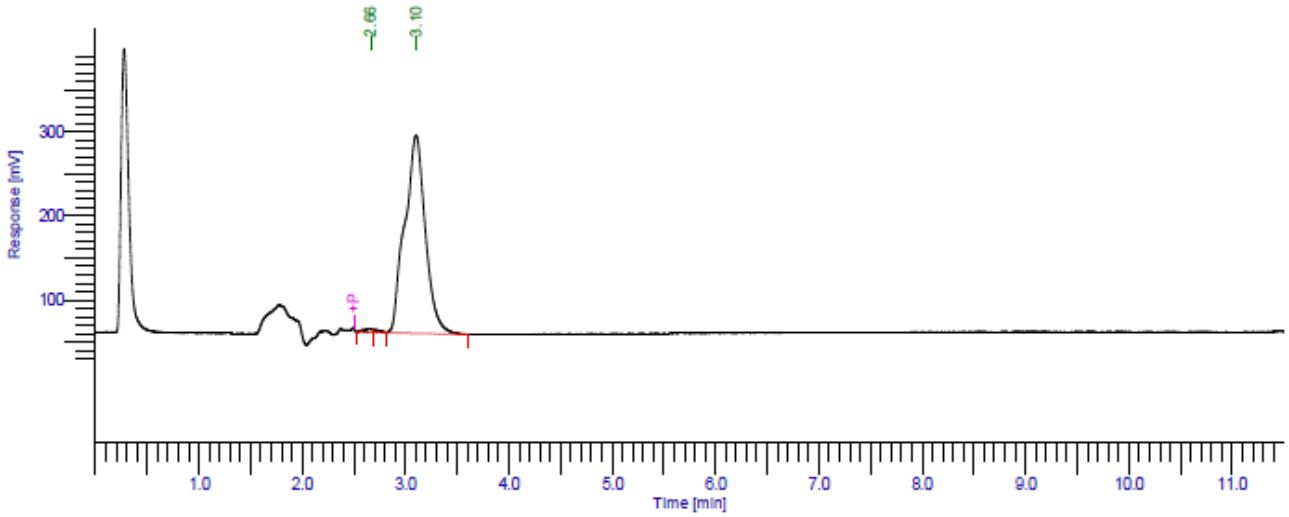


Figure 63: 95.4 mgL^{-1} MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.102	3263484.93	235328.15	99.30	99.30	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:47
Reprocess Number:	sci-42d4ee68db4: 5000	Data Acquisition Time:	17/02/2026 13:07:19
Sample Name:	190.8 mgL^{-1} MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/3	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	14		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_JD190.8MDPHP_17022026_003-20260226-114146.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

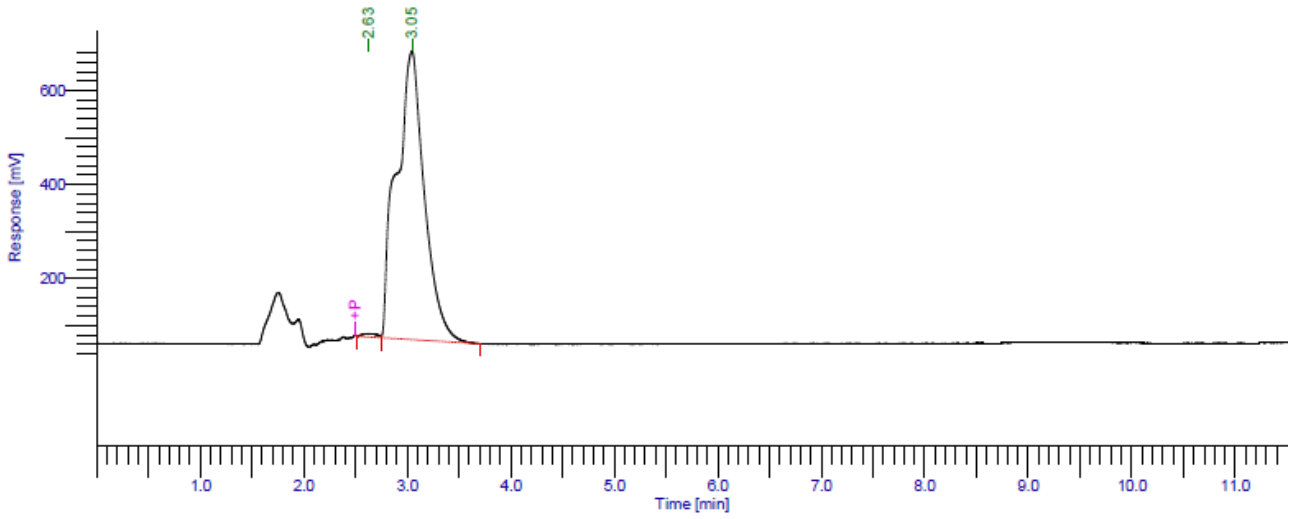


Figure 64: 190.8 mgL^{-1} MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	3.046	11485783.28	614008.21	99.40	99.40	VB

Software Version:	6.3.1.0504	Date:	26/02/2026 11:41:49
Reprocess Number:	sci-42d4ee68db4: 5000	Data Acquisition Time:	17/02/2026 15:46:03
Sample Name:	286.2 mgL^{-1} MDPHP	Channel:	A
Instrument Name:	HPLC1	Operator:	SCI-
Rack/Vial:	0/4	Dilution Factor:	1.000000
Sample Amount:	1.000000		
Cycle:	13		

Result File C:\Projects\Camilla Pesce 2025\Results\95%10mMAmF_JD286.2MDPHP_17022026_005-20260226-114144.rst
Sequence File: C:\Projects\Camilla Pesce 2025\Camilla Pesce2.seq
Sample Notes:
Based on UNODC method synthetic cathinones
ACE super C18 column. 150 x 4.6
10 mM Ammonium Formate pH 3.023 on A
Acetonitrile on B
Flow 0.6
30 degrees Celsius
UV 238 nm

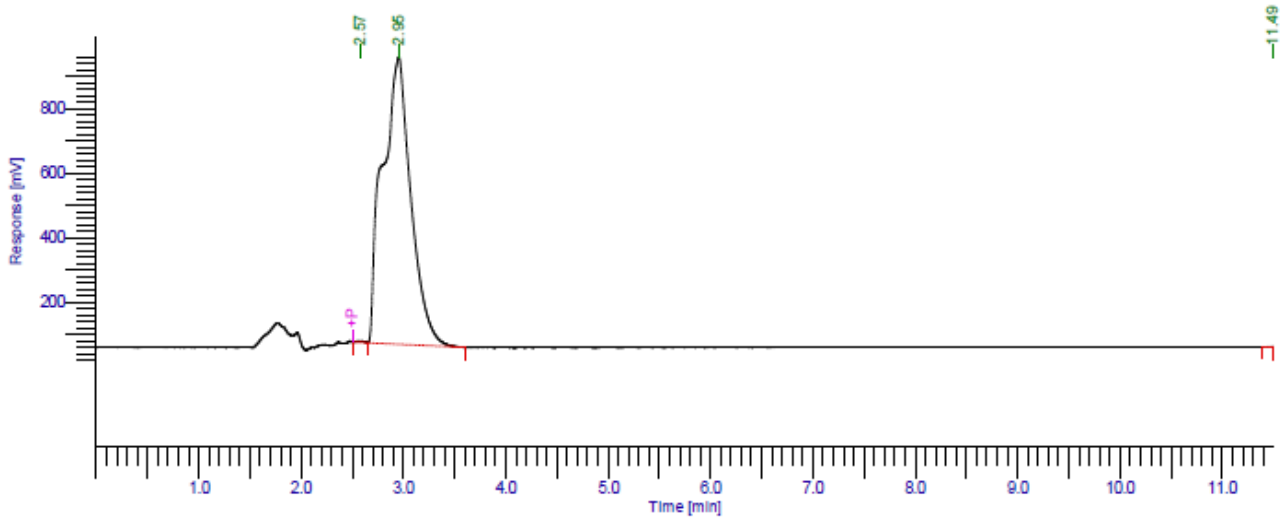


Figure 65: 286.2 mgL^{-1} MDPHP in MeOH Chromatogram

Peak #	Time [min]	Area [UV*sec]	Height [UV]	Area [%]	Norm. Area [%]	BL
2	2.954	17064295.64	889496.45	99.83	99.83	VB