



LISBON ADDICTIONS 2022

Metabolite profiling of synthetic cathinones: towards the identification of consumption biomarkers in forensic and clinical settings

Alexandra M. M. Antunes, Margarida Milhazes, Margarida Figueira, Raquel A. Ferro, Rita P. Lopes, Maria João Caldeira, Susana Santos, Helena Gaspar



New Psychoactive Substances

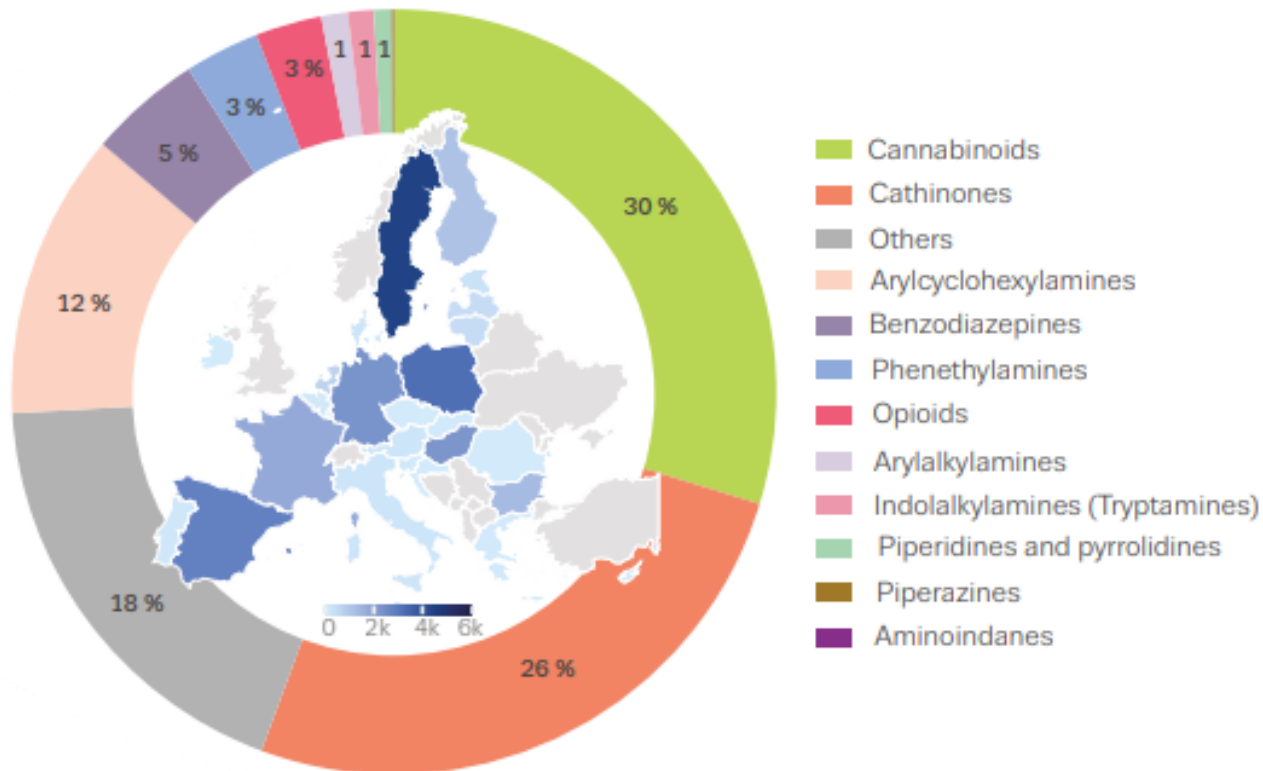


Not controlled by international drug conventions

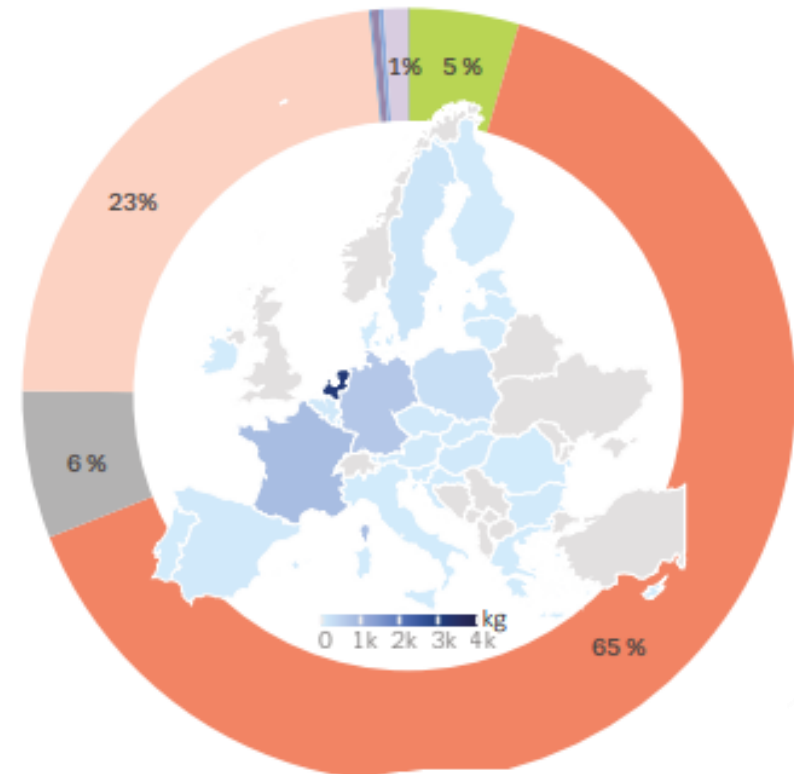
May pose a public health threat

Synthetic Cathinones: the second largest category of NPS monitored by the EU Early Warning System

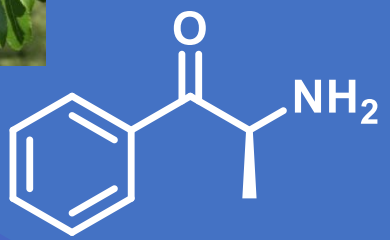
Number of NPS seizures by country and substance, 2020 (EU)



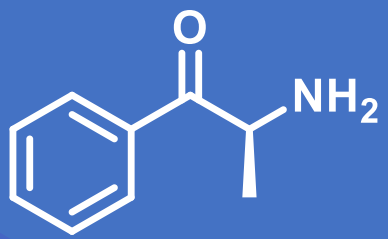
Quantity of NPS seized by country and substance, 2020 (EU)



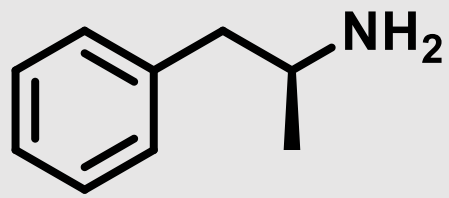
Source: EMMCDA 2022



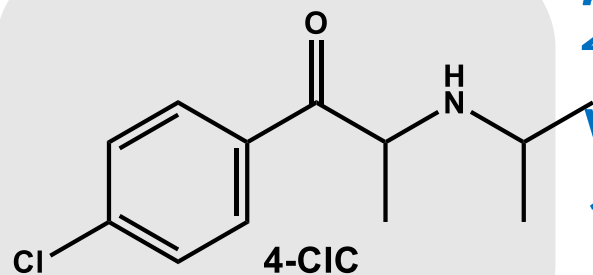
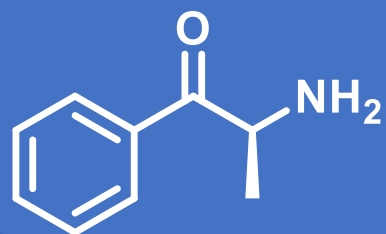
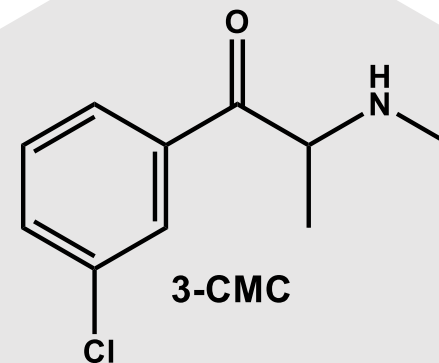
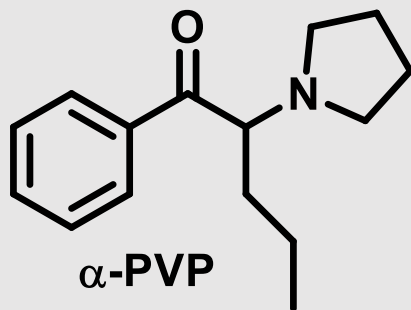
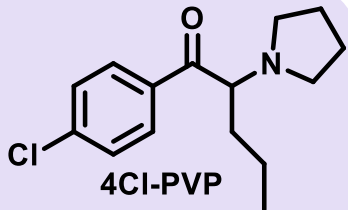
(S)-Cathinone



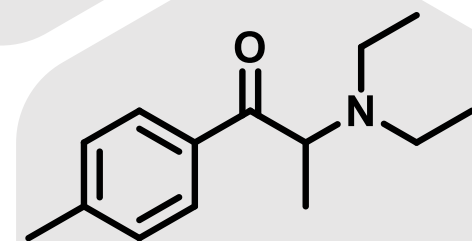
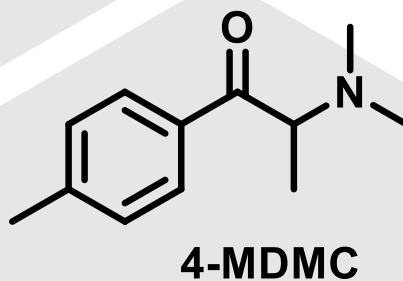
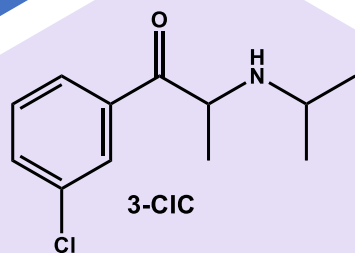
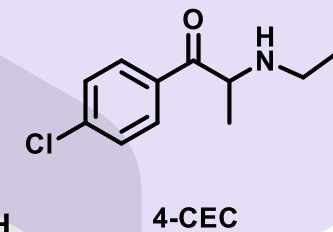
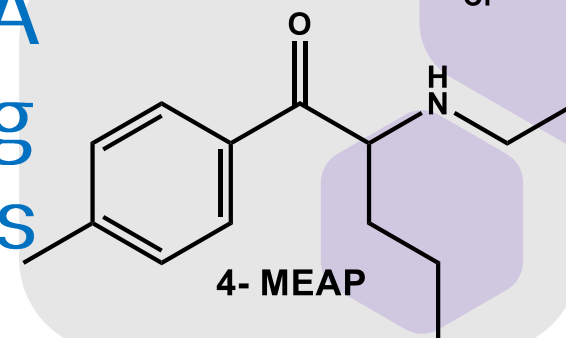
(S)-Cathinone



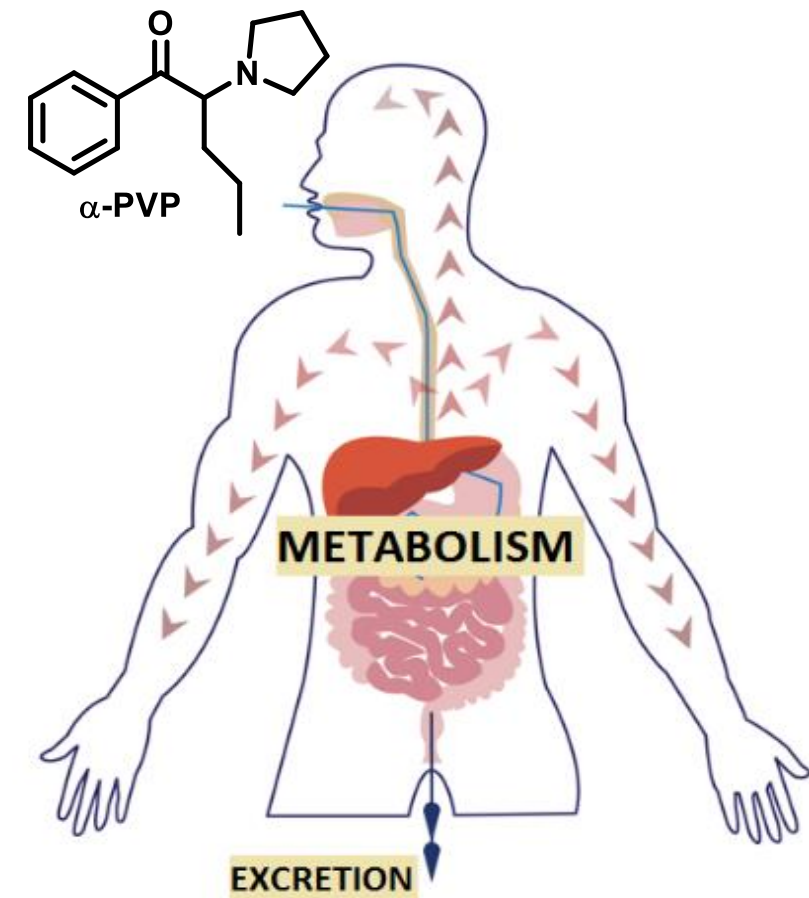
Amphetamine



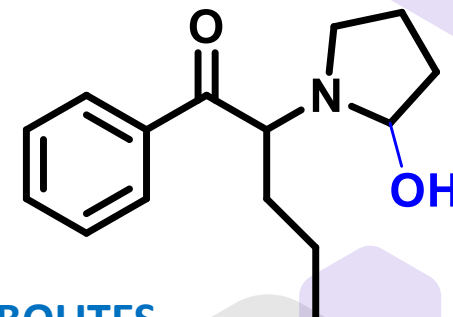
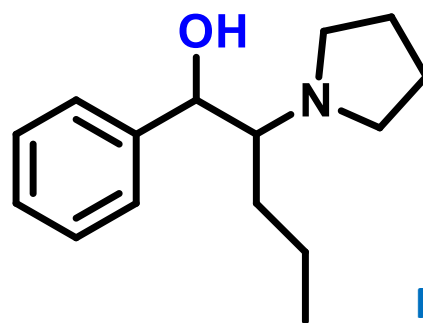
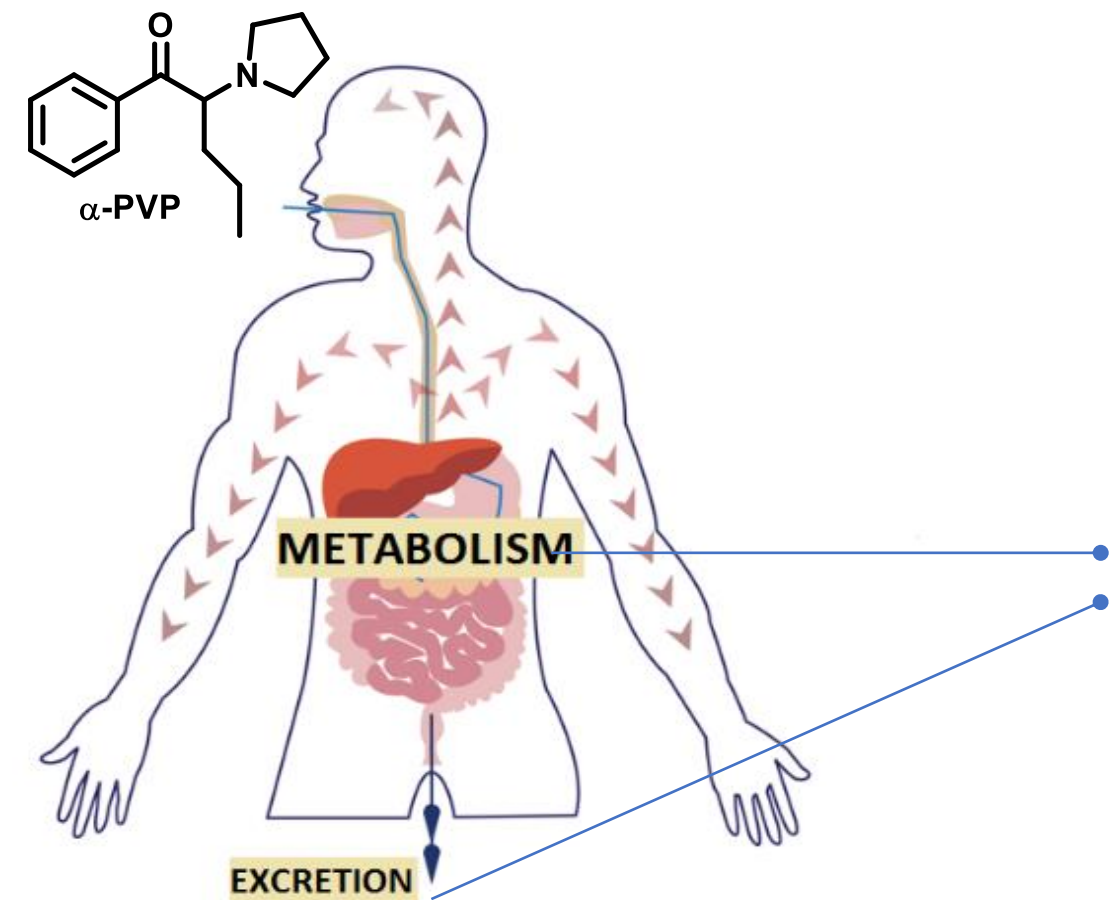
2021: EMCDDA
was monitoring
162 cathinones



METABOLITES: BIOMARKERS EXPOSURE/CONSUMPTION & TOXICITY



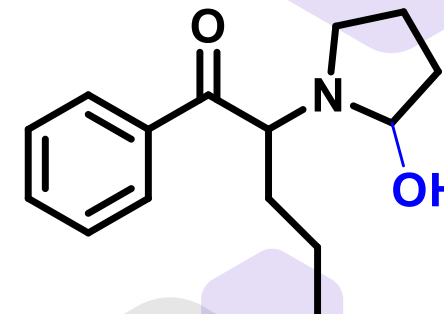
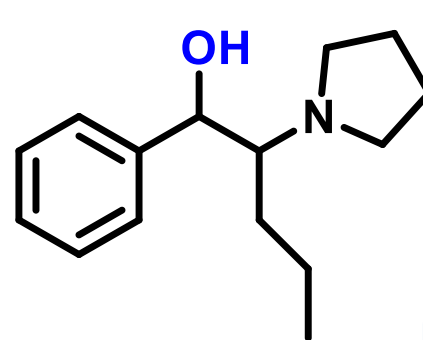
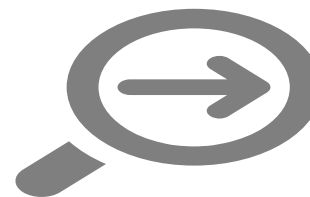
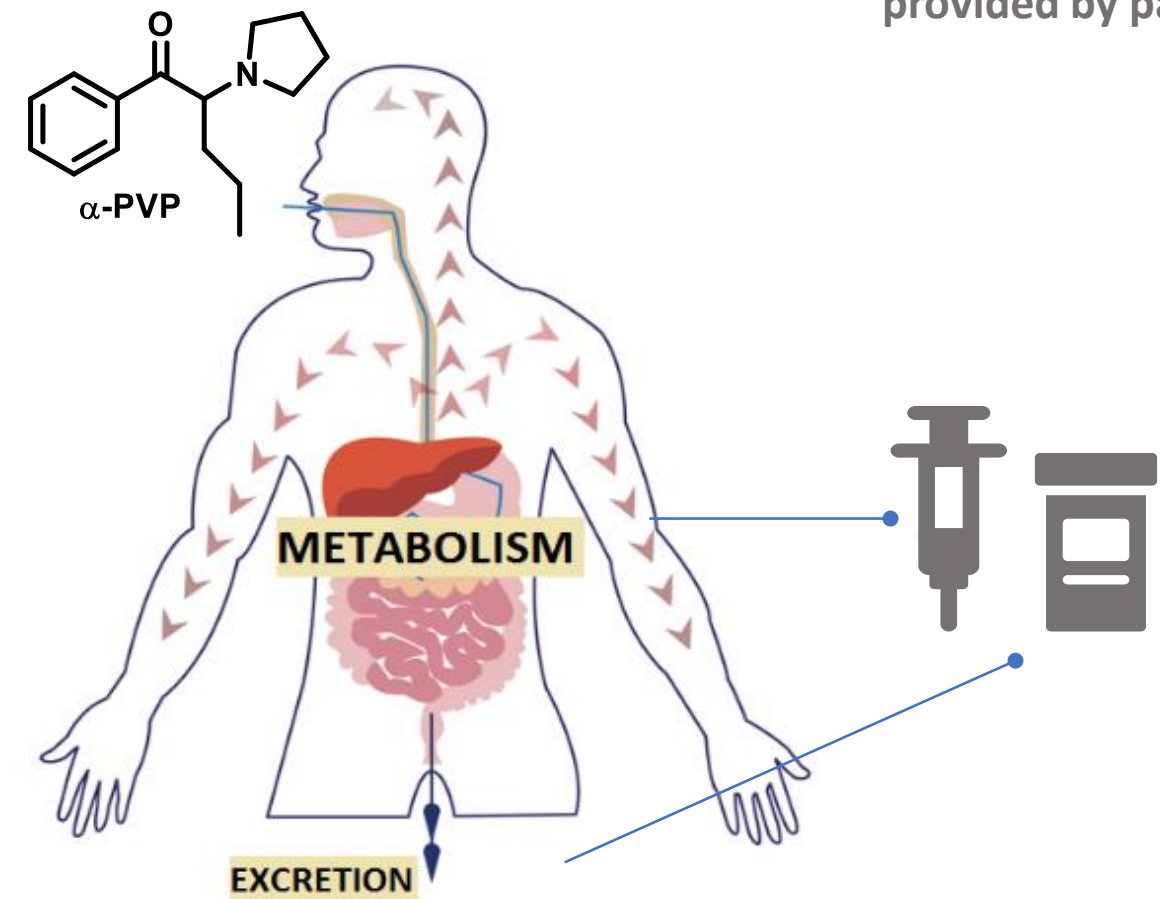
METABOLITES: BIOMARKERS EXPOSURE/CONSUMPTION & TOXICITY



METABOLITES

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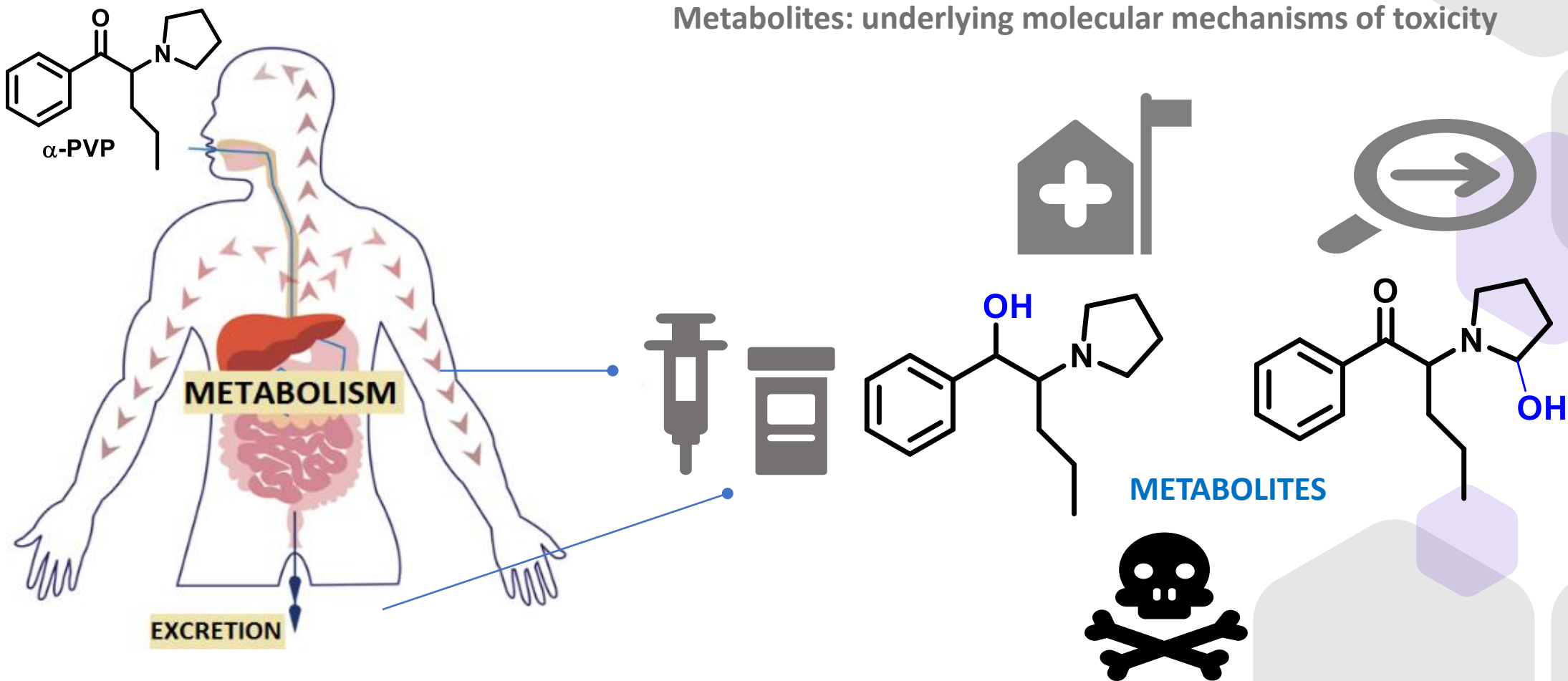
metabolites: suitable biomarkers to extend detection windows beyond those provided by parent cathinone



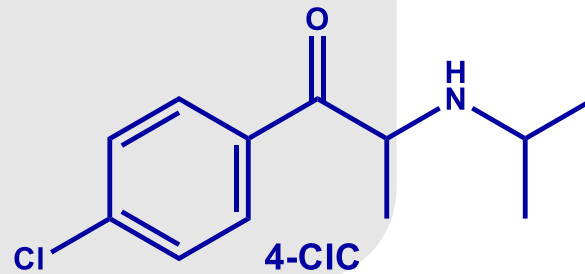
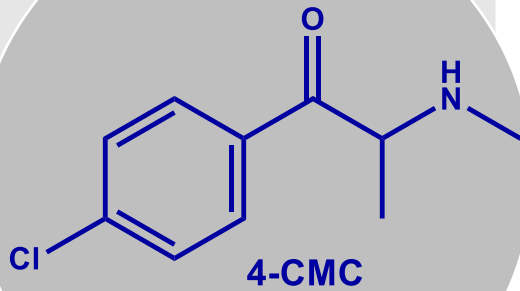
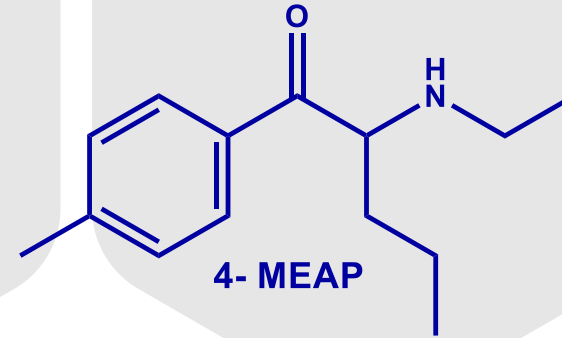
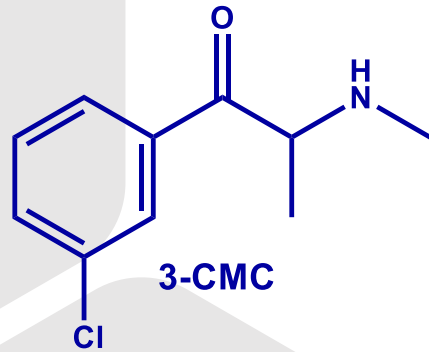
METABOLITES

METABOLITES: BIOMARKERS EXPOSURE/CONSUMPTION & TOXICITY

Metabolites: underlying molecular mechanisms of toxicity



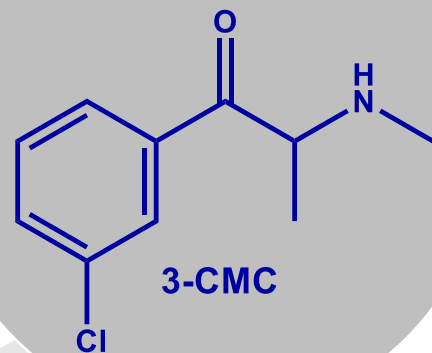
Cathinones Selected for this Study



Already reported in the Elicit Drug Market

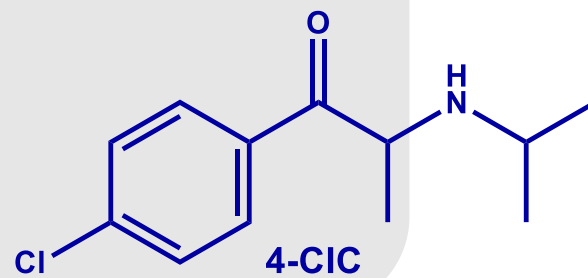
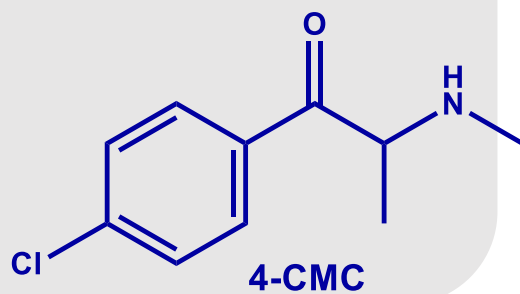
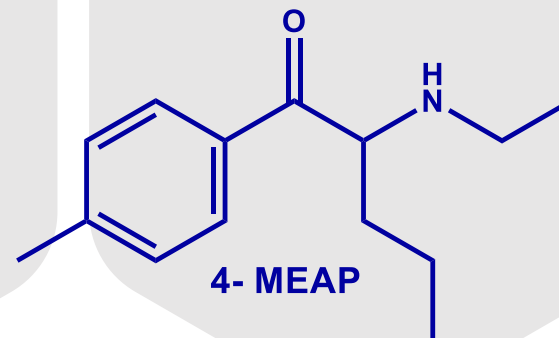
Drug covered by the 1971 United Nations Convention

Cathinones Selected for this Study



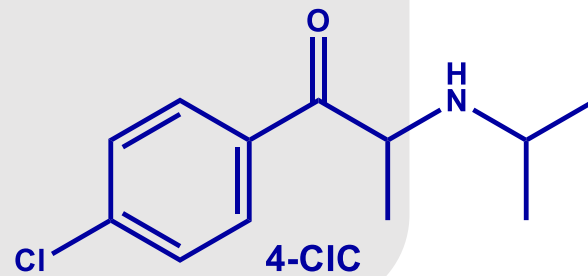
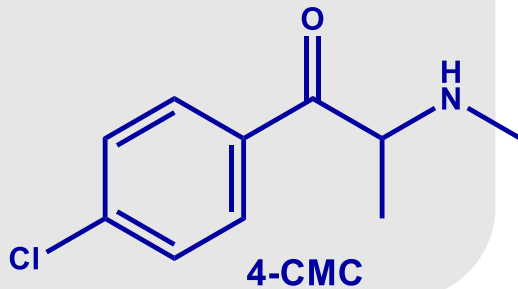
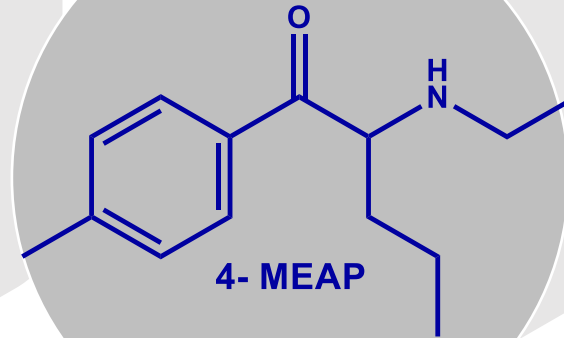
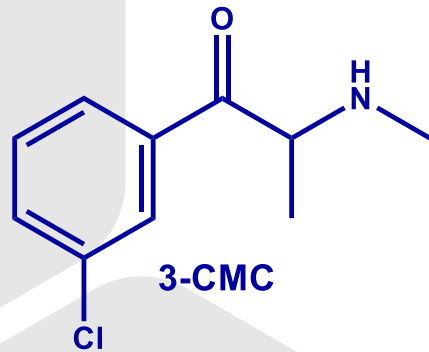
Accounts for almost a quarter of the quantity of powders seized during 2020

Risk Assessment Report, EMCDDA 2022



Already reported in the Elicit Drug Market

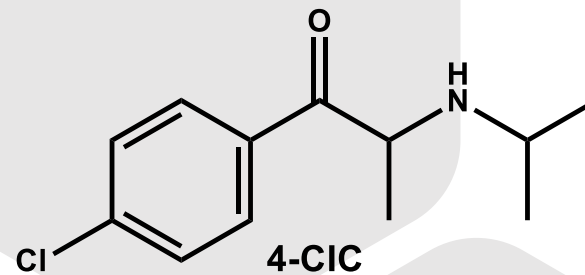
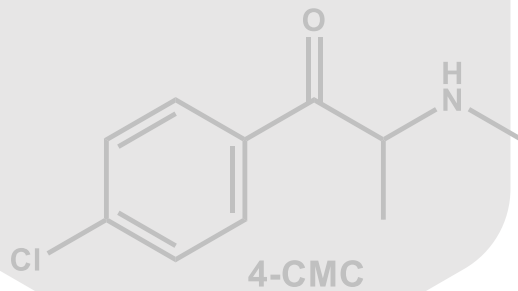
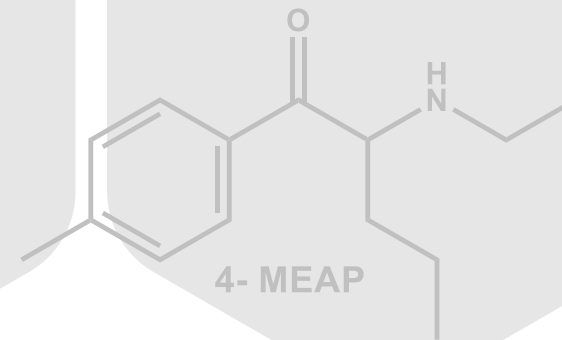
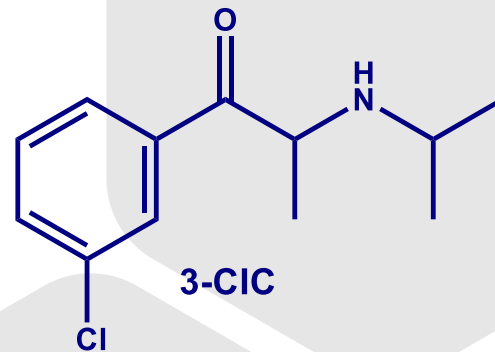
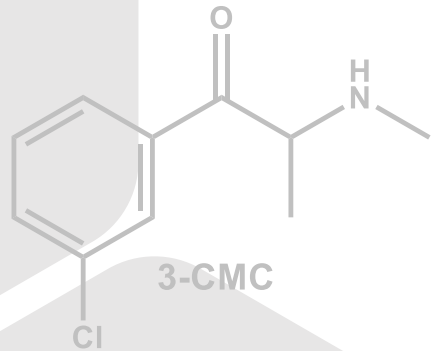
Cathinones Selected for this Study



Related to fatal cases of intoxication

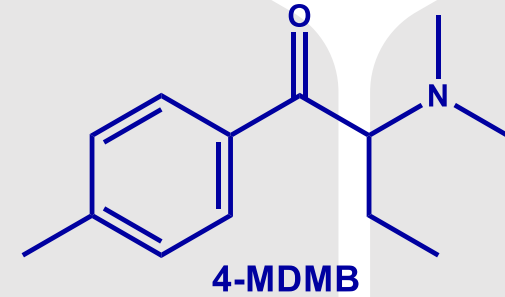
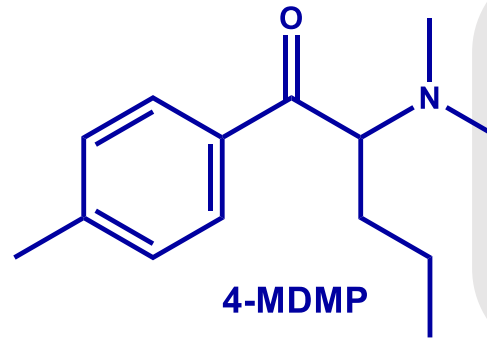
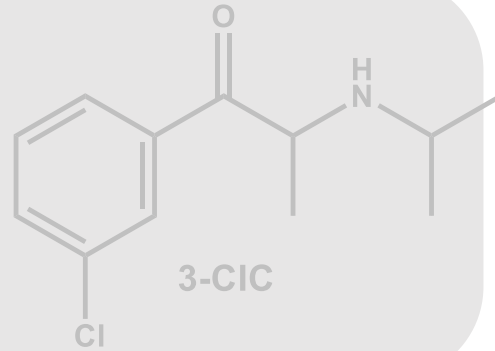
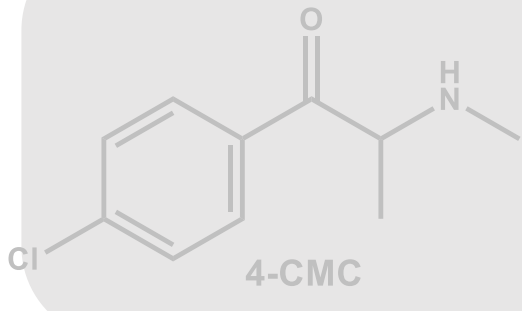
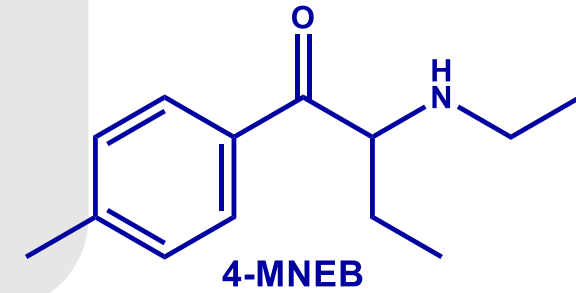
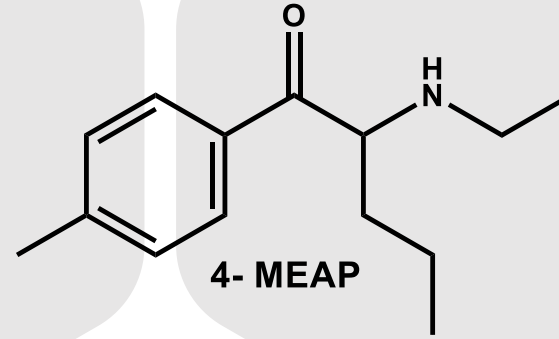
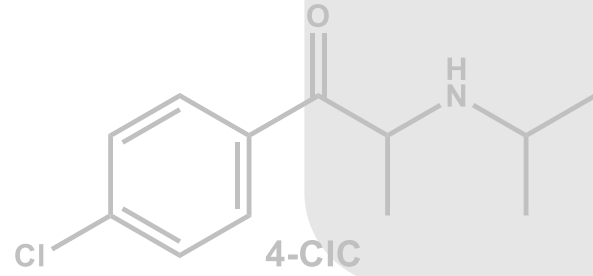
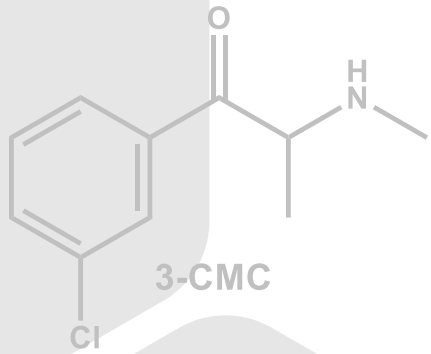
Already reported in the Elicit Drug Market

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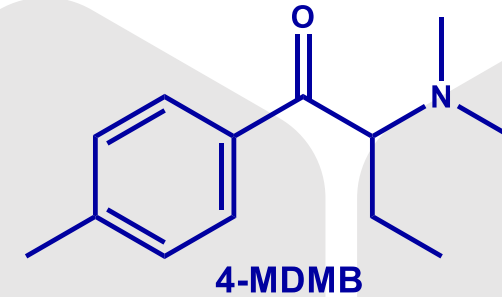
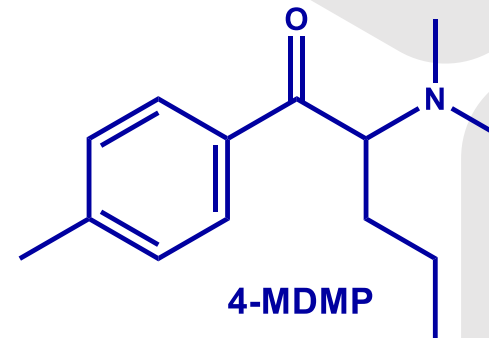
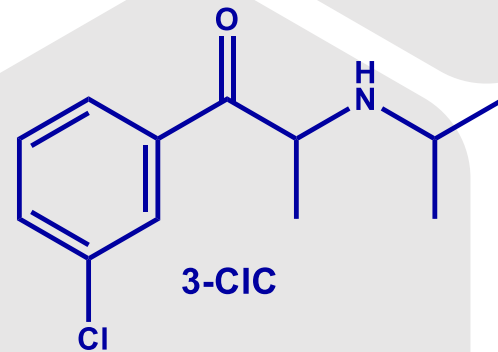
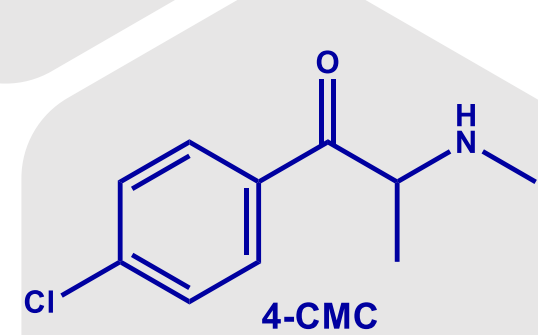
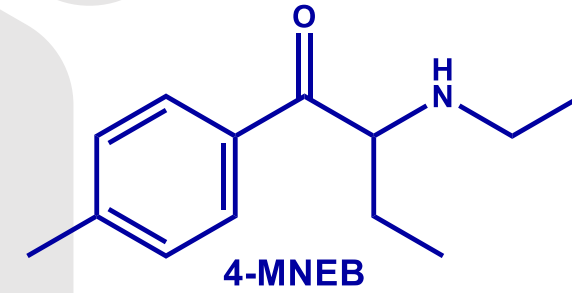
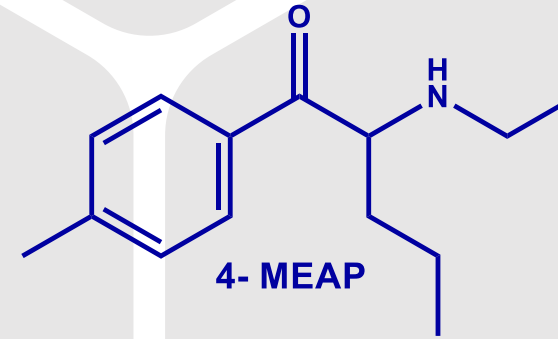
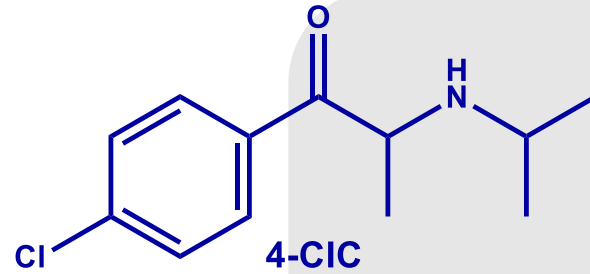
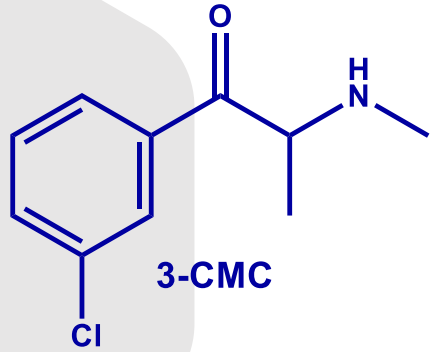
Anticipating new arrivals to elicit market

Cathinones Selected for this Study



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Cathinones Selected for this Study

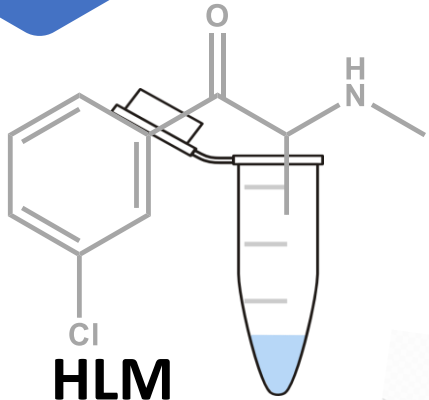


Metabolic stability
Metabolite Profile
Metabolite toxicity

GOALS



Experimental Design



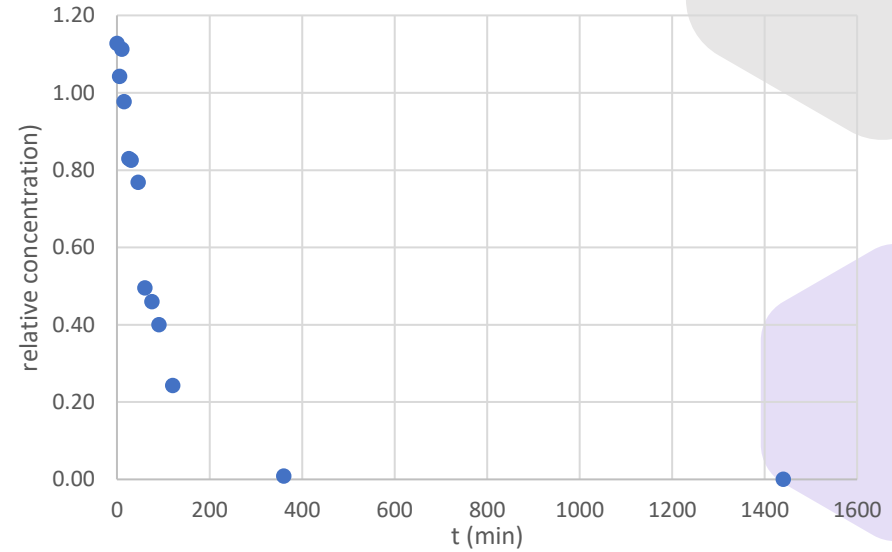
HLM
incubation



QTOF
HRMS/MS

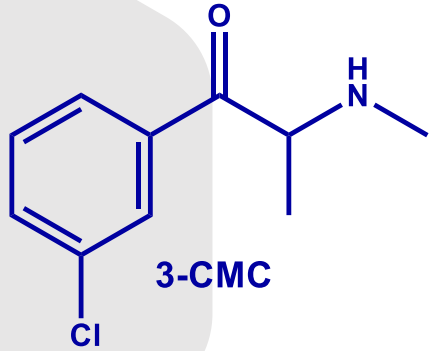


Consumption of cathinones over incubation time



Half-life & Intrinsic Clearance

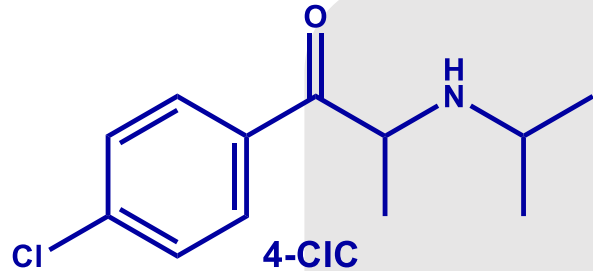
Metabolic Stability



3-CMC

$t_{1/2}$ 54 min

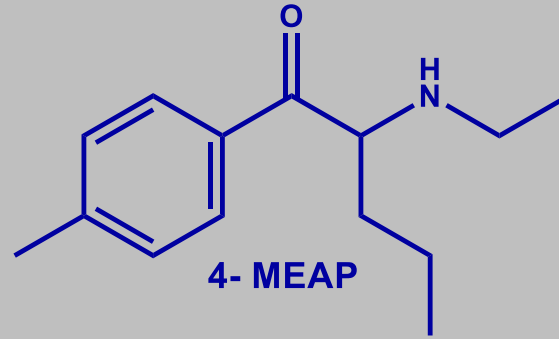
C_{int} 15 mLmin⁻¹kg⁻¹



4-CIC

$t_{1/2}$ 36 min

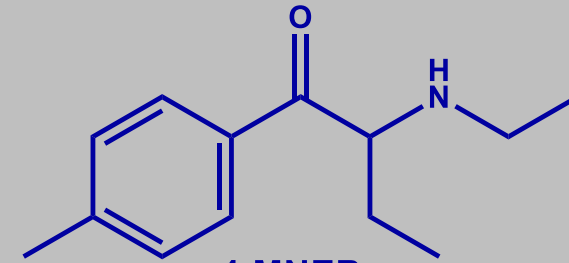
C_{int} 20 mLmin⁻¹kg⁻¹



4-MEAP

$t_{1/2}$ 16 min

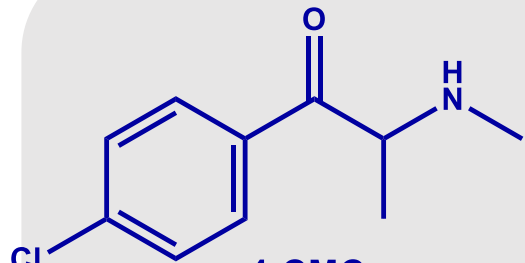
C_{int} 49 mLmin⁻¹kg⁻¹



4-MNEB

$t_{1/2}$ 32 min

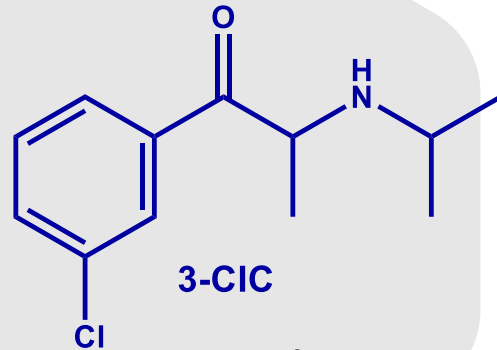
C_{int} 25 mLmin⁻¹kg⁻¹



4-CMC

$t_{1/2}$ 78 min

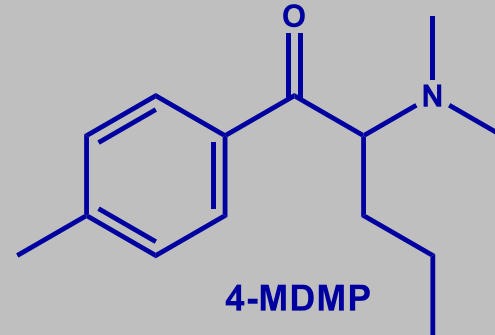
C_{int} 10 mLmin⁻¹kg⁻¹



3-CIC

$t_{1/2}$ 24 min

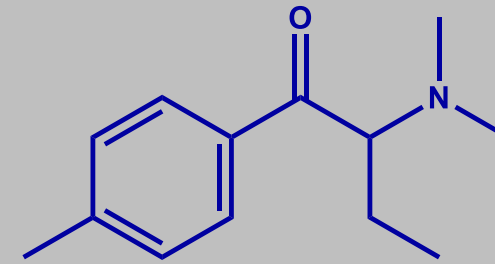
C_{int} 34 mLmin⁻¹kg⁻¹



4-MDMP

$t_{1/2}$ 23 min

C_{int} 36 mLmin⁻¹kg⁻¹



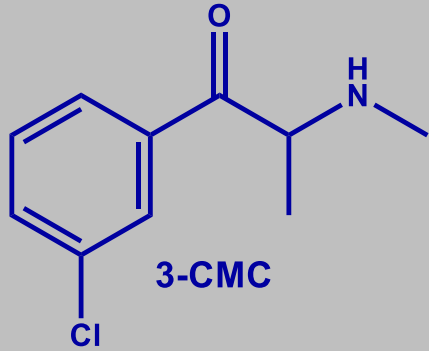
4-MDMB

$t_{1/2}$ 19 min

C_{int} 43 mLmin⁻¹kg⁻¹

Half-life & Intrinsic Clearance

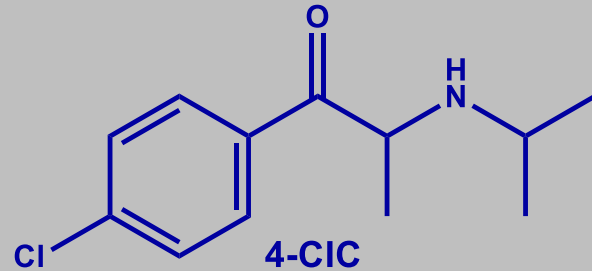
Metabolic Stability



3-CMC

$t_{1/2}$ 54 min

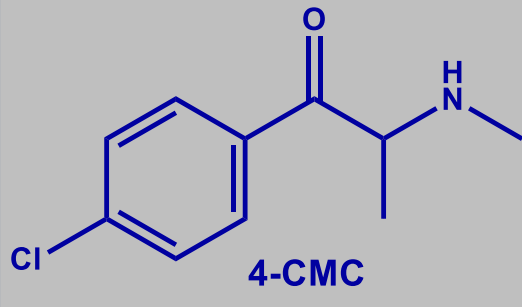
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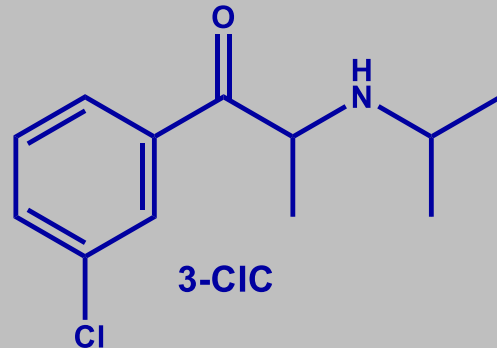
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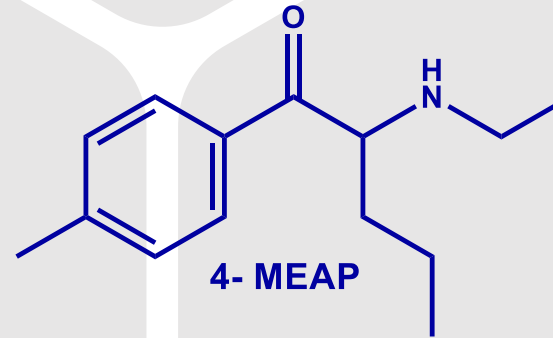
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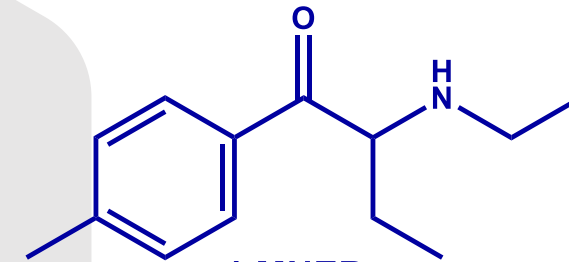
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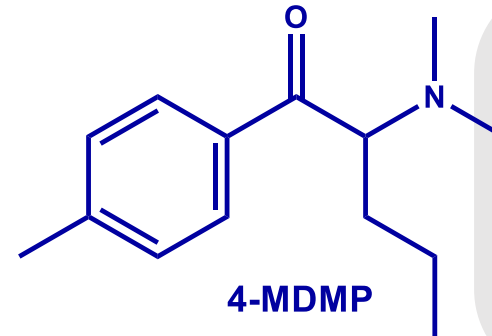
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4-MNEB

$t_{1/2}$ 32 min

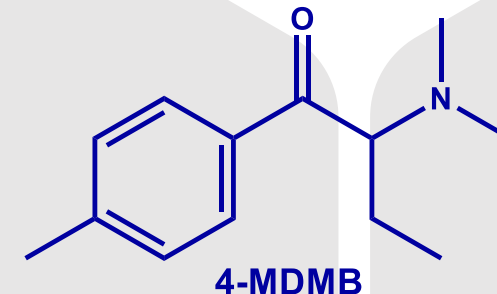
C_{int} 25 mLmin⁻¹kg⁻¹



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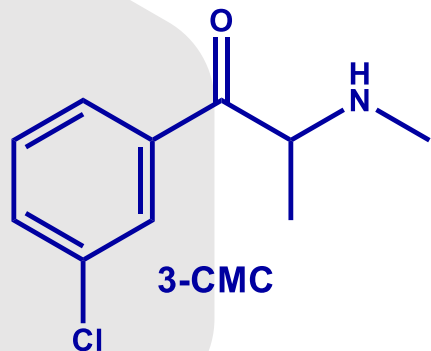
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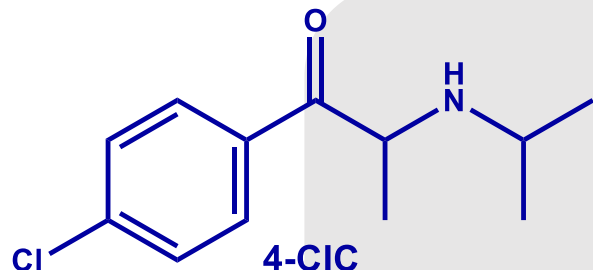
Metabolic Stability



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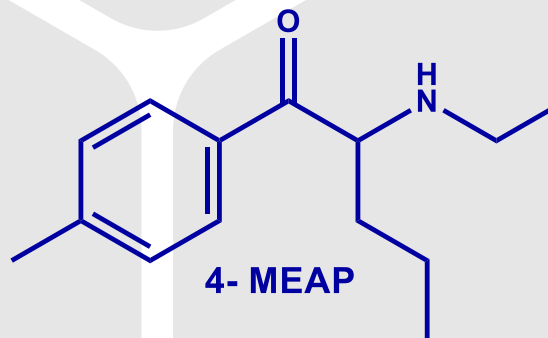
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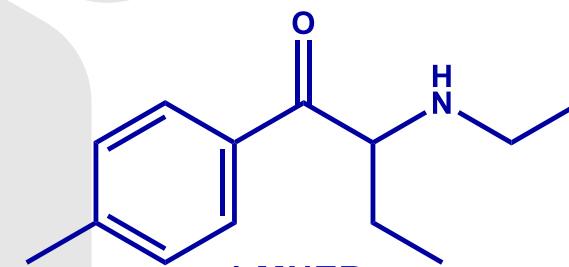
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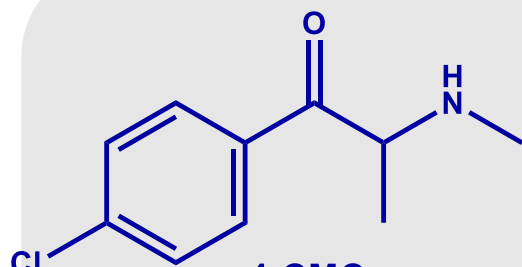
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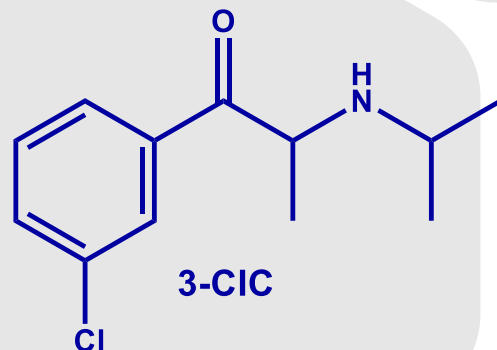
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4-CMC

$t_{1/2}$ 78 min

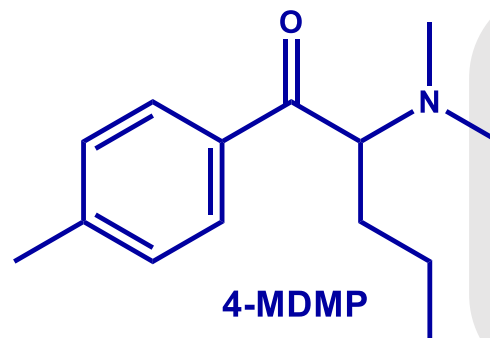
C_{int} 10 mLmin⁻¹kg⁻¹



3-CIC

$t_{1/2}$ 24 min

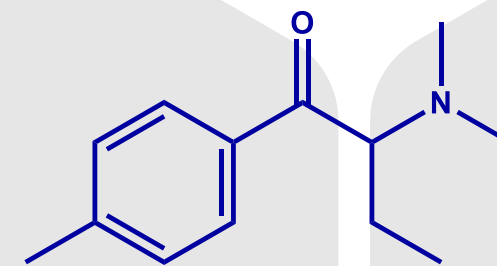
C_{int} 34 mLmin⁻¹kg⁻¹



4-MDMP

$t_{1/2}$ 23 min

C_{int} 36 mLmin⁻¹kg⁻¹



4-MDMB

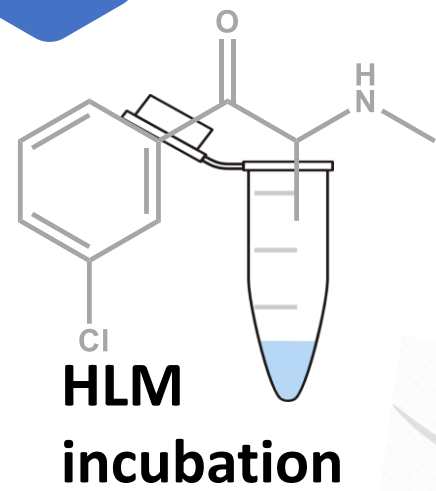
$t_{1/2}$ 19 min

C_{int} 43 mLmin⁻¹kg⁻¹

Half-life & Intrinsic Clearance



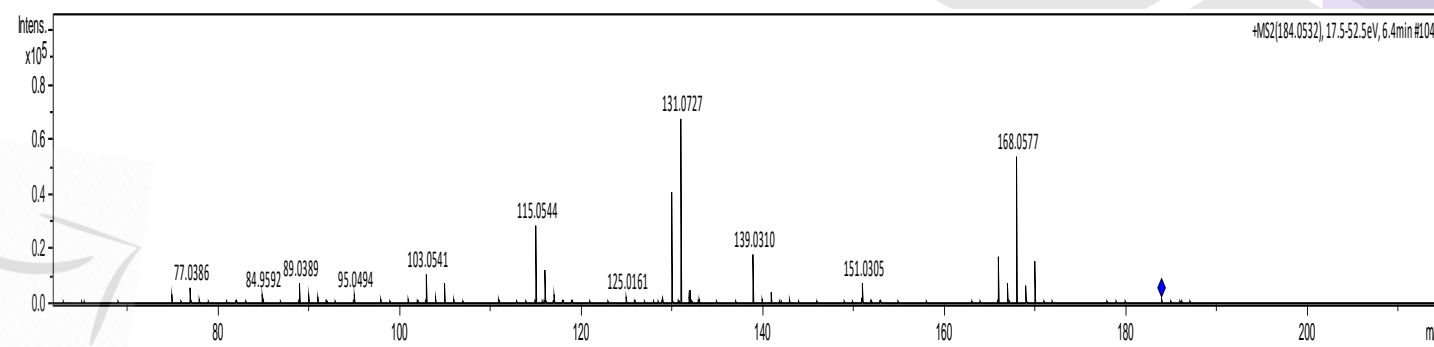
Experimental Design



QTOF
HRMS/MS

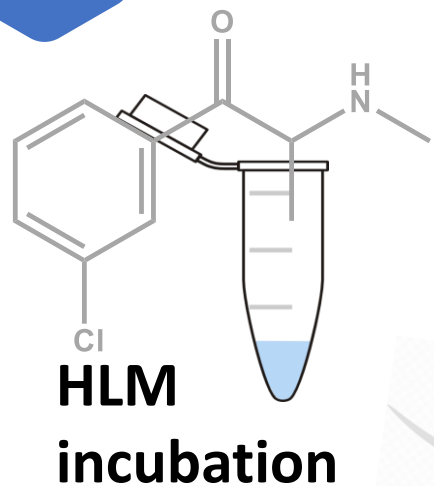
Metabolite Profile

MS/MS SPECTRA





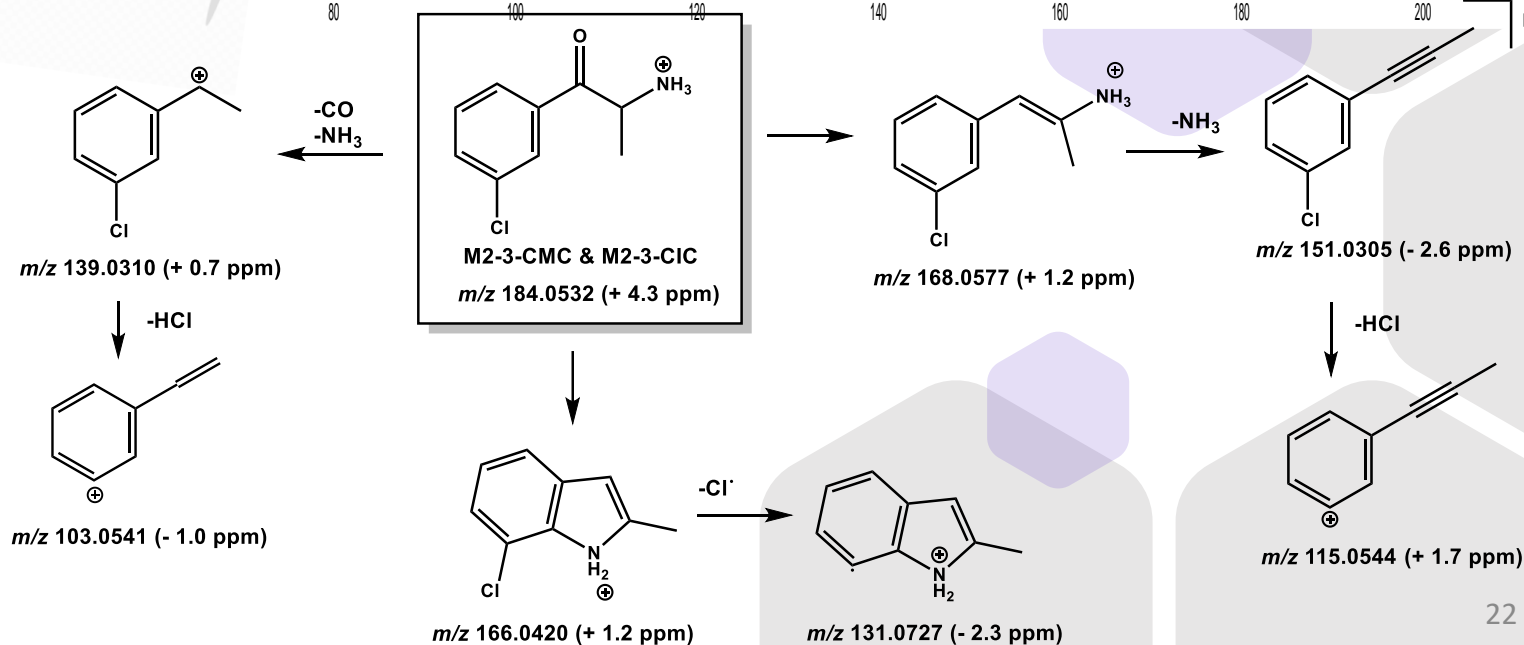
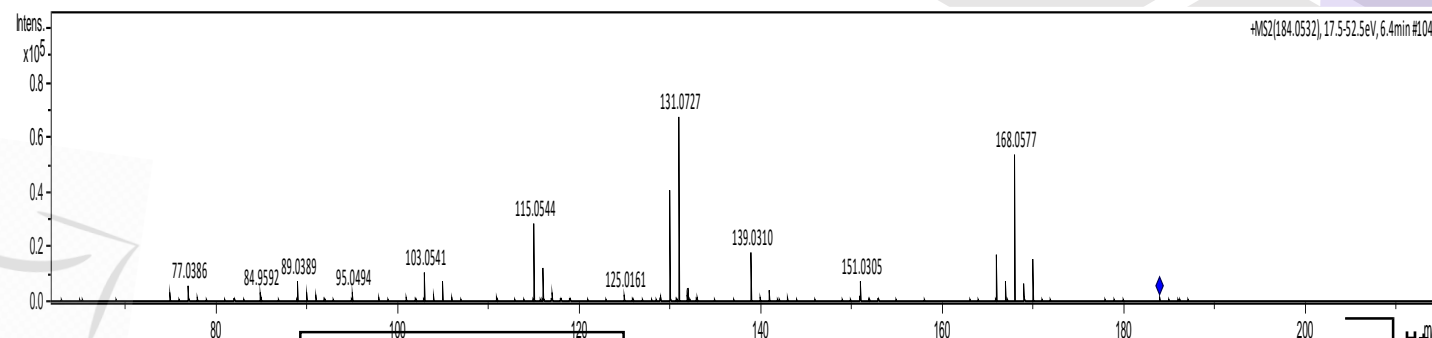
Experimental Design

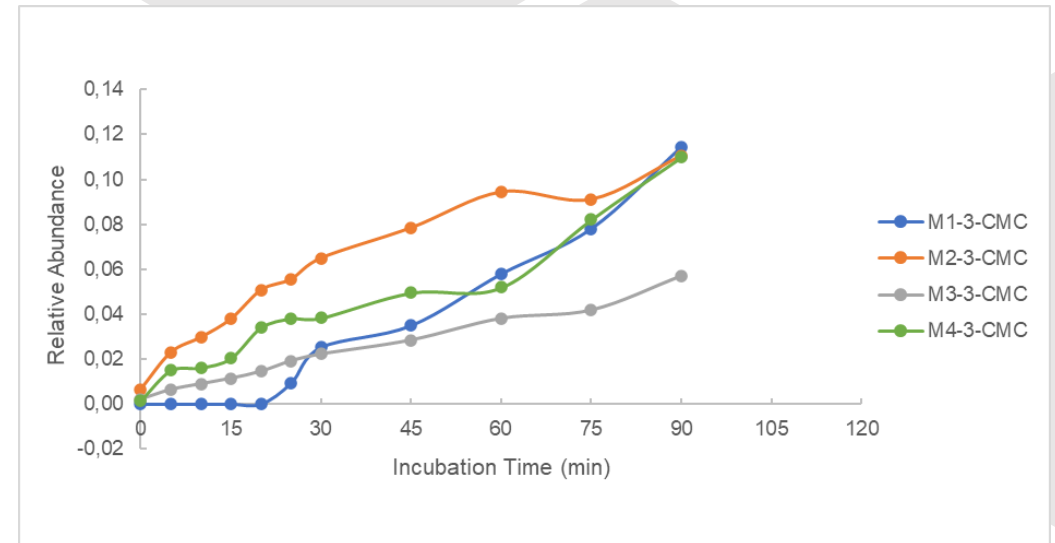
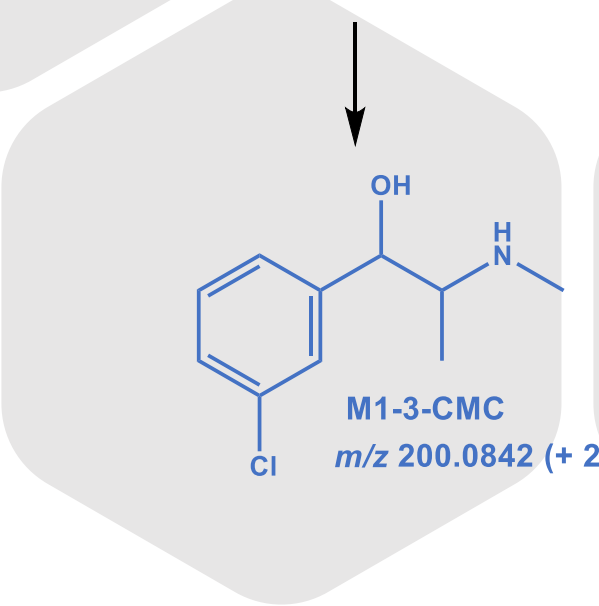
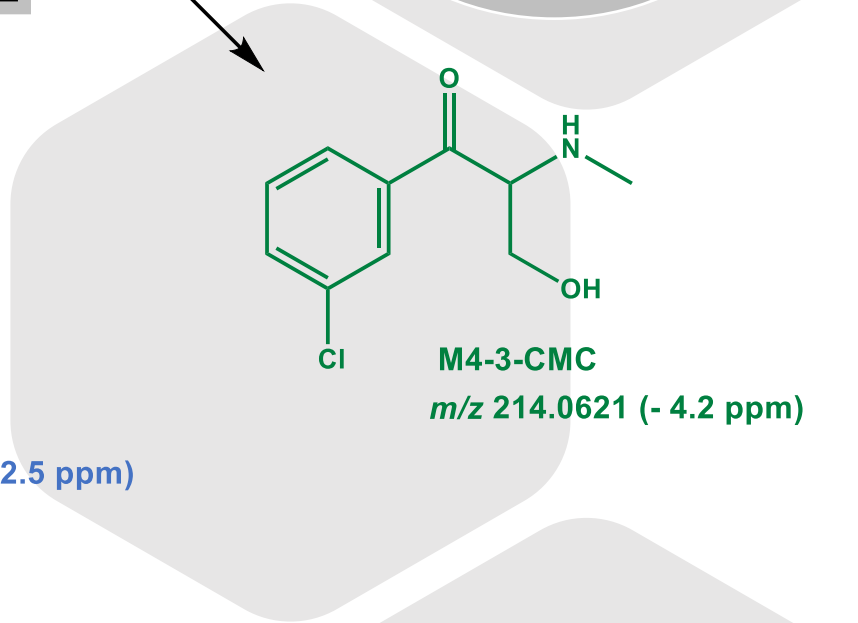
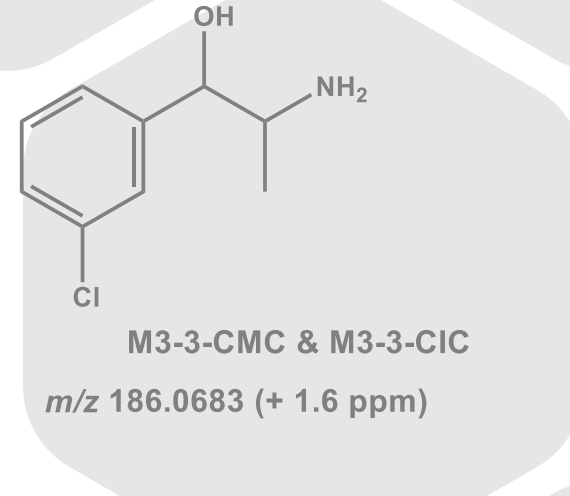
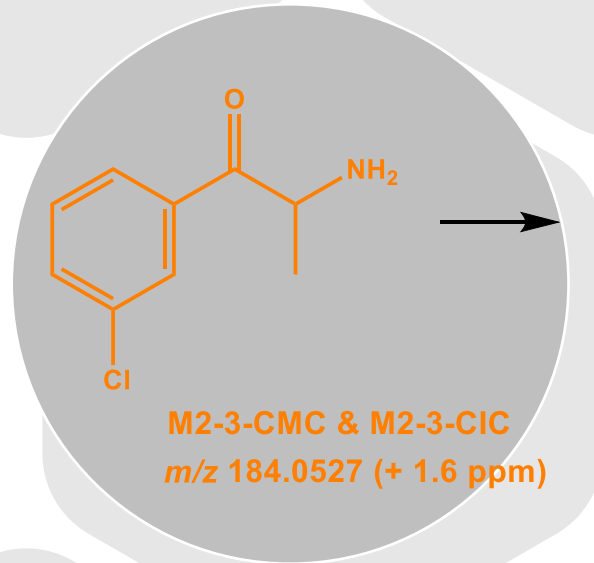
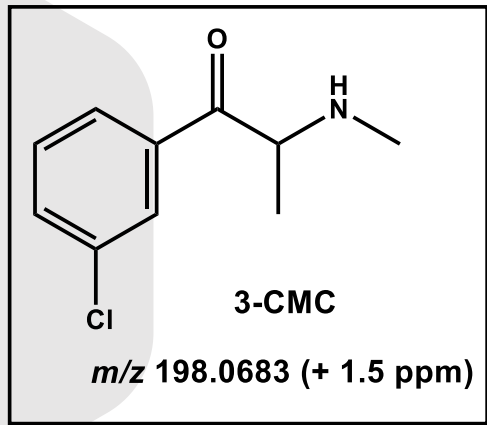


**QTOF
HRMS/MS**

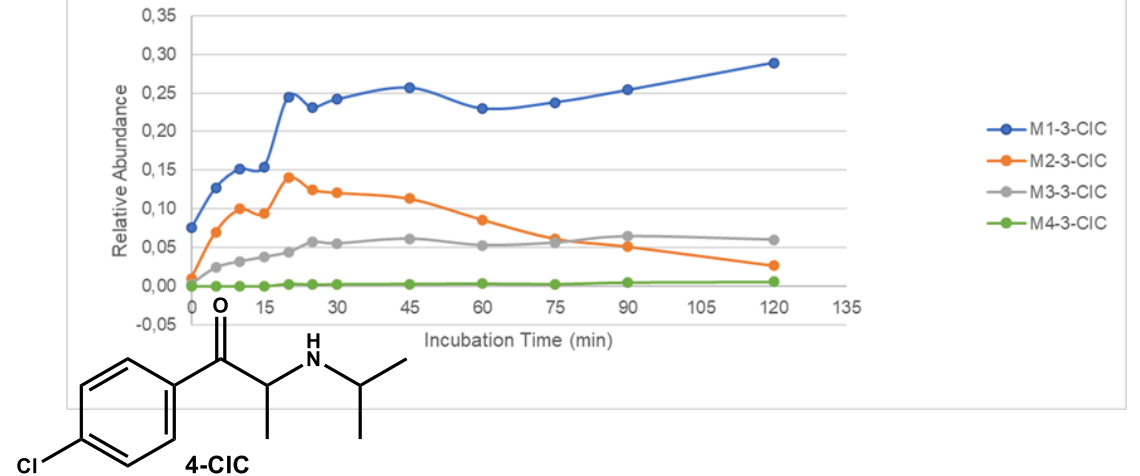
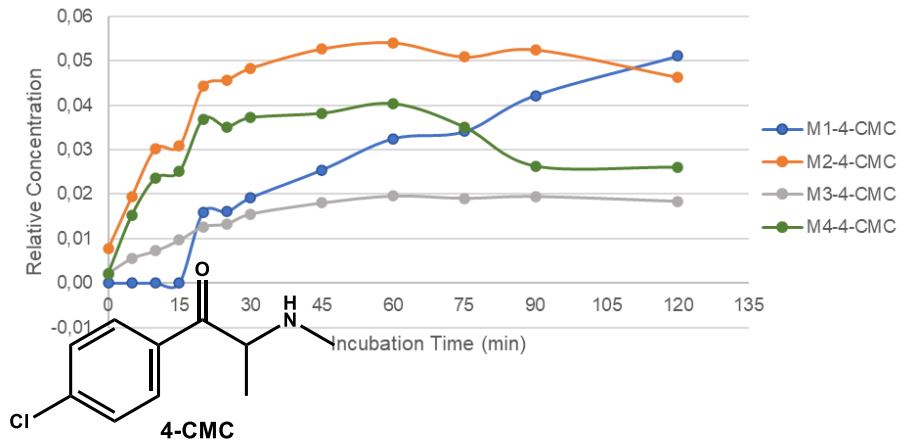
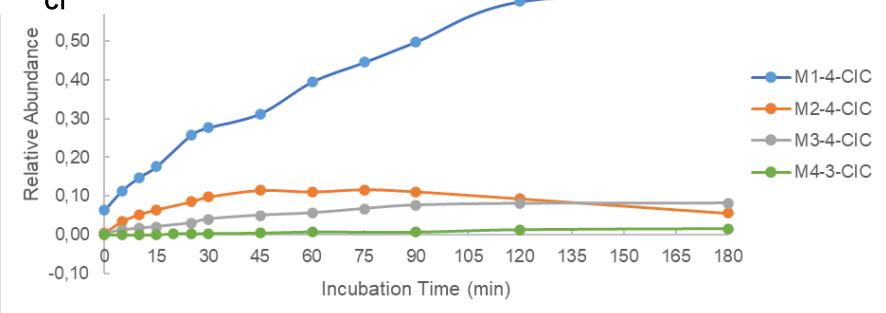
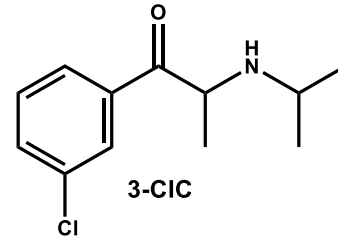
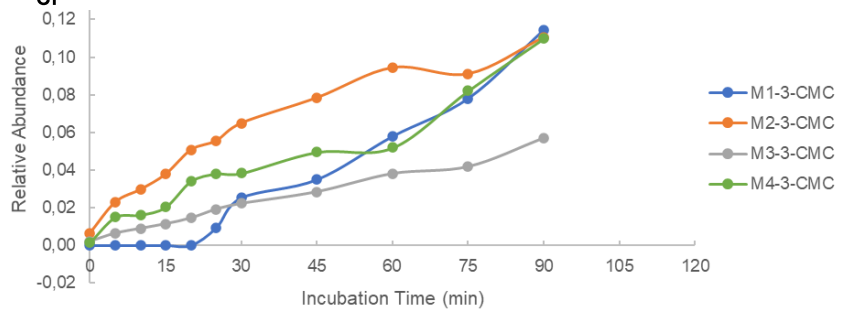
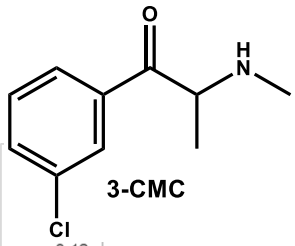
Metabolite Profile

MS/MS SPECTRA

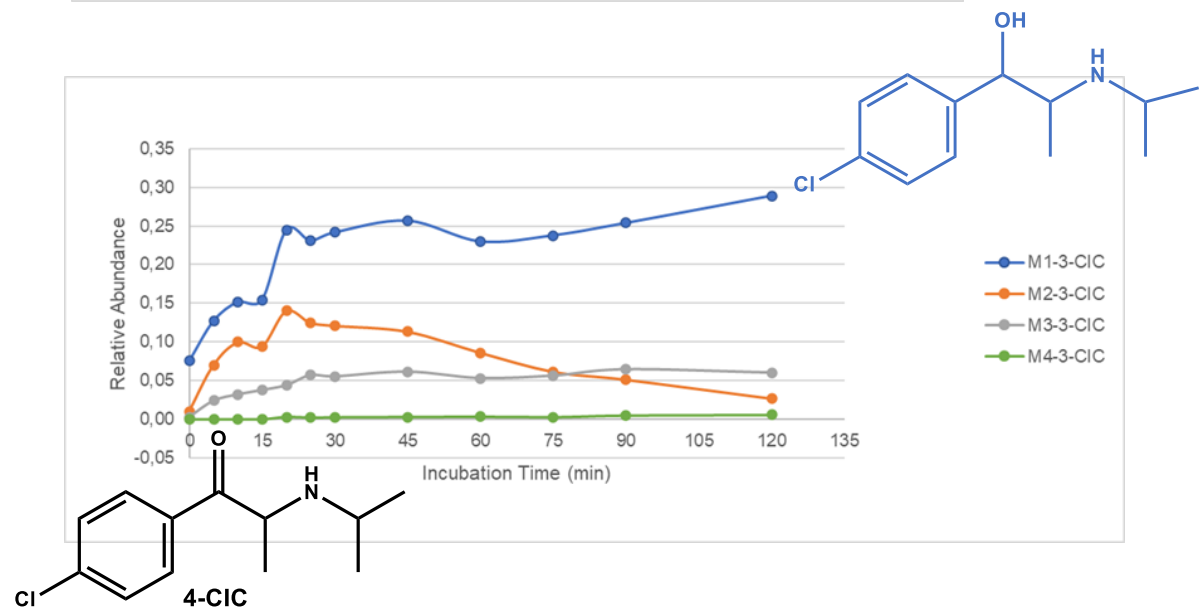
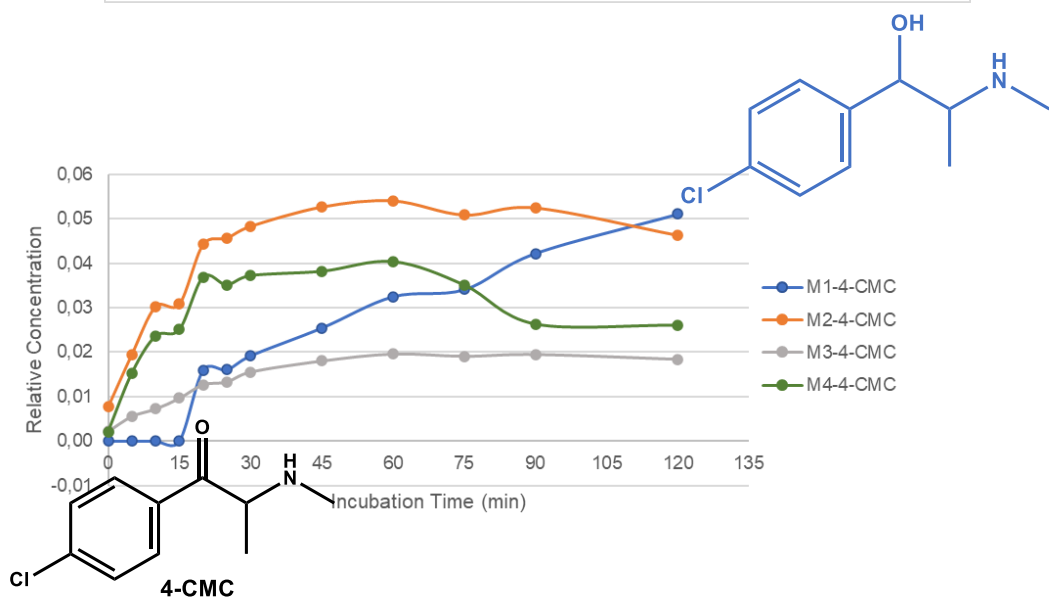
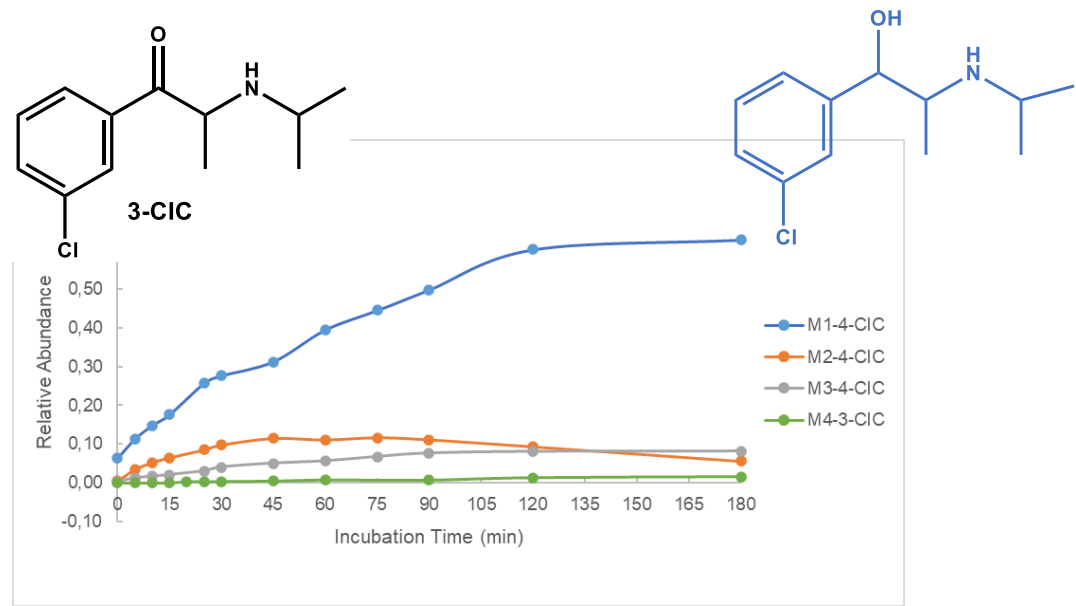
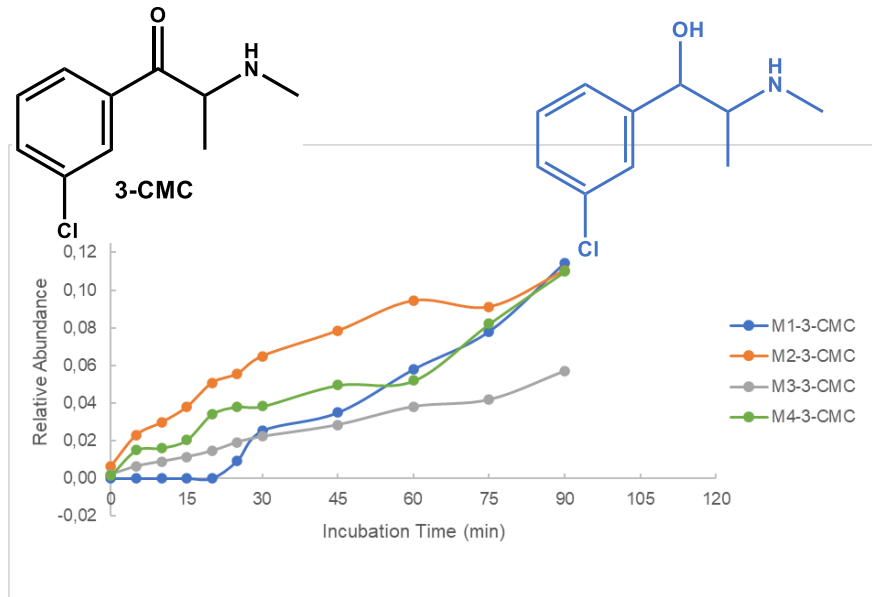




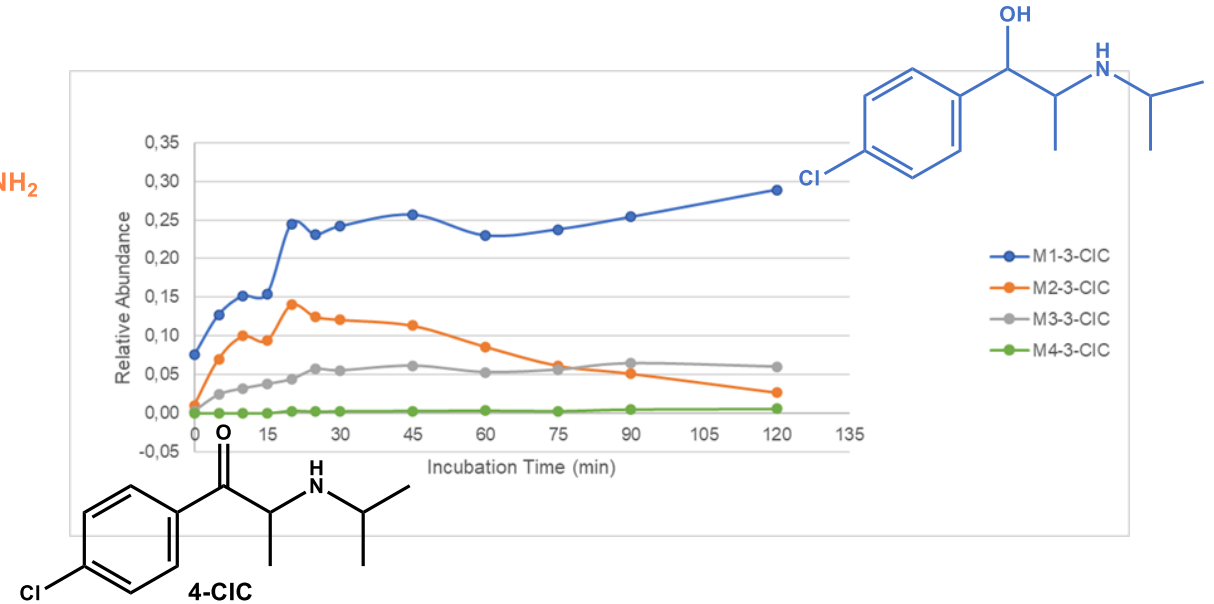
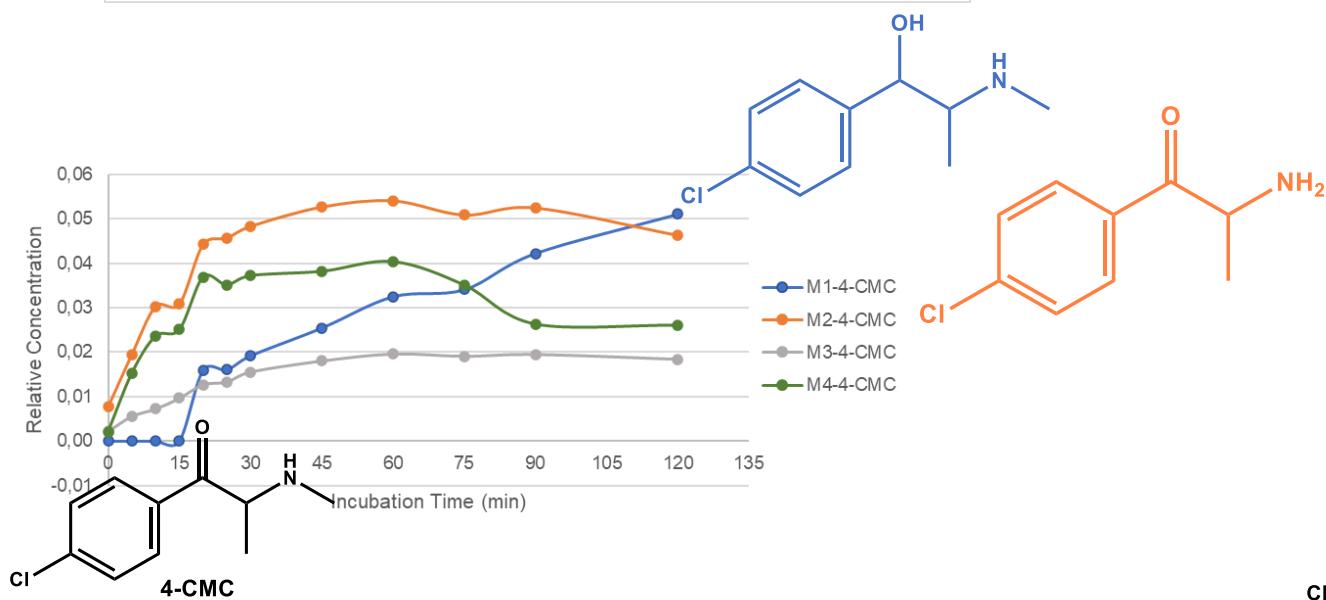
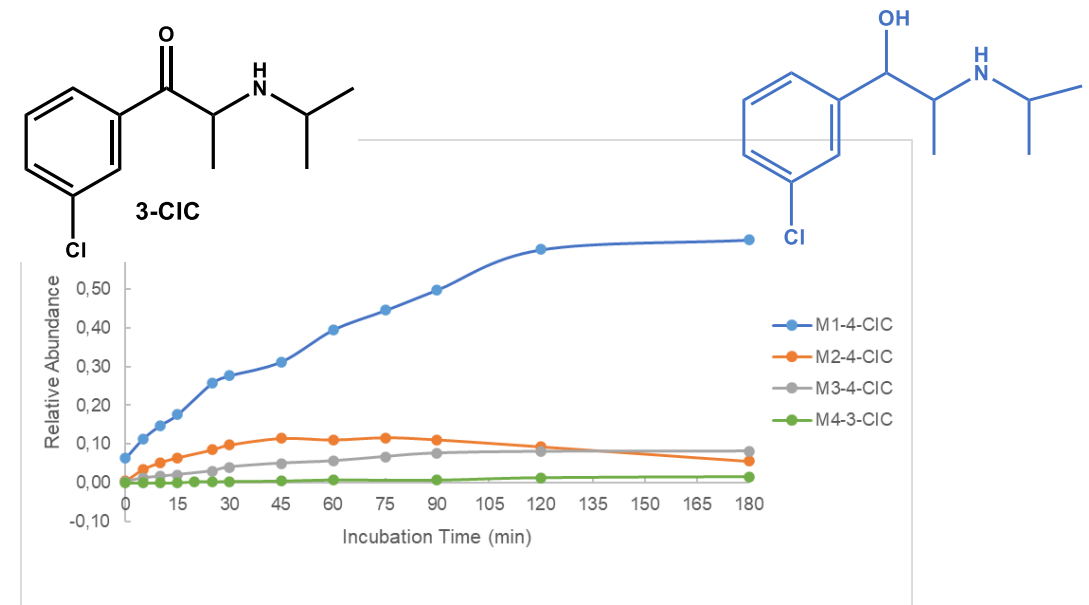
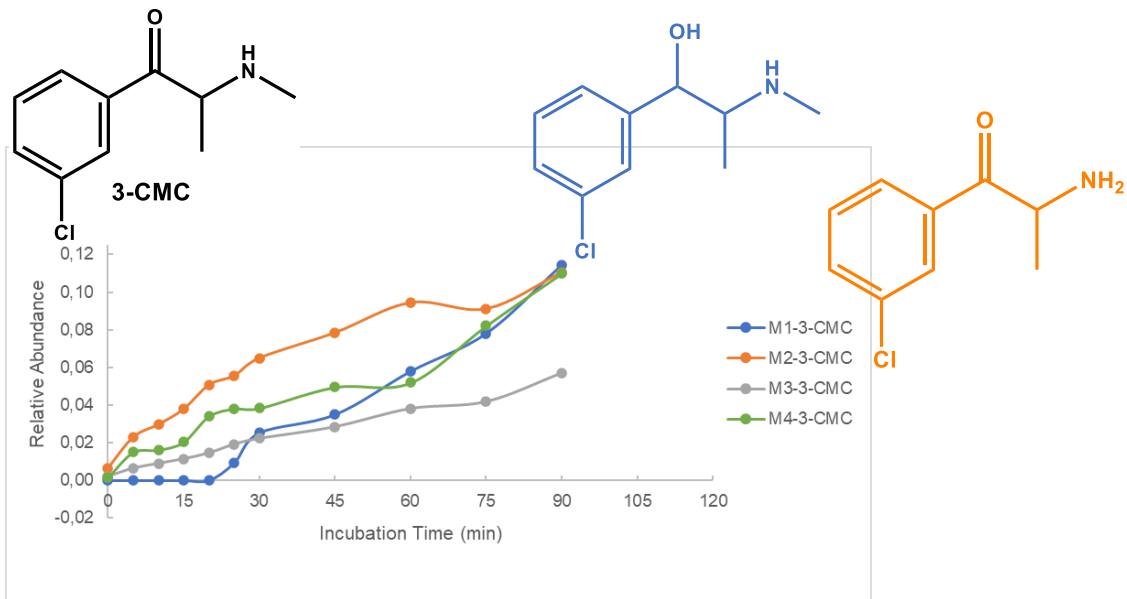
Metabolite Profile



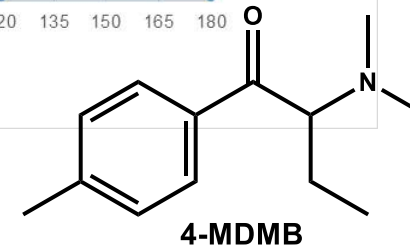
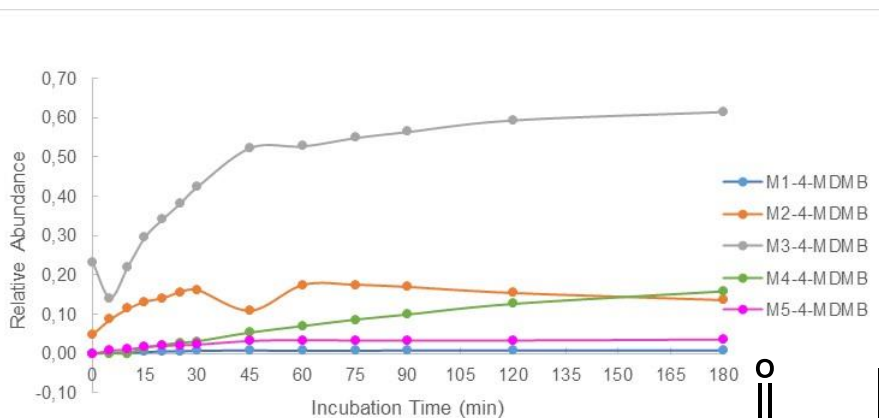
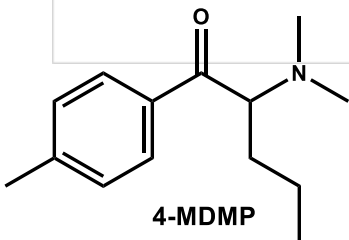
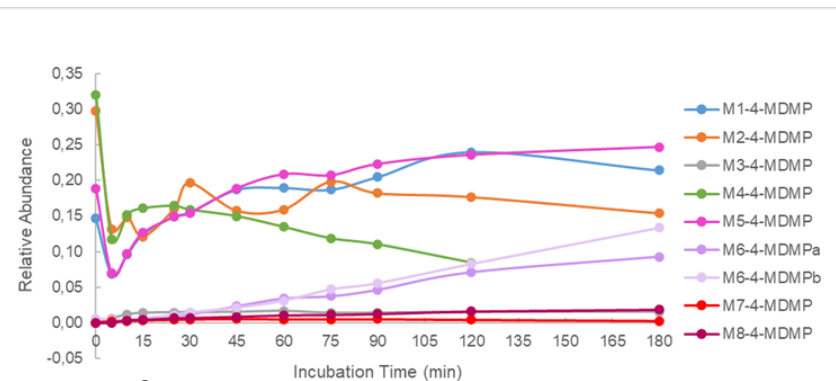
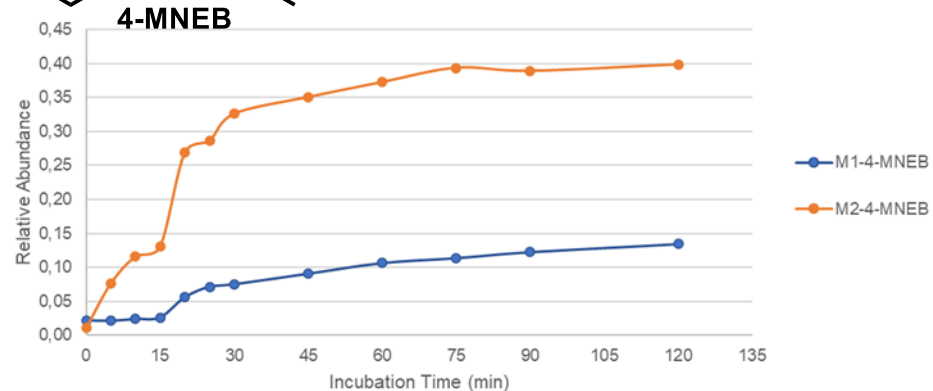
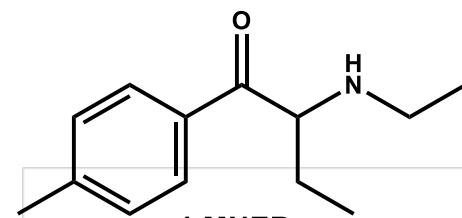
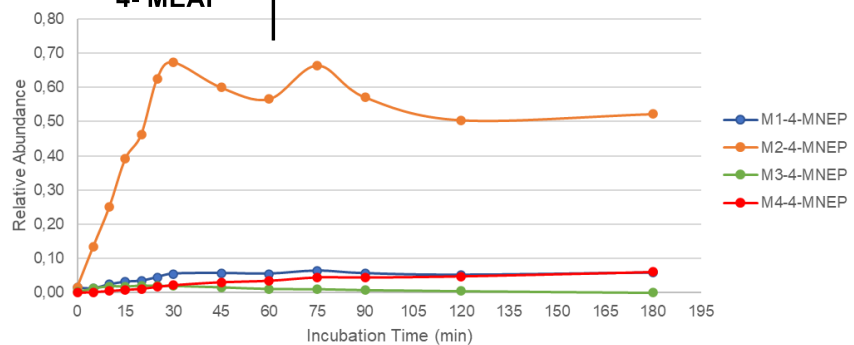
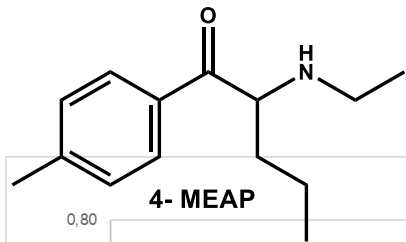
Metabolite Profile



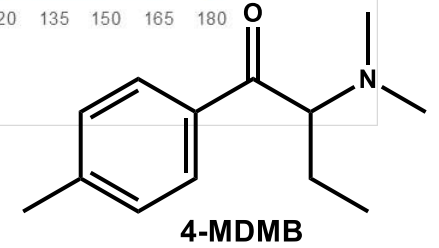
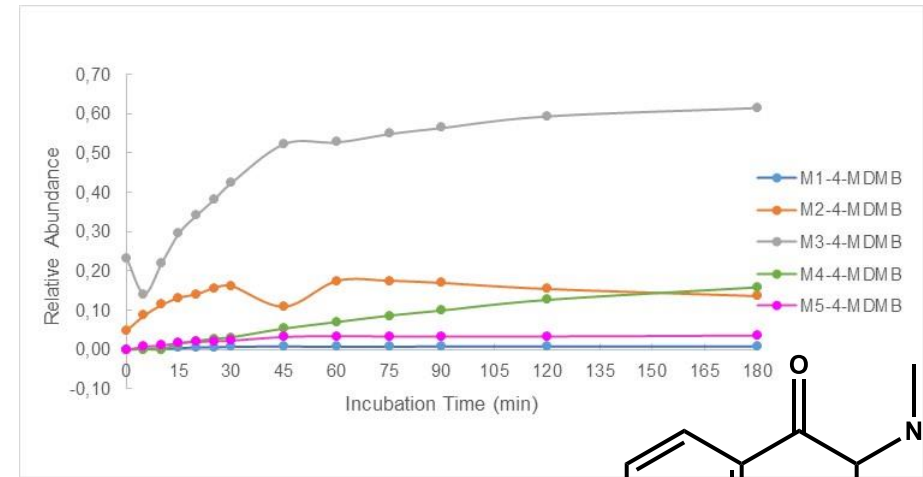
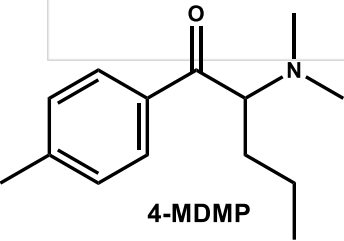
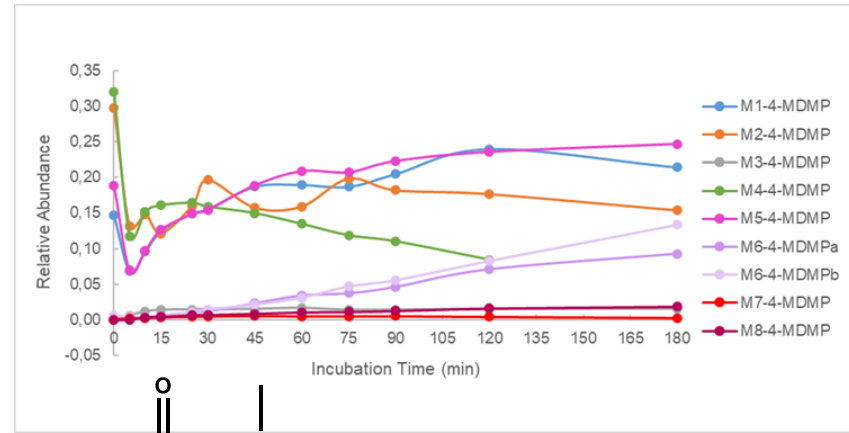
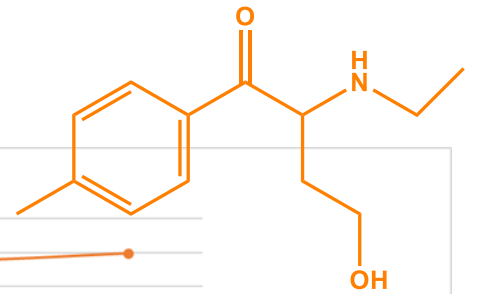
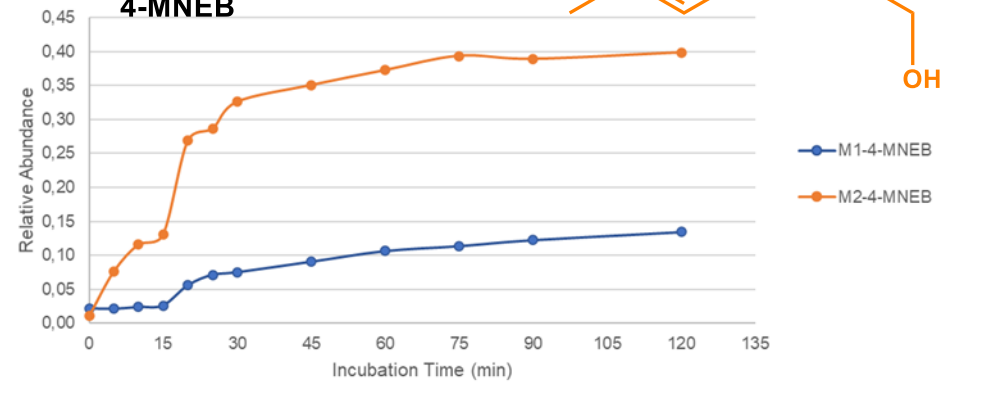
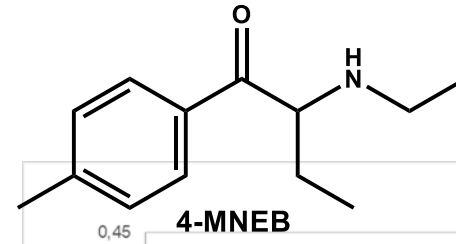
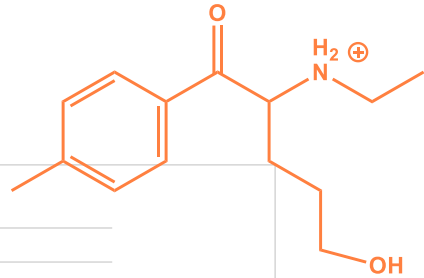
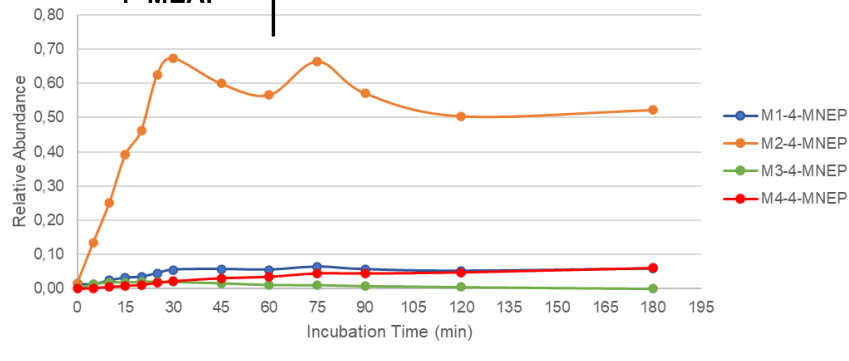
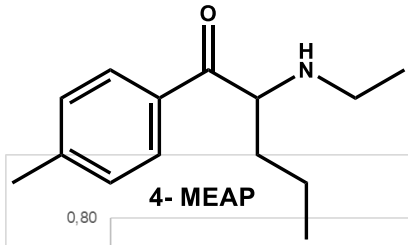
Metabolite Profile



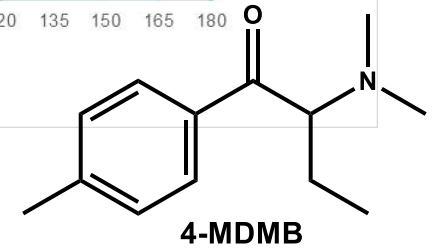
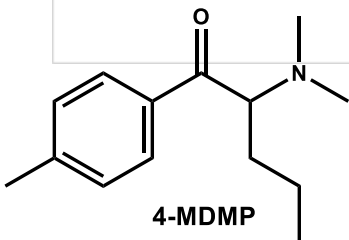
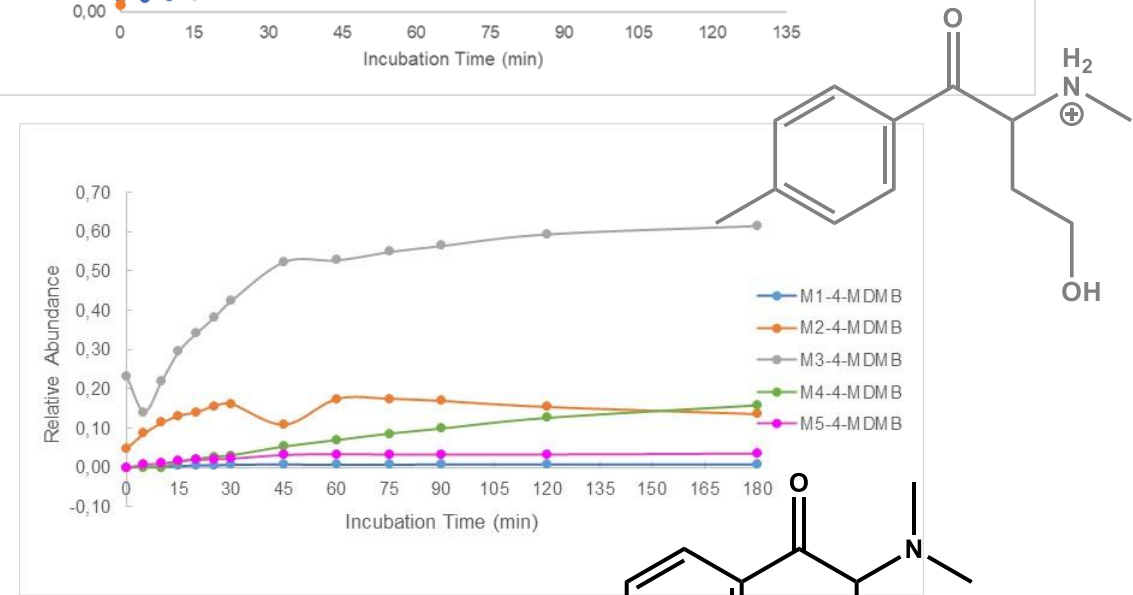
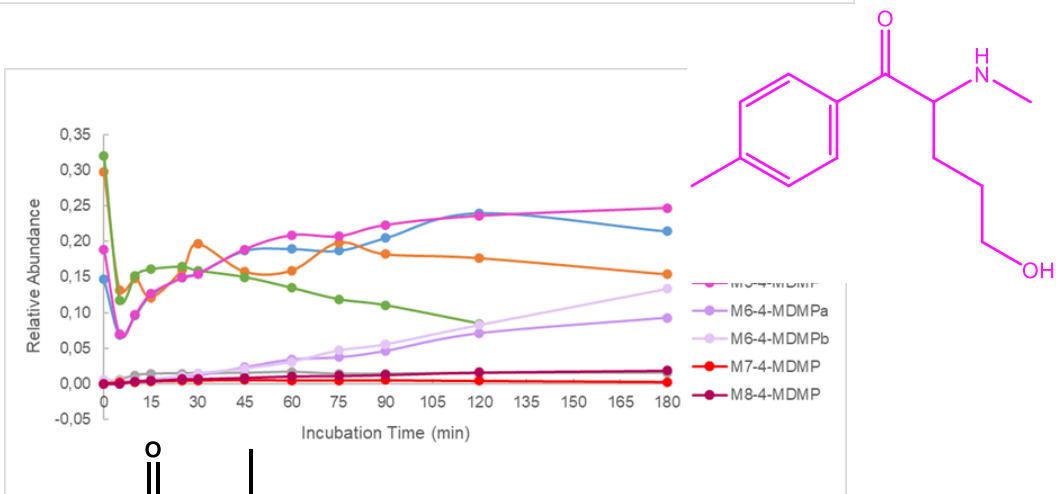
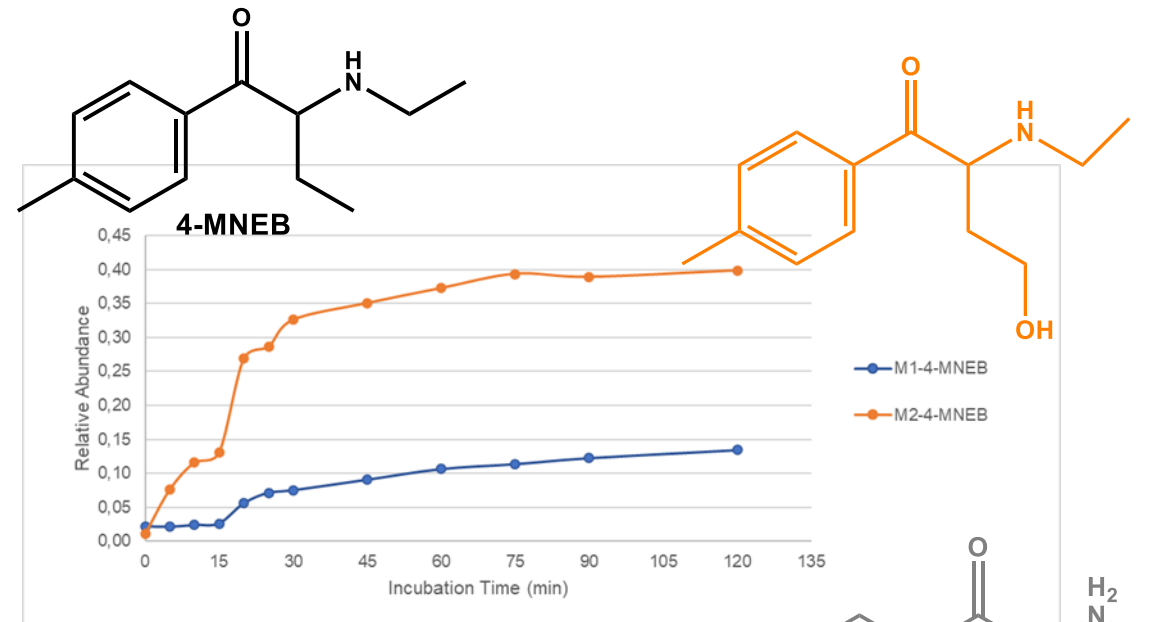
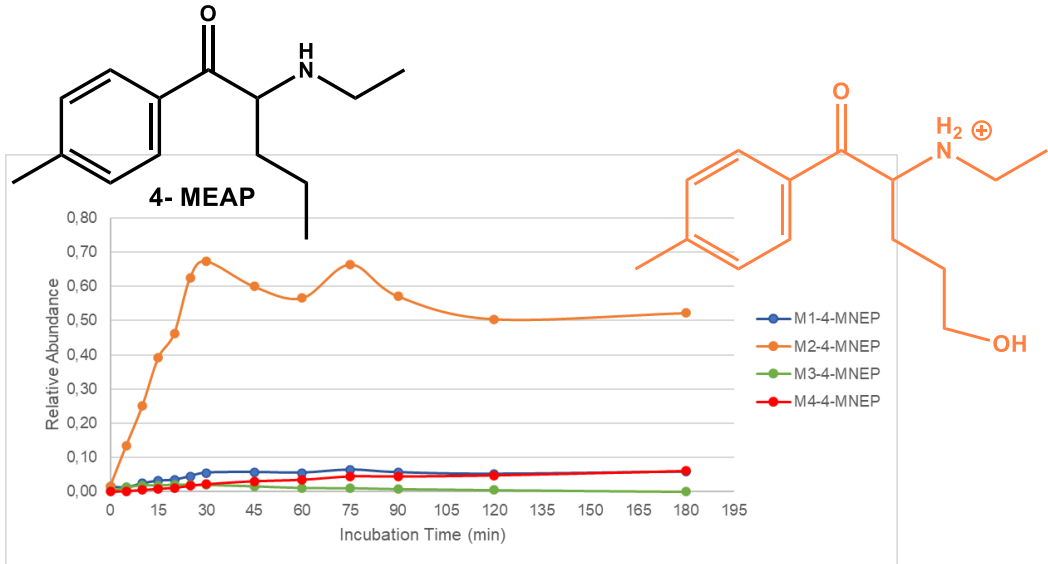
Metabolite Profile



Metabolite Profile



Metabolite Profile

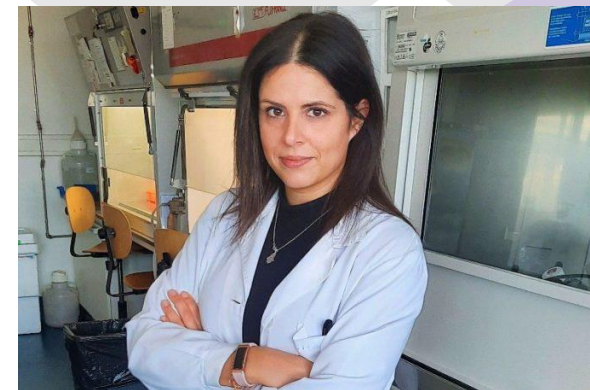


Metabolite Profile

Toxicity: Parent Drug vs Metabolite

SH-SY5Y cell lines

Cathinone	LD50 (mM)	Metabolite	LD50 (mM)
A	0.98	a	0.50
B	2.83	b	2.17
C	3.01	c	2.23



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Institute for Bioengineering and Biosciences





Conclusions

Selected cathinones displayed intermediate clearance: the key role of metabolites as suitable biomarkers to extend detection windows beyond those provided by parent cathinone





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Selected cathinones displayed intermediate clearance: the key role of metabolites as suitable biomarkers to extend detection windows beyond those provided by parent cathinone

The main Phase I metabolites of the 8 selected cathinones were characterized by HRMS: standards and analytical methods developed are now available

Metabolites were synthesized and tested in SH-SY5Y cell lines: more toxic than the parent drug

Acknowledgments



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Margarida Figueira
Rita Lopes



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Susana Santos



Maria João Caldeira



€€€€

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LA/P/0056/2020 (to IMS),
UIDP/04046/2020 (to BioISI)
UIDB/04292/2020 and
UIDP/04292/2020 (to MARE)
PTDC/QUI-QAN/32242/2017

Joint funding from FCT and the COMPETE Program through grant RNEM-LISBOA-01-0145-FEDER-022125 funding are also gratefully acknowledged.

Thank you!

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