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Metabolism of cathinones in functional hepatocyte-like cells derived from human neonatal mesenchymal stem cells: an enantioselectivity approach

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Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

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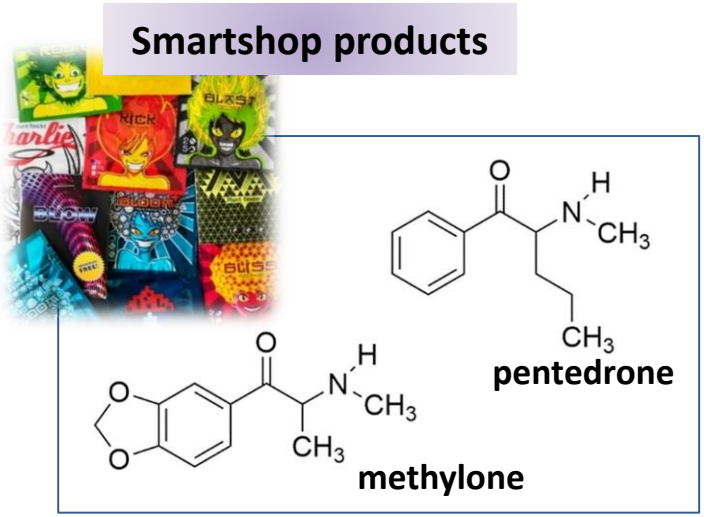
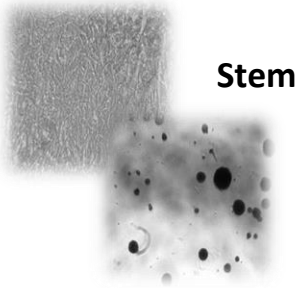
Metabolism of cathinones in functional hepatocyte-like cells derived from human neonatal mesenchymal stem cells: an enantioselectivity approach

Graphical Abstract



Chiral resolution by liquid chromatography

Enantioselectivity on toxicity/metabolism



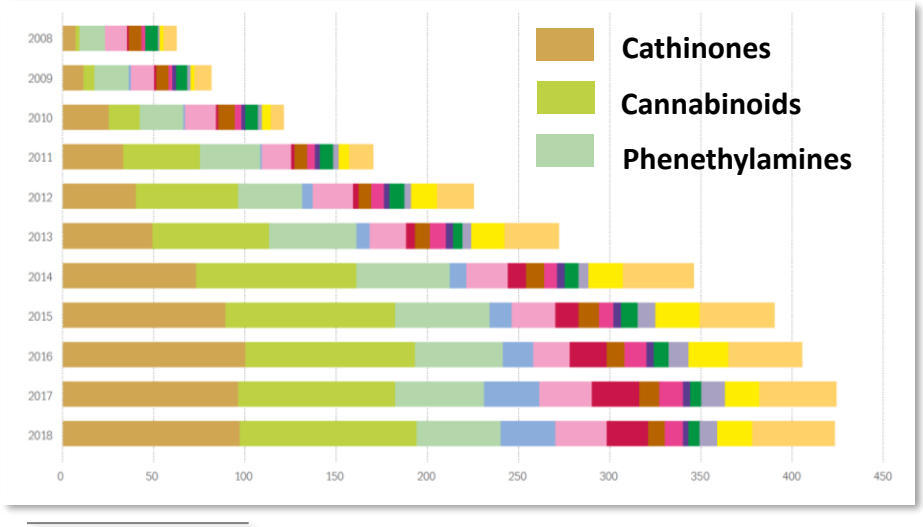
Stem cell-derived hepatocyte-like cells (HLCs)

Abstract

Liver damage is a common issue of synthetic cathinones abuse. Human stem cell-derived hepatocyte-like cells (HLCs) has been suggested to hepatotoxicity studies, by their ability to maintain hepatic-specific phenotype. Furthermore, all cathinone derivatives are chiral, and their biological effects can differ for each enantiomer. Thus, the aim of this work was to evaluate the cytotoxicity and metabolism of pentedrone and methylone enantiomers using HLCs models. Human neonatal mesenchymal stem cells were differentiated into HLCs by a three-step differentiation protocol and maintained under 2D and 3D culture conditions. Subsequently, pentedrone and methylone enantiomers were isolated by HPLC using a chiral stationary phase. Cell viability was evaluated through CellTiter-Glo assay and the formation of methylone and pentedrone metabolites was analysed by GS-MS. Racemates of pentedrone and methylone exhibited potential hepatotoxic in a concentration-dependent manner in both models. It was also observed a different cytotoxic profile for pentedrone enantiomers in HLCs 3D, being R-(-)-pentedrone the most cytotoxic. Concerning HLCs 2D metabolic assays, *S*-(-)-methylone was preferentially metabolized via N-demethylation, whereas the *R*-(+)-methylone by *O*-demethylation and *N*-hydroxylation. Although, in HLCs 3D, *R*-(+)-methylone was preferential metabolized by all metabolic pathways, except for O-demethylation. Regarding pentedrone enantiomers, metabolic pathways studied were more pronounced for *R*-(-)-pentedrone, namely *N*-demethylation and β-keto reduction in both models. Overall, this study revealed stereoselectivity on cytotoxicity and metabolism pathways for pentedrone and methylone.

Keywords: Enantiomers; HLCs; metabolism; methylone; pentedrone.

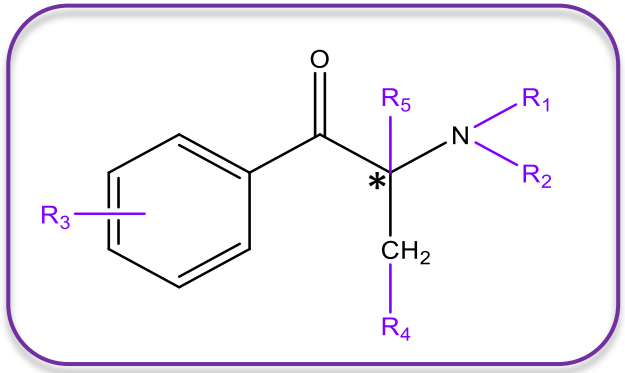
Introduction



European Drug Report 2020. EMCDDA

Sold as 'bath salts' or 'plant feeders'

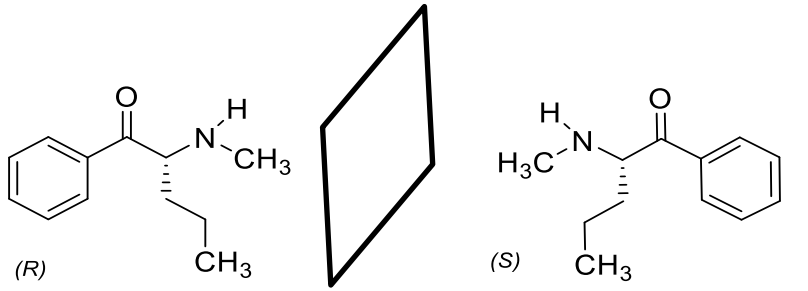
General chemical structure of synthetic cathinones



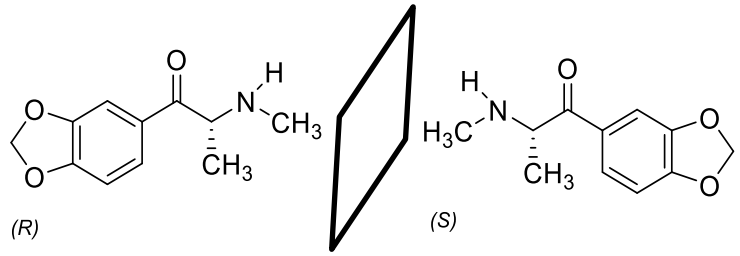
A.S. Almeida, B. Silva, P.G. Pinho, F. Remião, C. Fernandes, Molecules 2022, 27(7), 2057

Introduction

Pentedrone enantiomers



Methylone enantiomers



Pharmacological and toxic activity

Chiral discriminations

Metabolism stereoselectivity:

- different rates and/or different paths

Interaction with biological molecules
(plasmatic proteins, membranal transporters, metabolic enzymes or cellular receptors)

Different toxicokinetic properties and toxicological activities

B. Silva, C. Fernandes, P. Guedes-de-Pinho, F. Remião, J. Anal. Toxicol., 2018, 42(1), 17-24.

Results

Pentedrone and methylone isolation

Semi-preparative enantioresolution and determination of absolute configuration of pentedrone and methylone enantiomers

Smartshop products

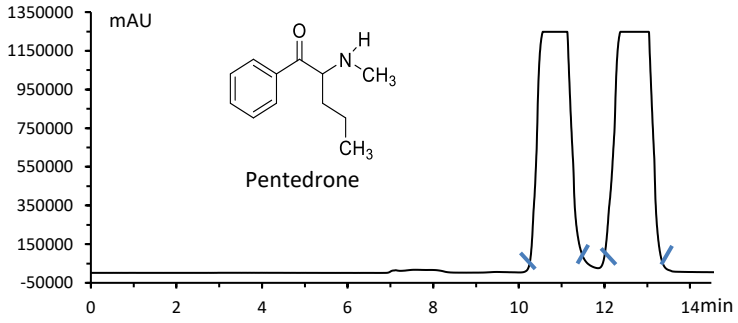
Chiral resolution by liquid chromatography

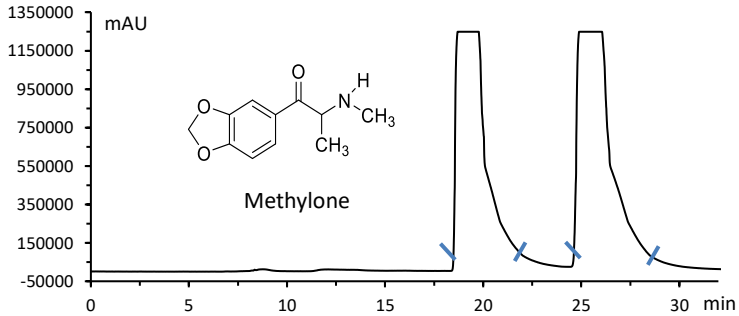
B. Silva, J.A. Pereira, S. Cravo, A.M. Araújo, C. Fernandes, M.M.M. Pinto, P. G. Pinho, F. Remião, J. Chromatogr. B, 2018, 1100-1101, 158-164.

Results

Pentedrone and methylone isolation

Chiral column: Chiralpak® AS-H
Mobile phase: Hex:2-PrOH
Flow rate: 2 mL/min
UV detection: 254 nm





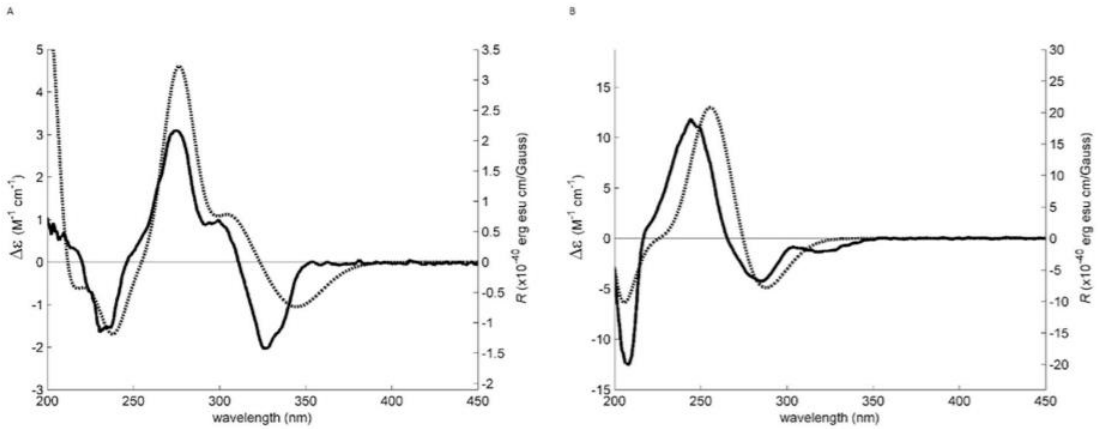
Elution order, specific rotation and enantiomeric ratios of pentedrone and methylone enantiomers at 25 °C.

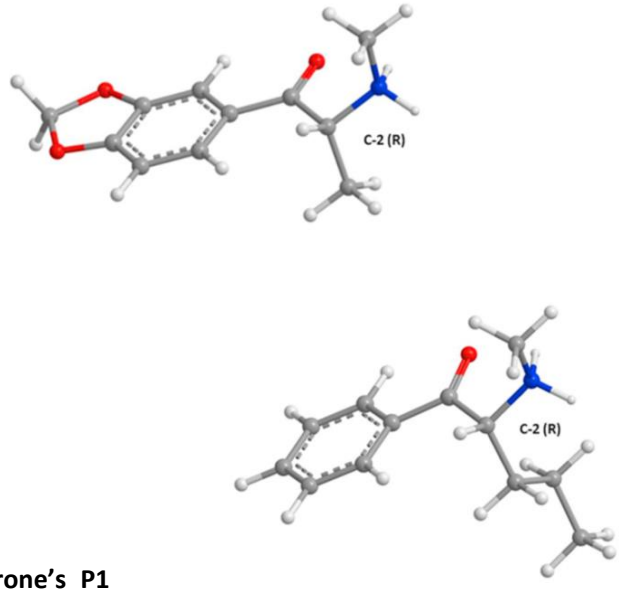
Enantiomer	Elution order	e.r. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(+)-pentedrone	First	98.4	+16 (2.5)	72
R-(−)-pentedrone	Second	97.8	−12 (2.5)	71
S-(−)-methylone	First	98.3	−20 (2.5)	80
R-(+)-methylone	Second	97.1	+24 (2.5)	79

[a] Specific rotation in EtOH (degrees mL/mg/dm) with c = concentration in mg/mL.

Results

Pentedrone and methylone isolation





Experimental ECD spectra (solid lines) of (A) methylone's M1 fraction and (B) pentedrone's P1 fraction, and simulated ECD spectra (dotted lines) of (A) methylone's C-2(S) and (B) pentedrone's C-2(S) model configurations, both in ethanol.

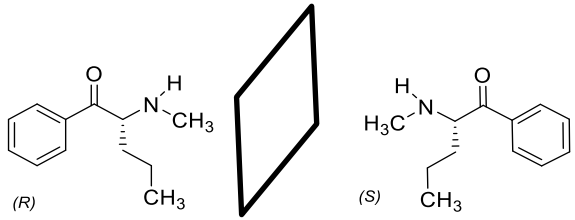
The absolute configuration of the enantiomers of both cathinones was determined as **(+)-(*S*) and (−)-(*R*)-pentedrone**, and **(−)-(*S*) and (+)-(*R*)-methylone**.

Results

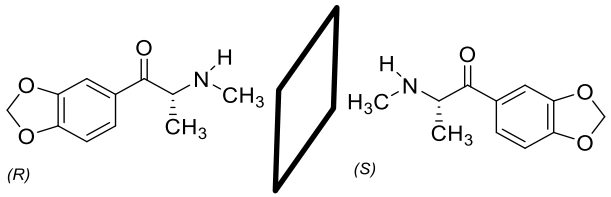
Pentedrone and methylone metabolism

Metabolic profiling of pentedrone and methylone enantiomers in 2D and 3D human hepatocyte-like cells

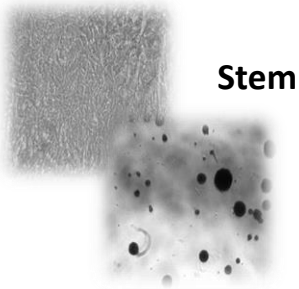
Pentedrone enantiomers



Methylone enantiomers



Enantioselectivity on toxicity/metabolism

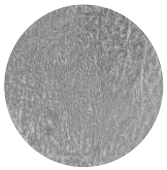


Stem cell-derived hepatocyte-like cells (HLCs)

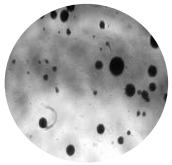
B. Silva, J.S. Rodrigues, J.P. Miranda, A.S. Almeida, A.R. Lima, C. Fernandes, P.G. Pinho, F. Remião, Pharmaceuticals, 2022, 15(3), 368.

Methods

Stem cell-derived hepatocyte-like cells (HLCs)



2D



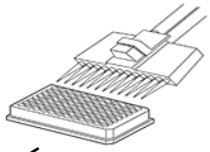
3D

racemate [0-20 mM]

enantiomers [0.5 and 1 mM]

24h

CellTiter-Glo®



Luminescence

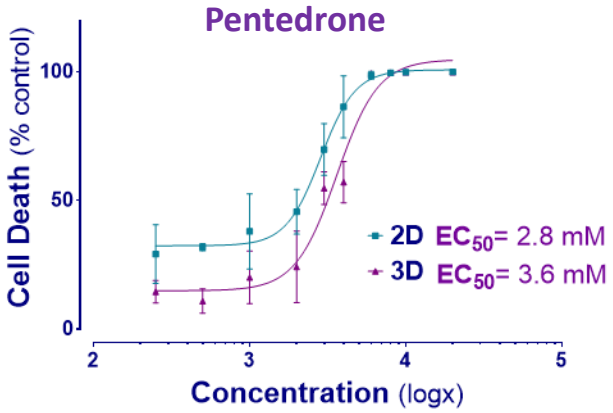
[1mM] of each enantiomer

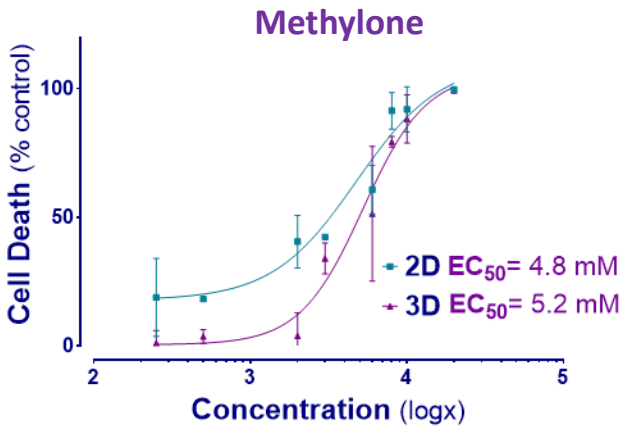
6h: scrapped with ice-cold acetonitrile

GC-MS

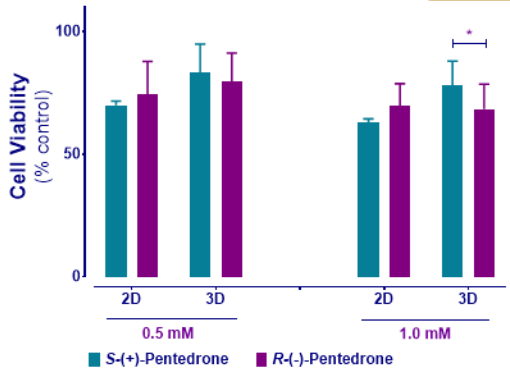
Results

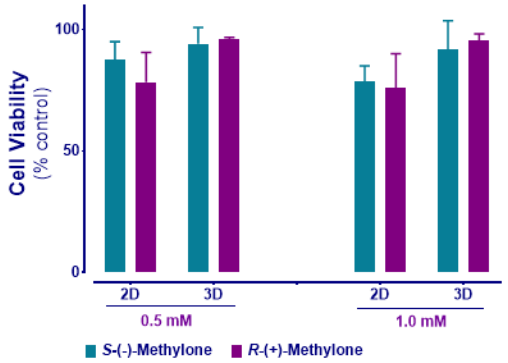
Racemate cytotoxicity





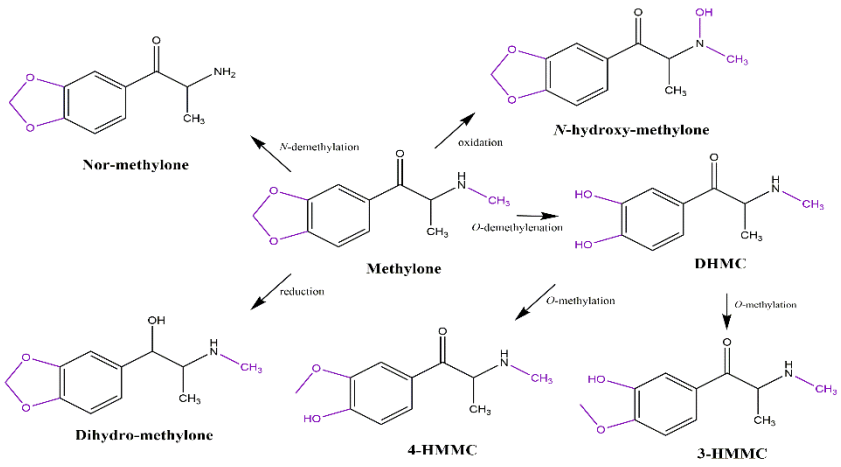
Enantiomer cytotoxicity



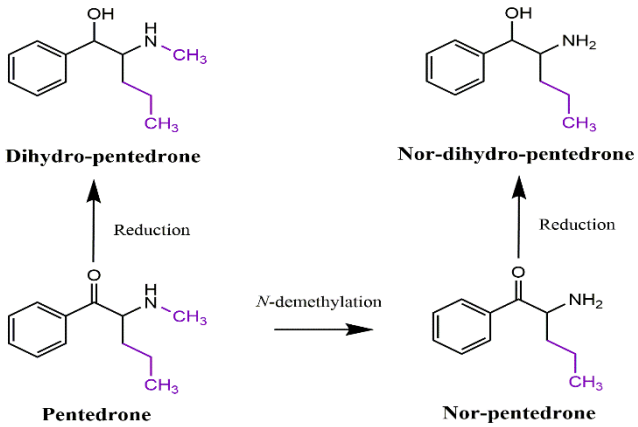


Results

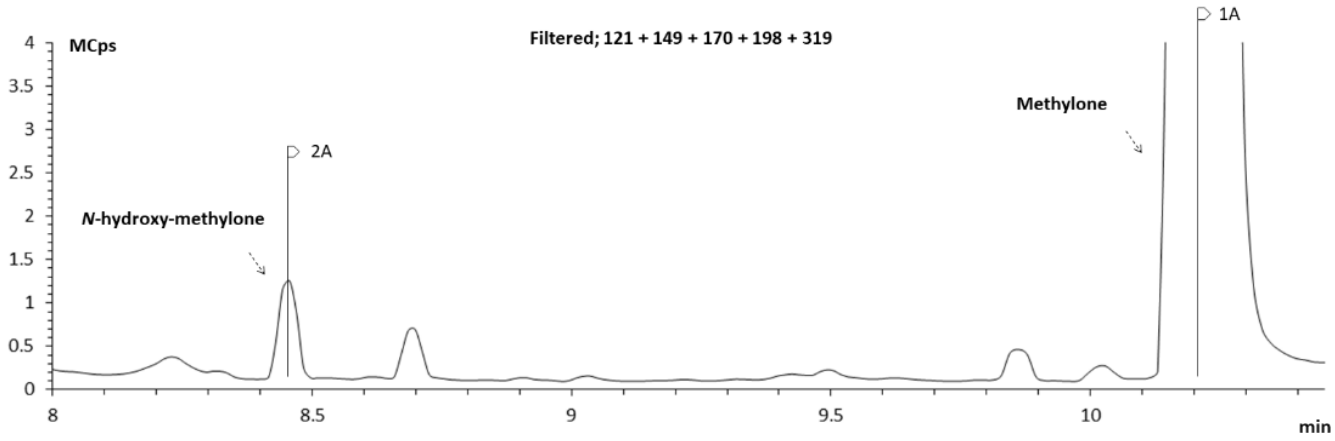
Methylone metabolism

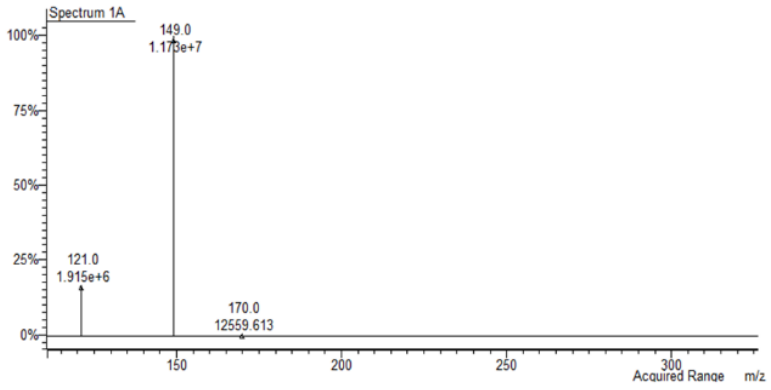


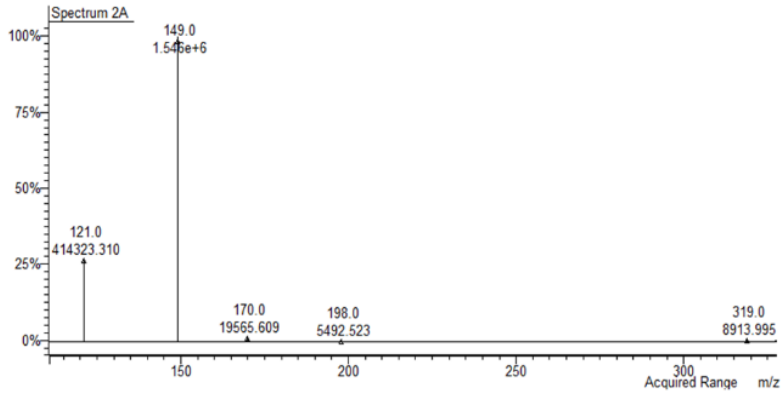
Pentedrone metabolism



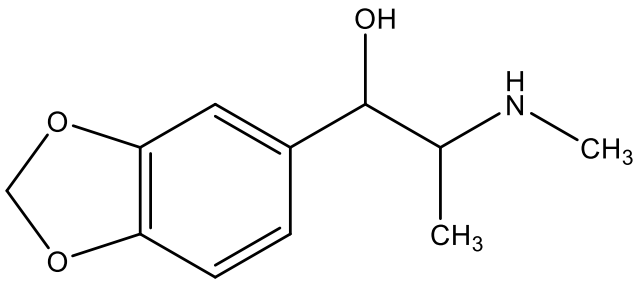
Results


MCps
Filtered; 121 + 149 + 170 + 198 + 319
1A
2A
Methylone
N-hydroxy-methylone
4
3.5
3
2.5
2
1.5
1
0.5
0
8
8.5
9
9.5
10
min

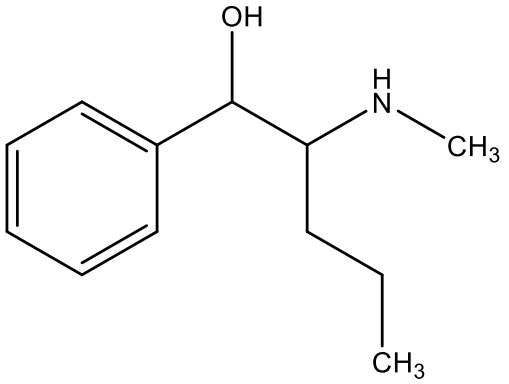

Spectrum 1A
149.0
1.173e+7
121.0
1.915e+6
170.0
12559.613
100%
75%
50%
25%
0%
150
200
250
300
Acquired Range
m/z


Spectrum 2A
149.0
1.545e+6
121.0
414323.310
170.0
19565.609
198.0
5492.523
319.0
8913.995
100%
75%
50%
25%
0%
150
200
250
300
Acquired Range
m/z

Results



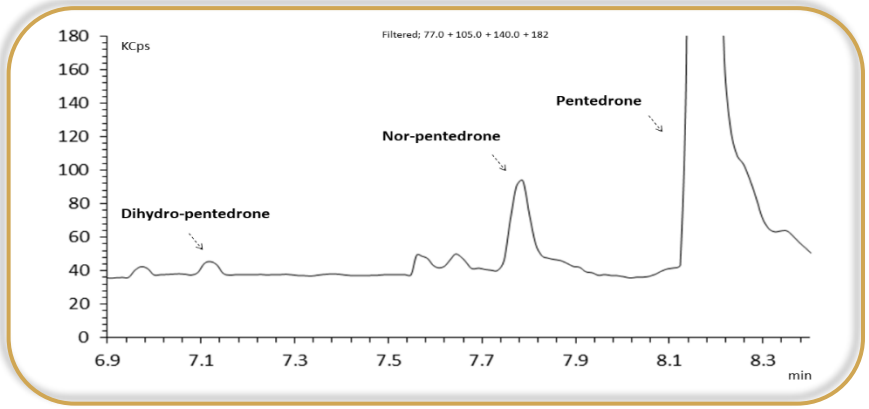
Dihydro-methylone



Dihydro-pentedrone

Dihydromethylone and **dihydropentedrone** was synthetized by keto group reduction of methylone and pentedrone, respectively, to the corresponding aminoalcohols. The synthetic strategy using sodium borohydride.

Results



Characteristics m/z:

Dihydro-pentedrone: m/z: 140, 182, 272 and 385

Nor-pentedrone: m/z: 77, 105, 26, 168 and 273

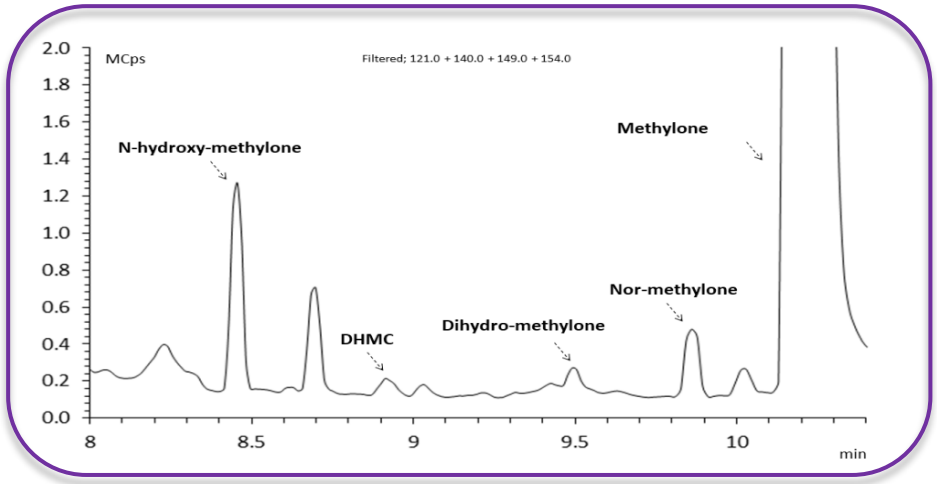
Characteristic m/z:

***N*-hydroxy-methylone:** m/z: 121, 149, 170, 198 and 319

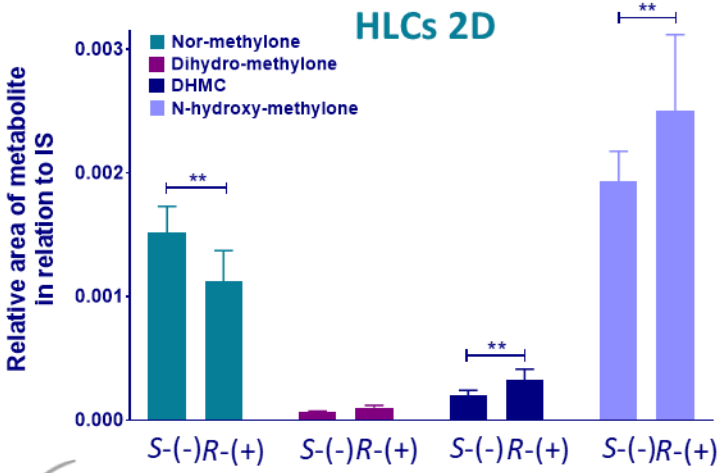
DHMC: m/z: 154, 182, 209, 237 and 391

Dihydro-methylone: m/z: 121, 154, 247, 280 and 401

Nor-methylone: m/z: 121, 140, 149, 168, 289 and 260



Results



Nor-methylone: *S*-(-) > *R*-(+)

Dihydro-methylone: *S*-(-) = *R*-(+)

DHMC: *R*-(+) > *S*-(-)

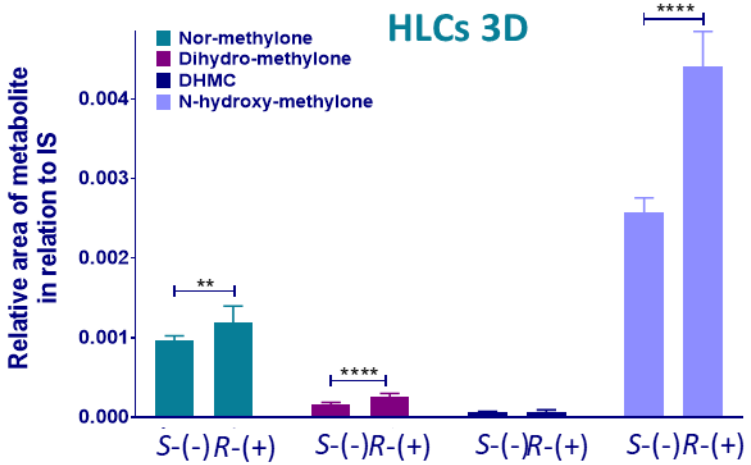
***N*-hydroxy-methylone:** *R*-(+) > *S*-(-)

Nor-methylone: *R*-(+) > *S*-(-)

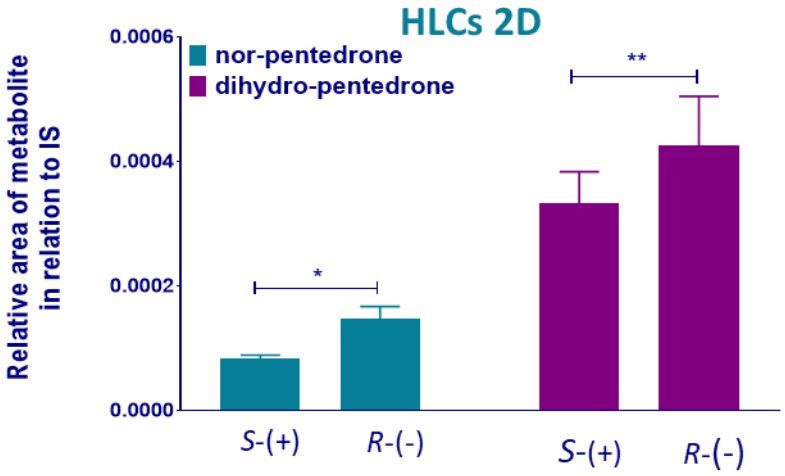
Dihydro-methylone: *R*-(+) > *S*-(-)

DHMC: *S*-(-) = *R*-(+)

***N*-hydroxy-methylone:** *R*-(+) > *S*-(-)



Results

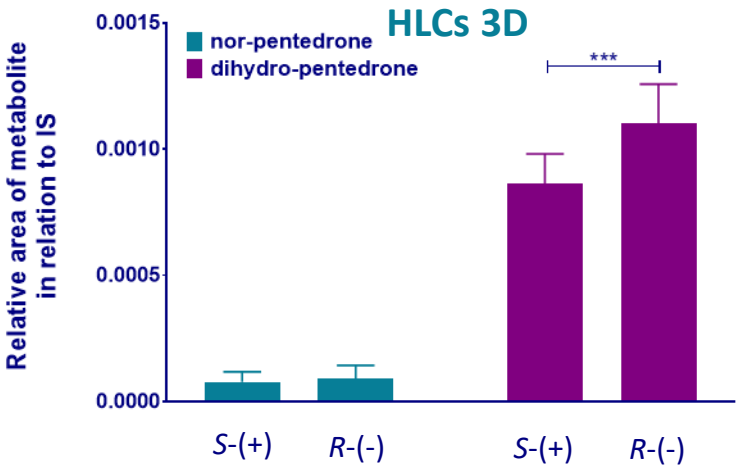


Nor-pentedrone: *R*-(-) > *S*-(+)

Dihydro-pentedrone: *R*-(-) > *S*-(+)

Nor-pentedrone: *S*-(+) = *R*-(-)

Dihydro-pentedrone: *R*-(-) > *S*-(+)



Conclusions

This study revealed stereoselectivity on the cytotoxicity for pentedrone in HLCs 3D, being the *R*-(-)-pentedrone the most cytotoxic.

It was also observed an enantioselective preference in the metabolism pathways for both enantiomers of pentedrone and methylone.

A differential cytotoxic and metabolic profiles between 2D and 3D culture systems was observed.

N-Hydroxy-methylone and dihydro-pentedrone, the compounds mostly formed in both models, are produced in greater quantity in the 3D model (about 2x time higher).

Acknowledgments

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