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Synthetic Cathinones: Chiral Resolution and Enantioselectivity Studies

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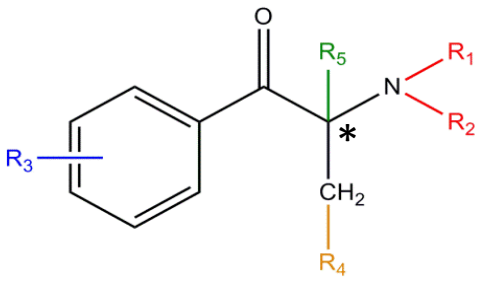
Synthetic Cathinones: Chiral Resolution and Enantioselectivity Studies

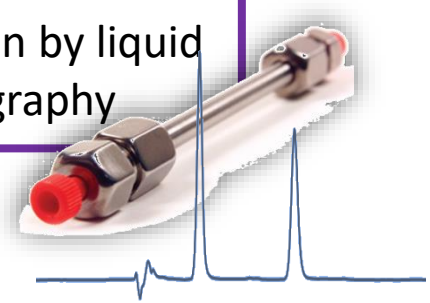
Graphical Abstract

smartshop products

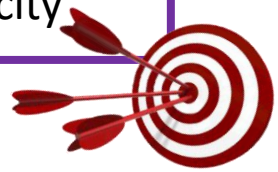
Synthetic Cathinones



Chiral resolution by liquid chromatography



Enantioselectivity on bioactivity/toxicity



Abstract:

Synthetic cathinones are interesting compounds and the most representative constituents of "legal highs". Their consumption has serious health concerns that may lead to acute liver and/or kidney failure. All the synthetic cathinone are chiral and, consequently, their biological activities could differ between enantiomers. Despite the interest regarding synthetic cathinones, there are only few studies concerning their potential enantioselectivity on bioactivity/toxicity.

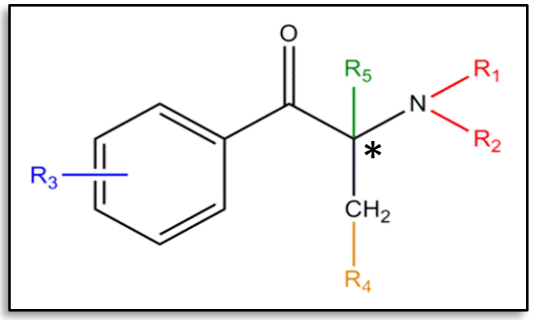
Recently, we reported the enantiomeric resolution of several synthetic cathinones by liquid chromatography using analytical chiral stationary phases based on polysaccharide derivatives. The enantioresolution of MDPV, pentedrone and methylone (three of the most commonly used synthetic cathinones worldwide) were scaled up to multi-milligrams for further enantioselectivity studies. All the cathinones enantiomers were isolated with high enantiomeric purity. Additionally, absolute configuration was determined by electronic circular dichroism (ECD) spectroscopy.

The toxicity of MDPV enantiomers was evaluated using primary cultures of rat hepatocytes, showing similar behavior. Nevertheless, enantioselectivity was observed for pentedrone and methylone enantiomers in dopaminergic SH-SY5Y cells cytotoxicity and reactive species production. Moreover, kinetic studies to evaluate the ability of pentedrone and methylone enantiomers to pass across the intestinal barrier model revealed a differentiated passage of the cathinones enantiomers.

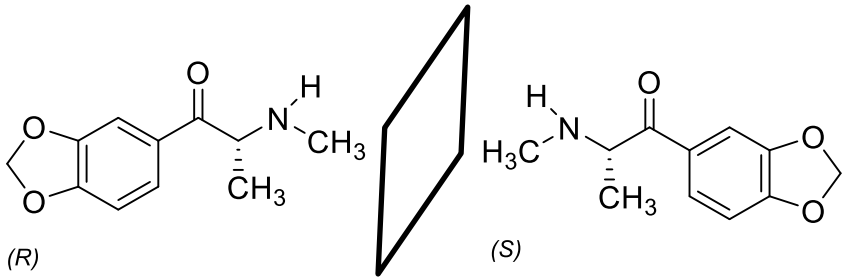
Keywords: Synthetic Cathinones; Chiral Resolution; Enantioselectivity; Bioactivity; Toxicity

Introduction

Synthetic cathinones – Chiral compounds

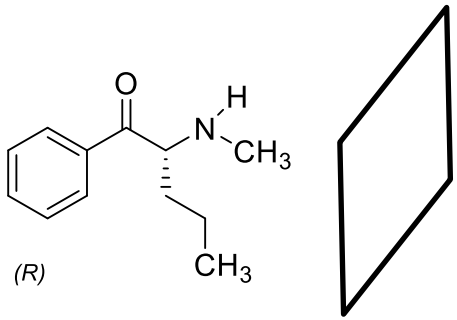


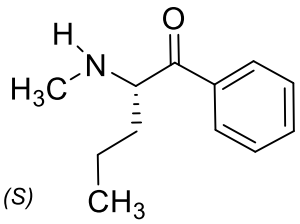
General structure of synthetic cathinones



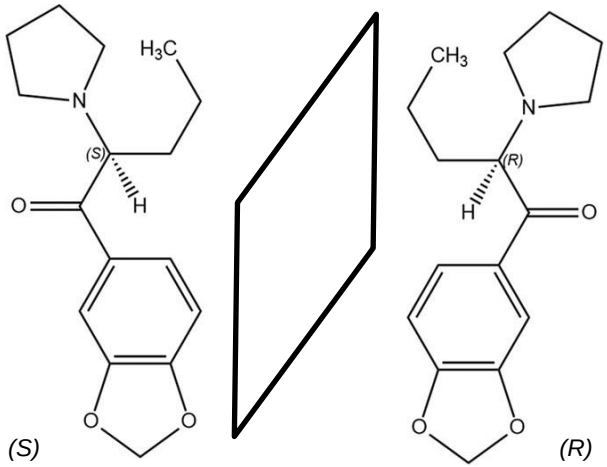
Methylone enantiomers

Examples:





Pentedrone enantiomers



MDPV enantiomers

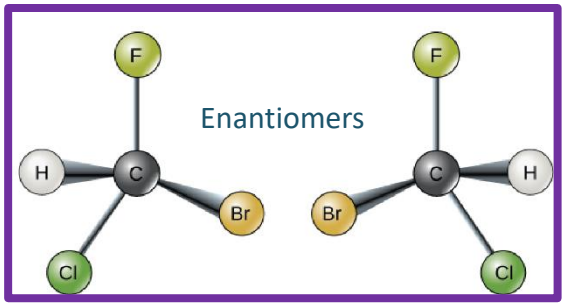
M.J. Valente, et al., *Khat and synthetic cathinones: a review.* Arch Toxicol, 2014. 88(1): p. 15-45.

Introduction

Enantiselectivity

Enantiomers

non-superimposable object/mirror image forms of chiral molecules

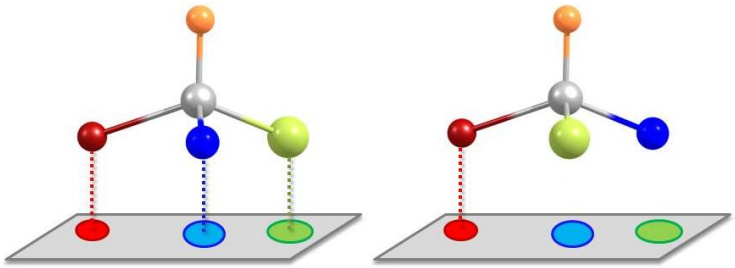


Differences in chiral environments

Similar physicochemical properties

Opposite optical activity

Chiral Recognition



Enantiomers may interact differently with biotargets

Enantioselectivity

M.E. Tiritan, A.R. Ribeiro, C. Fernandes, M. Pinto, Chiral Pharmaceuticals. In Kirk-Othmer Encyclopedia of Chemical Technology: John Wiley & Sons, Inc., 2016, 1-28.

Introduction

Enantiomers and biological activity

Examples of enantiomeric bioactive drugs and their biological activities

Drug	Biological effect	
	(*R*)-enantiomer	(*S*)-enantiomer
Ibuprofen	Inactive form	Anti-inflammatory
Thalidomide	Sedative	Teratogenic
Amphetamine	Less active	More active

Enantioselectivity

S.W. Smith, *Chiral toxicology: it's the same thing...only different.* Toxicol Sci, 2009. **110**(1): p. 4-30.

Introduction

Strategies to obtain single enantiomers

Enantioselective synthesis

Advantage in large scale production of one of the enantiomers

or

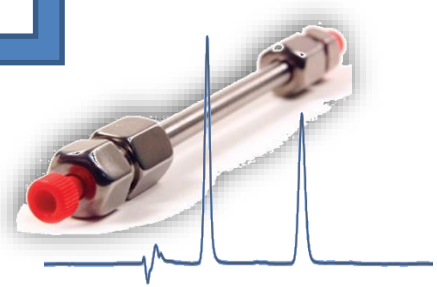
Enantioresolution

Direct method

Indirect method

Chiral selector	Chiral stationary phase (CSP)
Natural	Protein
	Cyclodextrin
	Polysaccharides
	Macrocyclic antibiotic
	Cinchona
Synthetic	Pirkle-type
	Ligand-exchange-type
	Crown ethers
	Synthetic polymers

Chiral liquid chromatography, among others



Derivatization with enantiomerically pure reagents required to obtain diastereoisomers

Conventional chromatography, crystallization, extraction, among others

C. Fernandes, M.E. Tiritan, and M. Pinto, Chromatographia, 2013. **76**: p. 871-897.

Introduction

Enantioresolution of cathinones

There are only few studies about synthetic cathinones and almost no information on their single enantiomers.

Journal of Analytical Toxicology, 2017;1–8
doi: 10.1093/jat/bkx074
Review

OXFORD

Review

Chiral Resolution and Enantioselectivity of Synthetic Cathinones: A Brief Review

Bárbara Silva[1,2,*], Carla Fernandes[2,3], Paula Guedes de Pinho[1], and Fernando Remião[1]

Enantioselective synthesis → 4 studies

Enantioresolution

- Indirect method → 2 studies
- Direct method → 1 study

analytical studies

↓

Enantiomeric separation

	Method	Nº
LC	Direct method	9
GC	Indirect method	5
CEC	Direct method	3
CE	Direct method	3

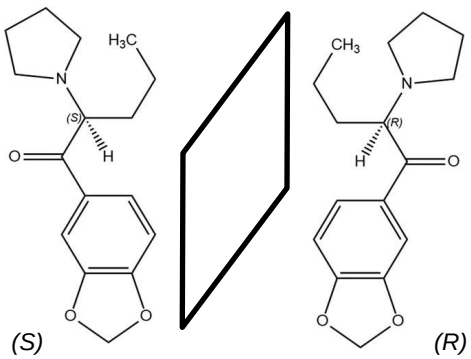
Six studies of biological enantioselectivity

LC: Liquid chromatography; GC: Gas chromatography; CEC: Capillary electrochromatography; CE: Capillary electrophoresis

Introduction

Cathinones: enantioselectivity in biological activity

Example:



MDPV enantiomers

Biological effect	*S*-(-)-MDPV	*R*-(+)-MDPV
Dopamine and norepinephrine reuptake inhibition	More potent	Less potent
Intracranial self-stimulation	Facilitator	Not change
Stimulant effect	More potent	Less potent
Locomotor stimulation	Effect	Not have effect

These data show that the neurochemical and behavioral effects related to drug abuse are stereoselective and *S*-(-)-MDPV is the most potent.

B. Silva, et al., *Chiral Resolution and Enantioselectivity of Synthetic Cathinones: A Brief Review*, J Anal Toxicol, 2018. 42: p. 17-24.

Results

Synthetic cathinones present in smartshop products
MDPV enantiomers

Forensic Toxicol
DOI 10.1007/s11419-016-0324-y

ORIGINAL ARTICLE

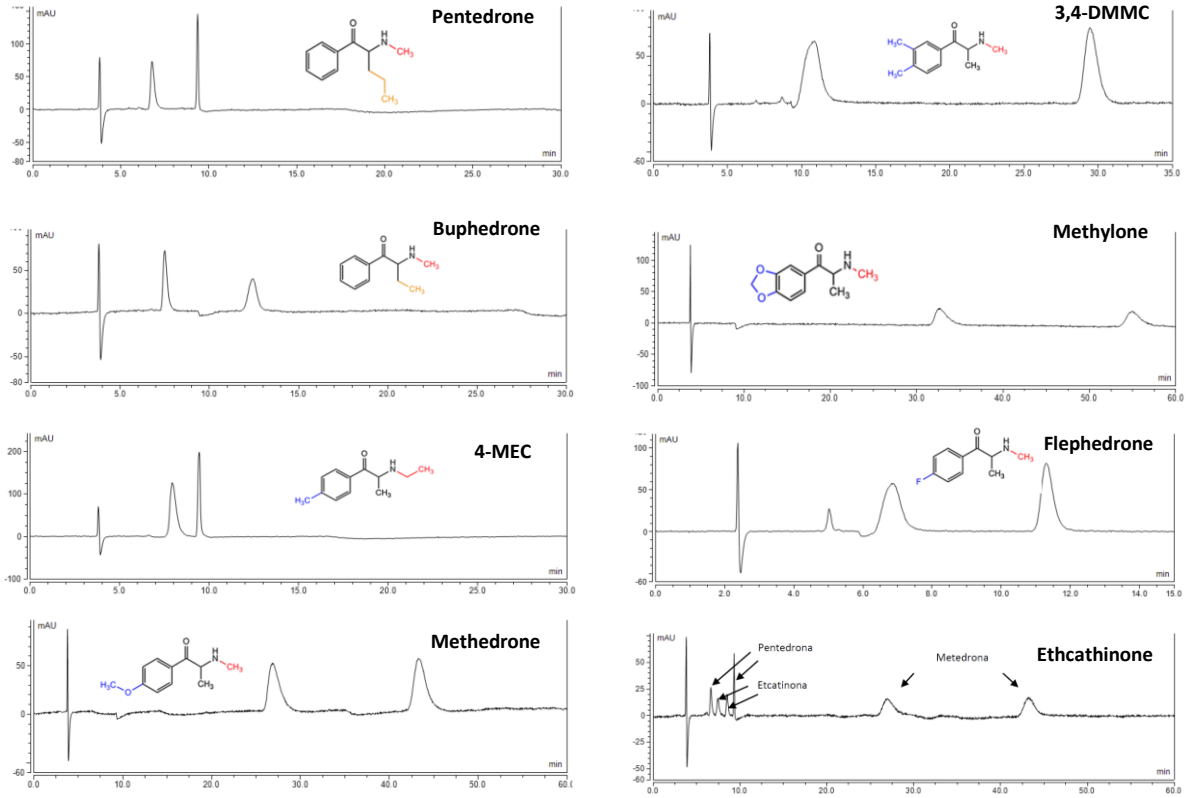
Chiral enantioresolution of cathinone derivatives present in "legal highs", and enantioselectivity evaluation on cytotoxicity of 3,4-methylenedioxypyrovalerone (MDPV)

Bárbara Silva[1,2] · Carla Fernandes[2,3] · Maria Elizabeth Tiritan[2,3,4] · Madalena M.M. Pinto[2,3] · Maria João Valente[1] · Márcia Carvalho[1,5] · Paula Guedes de Pinho[1] · Fernando Remião[1]

Received: 20 April 2016 / Accepted: 19 May 2016
© Japanese Association of Forensic Toxicology and Springer Japan 2016

Results

Enantioresolution of 8 synthetic cathinones present in smartshop products



Chromatograms of enantiomeric separation of synthetic cathinones

Samples: Smartshops products

Chiral column: Chiralpak® AS-H

Mobile phase: Hex:2-PrOH:TEA (97:3:0.1 *v/v/v*)

Flow rate: 0.5 mL/min

UV detection: 254 nm

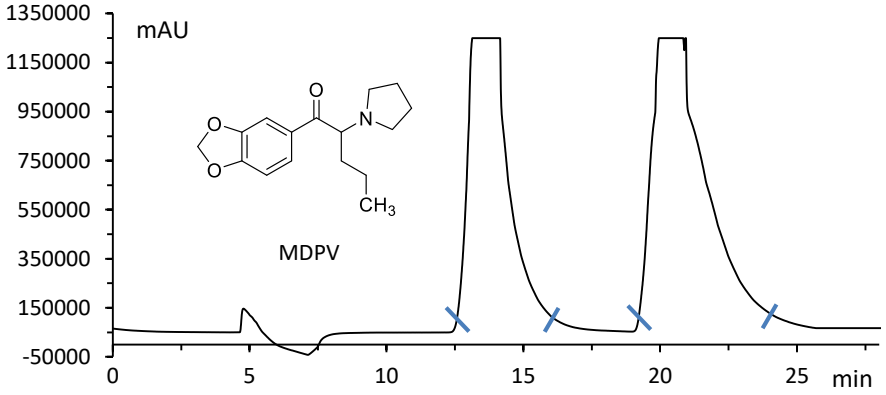
Cathinones: pentedrone, methedrone, methylone, 4-MEC, flephedrone, buphedrone, 3,4-DMMC, ethcatinone

All synthetic cathinones present in smartshop products were in **racemate form (50:50)**

Hex: Hexane; 2-PrOH: Isopropanol; TEA: Triethylamine

Results

Enantioresolution and isolation of MDPV enantiomers



Chiral column: Polysaccharide column (amylose *tris*-3,5-dimethylphenylcarbamate coated with APS-Nucleosil
Mobile phase: Hex:2-PrOH:TEA (97:3:0.1 *v/v/v*)
Flow rate: 1.5 mL/min
UV detection: 254 nm

Elution order, specific rotation and enantiomeric excess (e.e.) of MDPV enantiomers

Enantiomer	Elution order	e.e. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(-)-MDPV	First	99.1	-23 (10)	91.0
R-(+)-MDPV	Second	99.6	+23 (10)	82.6

[a] Specific rotation in ethanol (mg/mL)

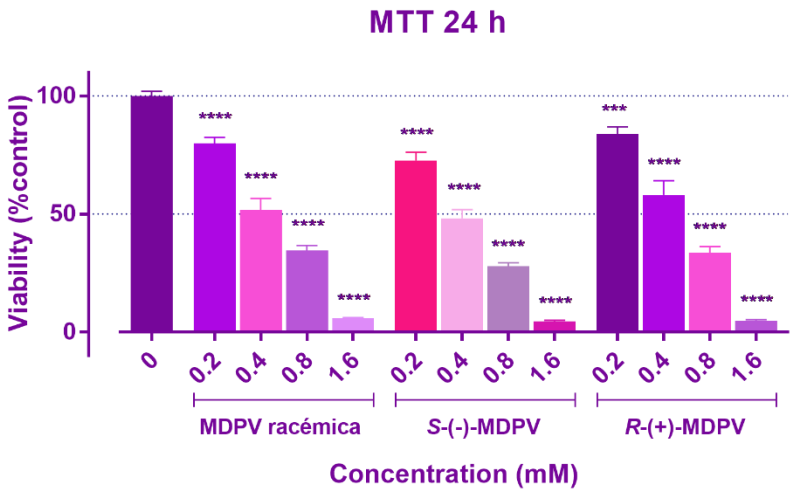
MDPV enantiomers were isolated, for the first time, by semi-preparative LC using a CSP with a high enantiomeric purity.

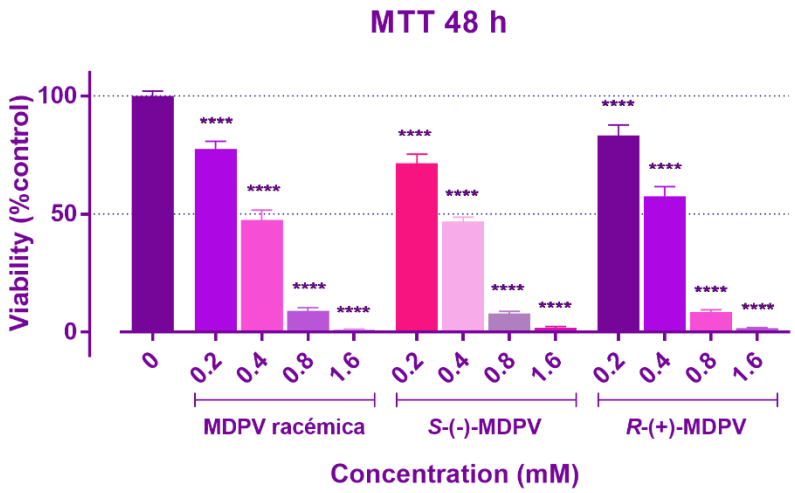
Hex: Hexane; 2-PrOH: Isopropanol; TEA: Trietilamine
e.e. enantiomeric excesso; LC: Liquid Chromatography; CSP: chiral stationary phase

Results

Citotoxicity of MDPV (racemate and enantiomers) in primary culture of rat hepatocyte

Primary culture of rat hepatocyte





MTT reduction by primary culture of rat hepatocyte exposed to MDPV (racemic form and enantiomers) for 24 and 48h. ***p < 0.001 vs control ****p < 0.0001 vs. control.

Same behaviour between enantiomers

Results

Pentedrone and methylone

Journal of Chromatography B 1100–1101 (2018) 158–164

Contents lists available at ScienceDirect

Journal of Chromatography B

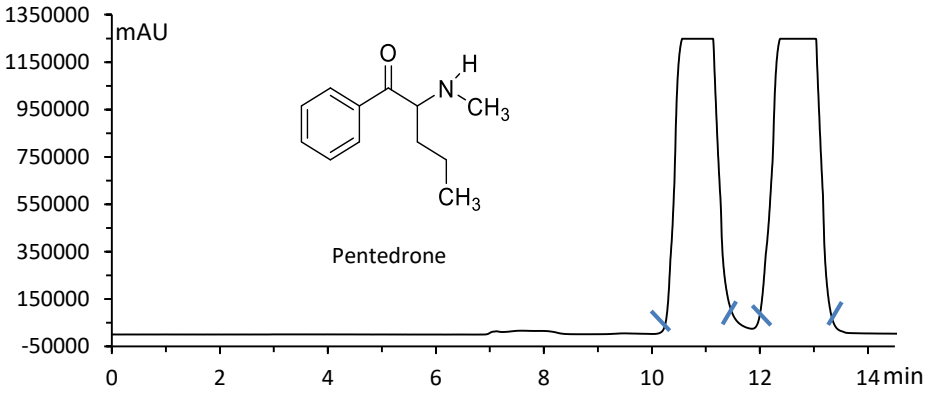
journal homepage: www.elsevier.com/locate/jchromb

Multi-milligram resolution and determination of absolute configuration of pentedrone and methylone enantiomers

Bárbara Silva[a,b], José A. Pereira[c,d], Sara Cravo[b,d], Ana Margarida Araújo[a], Carla Fernandes[b,d,*], Madalena M.M. Pinto[b,d], Paula Guedes de Pinho[a], Fernando Remião[a]

Results

Enantioresolution and isolation of pentedrone enantiomers



Chiral column: Chiralpak® AS-H
Mobile phase: Hex:2-PrOH (97:3 *v/v*)
Flow rate: 2 mL/min
UV detection: 254 nm

Elution order, specific rotation and enantiomeric excess (e.e.) of pentedrone enantiomers

Enantiomer	Elution order	e.e. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(+)-pentedrone	First	98.4	+16 (2.5)	72.0
R-(-)-pentedrone	Second	97.8	-12(2.5)	71.0

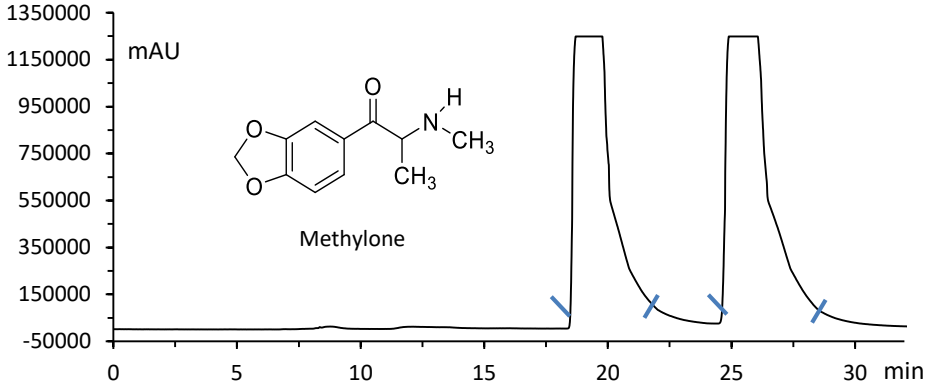
[a] Specific rotation in ethanol (mg/mL)

Pentedrone enantiomers were isolated, for the first time, by semi-preparative LC using a CSP with a high enantiomeric purity.

Hex: Hexane; 2-PrOH: Isopropanol

Results

Enantioresolution and isolation of methylone enantiomers



Chiral column: Chiralpak® AS-H
Mobile phase: Hex:2-PrOH (85:15 *v/v*)
Flow rate: 2 mL/min
UV detection: 254 nm

Elution order, specific rotation and enantiomeric excess (e.e.) of Methylone enantiomers

Enantiomer	Elution order	e.e. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(-)-methylone	First	98.3	-20 (2,5)	80.0
R-(+)-methylone	Second	97.1	+ 24(2,5)	77.0

[a] Specific rotation in ethanol (mg/mL)

Methylone enantiomers were isolated, for the first time, by semi-preparative LC using a CSP with a high enantiomeric purity.

Hex: Hexane; 2-PrOH: Isopropanol

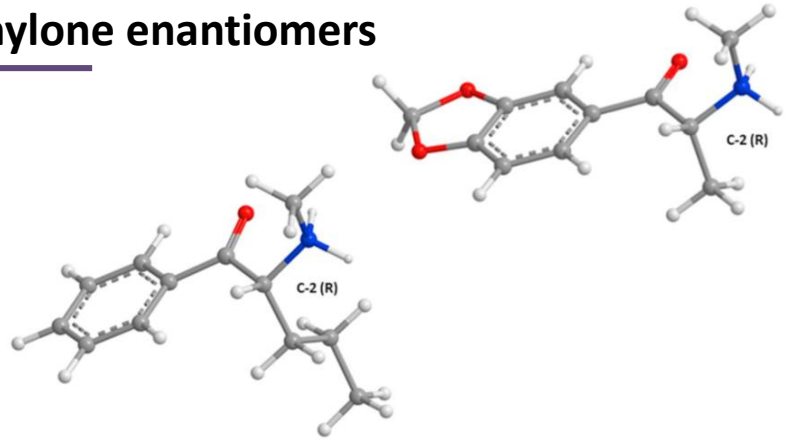
Results

Absolute configuration of pentedrone and methylone enantiomers

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

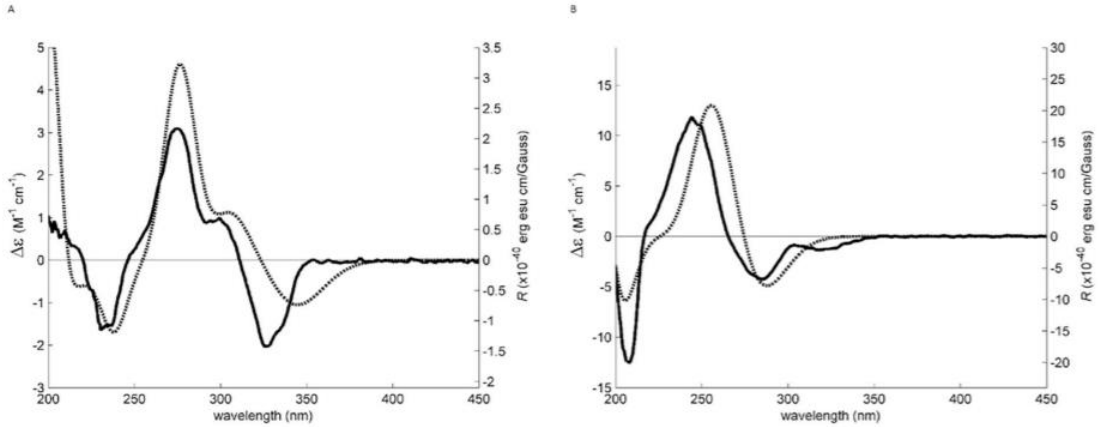
Enantiomer	Elution order	e.r. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(+)-pentedrone	First	98.4	+16 (2.5)	72
R-(−)-pentedrone	Second	97.8	−12 (2.5)	71
S-(−)-methylone	First	98.3	−20 (2.5)	80
R-(+)-methylone	Second	97.1	+24 (2.5)	79

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



Computational molecular modeling + electronic circular dichroism (ECD)

The absolute configuration of the enantiomers of both cathinones was determined for the first time by ECD spectroscopy, with the aid of theoretical calculations, as **(+)-(*S*) and (−)-(*R*)-pentedrone**, and **(−)-(*S*) and (+)-(*R*)-methylone**.

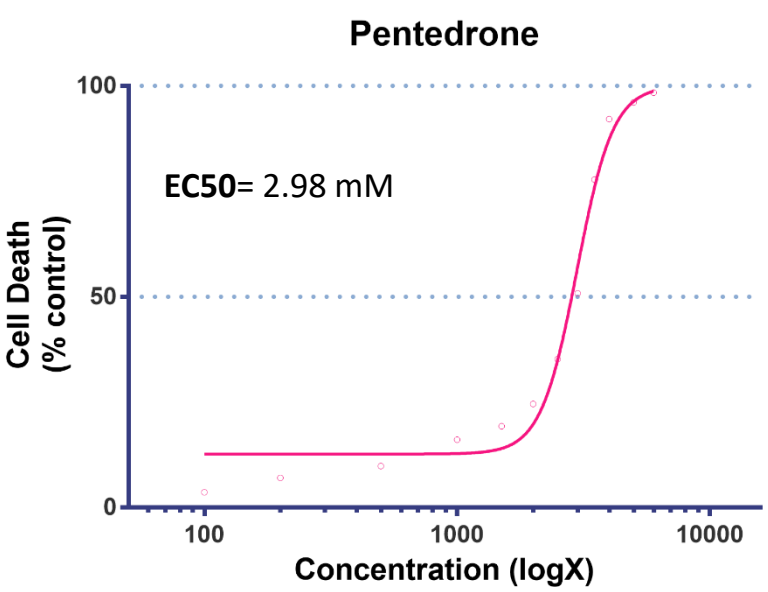


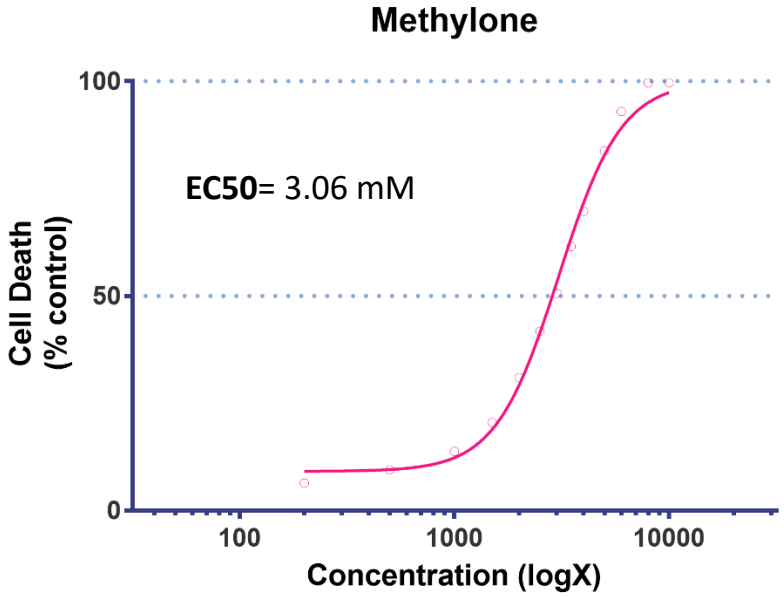
Experimental ECD spectra (solid lines) of (A) methylone's M1 fraction and (B) pentedrone's P1 fraction, and simulated ECD spectra (dotted lines) of (A) methylone's C-2(S) and (B) pentedrone's C-2(S) model configurations, both in ethanol.

Results

Pentedrone and methylone biological assays (ongoing)

Citotoxicity studies



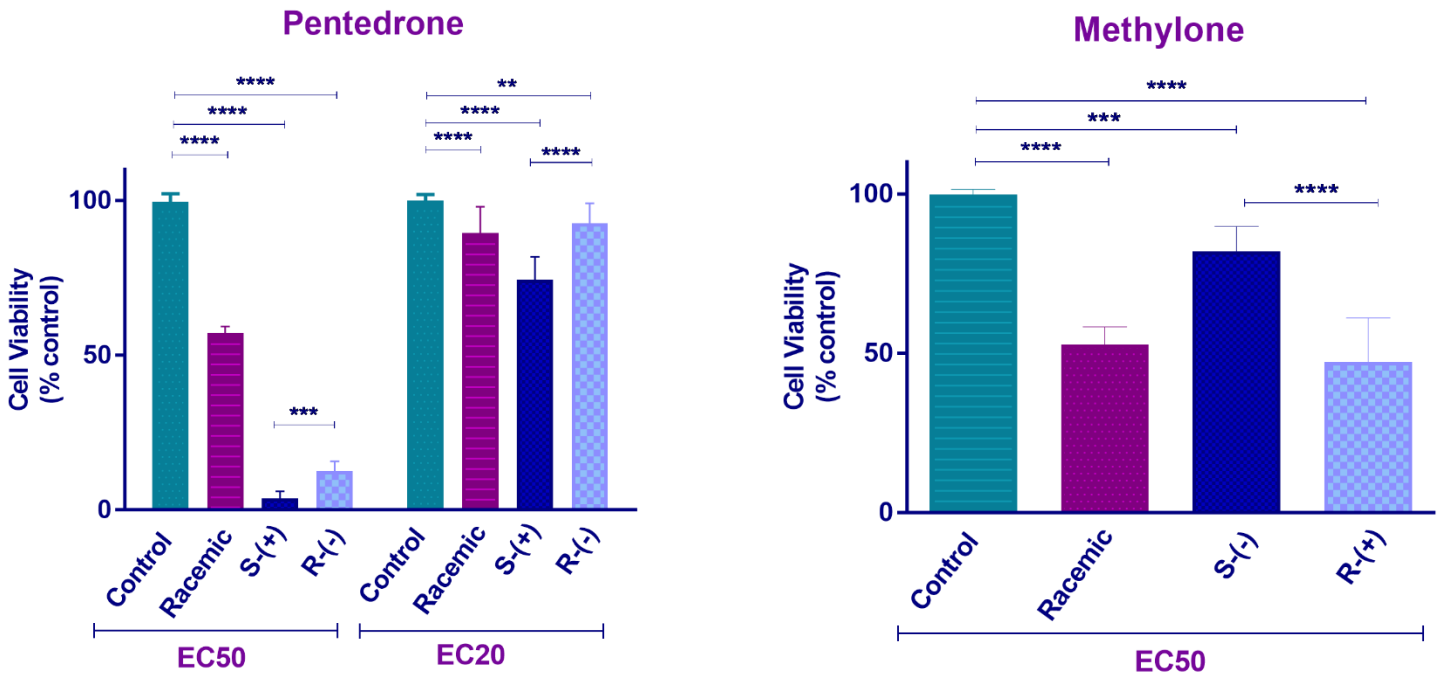


Nonlinear regression models for the cell death induced by pentedrone and methylone in dopaminergic SH-SY5Y cells, as evaluated by the MTT reduction assay after 24 h exposure. The mean effects were fitted to the logit function. Results were obtained from four independent experiments.

Results

Pentedrone and methylone biological assays (ongoing)

Citotoxicity studies



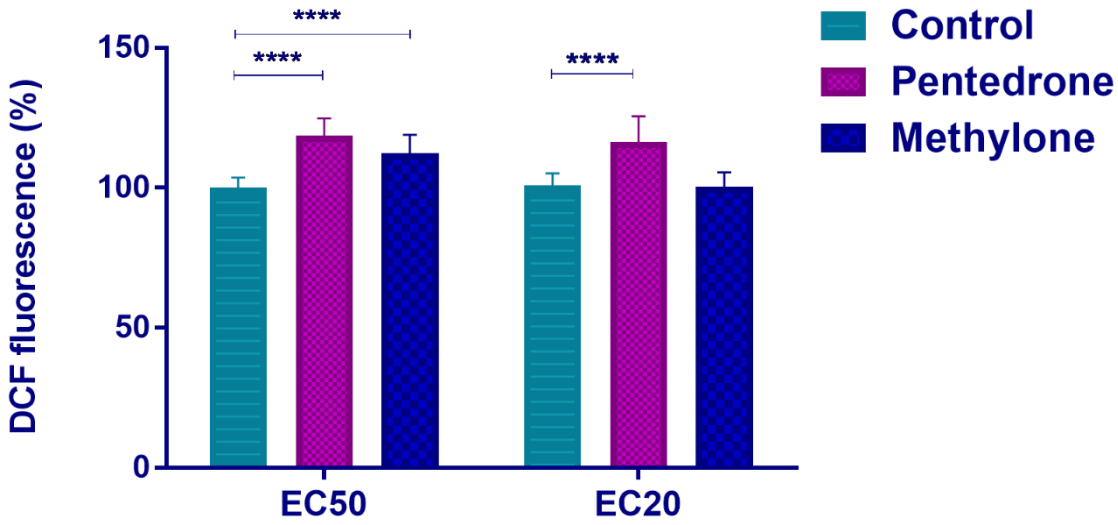
Citotoxicity in SH-SY5Y cells exposed to pentedrone and methylone enantiomers for 24 h. Results were obtained from four independent experiment, performed in triplicate. ****p < 0.0001, ***p < 0.001, **p < 0.01

***R*-(+)-methylone and *S*-(+)-pentedrone are the most potent**

Results

Pentedrone and methylone biological assays (ongoing)

ROS/RNS studies

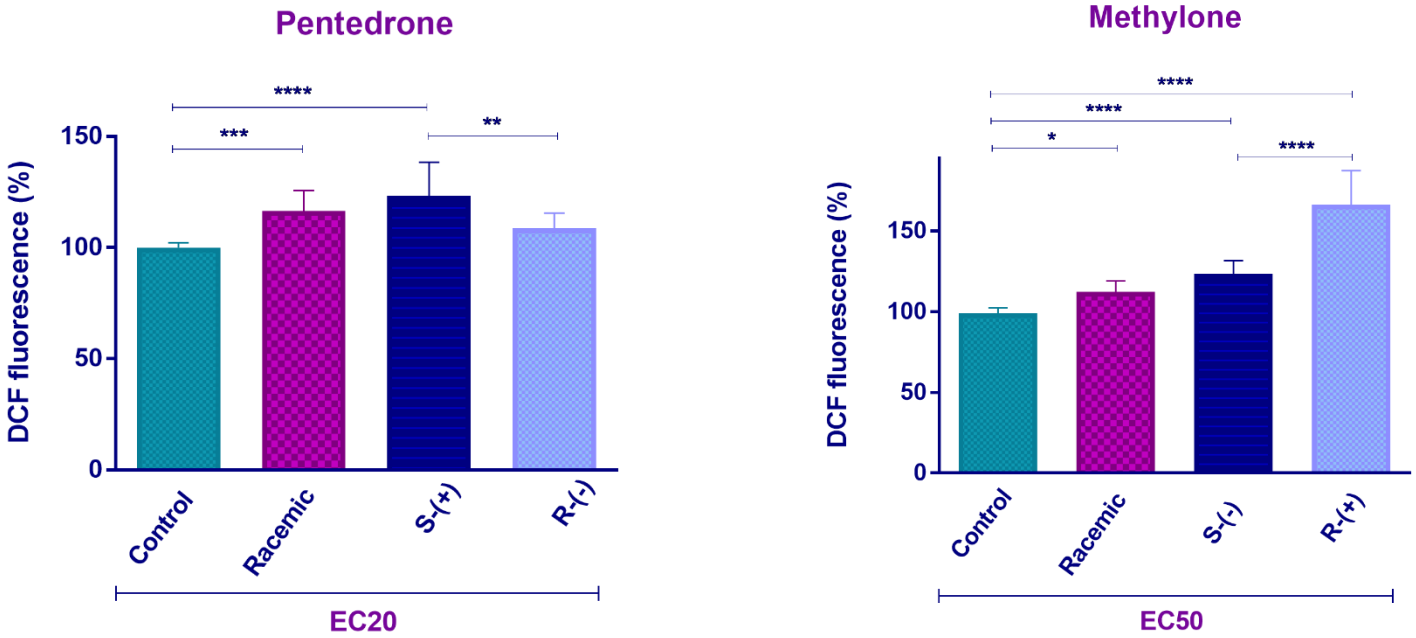


ROS and RNS production in cells exposed to EC20 and EC50 methylone or pentedrone for 24 h. Results are from four independent experiments. ****$p < 0.0001$

Results

Pentedrone and methylone biological assays (ongoing)

ROS/RNS studies



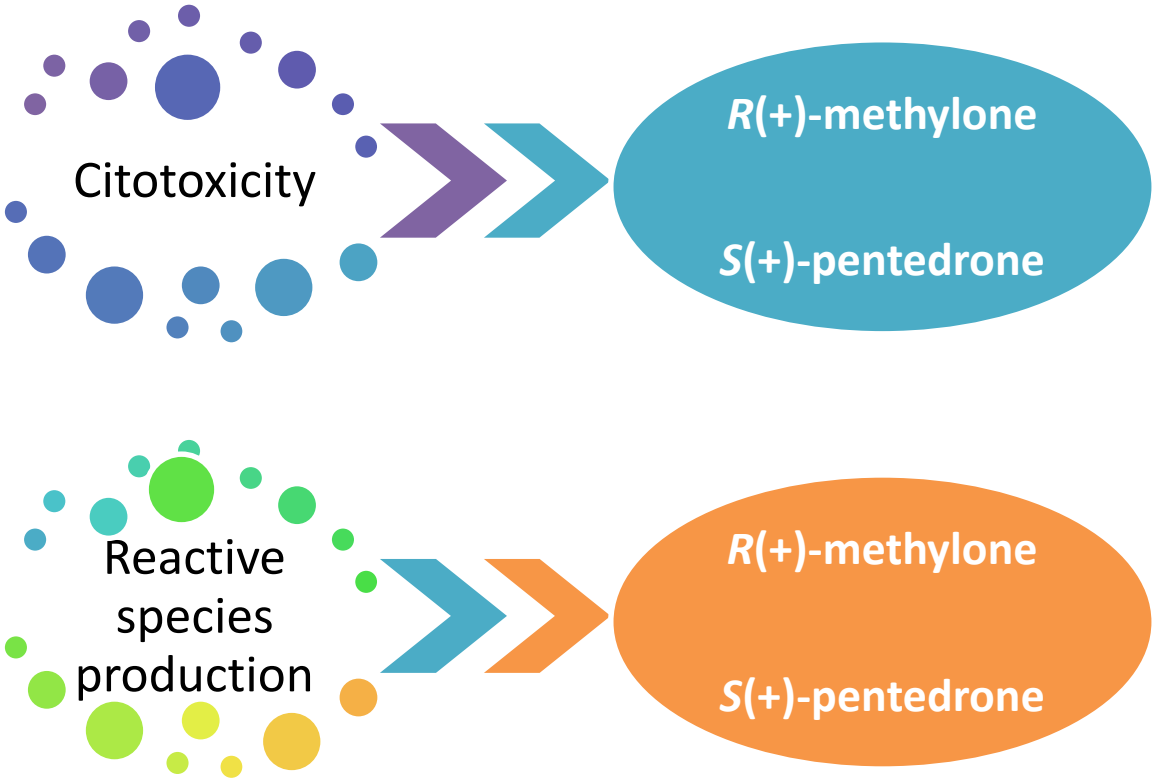
ROS and RNS production in cells exposed to EC20 pentedrone enantiomers and EC50 methylone enantiomers for 24 h. Results are from four independent experiments. *p < 0.1, **p < 0.01 ***p < 0.001, ****p < 0.0001

***R*-(+)-methylone and *S*-(+)-pentedrone are the most potent**

Results

Pentedrone and methylone biological assays (ongoing)

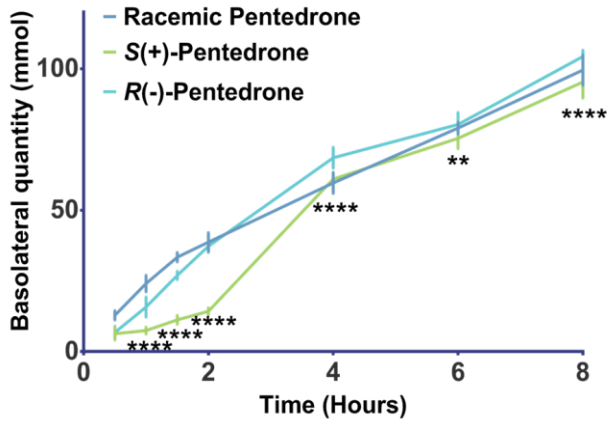
Summary

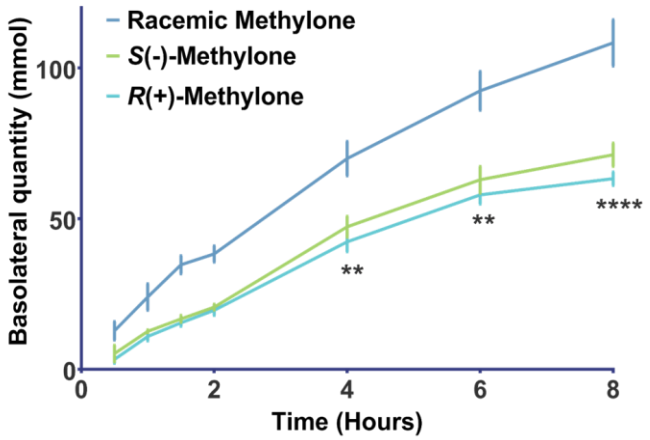


Development of an UHPLC-UV method to determine synthetic cathinones: Enantioselective absorption of cathinones using Caco-2 cell line

Results

Pentedrone and methylone (racemate and enantiomers) permeability





Permeability of Pentedrone and Methylone (500 µM) in Caco-2 cell line. Results are expressed as mean ± SD of 3 independent experiments (performed in triplicate). Statistical comparisons were performed using the two-way ANOVA parametric method.

It was possible to observe a differentiated passage of the cathinones enantiomers through Caco-2 cell monolayer. For pentedrone, this difference was observed after the first hour, being *R*(-)-pentedrone the most permeable compound. Concerning methylone, the difference was noted after the fourth hour, with *S*(-)-methylone presenting the highest permeability rate.

Conclusions

In the vast majority of situations, **enantiomers should be considered as different compounds**, as their biological behavior is differentiated.

- ✓ The effects of catinones **appear to be stereoselective**.
- ✓ **There are few studies on enantioselectivity of synthetic catinones** and more work needs to be done in this area.

Enantiomers remain a challenge to separate and research on specialized separation techniques continues to be developed to obtain single enantiomers.

The data available for enantioselectivity of synthetic cathinones has grown. However, **it is necessary to develop more work in this area** as the consumption of these compounds continues to increase and studies related with the repercussion of potential effects related to the enantioselectivity of drugs of abuse is a new area of concern.

Acknowledgments

Financial supported from Universidade do Porto/FMUP through FSE-Fundo Social Europeu, NORTE 2020-Programa Operaconal Regional do Norte (NORTE-08-5369-FSE-000011). Partially supported by the Strategic Funding UID/Multi/04423/2019 through national funds provided by FCT and ERDF, in the framework of the programme PT2020. Partially supported by FEDER funds through the Operational Programme for Competitiveness and Internationalisation (COMPETE 2020), Portugal, and. UID/MULTI/04378/2013- POCI/01/0145/FEDER/07728