Optimization of liquid chromatographic methods for the separation of 1,3-dimethylamylamine (1,3-DMAA) stereoisomers

Maria Mexia-de-Almeida[1,2,*], Virgínia M. Gonçalves[1,2,3], Alexandra Maia[1,2,], Diana Dias da Silva[1,4,5,6,7], Ricardo Jorge Dinis-Oliveira[1,2], Cláudia Ribeiro[1,2*]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, University Institute of Health Sciences – CESPU, 4585-116 Gandra, Portugal, mariaal97@hotmail.com, virginia.goncalves@cespu.pt, alexandra.maia@iucs.cespu.pt, dds@ess.ipp.pt, ricardo.dinis@iucs.cespu.pt, claudia.ribeiro@iucs.cespu.pt
[2]UCIBIO – Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal, mariaal97@hotmail.com, virginia.goncalves@cespu.pt, alexandra.maia@iucs.cespu.pt, ricardo.dinis@iucs.cespu.pt, claudia.ribeiro@iucs.cespu.pt
[3]UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS-CESPU), 4585-116 Gandra, Portugal, virginia.goncalves@cespu.pt
[4]REQUIMTE/LAQV, ESS, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 400 4200-072, Porto, dds@ess.ipp.pt
[5]UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal, dds@ess.ipp.pt
[6]Associate Laboratory i4HB – Institute for Health and Bioeconomy, University of Porto, 4050-313 Porto, Portugal, dds@ess.ipp.pt
[7]UCIBIO – Applied Molecular Biosciences Unit, Forensics and Biomedical Sciences Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal, dds@ess.ipp.pt
**Autor correspondente*

Background: 1,3-Dimethylamylamine (1,3-DMAA), also known as methylhexanamine, is a central nervous system stimulant with structural similarities with amphetamines and, therefore, presenting overlapping biological and detrimental effects [1]. Despite being banned, the presence of 1,3-DMAA in doping controls and dietary supplements continues to be of significant concern. This molecule has two stereogenic centres and, thus, four stereoisomers [2]. It is widely recognized that enantiomers may exhibit different biological activities, including pharmacokinetics, pharmacodynamics, and toxicity. Consequently, developing analytical methods for enantioselective separation of 1,3-DMAA is crucial to isolate and accurately determine the risks associated with each of these stereoisomers. **Objective**: To develop a semipreparative liquid chromatography with diode array detection (LC-DAD) method for separating the stereoisomers of 1,3-DMAA. **Methods:** For that, 1,3-DMAA was derivatized using the enantiomeric pure reagent (*R*)-(-)-*α*-methoxy-*α*-(trifluoromethyl)phenylacetyl chloride ((*R*)-MTPA-Cl) for the formation of diastereomers. Subsequently, the solution was evaporated, reconstituted in acetonitrile or mobile phase or 0.1% formic acid, and analyzed by LC-DAD. Different conventional and chiral analytical columns and chromatographic conditions were tested depending on the column used. Seventy-two tests were carried out with 6 different columns and under different conditions. **Results**: Preliminary results showed that the derivatization procedure allowed the formation of 4 diastereomers of 1,3-DMAA confirmed by gas chromatography. The best conditions allowed the separation of two pairs of diastereomers using the Luna 3µm PFP column with gradient elution, the Lux® 3µm Amylose-1 column with an isocratic elution, and the RP-18 LiChrospher® column with a gradient elution. **Conclusions**: Chromatographic conditions for enantioselective separation of the two stereoisomers of 1,3-DMAA by LC-DAD have been optimized. The isolation of each stereoisomer is crucial for assessing the differential pharmacokinetics and pharmacodynamics and, consequently, to unveil the perils associated with their presence in food supplements.

Keywords: enantioselectivity; dietary supplements; chromatographic analysis

Acknowledgments
This work was partially supported via institutional funding to associate laboratory UCIBIO (DOI: 10.54499/LA/P/0140/2020).

References
1. Rodrigues AN, Dinis-Oliveira RJ. Pharmacokinetic and Toxicological Aspects of 1,3-Dimethylamylamine with Clinical and Forensic Relevance. Psychoactives. 2023;2(3):222–41.
2. Vorce SP, Holler JM, Cawrse BM, Magluilo J. Dimethylamylamine: A drug causing positive immunoassay results for amphetamines. J Anal Toxicol. 2011;35(3):183–7.