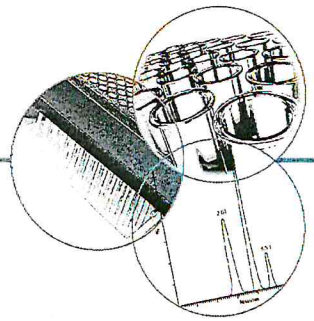
RESEARCH ARTICLE

MINI FOCUS: DRIED BLOOD SPOTS





# Pharmacokinetics of diphenylboroxazolidones of L-α-amino acids with activity on the CNS: quantification in rat DBS by UPLC–MS/MS

**Background:** A growing number of boron-containing compounds exhibit many important biological activities; of particular interest are the α-amino acid borinic derivatives with activity in the CNS. A validated, sensitive and specific UPLC–MS/MS technique for quantification of the diphenylboroxazolidones of glycine (DBPX-gly), L-aspartate (DPBX-L-asp) and L-glutamate (DPBX-L-glu) in dried blood spots (DBSs) is presented. **Results:** The most intense signal corresponds to compounds with $^{11}B$. The extraction procedure was liquid elution of 3.2-mm punched DBSs with acetonitrile:aqueous formic acid 0.1% (80:20 *v/v*). Assays proved to be linear, falling accurately and precisely within the range of 0.3–50 µg/ml for DPBX-L-asp and DPBX-L-glu and 0.1–5 µg/ml for DBPX-gly. Chromatograms exhibit clean 2.0-min running time peaks and S/N ratios for the LLOQ were approximately 15:1. The technique was used to evaluate the pharmacokinetics of the molecules and to correlate these with timecourse toxic effects. **Conclusion:** DBSs represent an advantage for the collection of small volumes of samples, and also in terms of processing and storage. UPLC–MS/MS allow us not only to identify the isotopic pattern of boron in DBPX, but also to quantify them with accuracy and specificity. Pharmacokinetics of these molecules exhibit a high apparent volume of distribution; it suggests a preference of DPBX-amino acids for fatty tissues such as the CNS.

The rapid development of boron-mediated reactions in synthetic chemistry has had a huge influence on the ability to design and synthesize new types of drugs, as well as biochemical tools for therapeutics, clinical diagnosis and medicinal chemistry. Thus, a growing number of boron-containing compounds may exhibit many important biological activities and are suitable for preclinical investigation [1]. In fact, some synthetic boron-containing molecules have demonstrated biological activity as antibiotics [2], apoptotic inductors [3] and for $^{10}B$-neutron capture during antineoplasic treatments [4]. A review on organo-boron compounds with biological activity has been written by Petasis [5].

Of particular interest among organo-boron compounds are the α-aminoborinic acid derivatives [6]. It is hypothesized that these entities mimic amino acids and allow their entrance to the CNS by acting as enzyme inhibitors or interacting with neuronal receptors. **Diphenylboroxazolidones** (2,2-diphenyl-1,2,3-oxaborolidin-5-ones) of L-amino acids (DPBX-L-aa) are neutral complexes obtained during the reaction of α-amino acids with diphenylborinic acid. These molecules possess a coordinate boron–nitrogen bond and exhibit effects on the CNS, such as increase in globus–pallidus trigger frequency, tonic-clonic seizures and motor activity increase [7].

Synthesis of DPBX of glycine (DPBX-gly), L-aspartate (DPBX-L-asp) and L-glutamate (DPBX-L-glu) was originally a strategy to avoid the zwitterionic character of these amino acids (FIGURE 1), and to increase the bioavailability of these neurotransmitters into the CNS; allowing evaluation of their inhibitory (gly) or excitatory (L-asp and L-glu) activity, as well as their toxic profile [6].

However, despite the diverse pharmacological activity shown by these molecules, there is no pharmacokinetic information, particularly concerning mammals, due to the lack of sensitive methodology available. In this regard, a HPLC–UV technique has previously been reported for separation and quantification of α-amino acids by boroxazolidone formation [8].

Thus, the aim of the present work was to develop a micro-technique for quantification of DPBX-gly, DPBX-L-asp and DPBX-L-glu in rat dried blood spots (DBSs) by UPLC coupled with MS/MS for its use in the pharmacokinetic description of these molecules.

**Alberto García González[1], Leticia Contreras Zavala[2], Alionka P Angeles Moreno[2], Eduardo Ramírez San Juan[1], José G Trujillo Ferrara[3], Liliana Rivera Espinosa[4] & Gabriel Marcelín Jiménez[†5]**

[1]Department of Physiology, Escuela Nacional de Ciencias Biológicas (ENCB), Instituto Politécnico Nacional (IPN), Mexico City, Mexico
[2]Global Bioanalytical Consulting, SC. Mexico City, Mexico.
[3]Department of Biochemistry, Escuela Superior de Medicina (ESM), IPN, Mexico City, Mexico
[4]Laboratory of Pharmacology, Instituto Nacional de Pediatría, Mexico City, Mexico.
[5]Clinical Pharmacology Research Service, Hospital General de México, Mexico City, Mexico
[†]Author for correspondence:
Tel.: +52 55 9157 7386
E-mail: gabmarcelin@pharmometrica.com.mx

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