

Challenges and novel strategies for quantification of marinobufagenin within the framework of preeclampsia

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Marinobufagenin (MBG), a bufadienolide cardiac inotrope, enjoys a growing interest in the early diagnosis of volume expansion-mediated hypertensive states such as preeclampsia (PE). This endogenous mammalian vasoconstrictive compound, is a selective inhibitor of the α_1 subunit of Na^+, K^+ -ATPase, leading to hypertension and natriuresis. Enhanced production of MBG has been described in preeclamptic patients prior the development of hypertension and proteinuria, leading to consider MBG as a biomarker for PE[1-3].

However, the role of MBG as a biomarker remains to be fully understood as well as its biosynthetic pathways.

The need has arisen for an accuracy and sensitive analytical method of MBG plasma levels in order to further investigate the implications of MBG in PE, and to help to establish a diagnosis for this syndrome.

Our aim is to develop a sensitive and specific analytical MBG dosage method allowing quantifications in the ng/ml range. A critical threshold value might be established by clinicians in the future, in order to discriminate normal pregnant from preeclamptic women.

Nowadays, two quantification methods based each on immunoassays with a limit of detection (LOD) at 5 pg/ml have been published. These techniques suffer from a lack of specificity due to cross-reactivity and tend to exhibit high variability at low concentrations [4].

This condition has led us to consider several liquid chromatography (LC) strategies coupled with different detection modes.

Given that the MBG standard compound is not commercially available, it forced us, in parallel, to develop an extraction method of MBG from the crystallized toad *Bufo Marinus* venom.

Pure MBG has been successfully extracted from the crystallized toad venom. The identity of the compound has been confirmed by MS and TLC.

Preliminary reversed-phase LC-UV method allowed quantifications of MBG in the $\mu\text{g/ml}$ range. In order to decrease the LOD, two different LC strategies will be presented: LC-Fluo method after derivatization of MBG with a fluorophore and LC-MS.

References:

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