Marinobufagenin and its applications in the diagnosis of preeclampsia

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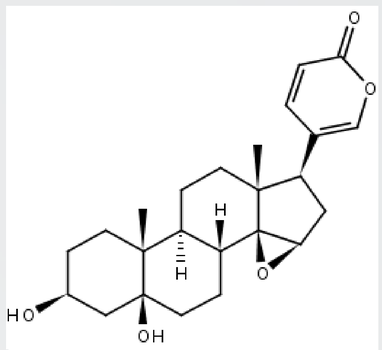
Introduction

- Marinobufagenin (MBG), an endogenous cardiotonic bufadienolide with vasoconstrictive activities, is a selective inhibitor of the α_1 subunit of Na^+,K^+-ATPase implicated in several pathophysiological circumstances that are characterized by hypertension and natriuresis, like in the preeclampsia syndrome (PE).
- PE is a pregnancy-related disorder that consists in the development of hypertension and proteinuria after 20 weeks of gestation. Increased plasma MBG has been observed in mammals (rat and humans) presenting a preeclampsia syndrome[1-3], leading us to consider MBG as a biomarker for PE.
- This consideration implicates an accuracy and sensitive analytical method for MBG plasma levels quantification in order to further investigate the implications of MBG in PE. The final aim is to provide better comprehension of the phenomenon and potential new trends to diagnose the syndrome.



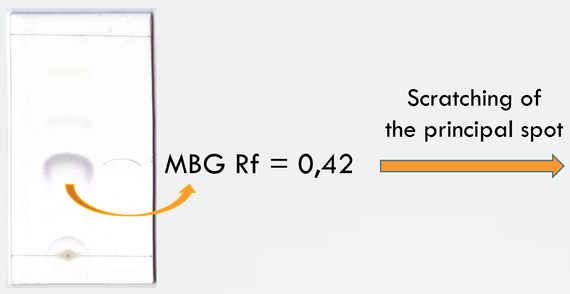
Marinobufagenin

Ref: [1] Vu, H.V., et al., *American Journal of Nephrology*, 2005. **25**(5): p. 520-528. [2] Agunanne, E., et al., *Amer J Perinatol*, 2011. **28**(EFirst): p. 509-514. [3] Lopatin, D.A., et al., *Journal of Hypertension*, 1999. **17**(8): p. 1179-1187.

Extraction of pure MBG

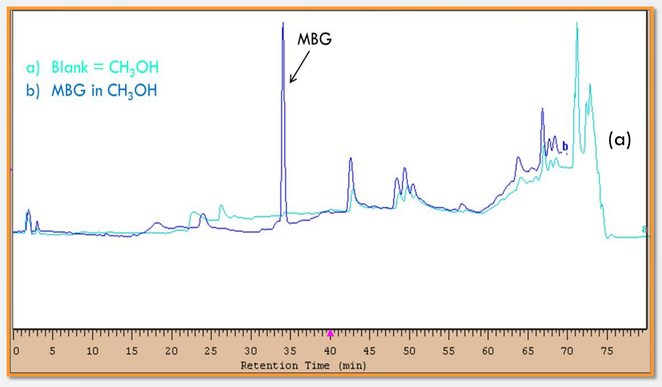
- Parotoid gland secretions of some toad species represent the main source of bufadienolides. Notably, MBG is the major cardiotonic steroid in the *Bufo Marinus* venom.
- *Bufo Marinus* is a toad species present in South America and introduced in Florida and Australia to control agricultural pests in sugar cane where it is currently become invasive.
- Given that no MBG standard is commercially available, we need to develop a successful extraction method to dispose of the reference compound. We made it from the crystallized form ot the venom.

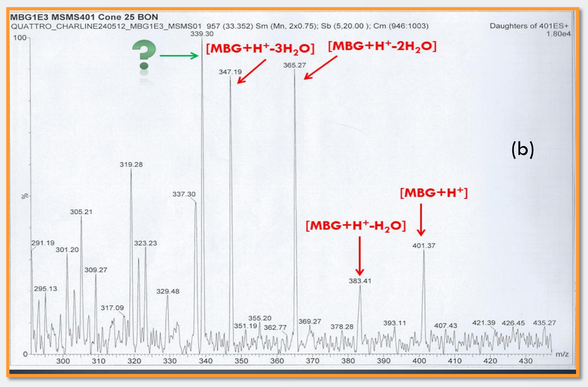
1) Preliminar step: Methanolic extraction and identification of MBG in *Bufo Marinus* venom



Scratching of the principal spot

TLC of the methanol extract of *Bufo marinus* venom (Mobile phase: Ethylacetate – Revelation by $SbCl_3$)





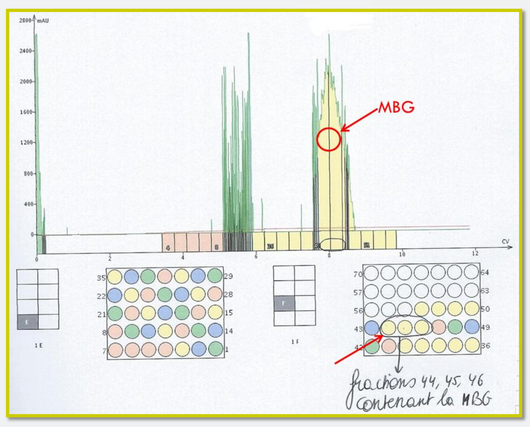
HPLC- UV (a) and MS/MS profile (b) of the principal spot at Rf 0,42

HPLC and MS/MS conditions	
Column	Atlantis dC18
λ UV-detection	296 nm
Mobile phase	Gradient acidified water and ACN
Mass MBG	400
Ion source and mode	Positive ion mode with ESI
Collision gaz and energy	Argon 30eV

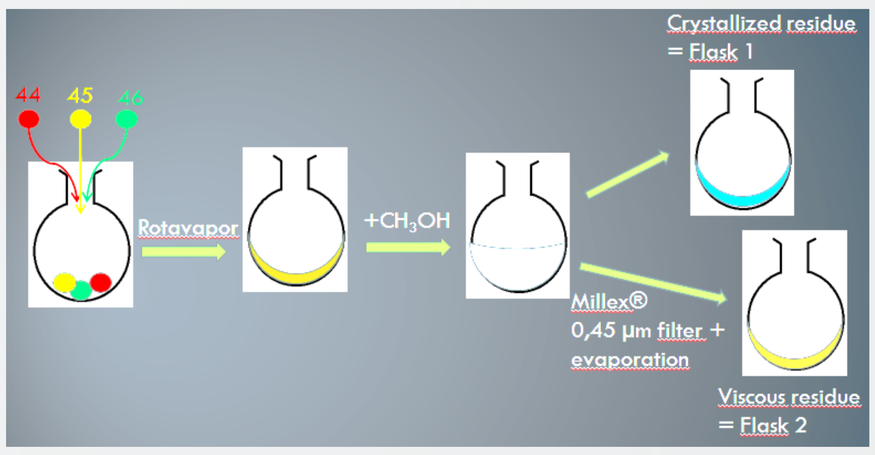
2) Quantitative extraction of MBG from *Bufo Marinus* venom

A Flash Chromatography device was used to isolate a consequent amount of pure MBG .

Flash Chromatography conditions	
Column	HP silice 25 µm
Gradient mobile phase	Hexane-Ethylacetate
Detection	ELSD-1080
Flow	50 ml/min

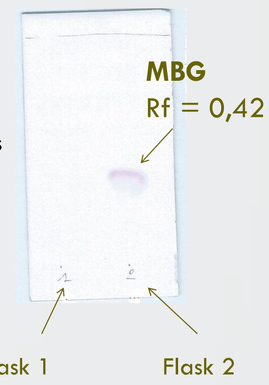


Purification of the isolated fractions



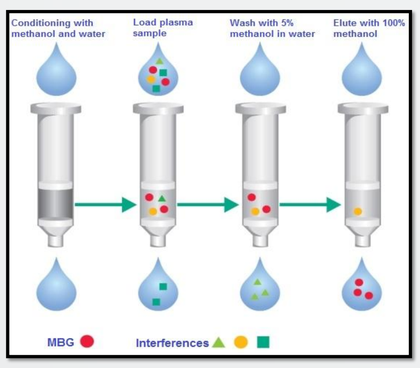
A Stock Solution of 1,16 mg/ml of MBG was made with the viscous residue

Comparison of the two residues by TLC

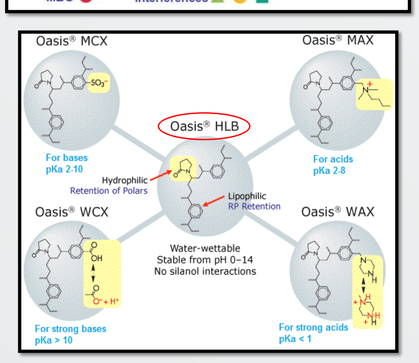


UPLC method development

1) Solid Phase Extraction (SPE) process



The setup of the sensitive dosage method of MBG plasma levels starts with an extraction from plasma samples by SPE. Several SPE sorbent phases were tested. MBG concentration is assessed via UPLC-UV.

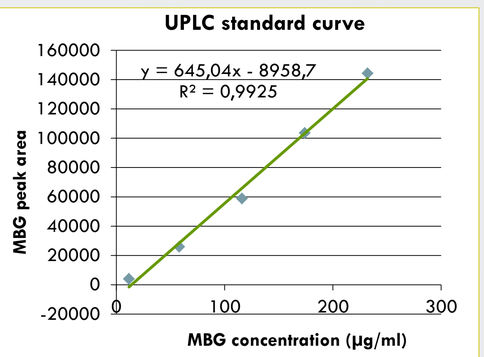


HLB presents the best extraction yield

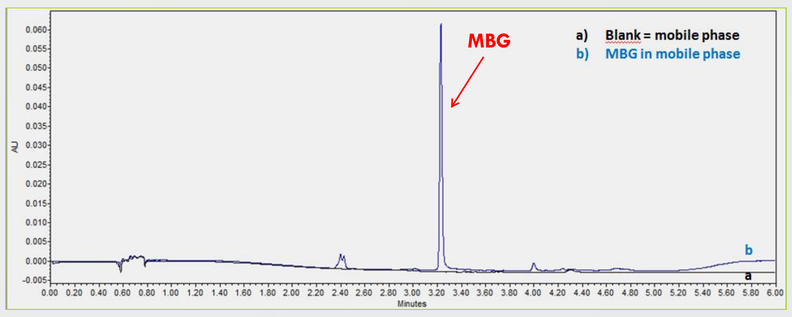
Sorbent cartridge	MBG Peak area	Concentration (µg/ml)	Extraction yield
SPE HLB	302051	823,34	88,72
SPE HLB optimized	259608	708,55	76,35
SPE MCX	253491	692,00	74,57
SPE WCX	230050	628,60	67,737
SPE MAX	285414	778,34	83,87
Liquid-Liquid extraction with mix of ethyl acetate - diethyl ether (4:1, v/v)	214726	587,16	63,27

2) UPLC caracterization

UPLC Conditions	
Column	BEH C18 1,7 µm 2,1 x 100 mm
Gradient mobile phase	Water wtih 0,1% formic acid and ACN
λ UV-detection	296 nm
Flow	0,4 ml/min



MBG UPLC -Chromatogram



Conclusion and Outlooks

- We have developed a successful extraction method of MBG from *Bufo Marinus* crystallized venom and isolated pure MBG as a standard.
- A pre-extraction step from rat and human plasma has been carried out through SPE HLB (hydrophilic lipophilic balanced) cartridge with an extraction yield of 88%.
- Knowing that MBG plasma levels in preeclampsia are in the ng/ml range, optimizations of the reversed-phase LC-UV method to allow quantifications of MBG in this range are currently under development.
- This dosage method once developed and validated will help to quantify MBG plasma levels of regular pregnant women and preeclamptic patients. By this, we will be able to elucidate some biological questions such as: the biosynthetic origin of MBG and/or new routes for the diagnosis of the PE syndrome.

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