Molecularly Imprinted Polymer for Solid Phase Extraction of Catecholamines and Metanephrines

Delphine Derrien[1], Florent Alix[1], Céline Perollier[1], Mégane Cloix[2], Bérengère Claude[2], Sami Bayoudh[1], Philippe Morin[2]

1 POLYINTELL- Voie de l'innovation Chaussée du Vexin Pharmaparc II 27100 Val de Reuil. www.polyintell.com contact@polyintell.com

2 Institut de Chimie Organique et Analytique (ICOA), CNRS FR 2708, UMR 6005, Université d'Orléans, Orléans.

www.polyintell.com

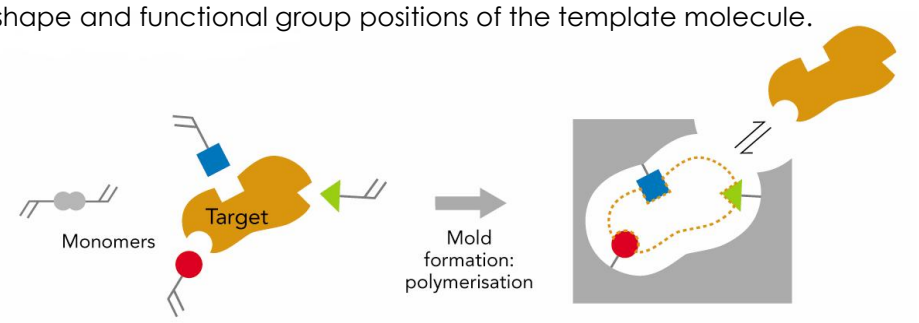
Introduction

Analysis of catecholamines and derivatives is fundamental for diagnostic of neurological diseases such as Parkinson's and Alzheimer's (dopamine (DA), 3-methoxytyramine (3 - MT), epinephrine (A), norepinephrine (NA)). Furthermore, quantification of plasma free metanephrine (MN) and normetanephrine (NMN) is considered to be the most accurate test for the clinical chemical diagnosis of pheocromocytoma.

The concentrations of these endogenous molecules are very low in serum and plasma (lower than 1nM). A clean up step is crucial in order to improve the sensitivity and the specificity before LC analysis. Current method involve non specific sample preparation. Solid phase extraction (SPE) based on molecular imprinted polymers (MIP) is the finest approach as it provides a high specificity and selectivity based on the recognition of form and interaction. We have successfully designed and synthesized a new polymer MIP specifically for catecholamines and metanephrines. In this poster, we will describe the results obtained for molecularly imprinted solid phase extraction (MISPE) for catecholamines and metanephrines from serum and plasma.

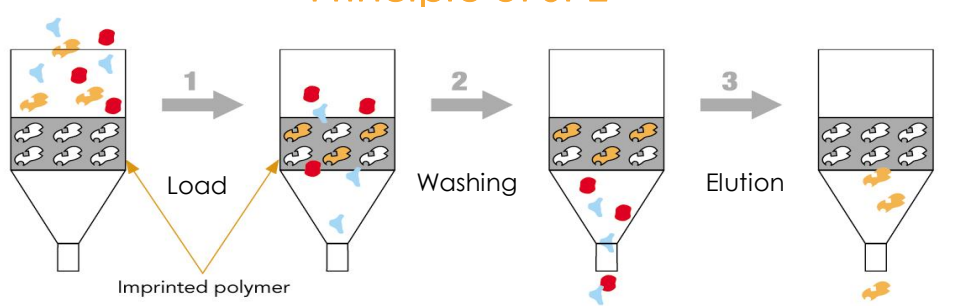
Principle of MIP

MIP is a process to create a three-dimensional network that as a « memory » of the shape and functional group positions of the template molecule.



This is a process where functional and cross-linking monomers are copolymerized in the presence of the target analyte (the imprint molecule). The functional monomers form a complex with the imprint molecule, and following copolymerization, their functional groups are held in position by the highly crosslinked polymeric structure subsequent removal of the imprint molecule reveals binding sites that are complementary is size and shape to the analyte .

Principle of SPE



Solvent able to develop specific interaction with low elution power

↓

Interfering compounds with no affinity with the sorbent will be eliminated.

Elimination of non specific interactions and interfering analytes

Disruption of specific interaction

↓

Desorption of target analyte

Protocol for serum and plasma

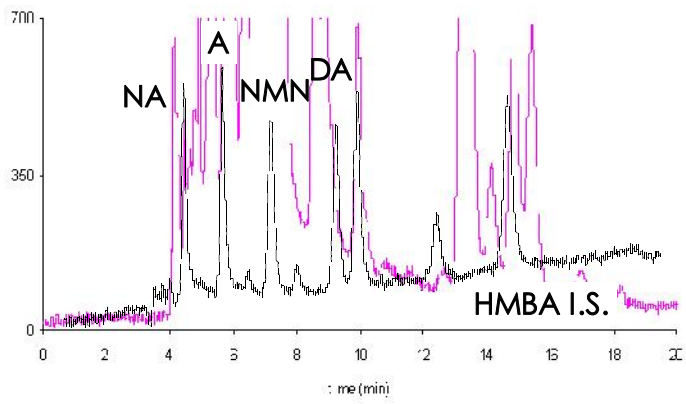
Step	Solvent
Loading	4mL of serum or plasma (pre-diluted by 10 with water)
Wash	4mL Water 2mL 85/15 Water/MeOH Dry 30 secondes 1mL MeOH
Elution	1,5mL MeOH-5% acetic acid

Advantages :

- Direct loading of diluted plasma or serum
- Dilution with water = no precipitation of protein and no step of centrifugation
- Concentration by 8

Extraction of catecholamines from serum

Analysis in LC-UV :



Serum spiked with catecholamines at 450nM

After MIP-SPE

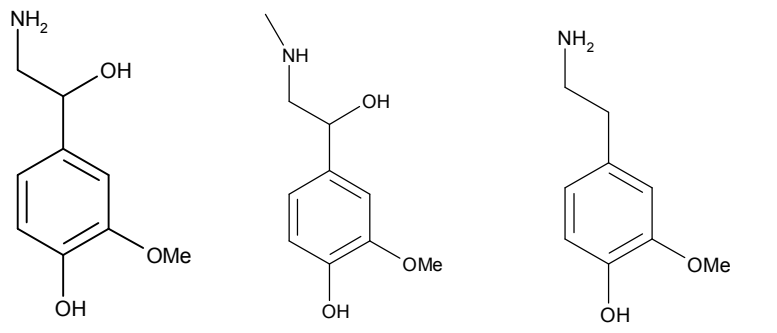
Column : Daiso C18B 250 X 4.6mm d.i. (5µm)
Mobile Phase : MeOH/Water-0.1%TFA 10/90 (v/v)
UV: 281nm, F= 0.7mL/min

Average Recoveries in serum

(*n* = 10)	R_{rel} (%)
NA	80.4
A	83.9
NMN	106.2
3-MT	101.9

Clean-up efficient
Test on 4 catecholamines
Recoveries > 80%
Good linearity : R^2>0.98

Analysis of metanephrine from plasma : diagnosis of pheocromacytoma

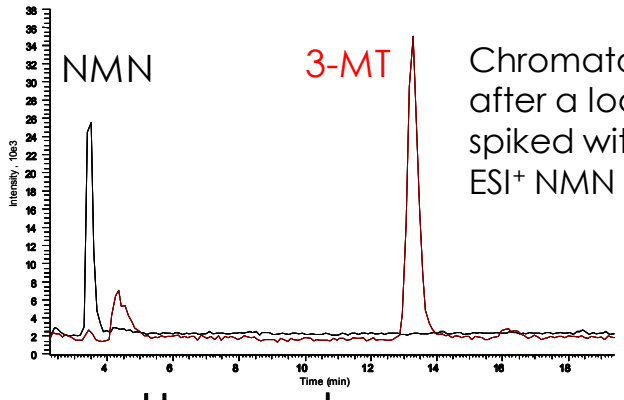


Normetanephrine NMN

Metanephrine MN

3-Methoxytyramine 3-MT

Analyse by LC-MS-ESI+ on Atlantis T3 column C18 150 X 2.1mm d.i. (3µm)
Mobile Phase : MeOH/Water-0.1%HCOOH 2/98 (v/v). F= 0.2mL/min.



Chromatograms of the elution fraction after a loading of 4mL of diluted plasma spiked with 60nM of NMN and 3-MT
ESI+ NMN : m/z = 166, 3-MT m/z=151

Human plasma

Recoveries > 70%
Linearity R^2>0.99
LC-MS : LOQ = 14nM

Conclusion

The detection of catecholamine and metanephrines in complex matrices at the trace level is a real interest to provide diagnostic. The synthesis of a MIP catecholamines and metanephrines was realised and shows a strong potential for the extraction of these molecules in various environment. 4 catecholamines were extracted specifically from serum spiked at concentration between 150 and 450 nM range. More than 70 % recovery were obtained with a factor of 8 of enrichment. MIPs superior cleanup and selectivity provide faster/simpler sample prep methods, better MS compatibility (reduced ion suppression) allowing scientist to reach lower detection limits and improved sensitivity.