TROUBLESHOOTING : Development of a LC-MS/MS method for trace-level analysis of salivary melatonin

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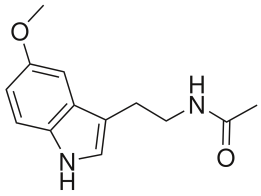
INTRODUCTION

Melatonin is an methoxyindole produced by the pineal gland. Due to its ubiquitous presence in animals, humans, plants and bacteria, melatonin is thought to be one of the first compound produced in accordance with the biological clock to coordinate basic events in life.

Melatonin is currently used as a marker of the circadian rhythm, providing information about potential disturbance and misalignment of circadian rhythms which is thought to be involved in a growing number of disease such as psychiatric diseases. Melatonin quantification can be also useful in the diagnosis of a rare cancer : the pituitary gland cancer.

As it is not stored in the pineal gland, melatonin levels in plasma and saliva provide a direct assessment of the gland activity.

Our goal is to develop a highly sensitive LC-MS/MS method for the measurement of salivary melatonin. However, during the development, several troubles were observed and had to be resolved.



Melatonin

$C_{13}H_{16}N_2O_2$

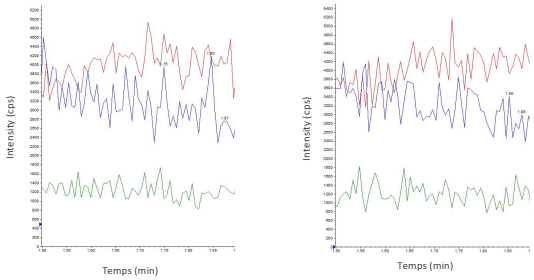
232,28 g/mol

CHALLENGES

1) CONTAMINATION OF THE SAMPLE BY PLASTIC CONSUMABLES

Procedural blanks showed a contamination with melatonin. To identify the exogen source, several experiment were conducted on reagents and plastics, as well as evaporation systems.

a) EVAPORATION

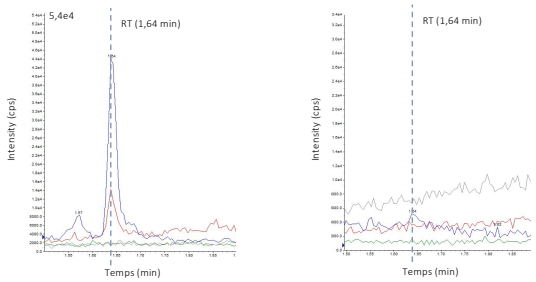


N_2 flow at 40°C

Concentrator Christ RVC 2-25 CD Plus

No differences can be spotted. Evaporation is not the source of the contamination

b) PLASTICS



Procedural blank with **UNWASHED PLASTIC CAPS**

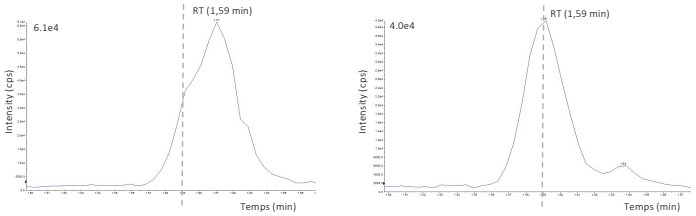
Procedural blank with **WASHED PLASTIC CAPS**

Plastic consumables must be avoided and non-avoidable plastic caps must undergo thorough wash with ethanol (sonication for 15 minutes) as previously said by others authors.[1,2]

2) INTERFERENCES IN REAL SALIVA SAMPLES

Passing drooling and hourly sampling was challenging because interferences coming from food, drinks, medication, … could be present.

Some saliva samples showed interferences. To remove them, the first approach was to modify the protocol of the liquid-liquid extraction (LLE) rather then modifying the liquid chromatography method. Different extraction solvents and different volumes were tested, as well as a washing step after the extraction.



Saliva sample **BEFORE** wash

Saliva sample **AFTER** wash

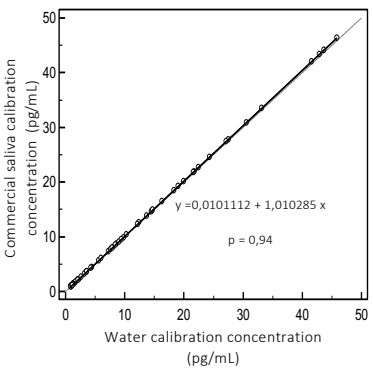
A washing step with 1 mL of water was added after the LLE because it was able remove the interference.

3) BLANK CALIBRATORS FOR HIGH SENSITIVITY

As human saliva naturally contains melatonin, commercially available blank saliva was purchased. Blank saliva showed low matrix effect thus tests were performed to assess if water could replace it.

Two kinds of calibrators were prepared in commercially available blank saliva and water.

The concentration of 58 saliva samples was calculated with all the calibration curves and compared the obtained results. Concentrations ranged from 0,93 pg/mL to 43,34 pg/mL.



All the Passing-Bablok regressions showed that all the calibration curves were providing statistically similar results for the same sample. Thus, calibration curve will be realised in water.

1. Khan, S. A. *et al.* Monitoring salivary melatonin concentrations in children with sleep disorders using liquid chromatography-tandem mass spectrometry. *Ther. Drug Monit.* **35**, 388–395 (2013).

2. Eriksson, K., Östin, A. & Levin, J. O. Quantification of melatonin in human saliva by liquid chromatography-tandem mass spectrometry using stable isotope dilution. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **794**, 115–123 (2003).