Validation of an ultra-sensitive method for the quantification of melatonin in human saliva

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Introduction :

Melatonin is a methoxyindole produced by the pineal gland currently used as a marker of the circadian rhythm. Melatonin quantification could also be useful in the diagnosis of a rare cancer: the pituitary gland cancer. As daytime melatonin levels in human saliva do not exceed 5 pg/mL, the aim of our work was to validate a sensitive LC-MS/MS method for the salivary melatonin quantification.

Material and Methods:

Saliva samples were exctracted by liquid-liquid extraction and analyzed by a Nexera X2 UPLC from Shimadzu (Shimadzu Corporation, Kyoto, Japan) coupled to a QT6500 mass spectrometer (Triple Quadrupole and Linear Trap analysers) by Sciex (AB Sciex, CA, USA). Validation of the method was realized according the European Medicines Agency (EMA) guidelines. As saliva naturally contains melatonin, quality controls samples (QCs) were commercially available cortisol depleted saliva, spiked at different levels : 0.8, 2.4, 40 and 80 pg/mL. Each QCs was analyzed 5 times per day on 3 different days to assess within and between-run coefficients of variation (CV). The calibrators (0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 pg/mL) were prepared in water by serial dilution. Linearity of the calibration curve was assessed by performing linear regression.

Results

After experiments, recovery rate was above 70% while matrix factor for the analyte was above 90%. Intra-run and inter-run accuracies were encompassed between 89% and 113%. Intra-run and inter-run CVs were all comprised between 1% and 7%. Accuracies and CV are reported in table 1. The limit of quantification (0.78 pg/mL) was determined as being the lowest concentration allowing an easy peak integration of the qualitative transition. The equation of the linear regression was y = 0,10528 x – 0,00769 (r= 0,99999). Calibration curve is represented in figure 1.

	DAY	CALCULATED CONCENTRATION (pg/mL)	INTRA-RUN PRECISIONS (%)	INTRA-RUN ACCURACY (%)	INTER-RUN PRECISION (%)	INTER-RUN ACCURACY (%)
QC LLOQ (0,8 pg/mL)	1	0,79 0,78 0,78 0,75 0,75	2,43	96,42	4,28	98,42
	2	0,8 0,86 0,81 0,81 0,84	3,05	103		
	3	0,74 0,75 0,78 0,79 0,78	2,82	96		
QC LOW (2,4 pg/mL)	1	2,45 2,44 2,28 2,24 2,35	3,98	98	4,45	102,06
	2	2,49 2,46 2,46 2,41 2,38	1,81	101,67		
	3	2,56 2,64 2,45 2,58 2,55	2,69	106,5		
QC MID (40 pg/mL)	1	40,78 41,95 44,20 39,20 42,08	4,42	104,11	4,31	108,67
	2	44,23 43,80 44,40 42,30 44,07	1,93	109,4		
	3	43,96 45,99 44,09 45,68 45,40	2,08	112,56		
QC HIGH (80 pg/mL)	1	86,50 82,66 81,86 80,03 79,13	3,49	102,55	4,38	107,48
	2	85,50 86,71 85,05 86,86 85,52	0,94	107,41		
	3	90,50 88,59 90,23 89,18 91,43	1,24	112,48		

Table 1. Intra-run precisions and accuracies and inter-run precisions and accuracies for each QCs

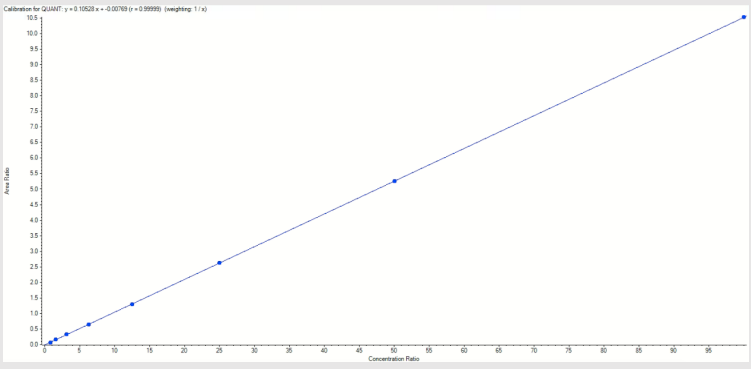

Figure 1. Calibration curve

Conclusions:

Our method was thus successfully validated and currently allows the determination of daytime melatonin levels with a high accuracy and selectivity.