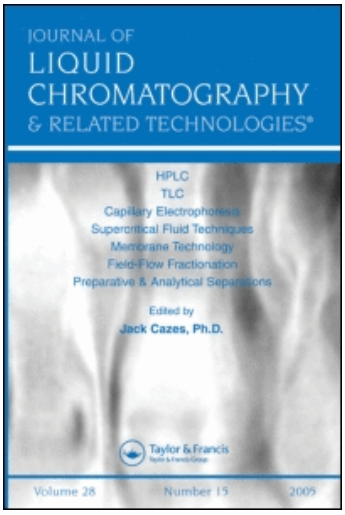

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Analysis of Melatonin in Dosage Formulation by Capillary Electrophoresis

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Analysis of Melatonin in Dosage Formulation by Capillary Electrophoresis

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Abstract: A simple, fast, and reproducible capillary electrophoretic method is developed and validated for analysis of melatonin in pharmaceutical preparations. The CE method was developed by using a fused silica capillary (60 cm × 50 μm I.D.), phosphate buffer (50 mM, 3.0 pH) as the background electrolyte (BGE), 20 kV applied voltage with UV detection at 214 nm, and at a working temperature of 23 ± 1°C. Linearity was observed in the concentration range from 10 to 100 μg/L with a correlation coefficient (R^2) of 0.9996. The limits of detection and quantification achieved were 10 and 15 μg/mL, respectively. The percentage recovery of melatonin from pharmaceutical preparations was 95.0. Validation parameters prove the precision of the method and its applicability for the determination of melatonin in pharmaceutical tablet formulations.

Keywords: Melatonin, Capillary electrophoresis, Pharmaceutical analysis, Pharmaceutical tablet formulation

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INTRODUCTION

Melatonin is a hormone, which is produced in the pineal gland at night and its secretion is stimulated and inhibited by dark and light, respectively. Melatonin is chemically known as *N*-[2-(5-methoxy-1H-indol-3-yl)ethyl] acetamide or *N*-acetyl-5 methoxytryptamine (Figure 1). This drug has a direct action on suprachiasmatic nuclei to influence circadian rhythms. Melatonin is metabolized in the liver and the main metabolite excreted is 6-sulphatoxy-melatonin. Melatonin is used for the treatment of jet lag (sleep disturbance, loss of appetite, reduced psychomotor efficiency, and general malaise).[1,2] Besides, this drug also provides better well being, strengthens the immune system, and reduction in free radicals in the body. Pharmacokinetic properties of melatonin have been studied,[3–6] and research is underway to determine it's effect as an antioxidant and immnomodulator in cancer therapy.

Drug testing is an integral part of pharmaceutical analysis and routine quality control monitoring of drug release characteristics. Therefore, pharmacokinetics and pharmacodynamics studies of melatonin are required, which involve the use of development of analytical methodologies. Some reports have been published on the analysis of melatonin by using liquid chromatographic[7–12] and capillary electrophoretic[13] approaches in various biological matrices, but there is no paper published on melatonin analysis in dosage formulations by using capillary electrophoresis (CE), which is a versatile technique of high speed, sensitivity, resolution, low sample requirement and running cost. In view of these points, attempts have been made to develop and validate a CE method for the analysis of melatonin in pharmaceutical preparations. The developed method was utilized for the analysis of melatonin in tablet formulation and the results of these findings are reported herein.

EXPERIMENTAL

Chemical and Reagents

Standard melatonin was obtained from Sigma Chemical Co., USA. Melatonin tablets of Natral Inc., USA, were purchased from a local market. Methanol of HPLC grade and *o*-phosphoric acid of A.R. grade were purchased from Fisher

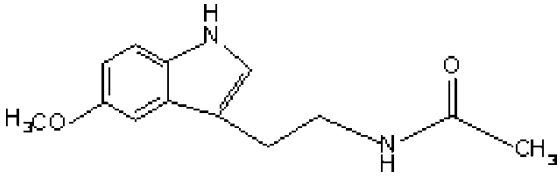

Figure 1. Chemical structure of melatonin.

Scientific (Fairlawn, New Jersey, USA). Sodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$) of A.R. grade was obtained from BDH Limited (Poole, England). Phosphate buffers were prepared by the standard methods.

Instruments Used

The capillary electrophoresis instrument used was Quanta 4000 E (Waters, USA) with Millennium 2000 Waters data station. The separation was carried out using a conventional fused silica capillary (60 cm × 50 μm I.D.), which was obtained from Waters, USA. pH was recorded using a pH meter (model 611, Orion Research Inc., USA). Purified water was prepared using a Millipore Milli-Q (Bedford, MA, U.S.A.) water purification system.

Capillary Electrophoretic Conditions

The CE instrument described in the instrumental section was used with the UV detector at the cathode side. The background electrolyte used in this study was phosphate buffer (50 mM, pH 3.0), which was prepared, filtered, and degassed on a daily basis before use. All the experiments were carried out at a temperature of 23 ± 1°C, using 20 kV as the applied voltage and detection by UV at 214 nm. Typically, the samples were loaded in 20 seconds by using a hydrostatic mode of injection at the cathode end of capillary. The data collection was carried out at 20 points per second. The electroosmotic mobility of BGE was ascertained by using benzene and was calculated by using a standard equation. The identification of melatonin in tablet formulation was carried out by running the electropherogram of the standard melatonin under the identical CE conditions.

Preparation of Standard Stock Solution

The stock solution of melatonin (100 μg/mL) was prepared by accurately weighing 5 mg of the drug and dissolving in methanol (50 mL). Serial dilutions (10 to 90 μg/L) were carried out using methanol to obtain the required concentration ranges.

Extraction of Melatonin from Tablet

Quantitative analysis of melatonin in tablets (400 mg containing 3 mg as melatonin) was carried out by extraction with methanol. Five tablets were ground to a fine powder and extracted with 50 mL methanol for 15 minutes by using a sonicator at 40°C. Methanol was decanted and the residue was again extracted similarly, twice with 50 mL methanol each time. Three

methanol extracts were combined together to give 150 mL volume. Methanol extract was filtered through a 0.22 μm membrane, diluted to various concentrations, and used for the electrophoretic studies.

Quantitation and Linearity

Equal volumes of melatonin standard solutions and the assay preparations were loaded onto the capillary electrophoresis instrument hydrostatically (20 seconds) and the electropherograms were recorded. Calibration standards of each concentration were analyzed in triplicate. Calibration curves of melatonin were constructed using the observed peak area versus concentrations of analyte.

Selectivity

The selectivity of the developed method was investigated by observing any interference encountered from excipients present in the formulations. It was observed that other components of the tablet, i.e., magnesium stearate, cellulose, silica, stearic acid, and gum do not interfere with the proposed method.

Validation

The limits of detection (LOD) and quantitation (LOQ) were determined as 3 and 5 times of the baseline noise, respectively, following the guidelines of United States Pharmacopoeia.[14] The results of the statistical analysis of the experimental data (CE, extraction, and stability), such as the standard deviation, correlation coefficients, and confidence limit were calculated by using a Microsoft Excel software program. Good linearity of the calibration graphs and negligible scatter of experimental points are clearly evident by the values of correlation coefficient and standard deviation.[15]

RESULTS AND DISCUSSION

Extraction from Tablet

The extraction of melatonin from tablets was carried out by using methanol and the percentage recovery achieved was 95.0. The reproducibility of extraction was determined by using three sets of extractions separately and independently, and results are given in Table 1. The data of Table 1 indicates ± 0.55, 0.9996 and 98.5 as the values of standard deviations, correlation coefficients, and confidence levels, respectively, indicating good efficiency of the extraction method. Attempts have also been made to extract melatonin from tablets by using ethanol, acetone, and buffers, but the maximum recovery

Table 1. Regression analysis data for the extraction of melatonin

Extraction set	Recovery (%)	Standard deivation	Correlation coefficient	Confidence level
I	95.0	± 0.55	0.9996	98.5
II	95.0	± 0.55	0.9996	98.6
III	95.0	± 0.55	0.9996	98.5

could be achieved with methanol, and, therefore, methanol was used as an extracting solvent for melatonin from the tablets.

Capillary Electrophoresis

CE parameters such as migration time, electrophoretic mobility, electroosmotic mobility, velocity, and number of theoretical plates for melatonin are calculated and given in Table 2. The values of the migration time, velocity, electrophoretic mobility, electroosmotic mobility, and theoretical plates were 10.2 minutes, 5.15 cm sec^{-1}, 0.93 $cm^2\ V^{-1}\ sec^{-1}$, 1.64 $cm^2\ V^{-1}\ sec^{-1}$, and 25200, respectively. The values of standard deviation, correlation coefficients, and confidence levels for these parameters were ± 0.85 to ± 0.86, 0.9995 to 0.9996, and 97.4 to 98.1 respectively, which indicate good precision and efficiency of the developed method. A typical electropherogram of melatonin in standard solution is shown in Figure 2.

To optimize capillary electrophoretic conditions, various buffers with different concentrations and pHs were tested. Some organic modifiers such as

Table 2. Capillary electrophoretic parameters for analysis of melatonin in pharmaceutical preparations

Parameters	Standard deviation (SD)	Correlation coefficient (R^2)	Confidence limit
t_{mig}	10.2 ± 0.85	0.9996	97.8
v	5.15 ± 0.85	0.9996	98.0
μ_{ep}	$0.93 \times 10^{-4} \pm 0.85$	0.9996	98.1
μ_{os}	$1.64 \times 10^{-4} \pm 0.85$	0.9996	98.0
N	25200 ± 0.86	0.9995	97.4

N: Number of theoretical plate, t_{mig}: migration time (min.), μ_{ep}: electrophoretic mobility ($cm^2\ V^{-1}\ s^{-1}$), μ_{os}: electroosmotic mobility ($cm^2\ V^{-1}\ s^{-1}$) and v: velocity (cm min^{-1}). Experimental conditions: Capillary: Fused silica (60 cm $\times$ 50 μm I.D.). BGE: Phosphate buffer (50 mM, 3.0 pH). Voltage: 20 kV. Detection: UV at 214 nm. Temperature: $23 \pm 1^{\circ}C$.

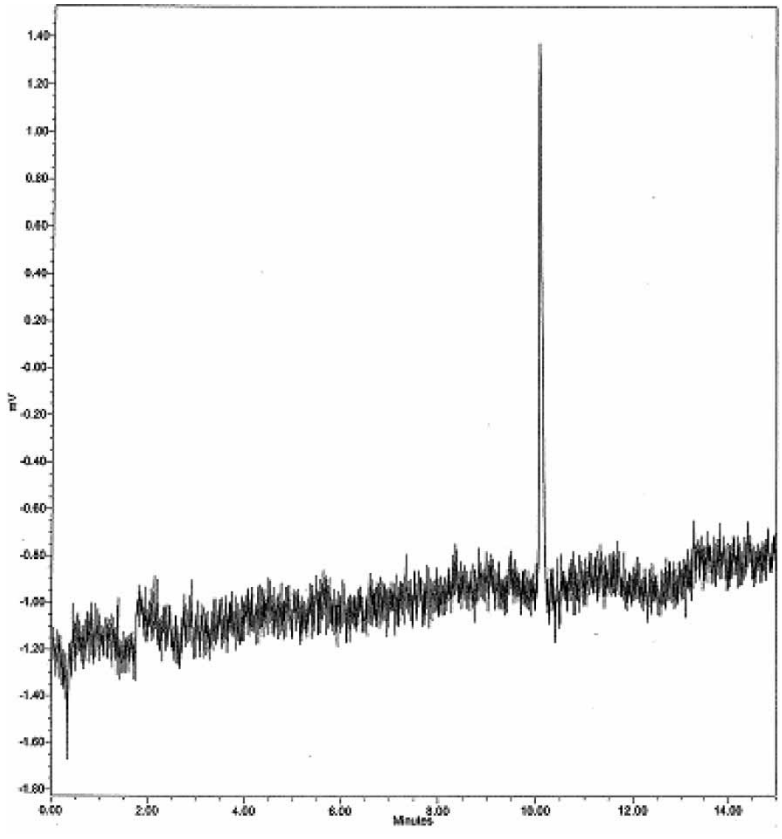

Figure 2. Electropherogram of melatonin (standard solutions, 100 μg/mL) using fused silica capillary (60 cm × 50 μm I.D.), phosphate buffer (50 mM, 3.0 pH) as BGE with 20 kV as the applied voltage and UV detection at 214 nm.

methanol, ethanol, trifluoroethanol additives were also tried. Preliminary investigations were directed towards the effect of various variables on the system suitability of the method. The parameters assessed include the variations in pH values, concentration of BGE, applied voltage, detection wavelength, type and quantity of organic modifiers. As a result of extensive experimentation optimized electrophoretic conditions were developed and reported herein.

The effect of pH on electrophoretic mobility and migration time of melatonin is carried out, and the findings are plotted in Figure 3. Observation of this figure indicates that electrophoretic mobility decreases with increasing pH value. The effect of BGE concentration on electrophoretic mobility and migration time was also studied. The results of this set of experiments are shown in Figure 4. It was observed that there was no marked effect on the electrophoretic mobility at the higher concentration of BGE, however, an increase

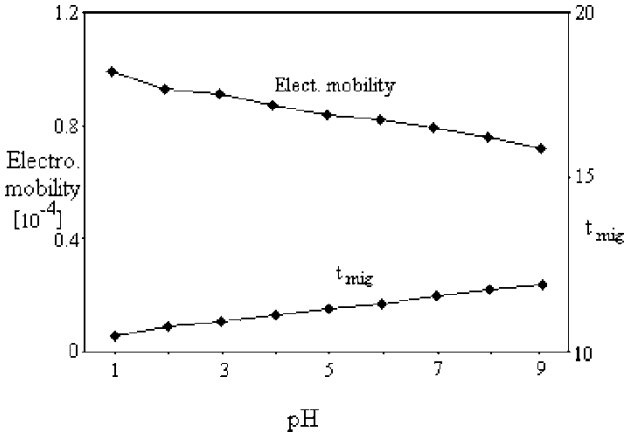

Figure 3. Effect of BGE pH on electrophoretic mobility ($cm^2\,V^{-1}\,sec^{-1}$) and migration time (mint.) of melatonin.

in migration time was observed. It was also noted that electroosmotic mobility increased at a high concentration of BGE, which is responsible for the higher migration times of melatonin. It is also interesting to mention, here, that the detection of melatonin becomes poor at higher concentrations of BGE. Consequently, the optimum CE conditions used in the proposed method including a BGE (phosphate buffer, 50 mM, pH 3.0), 20 kV as the applied voltage, 20 seconds sampling time, 214 nm detection wavelength at $23 \pm 1^{\circ}C$ were developed and applied for all the measurements.

The developed electrophoretic and electroosmotic mobilities forced the melatonin molecule to move towards the detector side. The used capillary

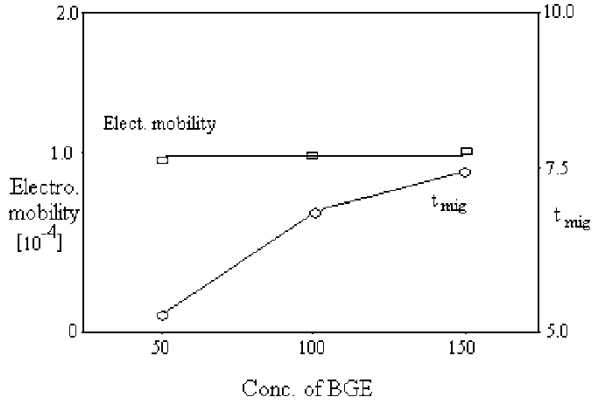

Figure 4. Effect of different concentrations of BGE (mM) on electrophoretic mobility ($cm^2\,V^{-1}\,sec^{-1}$) and migration time (mint.) of melatonin.

was made of fused silica having silanol groups on its surface. The studied molecule is attracted electrostatically by these silanol groups and, as a result of electrophoretic, electroosmotic forces and electrostatic attraction, melatonin moved towards the cathode. The charge size ratio also contributed to the migration velocity of this drug, since the greater the charge size ratio the higher the mobility with a lower migration time.

Analysis of Melatonin in Pharmaceutical Formulations

The validity of the developed method was applied to various concentrations taken from the pharmaceutical formulations for determining their content of melatonin. Qualitative and quantitative analyses of melatonin in tablet formulation (methanol extract) were carried out by using the developed CE method. An electropherogram of melatonin in tablet extract is shown in Figure 5. Melatonin in tablets were identified by comparing its migration time and electrophoretic mobility with those of the standard melatonin solutions. The confirmation of melatonin in tablets was ascertained by the internal addition method too. Quantitative analysis of melatonin in tablet formulation was carried out by comparing its peak area with the peak area of standard melatonin. For calculation of percentage recovery, three sets of CE experiments were carried out under identical conditions, and the recovery of melatonin from tablets was found to be 95.0%.

Selectivity

The selectivity of the developed method was investigated by observing any interference from excipients present in the pharmaceutical preparation, which include magnesium stearate, cellulose, silica, stearic acid, and gum. There was no interference from the ingredients using the proposed CE method, which indicates that the reported extraction and CE methods are selective (Table 3).

Linearity

The linearity of calibration curves (peak area vs. concentration) for melatonin in pure solution, as well as in dosage forms were checked over the concentration ranges of 10 to 100 $\mu g/mL$, with correlation coefficient (R^2) of greater than 0.9996, as determined by least squares analysis.

Limit of Detection (LOD), Limit of Quantitation (LOQ), and Accuracy

The limits of detection (LOD) and quantitation (LOQ) were calculated for the calibration graphs of melatonin as three and five times of the noise level for

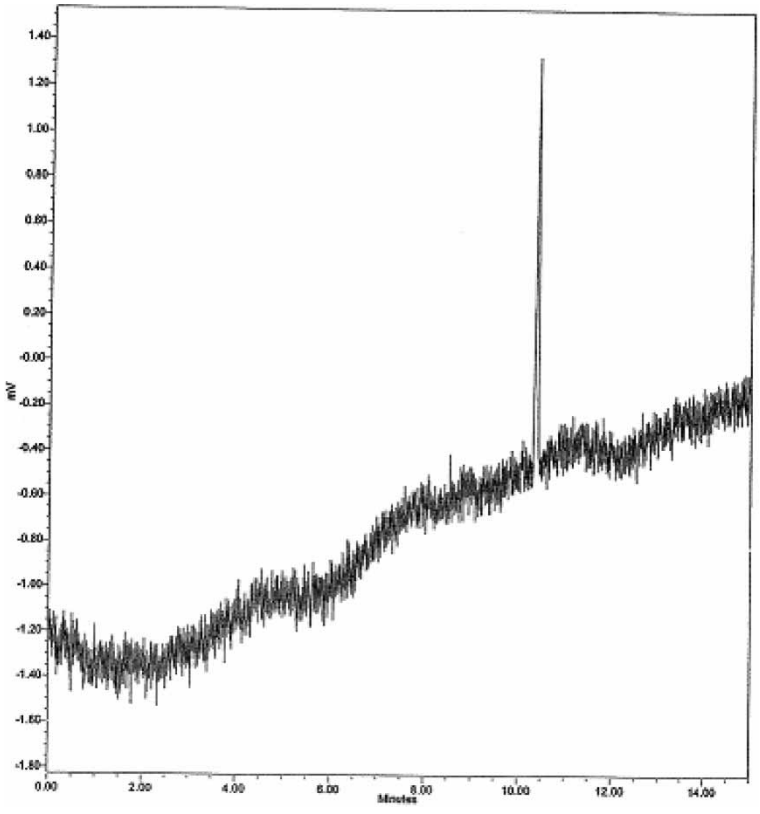

Figure 5. Electropherogram of melatonin (tablet extract, 100 μg/mL) using fused silica capillary (60 cm × 50 μm I.D.), phosphate buffer (50 mM, 3.0 pH) as BGE with 20 kV as the applied voltage and UV detection at 214 nm.

LOD and LOQ, respectively. The values for LOD and LOQ were 10 and 15 μg/mL, respectively. The accuracy of the method was tested by analyzing different samples of melatonin at various concentration levels in pure solutions. The results were expressed as percent recoveries of melatonin.

Stability of Analytical Solutions

Stabilities of sample solutions of melatonin were tested over a week's time. Freshly prepared and stored samples were analyzed by the optimized proposed CE method. Percent differences observed were in the range of 0.12 to 0.29 (Table 4), indicating the stability of the melatonin solution for one week. Standard deviation of the stability analysis was ± 0.54 to 0.55, while the values of correlation coefficient were in the range of 0.9996 to 0.9997. The confidence limits ranged from 99.0 to 99.1. The possible

Table 3. Effect of experimental parameters on the percent recoveries of melatonin

Parameter	Variations	Recovery (%)
Conc. of BGE	25 mM	94.0[a]
	50 mM	95.0
	100 mM	93.0
	150 mM	91.5[b]
pH of BGE	2	94.5
	3	95.0
	4	94.0
	5	92.0
	6	90.5
	7	88.0[a]
	8	85.0[a]
	9	82.0[a]
	10	80.6[a]
Wavelength (nm)	214	95.0
	254	80.0[b]
Voltage Applied (kV)	5	93.5[a]
	10	94.0[a]
15	94.5[a]	
20	95.0	
Sampling time (sec.)	5	93.0[b]
	10	94.0
	15	94.4
	20	95.0
	25	95.0
	30	95.0

[a]Broad peaks.
[b]Poor detection.

photodegradation of melatonin in aqueous solution and BGE was also studied by exposing various samples of melatonin to direct sunlight and darkness for 7 days. The samples kept in the dark showed full recovery without significant degradation. However, melatonin sample solutions exposed to sunlight showed a photodegradation of about 2.0%.

Robustness

To evaluate the robustness of the developed CE method, the optimum conditions for this method have been slightly modified for samples of

Table 4. Intra- and inter days data for melatonin (stability of melatonin within a week in aqueous solution of 0.1 mg/mL)

Days	Difference (%)	Standard deviation	Correlation coefficient	Confidence limit
1	0.12	±0.54	0.9996	99.1
2	0.15	±0.55	0.9997	99.0
3	0.19	±0.54	0.9996	99.1
4	0.23	±0.54	0.9997	99.0
5	0.25	±0.55	0.9997	99.0
6	0.28	±0.54	0.9996	99.1
7	0.29	±0.55	0.9997	99.0

n = 3.

Difference (%) = [quantity found in fresh solution—quantity found in stored solution] × 100/(quantity found in fresh solution).

melatonin. The small changes (0.1% magnitude) made included BGE composition, applied voltage, and sampling time. It was observed that there was no remarkable variation in CE results, which indicated a good robustness of the reported method. Considering the modifications in method suitability, parameters, and the selectivity of the method, as well as carrying out the experiment at room temperature, would conclude that the method conditions are robust.

CONCLUSION

A capillary electrophoretic method is developed and validated for the analysis of melatonin in pharmaceutical preparations. The method is inexpensive, fast, and reproducible. The limit of detection (LOD) and limit of quantitation (LQD) were 10 and 15 μg/mL, respectively. Linearity was observed in the concentration range of 10 to 100 μg/mL. The presented method can be used for the quality control of melatonin in pharmaceutical preparations.

REFERENCES

1. Arendt, J.; Aldhous, M.; Marks, V. Alleviation of jet-lag by melatonin: Preliminary results of controlled double-blind trial. Brit. Med. J. **1986**, *292*, 1170.
2. Claustrat, B.; Brun, J.; David, M.; Sassolas, G.; Chazot, G. Melatonin and jet-lag: Confirmatory result using a simplified protocol. Biol. Psych. **1992**, *32* (8), 705–711.

3. Mulchahey, J.J.; Goldwater, D.R.; Zemlan, F.P. A single blind, placebo controlled, across groups dose escalation study of the safety, tolerability, pharmacokinetics and pharmacodynamics of the melatonin analog beta-methyl-6-chloromelatonin. Life Sci. **2004**, *75* (15), 1843–1856.
4. Lissoni, P.; Rovelli, F.; Fumagalli, G.; Confalonieri, G.; Brivio, F.; Mariani, M. Pharmacokinetics of melatonin. Recenti progressi in medicina **1998**, *89* (7-8), 381–382.
5. Mallo, C.; Zaidan, R.; Galy, G.; Vermeulen, E.; Brun, J.; Chazot, G.; Claustrat, B. Pharmacokinetics of melatonin in man after intravenous infusion and bolus injection. Eur. J. Clin. Pharmacol. **1990**, *38* (3), 297–301.
6. Lane, E.A.; Moss, H.B. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. J. Clin. Endocrinol. & Metab. **1985**, *61* (6), 1214–1216.
7. Hamase, K.; Hirano, J.; Kosai, Y.; Tomita, T.; Zaitsu, K. A sensitive internal standard method for the determination of melatonin in mammals using precolumn oxidation reversed-phase high-performance liquid chromatography. J. Chromatogr. B: Anal. Technol. Biomed. & Life Sci. **2004**, *811* (2), 237–241.
8. Motoyama, A.; Kanda, T.; Namba, R. Direct determination of endogenous melatonin in human saliva by column-switching semi-microcolumn liquid chromatography/mass spectrometry with on-line analyte enrichment. Rapid Commun. Mass. Spectrom. **2004**, *18* (12), 1250–1258.
9. Almeida, E.A.; Klitzke, C.F.; Martinez, G.R.; Medeiros, M.H.; Di Mascio, P. Synthesis of internal labeled standards of melatonin and its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine for their quantification using an on-line liquid chromatography-electrospray tandem mass spectrometry system. J. Pineal Res. **2004**, *36* (1), 64–71.
10. Eriksson, K.; Ostin, 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. **2003**, *794* (1), 115–123.
11. Romsing, S.; Ulfberg, J.; Bergqvist, Y. Determination of melatonin in human plasma with solid-phase extraction, high-performance liquid chromatography and fluorescence detection. Scand. J. Clin. & Lab. Invest. **2003**, *63* (1), 81–88.
12. Tomita, T.; Hamase, K.; Hayashi, H.; Fukuda, H.; Hirano, J.; Zaitsu, K. Determination of endogenous melatonin in the individual pineal glands of inbred mice using precolumn oxidation reversed-phase micro-high-performance liquid chromatography. Anal. Biochem. **2003**, *316* (2), 154–161.
13. Kim, Y.O.; Chung, H.J.; Chung, S.T.; Kim, J.H.; Park, J.H.; Han, S.Y.; Kil, K.S.; Cho, D.H. Determination of melatonin in biological samples by capillary electrophoresis. J. Chromatogr. A **1999**, *850* (1-2), 369–374.
14. *The United State Pharmacopeia, 24th Ed.*; United States Pharmacopeial Convention: Rockville, MD, 2000; 2150–2151.
15. Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*; Wiley: New York, 1984.

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