CAPSULES notes and applications from BASi

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Determination of Melatonin in Commercially Available Products by LCEC and LC/MS/MS

Purpose

Determination of melatonin (MLTN) in commercially available products by liquid chromatography/electrochemistry (LCEC) and LC/MS/MS.

Existing Methods

Radioimmunoassay [1], GC/MS [2], and LCEC [3], and/or LC with fluorescence detection [4,5].

Conditions

LCEC System: BAS 200B chromatograph equipped with a low dead volume micro-injector (MF-4161) and a back-pressure column (UniJet C_{18} column, 5 µm, 100 mm x 1 mm I.D., MF-8901), before the injector, to raise the overall system pressure for optimal pump performance (2800-3500 PSI)

Column: UniJet C_{18} 5 µm, 150 mm x 1 mm I.D. (MF-8912)

Electrode: 3 mm glassy carbon working electrode (MF-1000)

Potential: +850 mV vs. Ag/AgCl

Temperature: 35°C

Mobile Phase: 20% acetonitrile; 80% (40.6 mM sodium citrate, 15 mM sodium perchlorate, 10 mM diethylamine hydrochloride, 2.15 mM sodium octylsulfonate, and 27 µM disodium EDTA)

Flow rate: 100 µL/min

Detection Limit: 3 pg MLTN on column

Sample Preparation

The dissolution of MLTN from the melatonin-containing formulations was obtained by dissolving the tablets and capsules in 20% acetonitrile in 0.1 N perchloric acid with ultrasonication. The filtrate of the resultant solution was separated on a 1 x 150 mm C_{18} microbore column and detected by electrochemical detection, and also separated on a 3.2 x 100 mm C_{18} column and detected by ion trap MS/MS.

Notes

A typical chromatogram of MLTN from tablet using LCEC is presented in F1.

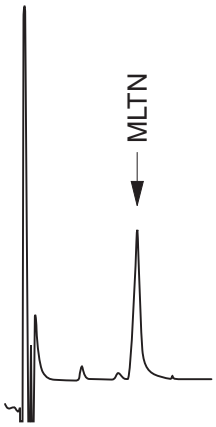

Figure 1. *Chromatogram of melatonin from a tablet using LCEC.*

The calibration curve for MLTN was linear over the range of 0.1 ng - 1 ng (r^2 = 0.9998). Four brands of tablets, products A, B, C, E, claiming MLTN contents of 0.2, 0.5, 1, and 3 mg per tablet, and one brand of capsule, product D, claiming a content of 2.5 mg, were analyzed using the developed methods. The results are shown in T1. As shown in F2, the agreement between LCEC and LC/MS/MS is excellent.

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Products	A		B		C		D		E	
Methods	LC/MS/MS	LCEC	LC/MS/MS	LCEC	LC/MS/MS	LCEC	LC/MS/MS	LCEC	LC/MS/MS	LCEC
mg of melatonin per tablet or capsule found	0.18 0.16 0.17 0.17 0.16 0.17 0.15 0.16	0.17 0.17 0.17 0.18 0.15 0.16 0.15 0.15	0.44 0.41 0.45 0.45 0.45 0.43 0.48 0.50	0.50 0.42 0.45 0.48 0.45 0.45 0.47 0.47	1.36 1.59 1.35 0.75 0.74 1.08 0.91 0.81	1.40 1.58 1.34 0.74 0.74 1.13 0.97 0.87	2.46 2.80 2.53 2.50 2.56 2.18 2.19 3.02	2.31 2.68 2.61 2.48 2.53 2.17 2.15 2.99	3.27 3.37 3.17 3.26 2.97 3.04 3.09 3.25	2.91 3.26 2.97 3.15 3.06 2.88 2.94 3.30
Mean	0.17	0.16	0.45	0.46	1.07	1.10	2.53	2.49	3.18	3.06
S.D.	0.01	0.01	0.03	0.02	0.32	0.32	0.28	0.28	0.13	0.16
R.S.D.(%)	5.9	6.3	6.7	4.3	30	29	11	11	4.1	5.2
Claimed content	0.2		0.5		1		2.5		3	
% of claimed content	85	80	90	92	107	110	101	99.6	106	102

Table 1. *The content of melatonin found and claimed in commercial products.*

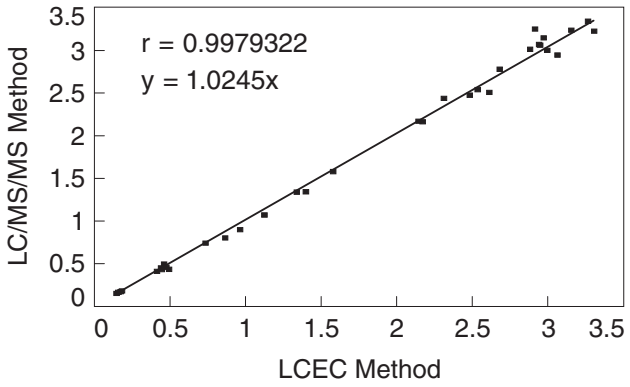

Figure 2. *Correlation between LCEC and LC/MS/MS for the determination of melatonin in 40 samples of melatonin-containing products.*

References

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