Optimization of dispersive liquid–liquid microextraction for trace analysis of salivary melatonin as a biomarker of circadian rhythm related to the shift work

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Abstract

"Melatonin" (N-acetyl-methoxytryptamine) is secreted from a part of the brain called suprakiasmic nuclui. This hormone has the highest level of secretion at night and the lowest during the day. The biological clock of the body or the circadian rhythm is regulated through the secretion of hormones such as melatonin and cortisol. Disturbance in circadian rhythm is specifically connected with shift working. Melatonin is known as a good biomarker to show circadian rhythm irregularities. So, in most studies it is used to determine the degree of disturbance in circadian rhythm due to shift working in occupational health. Through this study, the dispersive liquid–liquid microextraction (DLLME) was optimized for the analysis of human salivary melatonin. Different variables including extracting solvent, dispersing solvent, volume of extracting solvent, volume of dispersing solvent, sample pH, sample ionic strength, extraction time, and centrifugation time were screened using one factor at a time design (OFAT) and then, the significant variables were selected as optimum values. Optimum conditions were: extracting solvent (CCl_4) 200 ml, dispersing solvent (AcN) 2 ml, sample pH 7.00, sample ionic strength 5% , extraction time 3 min, and centrifugation time at 4500 rpm 6 min. To validate achieved procedure, accuracy and precision of the optimized method was evaluated at three concentrations of 50, 100, 250 $pg.ml^{-1}$. Based on this evaluation, CV% of 6.29, 2.39, 1.82 were achieved respectively for day-to-day reproducibility as well as CV% of 4.49, 2.68, 1.83 at the same three concentrations respectively for within-day reproducibility. Considering the appropriate results of the study, dispersing liquid-liquid microextraction procedure (DLLME) can be used for preparation of trace melatonin hormone in saliva samples as a biomarker of circadian rhythm of shift workers in field of occupational health.

Keywords: melatonin, circadian rhythm, dispersive liquid–liquid microextraction, one factor at a time, saliva, HPLC