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Development and validation of an on-line SPE coupled to liquid chromatography with fluorescence detection method for the quantification of bisphenol A in human saliva

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Abstract

Background: Resin-matrix composites, commonly used in dentistry, often contain derivatives of Bisphenol A (BPA) such as Bisphenol A-Diglycidyl Methacrylate and Bisphenol A-Dimethacrylate in their organic matrix composition [1]. Although BPA is not a direct ingredient of these composites, it may be present as a contaminant or degradation product, potentially released into the oral cavity following dental restoration. BPA is an endocrine disruptor that can interfere with the body's hormone-regulating endocrine system. Thus, the development of sensitive and specific analytical methods for its detection and quantification of BPA in biological matrices is crucial due to its prevalence and potential impact on human health [2]. **Objective:** This study aimed to develop and validate an on-line solid phase extraction method coupled with liquid chromatography and fluorescence detection (SPE-HPLC-FD) for quantifying BPA in human saliva. **Methods:** Prior to on-line SPE-HPLC-FD analysis, 6 mL of acetonitrile were added to a 2 mL saliva aliquot to precipitate proteins. The supernatant was then evaporated and reconstituted in a 2 mL solution of 5% ethanol. A 500 µL aliquot was directly injected into the HPLC system. The initial step involved sample clean-up in the on-line SPE column (Restricted Access Media column; RP-18 ADS-20 mm × 4 mm; 25 mm) in the first dimension, the retained components were automatically transferred to the analytical column (Luna PFP (2) column-150 mm × 4.6 mm; 3 mm, 100 Å) for component resolution and subsequent fluorescence detection in the second dimension. **Results:** The developed method was validated according to the ICH Guidelines [3] and revealed to be linear over the dynamic range of 5.0 ng/mL and 50 ng/mL, with accuracy and precision ranging between 89.9-104.9% and 1.1-6.7%, respectively. Recoveries ranged from 91.5% to 97.1%. **Conclusions:** The developed method required low sample volume and allowed for automation, simplification, short time analysis and low solvent consumption. This method represents a significant advancement in the detection and quantification of BPA in biological matrices.

Keywords: on-line solid phase extraction (SPE); liquid chromatography; bisphenol A; dental composites; saliva

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