

# HPLC quantitation of salivary vitamin B<sub>12</sub> after administration of sublingual tablets and oral sprays by fabric phase sorptive microextraction

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Fabric phase sorptive extraction (FPSE) has established itself as a powerful tool in bioanalysis. In this study, a simple and rapid FPSE protocol was developed for the extraction of salivary vitamin B<sub>12</sub> after administration of its sublingual tablets and oral sprays. The separation and detection were performed using high performance liquid chromatography-ultraviolet detection (HPLC-UV). Different sol-gel coated FPSE membranes were evaluated towards their extraction efficiency and sol-gel Carbowax 20 M coated polyester membranes showed the optimum performance. The extraction procedure was optimized using face-centered central composite design (FC-CCD) and one-factor-at-a-time" (OFAT) method. Method validation was conducted according to the FDA guidelines for bioanalytical methodologies. The lower limit of quantification for vitamin B<sub>12</sub> was 0.10 µg mL<sup>-1</sup> and the linear range was 0.10-10.0 µg mL<sup>-1</sup>. The relative recoveries for intra-day study were 87.5-113.8% and for inter-day study were 88.2-119.2%. In all cases, the relative standard deviation was better than 8.2%, demonstrating good method precision. The sol-gel coated FPSE membranes were reusable for up to 25 times. At a final step, the developed method was successfully used for the quantitation of salivary vitamin B<sub>12</sub> in saliva samples obtained by volunteers following the administration of different formulations containing the vitamin.

## Determination of Vitamin B<sub>12</sub> in saliva samples using FPSE/HPLC-UV

### 1 Saliva sampling



#### Use of Salivette

Insert the cylindrical polyester pad into the mouth after the intake of the B<sub>12</sub> supplement and roll it for 2 minutes. Repeat every two minutes. Centrifuge the pad.

### 2 FPSE



#### Fabric Phase Sorptive Extraction

Insert the FPSE media into a vial, containing the centrifuged saliva and a bar magnet. Stir the solution for 35 minutes at 200 rpm.

### 3 Elution



#### Elution with MeOH/H<sub>2</sub>O

Place the FPSE media into the extraction vial (500µL eluent) for 5 minutes

### 4 Analysis



#### High Performance Liquid Chromatography

Injection of the eluent into HPLC-UV for a 37-minute gradient elution process