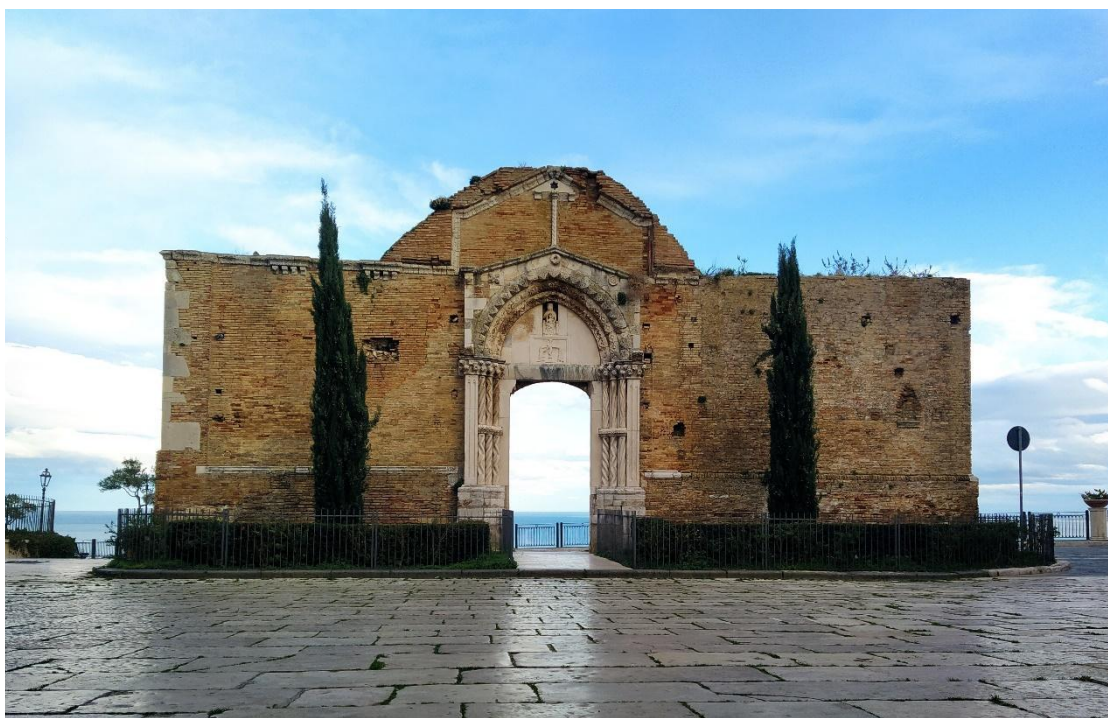


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ANALYTICAL METHOD FOR BISPHENOL A (BPA) CONJUGATED/FREE DETERMINATION IN BIOLOGICAL FLUIDS SAMPLES

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The method here described is developed for the analysis of bisphenol A (BPA), a compound with endocrine disrupting action, critical for human and environmental health^{1,2}, in samples of biological fluids (serum, but the method is also appropriate for milk). The method leads to the determination of the free/conjugated fraction of BPA and, for this reason, two complete analyses are carried out in parallel on the same sample. Enzyme glucuronidase was added to a first aliquot of 0.5 ml of serum sample and were placed in an incubator at 37 °C for 12 hours. The incubation of the sample with the glucuronidase enzyme allows the evaluation of the total concentration of the contaminant, including free BPA and conjugated BPA, i.e. already metabolised. A second aliquot of sample undergoes only the procedure described below, without the preliminary step of deconjugation, therefore the result represents only free BPA. Ethyl ether was added to both sample aliquots, shaken and subsequently centrifuged. Thus constituted a subnatant and a supernatant, the latter was collected in a new test tube in "BPA-free" polyester. This procedure was repeated three times, and once the collection volume was reconstituted, the latter was evaporated under nitrogen flow and reconstituted in acetonitrile. Finally, it was filtered with a solvent-resistant nylon filter and brought to a final volume of 0.5 ml with acetonitrile in autosampler vials. For the analytical determination of BPA, each aliquot was injected into a LC-ESI-MS system, which uses a Betasil C18 50x2.1 mm column at a flow rate of 250 µL/min. The mobile phases used were acetonitrile and ammonium acetate, according to a defined solvent program. The ESI-MS system operates in negative mode. The negative ion useful for the identification of BPA was obtained by fragmentation of the 227 ion with a collision energy of 35 eV and production of the ion (m/z) 212. Several tests were carried out to calculate the recovery percentage of the process, that resulted between 85 and 90%. The limit of detection (LOD), defined as 3 times the signal-to-noise ratio, were equal to 0.5 ng/ml for BPA. The results obtained for each sample, by subtracting the unconjugated BPA value (aliquot without enzyme addition) from the total BPA value (aliquot with enzyme addition), give a crucial indication for the interpretation of the exposure data in biological samples. For this reason, this method can be usefully employed in analyses in biomonitoring and in studies of exposure to this environmental contaminant of priority interest³.

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