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Determination of Bisphenol A and Bisphenol A- β -Glucuronide in Human Urine

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Abstract:

Methods have been developed for the determination of bisphenol A (free-BPA) and BPA- glucuronide in human urine. One method was for free-BPA and the other was for the total amount of free-BPA and BPA-glucuronide. Both of the methods were based on the use of solid phase extraction (SPE) on a C1 8 cartridge and GC-MS analysis of trimethylsilylated BPA. Enzymatic deconjugation was performed prior to the SPE to determine the total amount of free-BPA and BPA-glucuronide. Some human urine samples were investigated with these methods.

Methods:

Urine samples were collected from healthy volunteers and were stored at－5℃ until used. ^{13}C-BPA was added to each 10ml urine sample as a surrogate and the samples were filtered prior to the enzymatic deconjugation or the SPE.

For free-BPA analysis, the filtered samples were applied to C18 cartridges and washed with purified water and acetone/water (2:3v/v) and the cartridges were sucked dry, followed by washing with hexane. The compounds were eluted with ethyl acetate/hexane (1:4v/v). the ethyl acetate/hexane fractions were dried with anhydrous sodium sulphate, further concentrated using a rotary evaporator at reduced pressure and dried under a flow of nitrogen. 100 μ l of *N*, *O*-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) were added to each extract and the trimethylsilylated BPA was analysed by GC-MS.

For the analysis of the total amount of BPA and BPA-gluctronide, phosphate buffer and β -glucuronidase were added to the filtered samples and incubated. After being applied to C18 cartridges and washing with purified water and acetone/water (2:3v/v), the samples were eluted with acetone. The acetone was evaporated and ethyl acetate/hexane (1:4v/v) was added to the residue to extract free-BPA. The extracted solution was dried with anhydrous sodium sulphate, further concentrated using a rotary evaporator and dried under a flow of nitrogen. 100 μ l of BSTFA were added to the extracts and the trimethylsilylated BPA was analysed by GC-MS.

Results and Discussion:

For free-BPA analysis, recoveries and RSD were 100～161% and 7.5%, respectively, at a spike level of 50 pg/ml (n=6), with a method detection limit of 7.6 pg/ml (n=8). For the analysis of the total amount of BPA and BPA-glucuronide, recoveries and RSD were 92～185% and 3.7%, respectively, with no spike (n=7), with a method detection limit of 15.9 pg/ml (n=6).

For some human urine samples under investigation with these methods (n=12), concentrations of free-BPA ranged from 8.3 to 55 pg/ml and total-BPA from 61pg to 2.3ng/ml, with 0.9～17% of the total BPA being free-BPA, which suggests that most of BPA exist as a conjugated form in human urine although the percentages differed from individual to individual. In this study, free-BFA and BPA-glucuronide were analysed but it is possible that other metabolites exist, so that further study might be necessary.