Analysis and quantification of DNA photoadducts by HPLC/ion trap mass spectrometry

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DNA is known as the genetic material in cells. Various environmental factors can cause DNA damages. One of them is sunlight. The life on earth depends on the sunlight, but on the other hand, the UV light in sunlight can cause skin DNA damages. When these damages are not fully repaired before replication, they can lead to mutations of oncogenes and tumour suppressor gene and result in photo carcinogenesis, in the end, skin cancer.

Pyrimidine dimers and 8-oxodG are major DNA damages when skin cells are exposed to UVA, UVB or UVC. Two different kinds of quantification methods were used to quantify these main DNA photoadducts.

1. When in the rare cases, the substances of adducts, such as 8-oxodG were commercially available, we used Uridine as internal standard and SRM mode in HPLC-MS/MS to quantify the adducts in DNA.
   In the last years, efforts have been made to quantitatively determine the 8-oxodG level in DNA and other biological fluids. HPLC-EC, GC-MS and HPLC-MS/MS are the most frequently used methods. Among them HPLC-MS/MS is particularly favoured because it combines the advantages of an efficient HPLC separation with a sensitive and specific tandem mass spectrometric detection. Typically isotopically labelled 8-oxodG has been used as the internal standard. Here we present our quantification method with uridine as internal standard using HPLC-MS/MS to measure 8-oxodG in UVA treated calf thymus DNA. The advantages of using uridine as internal standard are that it is easily available and economic to use in comparison to isotopically labelled substance. It has very similar structure and properties as 8-oxodG.

2. When in most of cases, the pure adducts were not commercially available, we used our two steps quantification method. Firstly, we quantified the target analytes in the simple model system by ICP/MS and then secondly we quantified the target compounds in DNA samples with HPLC-MS/MS.
   This method combines the advantages of both ICP-MS and HPLC-MS/MS. ICP-MS has commercially available standard, but need substances to be fully separated. Target compounds in relatively simple model samples can be quantified. HPLC-MS/MS SRM can monitor the substances with the same masses but with different fragment ions. Substances don't need to be fully

separated. Thus analytes in more complicated DNA samples can be quantified. This method can avoid the tedious procedure to get pure substances for each individual standard. It is possible to quantify several adducts at the same time.