

ANALYSIS OF ESTROGEN-DNA-ADDUCTS WITH NANO-LC-ES MS/MS AND COLUMN SWITCHING

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Interaction of xenobiotics or endogenous compounds with DNA may lead to the formation of DNA-adducts. Recently it was statistically shown that a correlation seems to exist between the administration of estrogens (+ progestins) and an enhanced risk for the development of breast cancer (1). In the body, estrogens are metabolized to 4-hydroxy-estrogens which are further oxidized to their quinon-form which interact with DNA (2,3). In this context the interaction of metabolites of estrogens was studied by the coupling of nano-liquid chromatography (nano LC) hyphenated to nano-electrospray tandem mass spectrometry (nano-ES MS/MS) . The chromatography was studied and the fragmentation patterns obtained during low -energy CAD conditions were studied with the aid of deuterated compounds .

For the *in vitro* study adducts were prepared of 2'-deoxynucleosides with 4-hydroxyequilenin (4OHEN) according to Shen *et al.* (4). These authors already reported the occurrence of rather unusual adducts of 4OHEN due to the formation of two covalent bonds between the metabolite and the 2'-deoxynucleoside. The adducts of the 3,4-quinone of 17- α -ethynylestradiol (4OHEEQ) were synthesized according to Van Aerden (5).

These preparations were repeated by us in order to obtain the necessary reference compounds which enabled us to develop a highly sensitive nano LC -nano ESMS/MS method ,which combined with a column switching approach , should allow us to detect such adducts from *in vivo* sources.

A pre-column (300 μ m * 5mm, C18) was loaded with a crude reaction mixture resulting from the interaction of a 2'-deoxynucleoside and 4OHEN. The unmodified nucleosides were discarded , while the more lipophylic adducts were captured at the top of the pre-column. After 3 to 5 minutes the pre-column was back-flushed and the adducts were sent to the analytical nano-column (75 μ m * 150 mm, C18) . With gradient elution, in each reaction mixture more than one adduct was found.

Using the low energy CAD product ion spectra we were able to confirm that diastereomeric 4OHEN/deoxynucleoside-adducts were formed. An explanation for the occurrence of some identical fragmentation patterns is given by taking into account the orientation in space of some of the atoms involved during a rearrangement process . Fragmentation processes were proven by the use of deuterated compounds.

Sensitivity tests were performed in order to measure the detection limits. For this purpose a 2'-deoxyguanosine/equilenine (dG/4OHEN) adduct was isolated in a semi-preparative way.

A injection of 10 μ L of a $3,5 \cdot 10^{-11}$ M solution of this dG/4OHEN-adduct on the pre-column (197 fg; 350 amole) still gave a response with a S/N of 8.4 under Single Reaction Monitoring (SRM) conditions ([MH]⁺ = 564 fi m/z = 448). Detection limits under Full Scan- and SIR- and SRM mode are summarized in Table 1.

	Concentration	Amount injected	S/N
Full Scan	$1.75 \cdot 10^{-9}$ M	9.9 pg (17.5 fmole)	3.9
SIR	$2.19 \cdot 10^{-10}$ M	1.2 pg (2.18 fmole)	1.6
SRM	$3.50 \cdot 10^{-11}$ M	197 fg (350 amole)	8.4

Table1 : Detection limits of a 2'-deoxyguanosine-4OHEN-adduct under Full scan, SIR en SRM conditions (10 μ L injection, nano-LC-ES MS column-switching system).

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