Analysis of benzo[a]pyrene diol epoxide DNA adducts by capillary zone electrophoresis-nano-electrospray mass spectrometry

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Introduction (1)

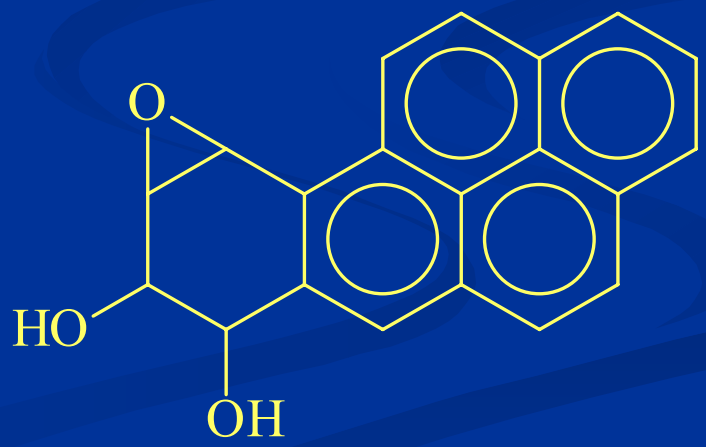
- Chemicals → electrophilic → DNA attack → DNA adduct → cancer ?

- Benzo[a]pyrene: prototype PAH

  → activation

  → diol epoxide

  → DNA adduct



Introduction (2)

- Amount DNA adduct ↓↔ nucleotides ↑↑↑
- Method:
  - sensitive
  - selective
  - structural informative
- Goal: identification

Introduction (3)

- CZE
  - charged molecules
  - miniaturization → sensitivity

    (sample introduction !)
- Q-TOF
  - mass spectrometric selectivity
  - identification (MS/MS)
  - “full scan” sensitivity

Materials & Methods (1)

- Samples:

  - nucleotides (per ml)

    (2'-deoxy)nucleotide (5 mg) + BPDE (0.1 mg)

    (dAMP, dCMP, dGMP, TMP)

    chloroform extraction (3x)

  - DNA hydrolysate (per ml)

    calf thymus DNA (10 mg) + BPDE (10 mg)

    ethanol precipitation

    DNA hydrolysis: DNA-ase I, nuclease P1, SVP

  - SPE: Chromabond HR-P

Materials & Methods (2)

- CZE conditions:
  - column: 1 m x 50 μm i.d. fused silica
  - buffer: 20 mM ammonium acetate (pH 9.5)
  - hydrodynamic injection: 80 mbar, 1.5 min
  - electrophoresis: 23 kV, 20 mbar
  - sample stacking → low amount
    - → nucleotide adducts: 100 mbar, 0.6 min
    - → DNA hydrolysate adducts: 100 mbar, 1 min

Materials & Methods (3)

- MS conditions:

  - nano-ESI interface

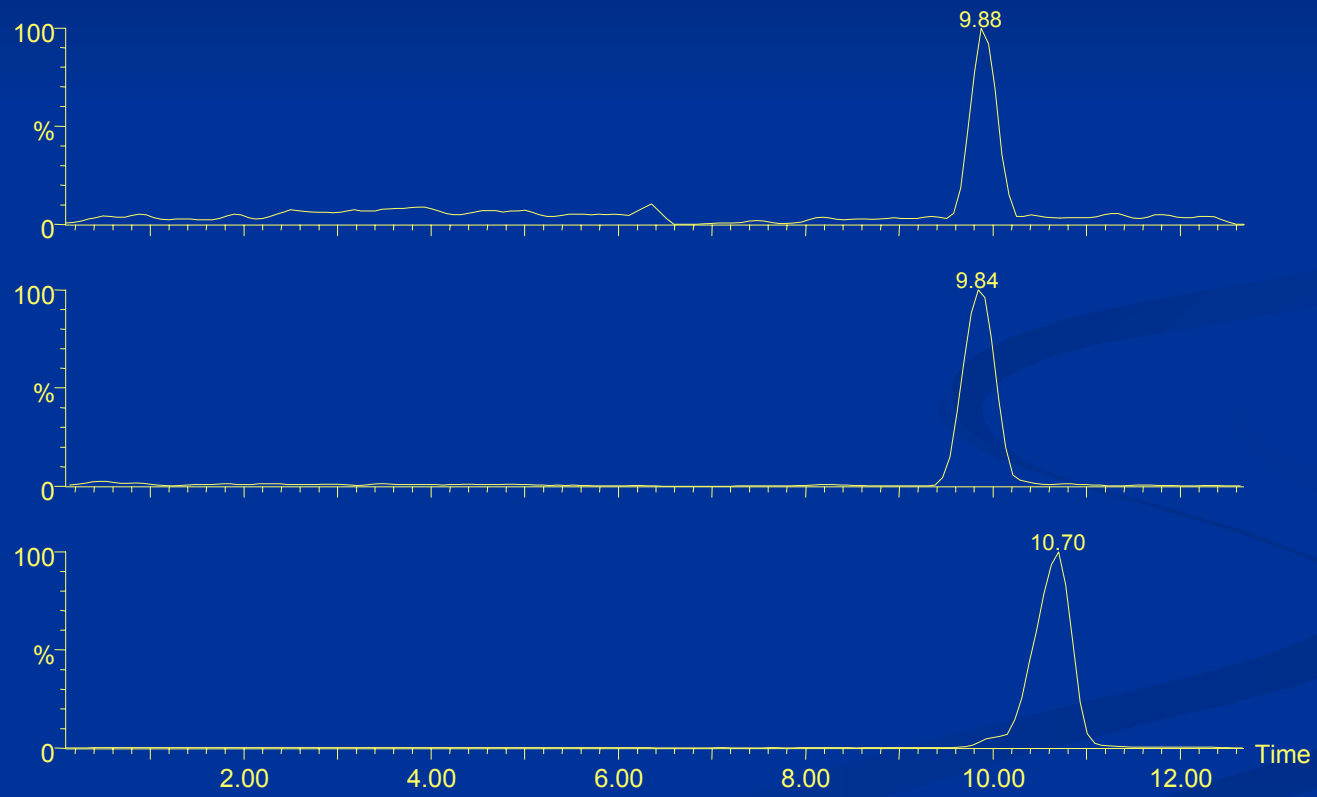
  - sheath flow:

    - neg. mode: 80/20 isopropanol/water
    - pos. mode: 50/50 methanol/water

  - capillary: -/+ 3.5 kV

Results (1)

- CZE-MS: electropherogram



BPDE-dCMP adduct m/z 608

BPDE-dAMP adduct m/z 632

BPDE-dGMP adduct m/z 648

Results (2)

- CZE-MS/MS: MS/MS spectrum

  - negative mode

    → phosphate alkylation ?

    → base alkylation ?

  - positive mode

    → exact place for base alkylation

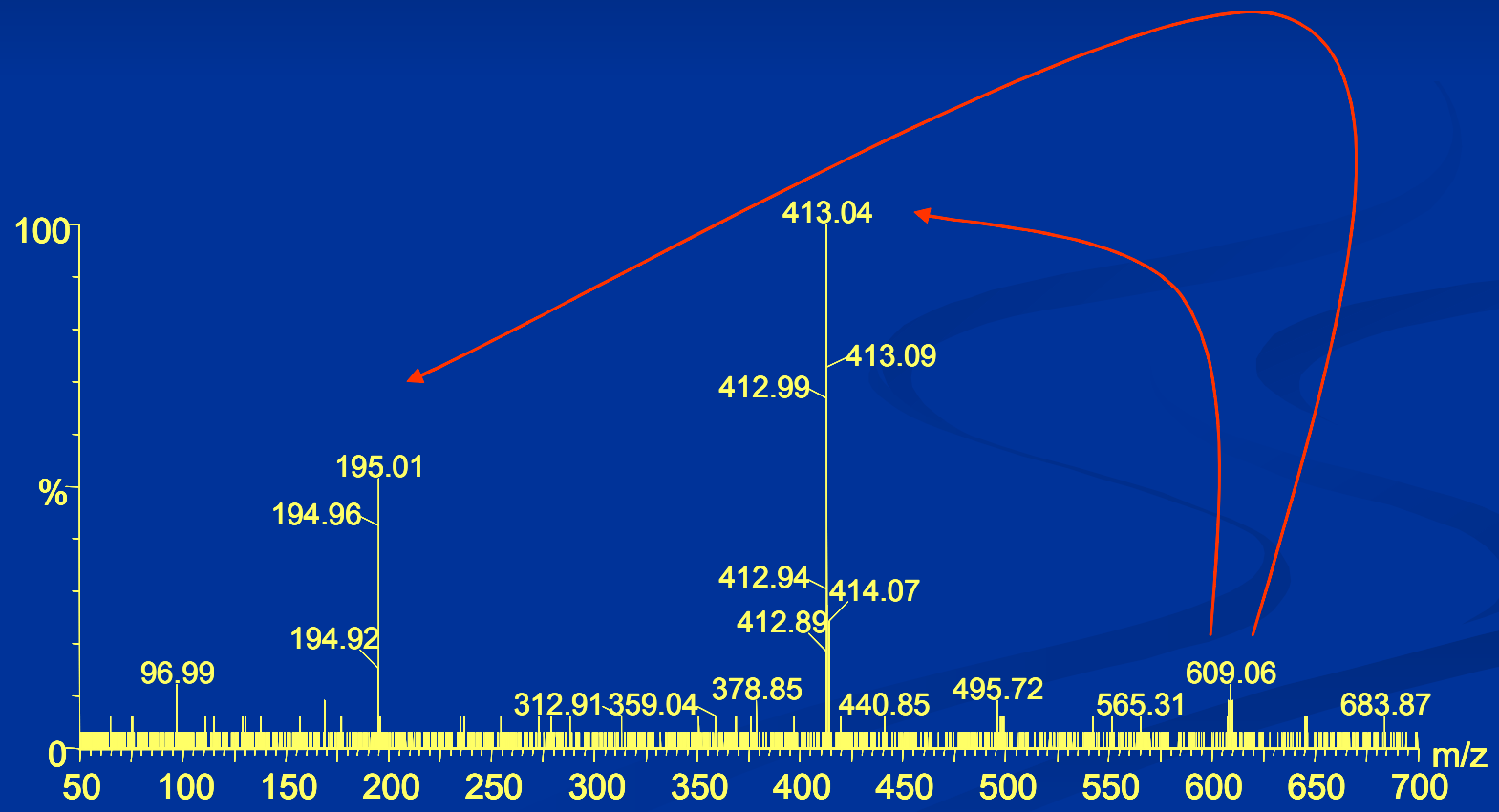
Results (3)

- Incubation of BPDE with (2'-deoxy)nucleotides

BPDE adduct	dAMP (MM 633)	dCMP (MM 609)	dGMP (MM 649)	TMP
RT (min)	10.3	10.4	10.9	-
neg. mode	m/z 632 base-alk ($N1/N^6$)	m/z 608 base-alk ($N3/N^4$)	m/z 648 base-alk ($N^2/N7$)	-
pos. mode	m/z 634 N^6-alk	m/z 610 N^4-alk	m/z 650 N^2-alk	-

Results (4)

- Incubation of BPDE with dCMP (- mode)



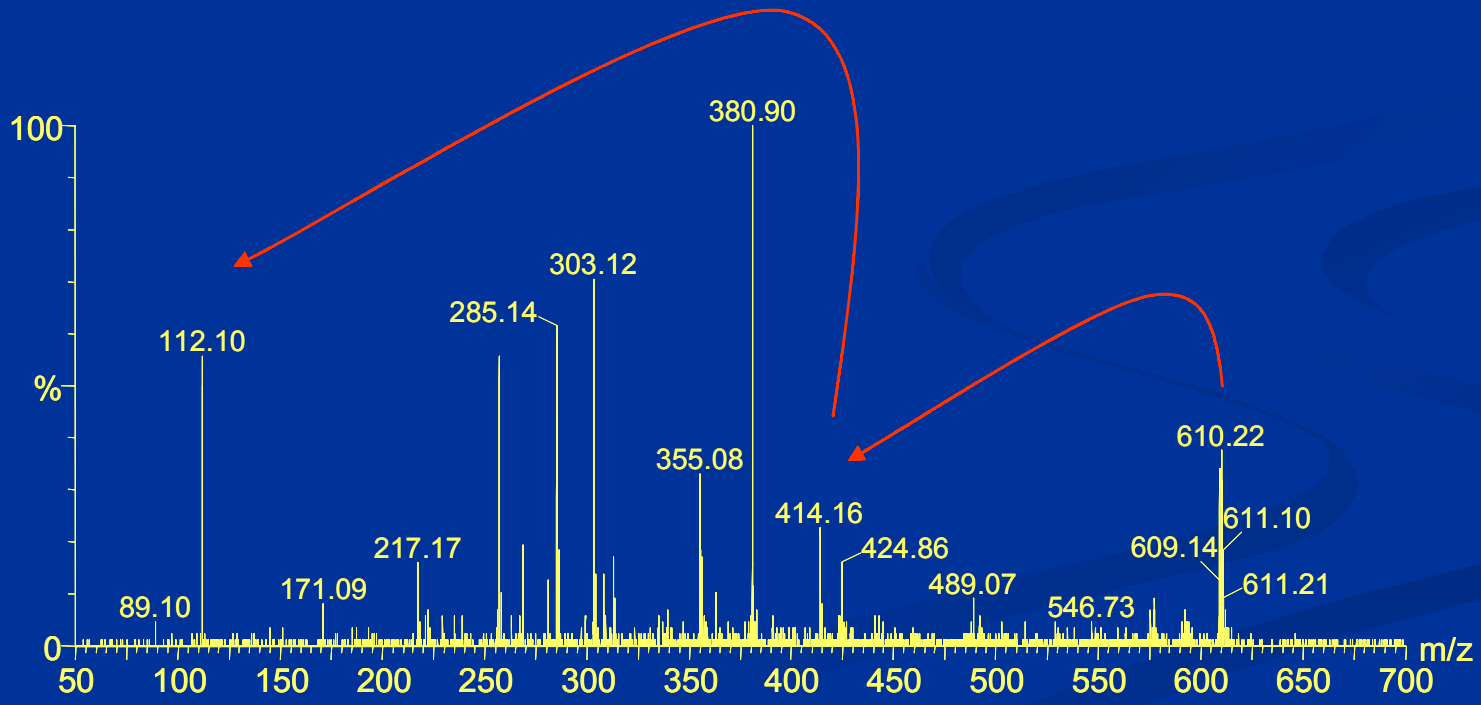
Results (5)

- Incubation of BPDE with calf thymus DNA

BPDE adduct	dAMP	dCMP	dGMP	TMP
RT (min)	9.8	9.9	10.7	-
neg. mode	m/z 632 base-alk	m/z 608 base-alk	m/z 648 base-alk	-
pos. mode	m/z 634 N^6-alk	m/z 610 N^4-alk	m/z 650 N^2-alk	-

Results (6)

- Incubation of BPDE with calf thymus DNA (+ mode): BPDE-dCMP



Conclusion

- CZE (negative compounds) → OK
- Coupling CZE-ESI-MS → proved straightforward in our hands
- Sample stacking → small amounts
- DNA adducts with BPDE: BPDE-N^6-dAMP, BPDE-N^4-dCMP, BPDE-N^2-dGMP