

Benzopyrene Analysis in Edible oil and fat

1. Preparation of Reagent and Standard sample

Solvent : ACN & Water (HPLC grade)

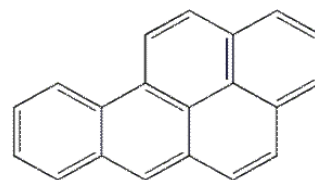
other solvents - N,N-DMF, Dichloromethane, n-Hexane

Standard sample : Benzopyrene standard quality 100ug/ml(ACN)

=> Dilute and make a calibration curve

Internal standard sample : 3-methylcholantrene standard quality 100ug/ml(ACN) => Dilute

Frorisil cartridge(for SPE), membrane filter(PTFE, 0.45um)



Benzo[a]pyrene

2. Analytical Condition

YL 9100 Series HPLC

Mobile Phase : ACN:Water=80:20

Flow Rate : 1.0ml/min

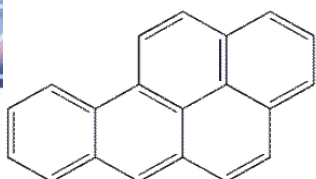
Column Oven : 35°C

Detector : FLD Ex 294nm Em 404nm

Column : Sunfire C18 (or PAH) [4.6*250mm, 5um]

Injection Volumn : 20ul sample loop

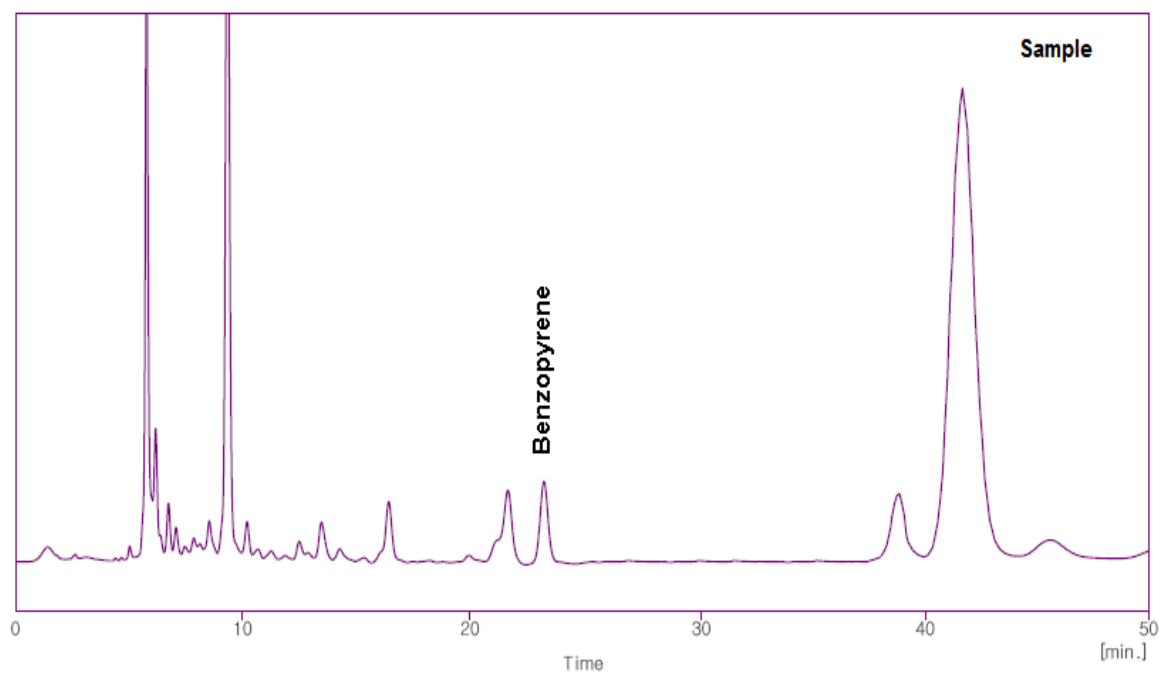
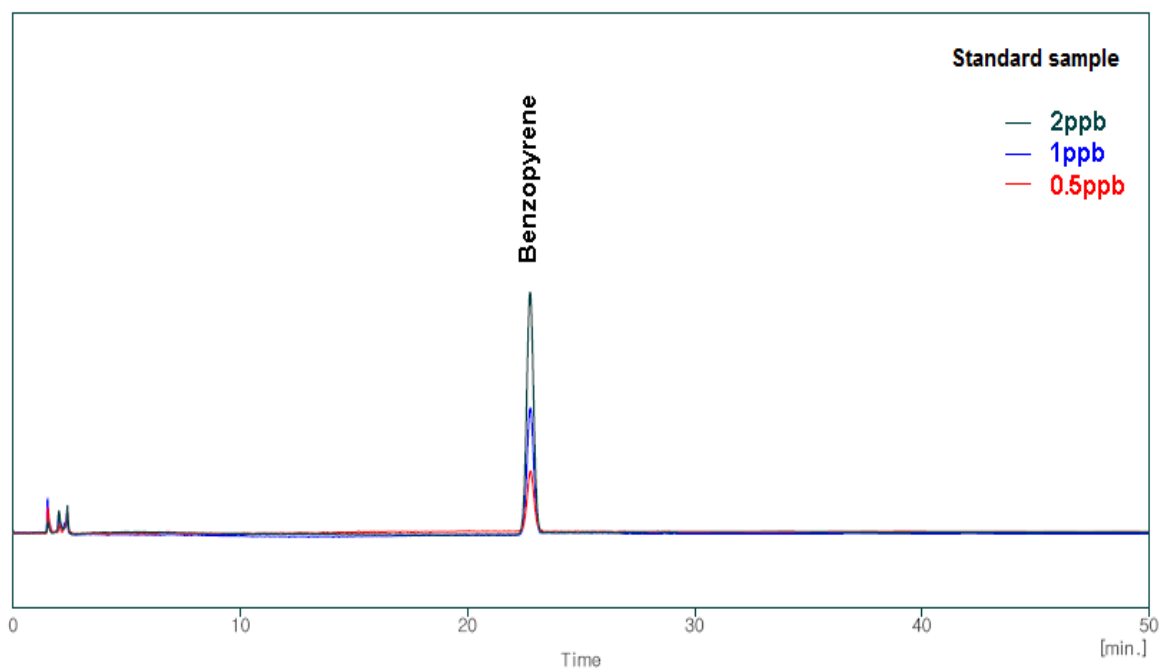


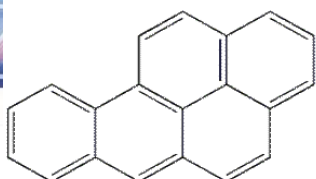


Benzo[a]pyrene

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3. Chromatogram





Benzo[a]pyrene



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4. Preconditioning of sample

<I> Homogenized specimen 10g + n-Hexane 100ml + DMF-Water(9:1) 50ml + ISTD 1ml

Shaking for 1 min



After separating water layer, move to <II> ,

At Hexane layer of <I> , Add DMF-Water(9:1) 25ml, Extract twice repeatedly

=> Add water layer to <II>



Extraction

Add 1% Na₂SO₄ 100ml + n-Hexane 50ml at <II>=> Mix => stay for 1 min

Add to Hexane layer <III> , Extract twice repeatedly with hexane 35ml at water layer

=> Add hexane layer to <III>



Add water 50ml to <III> then mix, vent the water layer twice repeatedly



Dewatering filtration Na₂SO₄(15g) at hexane layer of <III> , evaporate to 2ml at 40 °C

Florisil cartridge : Dichloromethane 10ml-> Vitalization with Hexane 20ml



Add concentrate with the flow rate of 1ml/min at cartridge



Refinement

Elute with hexane 5ml, hexane/dichloromethane(3:1) 15ml each



Concentrate the effluent at below 40 °C with nitrogen gas



Dissolve the residue with ACN 1ml => filtering 0.45um => analyze with HPLC/FLD

