

ELSD detektor HPLC's

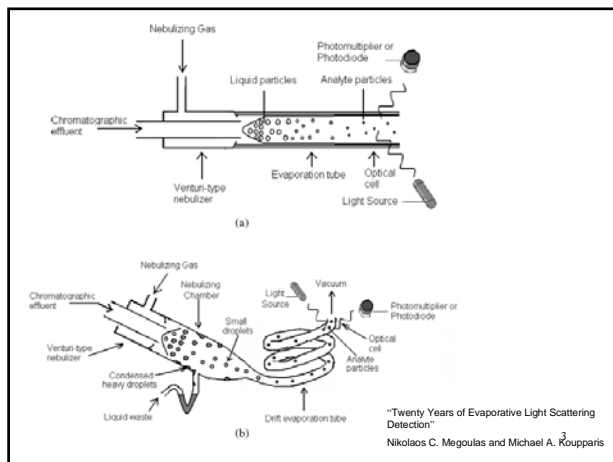
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Tööpõhimõte

- Efluent pihustatakse gaasivoolu
- Gaasivool kannab efluendi kuivatustorru, kus eluent aurustub
- Alles jäänud analüüdi osakestest koosnevat udu valgustatakse ning hajunud valgus detekteeritakse

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Nõuded analüüdile

- Analüüt peab olema mittelenduv või pooleldi lenduv
 - Selle hindamiseks kasutada mitte keemistemperatuuri vaid aururõhku ja aurustumissoojust

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Võrreldes teiste detektoritega

- Ei tugine optilistel omadustel, ainus tingimus on analüüdi mittelenduvus
- Tundlikkus kõigi analüütide juures pea sama
- LOD nanogrammi suurusjärgus, uuematel aparaatidel ka paarisaja pikogrammi juures
 - Väiksemad LOD'd nõuavad väikseid voolukiirusi
- Gradiendi puhul jääb baasijoon muutumatuks (eluent ei mõjuta signaali)
 - Võimalikud järsud gradiendid

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Võrreldes

UV'ga	RI'ga	MS'iga
Vähem derivatiseerimist (ei sõltu kromofoorist)	Mittetundlik väikestele temperatuuri kõikumistele	Odavam
Näitab rohkem komponente	Gradient	Kerge kasutada
Solvendi piigid puuduvad	Parem LOD	Solvendi piigid puuduvad
Solvent ei "löika"	Solvendi piigid puuduvad	Töötab atmosfäärirõhul
Response sama	Parem lineaarsus	
Suurem valik solvente?	Laiem dünaamiline ala	
Järsemad gradiendid	Laiem solventide ja modifikaatorite võimalus	
	Sarnane signaal sama ehitusega molekulidele	
	Ainult positiivne signaal	
	Pumba mõju puudub	

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Probleemid ELSD juures

- Solvendi lenduvuse nõue
 - Limiteerib kasutatavate solventide hulka oluliselt
 - Mitmed happed, alused, puhvrid jaioon-paar modifikaatorid jäävad kasutusest välja (nt. fosfaatpuhvrid)
 - Seega raske olemasolevaid LC-UV metoodikaid kasutada ELSD juures

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Lubatud eluendi modifikaatoreid

	pKa	pKb	pH vahe	Keemistemp.	Sulamistemp.
Happed					
Trifluoroacetic Acid	0.3	13.70		72.4°C	
Formic Acid	3.75	10.25		100.7°C	
Acetic Acid	4.75	9.25		116.0°C	
Carbonic Acid	6.37	7.63		-	
Alused					
Ammonia	9.25	4.75		-33.35°C	
Methylamine	10.81	3.19		16.6°C	
Ethylamine	10.66	3.34		-6.3°C	
Triethylamine	11.01	2.99		89.3°C	
Puhvrid					
Ammonium Formate			3.0-5.0		120°C
Pyridinium Formate			3.0-5.0		
Ammonium Acetate			3.8-5.8		111°C
Ammonium Carbonate			5.5-7.5 & 9.3-11.3		
Ioon-paar reagentid					
Pentafluoropropionic Acid		~0.6		97°C	
Heptafluorobutyric Acid		~0.6		120°C	
Nonafluoropropionic Acid		~0.6		140°C	
Pentadecafluorooctanoic Acid		~0.6		189°C	
Tridecafluoroheptanoic Acid		~0.6		175°C	

<http://www.polymerlabs.com/products/000405.html>

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Probleemid ELSD juures

- Lineaarsuse puudumine
 - Põhjus: osakeste suurus ei ole reprodutseeritav
 - Lahendus:
 - Mittelineaarne regressioon
 - Topeltlogaritmilist skaalat kasutades lineaarsus 2-3 suurusjärku

$$A = am^b$$

A – piigi pindala

$$\log A = b \log m + \log a$$

a ja b – ELSD ehitusest sõltuvad koefitsiendid

$$A^{1/b} = a^{1/b} m = km$$

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Probleemid ELSD juures

- Destruktiivne
- Detekteerib kolonni fooni

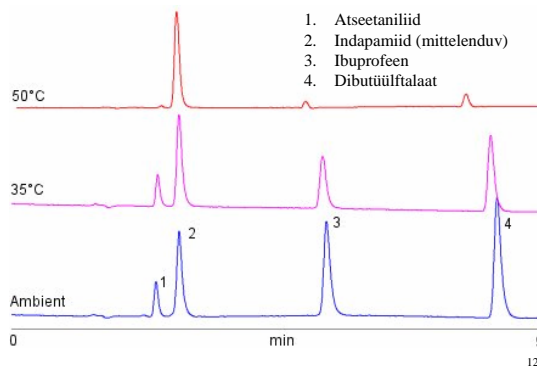
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Temperatuur (10 – 300)°C

- Varasemalt oli limiteerivaks faktoriks eluendi keemistemperatuur
- Praegu levinud madalatel temp'idel töötavad ELSD'd
 - Saab avastada poollenduvaid analüüte
 - Maksimaalseks signaal-müra suhteks valida siiski võimalikult kõrge temperatuur, samas mida madalam temp. seda rohkem komponente näeb

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Temperatuuri mõju



<http://www.polymerlabs.com/products/000095.html>

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Kasutusala

- Farmaatsiatööstus
 - Gingenosidid
 - Steroidid
 - Polüetridid
- Toitude ja jookide koostis
 - Süsivesikud
 - Lipiidid

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Farmaatsiatööstus

Compound	Matrix	Column	Mobile phase
Aminoglycosides	Raw materials, formulations, biological fluids	ODS-2 C18 Spherisorb (250 mm × 4.6 mm, 5 μm)	water-acetonitrile (55:45 v/v), containing 1.5 ml HFBA per liter
Ginsenosides	Ginseng roots	ODS-2 C18 Spherisorb (250 mm × 4.6 mm, 5 μm)	water-acetonitrile (gradient from 75:25 to 10:90 v/v)
Steroid conjugates	Standard solutions	Alltima C18 (150 mm × 2.1 mm, 5 μm)	water-methanol (gradient from 2:3 to 0:1) containing 0.1% TFA
PEGs, methyl and dimethyl ethers	Synthesized samples	Spherisorb ODS-2 S3W (100 mm × 4.6 mm, 3 μm) and Spherisorb S5X C18 (250 mm × 4.6 mm, 5 μm)	water-methanol (from 20:80 to 40:60)
Sodium carbonate	Cefazidime for injection	Hypersil SCX (250 mm × 4.0 mm, 5 μm)	ammonium acetate (0.02 M, pH 6.0)-acetonitrile (80:20 v/v)
Polysorbate 80	Parenteral formulations	Alltima C18 (250 × 4.6 mm, 5 μm)	methanol-water (gradient from 30:70 to 90:10 v/v)
Midecamycin and related impurities	Raw materials	Diamonsil C18 column	acetonitrile-ammonium formate (0.2M, adjusted to pH 7.3 with triethylamine) (52:48 v/v)
Saponin and alkaloids	Caustophyllum thalictroides (blue cohosh)	Synergi Max-RP 80A (150 mm × 4.6 mm, 4 μm)	ammonium acetate buffer (pH 8)-acetonitrile (gradient from 90:10 to 40:60 v/v)
Terpene lactones and flavonoid aglycones	ginkgo biloba	Supelco Discovery C18 (250 mm × 4.6 mm, 5 μm)	methanol (containing 0.05% TFA)-water (containing 5% methanol and 0.05% TFA) (gradient from 25:75 to 90:10 v/v)
Organic acids	Hygroscopic pharmaceutical herbal dry extracts	Aminex HPX-87-H strong cation-exchange resin column (300 × 7.8 mm), fitted with an ion-exclusion Micro-Guard retil cartridge	0.02 M TFA

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Toiduained ja joogid

Compound	Matrix	Column	Mobile Phase
Carbohydrates (fructose, glucose, lactose, maltose, raffinose, sucrose)	Honey, milk powder, pineapple-juice	Silica gel	water-acetonitrile containing 0.05% (v/v) ethanolamine and 0.05% (v/v) triethylamine.
Paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide	Migration of lubricants from food packaging	LiChrospher R Diol (125 mm × 3 mm, 5 μm, 100 Å)	Isooctane-tert-butyl methyl ether containing 0.1% acetic acid
Triacylglycerol (cocoa butter equivalents)	Chocolate	LiChrospher 100-5-RP18 (500 mm × 4 mm, 5 μm)	acetonitrile-chloroform (40:60 v/v)
Fatty acids	Grape seed oil	Phenomenex Luna C18 (150 mm × 4.6 mm)	methanol (1% acetic acid)-water (1% acetic acid) (95:5 v/v)
Lactose, fat, and total protein	Milk	Autosampler based on AluSphere Al 100 alumina (125 mm × 4 mm, 5 μm)	hexane (THF 1%)-isopropanol (gradient from 100:0 to 95:5 v/v)
Wax esters, sterol esters and FAME	Margarines and vegetable oils	Used frying oils	Cyano Nucleosil (30 mm × 4.6 mm, 10 μm)
Polar compounds	Infant formulae	Spherisorb ODS-2 (250 mm × 4.6 mm, 5 μm)	hexane-hexane:ethanol: water, (50:50:1 v/v/v) (gradient from 100:0 to 0:100 v/v)
Sn-2 monopalmitin			acetonitrile-acidified water (99:1 v/v)

Kasutusala

- Loodustooted ja bioloogilised proovid
- Polümeerid

Compound	Matrix	Column	Mobile Phase
Telechelic poly(methyl methacrylate)	Synthesized and commercial samples	Packed-in-house with Hypersil Silica (150 mm × 4.6 mm, 3 μm 100 Å)	acetonitrile and dichloromethane of varying composition
Polyethylene glycol	Low-density polyethylene	Kromasil C18, 100 Å (100 mm × 0.5 mm, 3 μm)	acetonitrile-THF-water (40:5:55 v/v/v)
Polyamide-6	Synthesized samples	Two Nucleosil 50-5 (200 mm × 4 mm)	81.6% (w/w) formic acid in propanol
Poly(butylene glycol) and derivatives	Synthesized samples	ODS C18	Binary or ternary gradient systems of acetonitrile, THF, water and methanol

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Loodustooted ja bioloogilised proovid

Compound	Matrix	Column	Mobile Phase
Hepatotoxic pyrazolidine alkaloids	Plant extracts	Waters XTerra RP18 (150 mm × 4.6 mm, 5 μm)	0.015 M ammonium acetate-acetonitrile (gradient from 95:5 to 50:50 v/v)
Bile acids	Human serum and urine	integrated flow injection-LC system	acetonitrile-methanol (65:35 v/v)
Ceramides	Yeast	CN (150 mm × 4.6 mm, 5 μm)	hexane-ethanol (99:1 v/v)
Profiling of constituents	Sarcostemma hirtellum	Betasil C18 (50 mm × 4.6 mm, 3 μm)	water (0.1% formic acid)-acetonitrile (0.1% formic acid) (gradient from 95:5 to 5:95 v/v)
Phytosterols	Plant matrices	Hypersil BDS RP18 (250 mm × 3.4 mm, 5 μm)	methanol-acetonitrile-water (75:15:10 v/v/v)
Phosphatidylserine molecular species	Biological tissues	Carotenoid C30 (250 mm × 4.6 mm, 5 μm)	2-propanol-THF-ammonium formate (55:15:30 v/v/v)
Maltodextrins	Plant extracts	octadecyl-bonded silica and amino-bonded polymeric end-capped C18	aqueous methanol-aqueous acetonitrile gradient of acetonitrile-water-acetic acid.
Apoliar low molecular weight constituents	Wood		
Hydrocarbons, wax esters, sterol esters, triacylglycerols and sterols	Zooplankton	LiChroCART column (12.5 cm × 4 mm) packed with AluSphere (alumina) particles (5 μm)	0.5% THF in hexane-70% THF/propan-2-ol/hexane (1:1:3 v/v/v) (gradient from 100:0 to 30:70 v/v)
Aminoacids	Human parathyroid hormone	Dionex IonPac CS-10 cation-exchange column (250 mm × 4 mm)	0.1% TFA-ammonium acetate (0.1M (a ternary system with increasing pH gradient steps)

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ELSD kasutusvaldkonnad väljaspool HPLC'd

- Gel Permeation Chromatography (GPC)
 - Polümeerid
- Supercritical Fluid Chromatography (SFC)
 - Pindaktiivsed ained
 - Ravimid

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Arengusuunad

- Mõlema aurustamistüübi samaaegne kasutamine (Alltech)
- Linearse ala kasvatamine
- Korruga mitme erineva kolonni jaoks (Sedere)
- Üha madalamad temperatuurid

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Kasutatud materjalid

- Tootjate brošüürid (Sedere, Alltech, Polymer Laboratories, Shimadzu)
- Artikkel

"Twenty Years of Evaporative Light Scattering Detection"
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