

LC/MS: OPTIMIZATION OF PARAMETERS

ANNELI KRUBE

IONIZATION TYPE

- ESI
- APCI
 - Also a combination of ESI and APCI is available
- APPI
 - Not too common

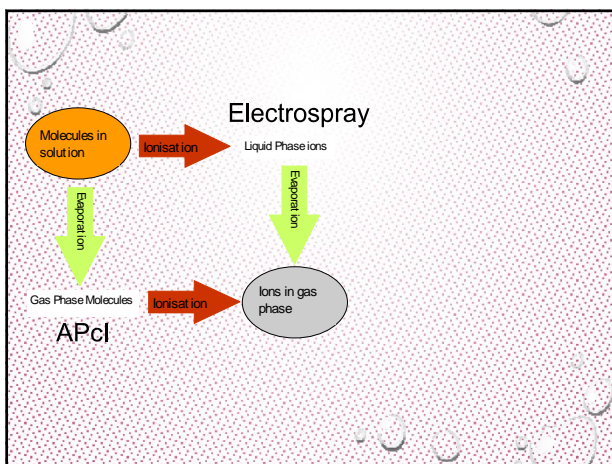


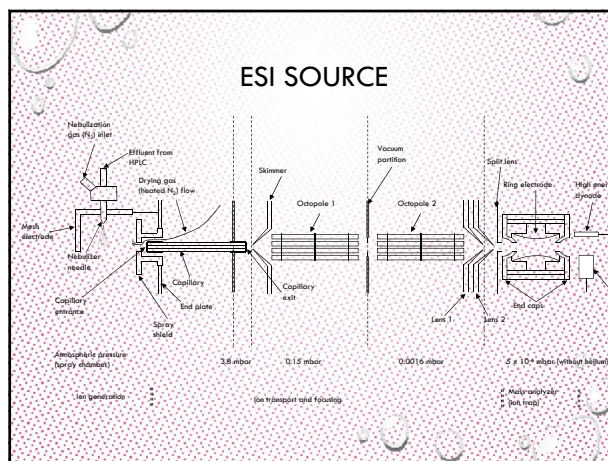
Table 1. Response Factors of Various Classes of Pesticides in Optimized Mobile Phases Using LC/MS in Full-Scan Mode*

compound	APCI+	APCI-	ESI+	ESI-
phenylurea herbicides	64	0	0	4
chlorobenzene	57	10	7	5
thiametam	44	7	6	4
thiazolam	190	0	14	0
imazam	14	7	7	5
nitroar herbicides				
atrazin	190	0	7	0
desmethylatrazin	20	0	3	0
chlorobutylatrazin	25	0	2	0
cyromazine	21	0	2	0
trifluralin	190	0	14	0
propazine	190	0	9	0
simazine	71	0	5	0
terbutylatrazin	190	0	9	0
nitroar herbicides				
chloromugil	0	0	6	0
metoprolol	0	0	10	0
diquat	0	0	7	0
patoparg	0	0	7	0
acetanilide derivatives				
acetochlor ECA	0	0	0	11
acetochlor ECA	0	0	0	11
acetochlor ECA	0	0	0	14
acetochlor OXA	0	0	0	14
acetochlor OXA	0	0	0	14
acetochlor OXA	0	0	0	14
chlorophenoxycid herbicides				
2,4-D	0	14	0	50
2,4-DP	0	14	0	50
2,4,5-T	0	14	0	50
chlorophenoxycid herbicides				
glyphosate	0	0	0	0
2,4-dimethylphenoxy	0	0	0	14
2,3,6-trimethylphenoxy	0	14	0	14
4-chloro-3-methylphenoxy	0	14	0	14
2,4,5-trimethylphenoxy	0	50	0	7
alkyl sulfonate surfactants				
sodium dodecyl sulfate	0	0	0	30

*Thurman 2001 Anal. Chem. 73, 5441-5449

ESI VS APCI

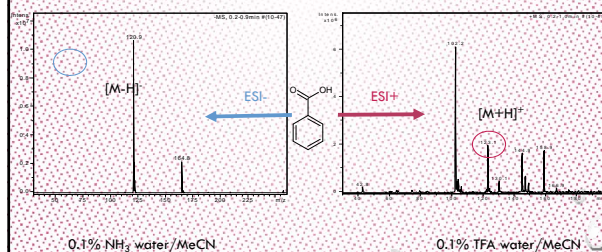
<p>ESI</p> <ul style="list-style-type: none"> • pKa • electronafinity • hydrophobisty 	<p>APCI</p> <ul style="list-style-type: none"> • volatility • Gas phase proton afinity
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IONIZATION SOURCE PARAMETERS

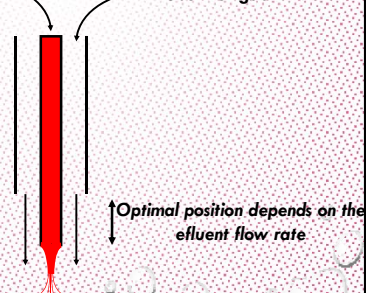
- Polarity ESI+ or ESI-
- Nebulizer gas
 - Curtain gas
- Drying gas
- Capillary voltage

ESI+ OR ESI-



ESI NEBULIZER

Effluent from LC Nebulizer gas



- 1). Fixed length (more robust)
- 2). adjustable (possible to optimize sensitivity)

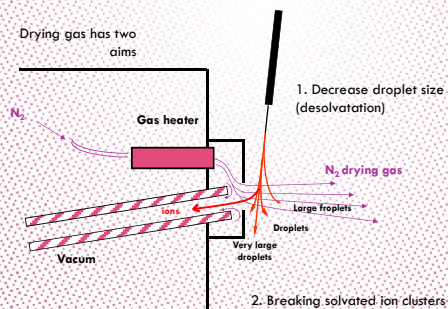
SUGESTED PARAMETERS

Electrospray Ionization¹

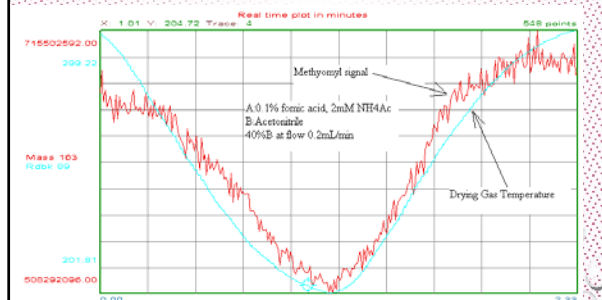
HPLC Flow Rate (ul/min)	Nebulizer Spacer ²	Nebulizer Pressure (psi)	Drying Gas Flow (l/min)	Drying Gas Temp (°C)	Capillary Voltage (V)
1 – 10	Not Installed	10 – 15	4	325	3500 ³
10 – 50	Not Installed	15 – 20	5	325	
50 – 200	Installed ³	20 – 40	8	350	
200 – 500	Installed	30 – 50	8 – 10	350	
500 – 1000	Installed	50 – 70	10 – 12	350	

DRYING GAS

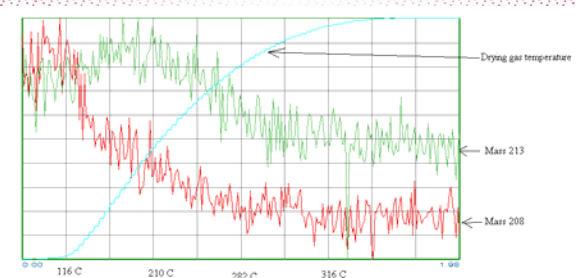
Drying gas has two aims



EFFECT OF DRYING GAS TEMPERATURE

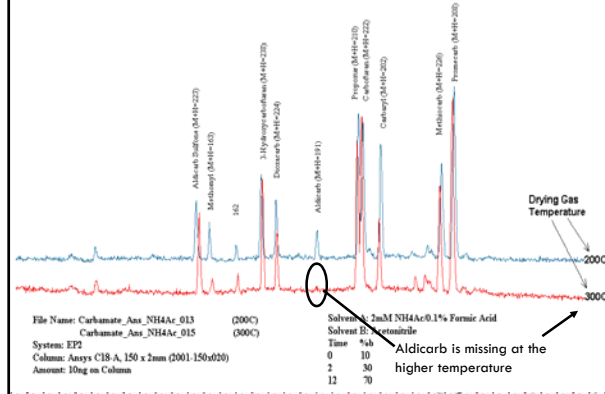


EFFECT OF DRYING GAS TEMPERATURE

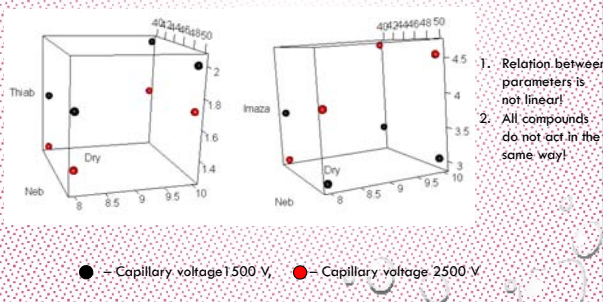


LC/MS Analysis of Carbamates Mixture

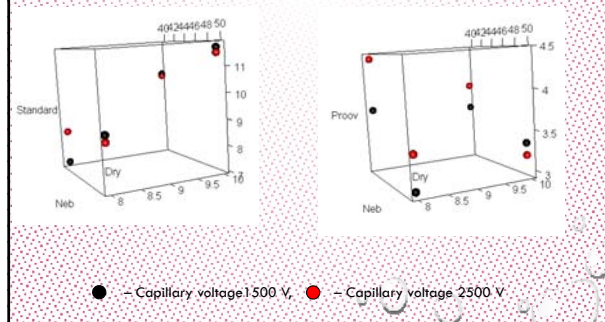
(Comparison of Drying Gas Temperature)



IT IS WISE TO STUDY SEVERAL PARAMETERS TOGETHER



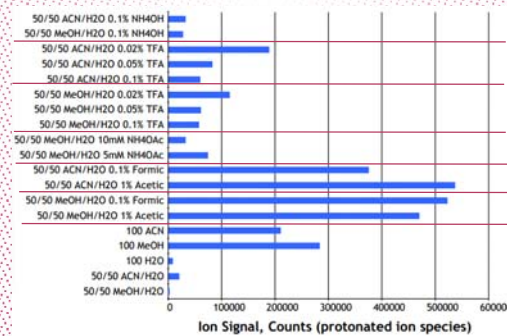
Optimizing in solvent or in real samples?



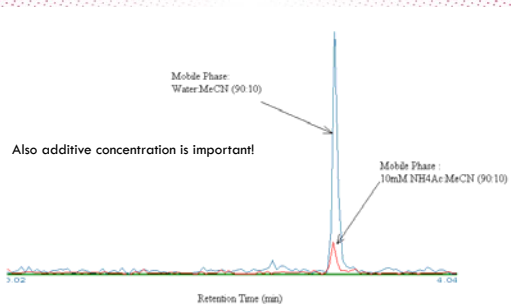
ADDITIVES

- pH
 - Volatile buffer
 - According to analyte pKa
- Decreasing surface tension
- LC method may need to be reoptimized
 - Better - optimize ionization before and then chromatography!

ADDITIVES



ADDITIVES



Sample: 8-Hydroxy-2'-deoxyguanosine
Amount: 10µL, 2µg/µL