Accelerate method development using fast screening of mobile phases additives and solvents for optimum sensitivity in LC-MS

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1: Introduction

It is now well-known that mobile phase components (*i.e.* additives and solvents) play a major role in ionization efficiency. Laboratory facing challenges in fast method development and high sensitivity are often condemned to use generic mobile phases and to invest in expensive high-end mass spectrometers. Furthermore, recent developments in HPLC columns allow more flexibility in the use of acidic or basic additives as well as viscous solvents .In this study, we propose a rapid and systematic methodology to quickly optimize HPLC mobile phase recipe from a MS sensitivity point of view.

2: Materials and Methods

Model compounds representing a wide panel of chemical classes (table 1) were dissolved in several mobile phase mixtures. Compounds were chosen with different chemical moieties and hydrophobicity. They were also chosen in order to have both compounds ionized in positive or negative mode and some of them could only be ionized forming salt adducts.

Mobile phase mixtures were elaborated using a rational combination of solvents with water and several additives including organic acids, bases and salts (buffered or not) (Table 2). Each tested solvent was mixed in equal proportion with each aqueous buffer or additive solution. The total number of combination was 60.

Table 1: Studied compound list

Compound	Mol. weight	pKa	logP	Ionisation mode	MRM
Fructose	180.2	12.2	-1.03	ESI -	179.00>89.00
Pyridoxine	169.2	pKa1 5.6 pKa2 8.6	-0.77	ESI+	169.95>134.00
Leu-**Enkephalin**	555.6	N/A	1.22	ESI+	556.20>120.10
Chlorzoxazone	169.6	8.3	1.6	ESI-	168.10>131.90
4-nitrophenol	139.1	7.08	1.91	ESI-	138.00>108.00
Nifedipine	346.3	3.9	2	ESI+	347.00>314.90
Digoxin	780.9	12.98	2.2	ESI-	[M-H]- 779.30>84.90 [M+2Na-H]- 825.20>779.10
Buspirone	385.5	1: 1.22 2: 7.32	2.3	ESI+	386.15>122.10
Amantadine	151.2	10.8	2.3	ESI+	152.10>135.00
Capsaicin	305.4	9.5	3.81	ESI+	305.70>137.00
Warfarin	308.3	5.08	3	ESI+ ESI-	309.00>162.90 307.10>160.95
Propranolol	259.3	9.5	3	ESI+	260.00>155.00
Papaverine	339.4	5.9	3	ESI+	340.05>324.00
Reserpine	608.7	6.6	3.2	ESI+	608.90>194.90
Indomethacin	357.8	4.5	3.4	ESI-	356.20>311.80
Ibuprofen	206.3	4.91	3.6	ESI-	204.90>160.90
Dextrometorphan	271.4	8.3	3.6	ESI+	271.80>171.00
Cyclosporin A	1202.6	N/A	3.64	ESI+	[M+NH4]+ 1219.70>1202.40
Amodiaquine	355.9	1: 7.1 2: 8.1	3.7	ESI+	356.10>283.00
Verapamil	454.6	8.92	4.7	ESI+	455.20>165.10
Difenacoum	444.5	4.5	7.6	ESI-	443.10>134.90
Propamocarb	188.3	9.5	0.84	ESI+	188.90>102.10

These mixtures were then injected using flow injection analysis and a dummy mobile phase carrier. An air gap was introduced before and after the injected sample to prevent mixing with the dummy mobile phase (figure 1). The impact of the air gap volume, of the injection volume and of the flow rate was evaluated.

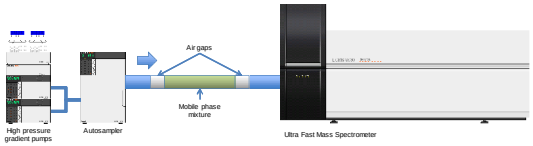

Figure 1: Schematic of th experiments

To prevent any unstability and to accelerate the most tedious task, mixtures were prepared and spiked with the compound stock solution on the autosampler rack using the sample pretreatment function just before injection.
All compounds were injected simultaneously at a final concentration of 50 ng/mL.
Each MRM dwell time was set to 10 ms. The pause time was set to 1 ms. The polarity switching time was of 15 ms. The duty cycle time of the MS was then of 294 ms. For comparison purpose, when the number of MRM was reduced, the dwell time was increased to maintain the MS cycle time.

Table 2 : Used solvents, buffers and additives

Aqueous solution or buffer	Organic solvent
Water Formic acid 0.1% (v/v) Acetic acid 0.1% (v/v) Ammonia (NH_4OH) 0.1% (v/v) Pyrrolidine 0.2% (v/v) 4-Methylmorpholine 10mM Ammonium Acetate 10mM Ammonium Formate 10mM Ammonium Fluoride 0.2mM Ammonium Bicarbonate 10mM Ammonium Acetate 10mM pH 5 Ammonium Acetate 10mM pH 10 Ammonium Formate 10mM pH 3 Ammonium Formate 10mM pH 10 Ammonium Bicarbonate 10mM pH 10	Methanol Acetonitrile 2-propanol Acetone

Table 3: Analytical conditions

LC	
LC system:	Nexera (Shimadzu, Japan)
Analysis Column:	None
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Gradient Program:	50% A / 50%B
Flow rate:	0.3 mL/min
Column Temperature:	Ambient
Injection Volume:	5 µL
MS	
MS system:	LCMS-8030 (Shimadzu, Japan)
Ionization:	ESI (positive/negative)

3: Results

3.1: Experimental conditions

Using warfarin as a model compound, experimental conditions including flow rate, air gap volume and injection volume were optimized. Three injections per condition were performed.
Results showed that :

- Mobile phase component effect was more visible using a air gap to prevent mixing with the carrier,
- An air gap of 1 µL is sufficient,
- Higher air gap induced spray disturbances leading to higher result dispersion,
- The combination of a flow rate of 300 µL/min and injection volume of 5 µL gives enough time to the sample in the source to show dramatic ionization yield differences.

(Data not shown)

3.2: Dwell time impact

The effect of the dwell time on result validity was evaluated using 10 or 100 ms. The figure 2 shows that no significant impact on the mobile phase effect was measured.

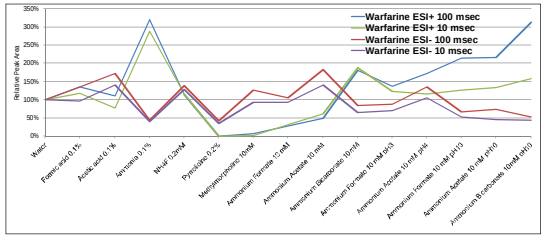

Figure 2: Effect of Dwell time on Warfarine behaviour in various buffer mixtures with methanol.

3.3: All compounds mixture

The Table 4 reports best and worst mobile phase for each tested compound during this study. All effects were normalized by comparing the peak area measured versus the area measured in water / methanol. Peak area was used to take the noise into account.

Table 4: Results

Compound	Best mobile phase (area ratio)	Worst mobile phase (area ratio)
Fructose	Methylmorpholine 10mM / Methanol (798%)	Ammonium Formate 10 mM pH3 / Acetonitrile (9%)
Pyridoxine	Ammonia 0.1% / 2-propanol (671%)	Pyrrolidine 0.2% / Acetonitrile (1%)
Leu-**Enkephalin**	Ammonia 0.1% / 2-propanol (854%)	Water / Acetone (0%)
Chlorzoxazone	Ammonium Acetate 10 mM / 2-propanol (163%)	Ammonium Bicarbonate 10mM pH10 / Acetonitrile (5%)
4-nitrophenol	Ammonium Acetate 10 mM / 2-propanol (117%)	Ammonium Bicarbonate 10mM pH10 / Acetonitrile (7%)
Nifedipine	Ammonia 0.1% / 2-propanol (2214%)	Pyrrolidine 0.2% / Acetone (0%)
Digoxin [M-H]- [M+2Na-H]-	Ammonium Bicarbonate 10mM / Methanol (1752%) Ammonium Formate 10 mM / 2-propanol (407%)	Ammonium Formate 10 mM / 2-propanol (1%) Ammonium Bicarbonate 10mM / 2-propanol (0%)
Buspirone	Ammonia 0.1% / 2-propanol (279%)	Pyrrolidine 0.2% / Acetone (1%)
Amantadine	NH4F 0.2mM / 2-propanol (230%)	Pyrrolidine 0.2% / Methanol (0%)
Capsaicin	Ammonia 0.1% / 2-propanol (767%)	Any pyrrolidine or methylmorpholine mixture (1-5%)
Warfarin ESI+ ESI-	Ammonia 0.1% / 2-propanol (504%) Ammonium Acetate 10mM / 2-propanol (253%)	Pyrrolidine 0.2% / Acetone (0%) Ammonia 0.1% / Acetone (21%)
Propranolol	Ammonia 0.1% / 2-propanol (231%)	Pyrrolidine 0.2% / Acetonitrile (3%)
Papaverine	Ammonia 0.1% / 2-propanol (275%)	Pyrrolidine 0.2% / Acetone (1%)
Reserpine	Ammonia 0.1% / 2-propanol (349%)	Pyrrolidine 0.2% / Acetone (6%)
Indomethacin	Ammonium Bicarbonate 10mM / 2-propanol (165%)	Pyrrolidine 0.2% / Methanol (1%)
Ibuprofen	Methylmorpholine 10mM / Acetone (114%)	Formic acid 0.1% / Acetonitrile (1%)
Dextrometorphan	NH4F 0.2mM / 2-propanol (145%)	Pyrrolidine 0.2% / Acetonitrile (2%)
Cyclosporin A	Ammonia 0.1% / 2-propanol (3945%)	Any pyrrolidine or methylmorpholine mixture (0%)
Amodiaquine	Water / Acetonitrile (275%)	Pyrrolidine 0.2% / Acetonitrile (0%)
Verapamil	Ammonia 0.1% / 2-propanol (173%)	Pyrrolidine 0.2% / Acetone (0%)
Difenacoum	Ammonium Acetate 10 mM / 2-propanol (147%)	Pyrrolidine 0.2% / Methanol (10%)
Propamocarb	Ammonia 0.1% / 2-propanol (278%)	Pyrrolidine 0.2% / Acetone (14%)

4: Conclusion

It is possible to quickly screen solvents, salts , pH and additives mixtures to choose the mobile phase leading to the highest sensitivity in LC-MS. This screening must be performed in normalized conditions. Autosampler features like sample pretreatment and air gap addition even increase the ease, speed and reliability of this screening. For multiple compound simultaneous optimization it is necessary to have an ultra fast MS to have a complete overview of the mobile phase possibilities without sacrificing data quality.
This stage of method development can be performed very quickly (about 15 min to test all combinations). Compared to the tedious task of manual infusion, the benefits of this approach are evident.
Popular buffers and solvent (*e.g.* ammonium acetate and acetonitrile) are not always the best choice for sensitive assays. In this study, ammonia and 2-Propanol were clearly the best choice for positive ESI. Nowadays, LC columns allow the use of such combinations.