Accelerate Method Development using Fast Screening of Mobile Phases Additives and Solvents for Optimum Sensitivity in LC-MS

Mikaël LEVI, Maureen RAMERO, Stéphane MOREAU
SHIMADZU France, le Luzard 2, Bd Allende, 77186 Noisiel, FRANCE.

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.

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 (not buffered) (Table 2). Each tested solvent was mixed in equal proportion with each aqueous buffer or additive solution. The total number of combination was **50**.
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. To prevent any instability 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 20 ng/mL.
Each MRM dwell time was set to 5 ms. The pause time was set to 3 ms. The polarity switching time was of 15 ms. The duty cycle time of the MS was then of 326 ms. For comparison purpose, when the number of MRM was reduced, the dwell time was increased to maintain the MS cycle time.

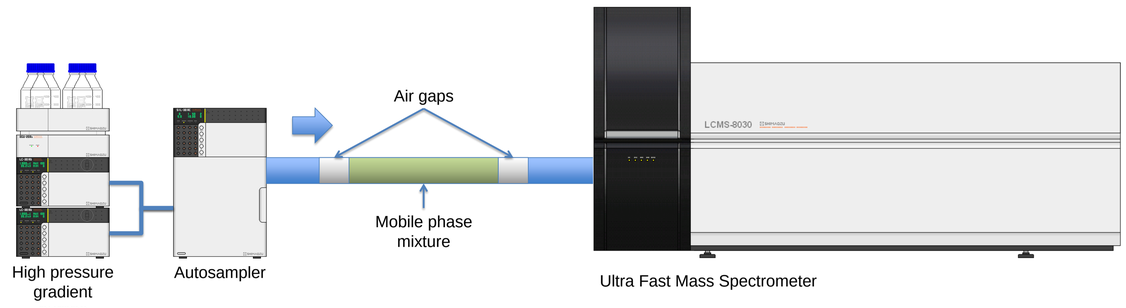

Figure 1: Schematic of the experiments

Table 1: Studied compound list

Compound	Mol. weight	pKa	logP	Ionisation mode	MRM
4-nitrophenol	139.1	7.08	1.9	ESI-	138.1 > 108.2
Acetaminophen	151.2	9.38	0.4	ESI+	151.85 > 110.05
Amantadine	151.2	10.8	2.3	ESI+	152.15 > 135.25
Amodiaquine	355.9	7.7	3.7	ESI+	356.35 > 283
Androstenedione	286.4	N/A	2.7	ESI+	287.2 > 97.1
Angiotensine	1296.5	N/A	N/A	ESI+	649 > 110.2
Aniline	93.1	4.62	0.9	ESI+	94.15 > 77.05
BADGE	340.2	N/A	3.7	ESI+	358 > 285
Buspirone	385.5	N/A	2.3	ESI+	385.9 > 122.1
Caffeine	194.2	10.4	-0.5	ESI+	195.1 > 138.1
Capsaicin	305.4	9.5	3.8	ESI+	306 > 137.1
Chlorzoxazone	169.6	8.3	1.6	ESI-	168.05 > 132.15
Cyclosporin A	1202.6	N/A	3.6	ESI+	1219.8 > 1202.8
Dextrometorphan	271.4	8.3	3.6	ESI+	272.1 > 214.15
DHEA	288.2	N/A	3.2	ESI+	289.95 > 249
Difenacoum	444.5	4.5	7.6	ESI-	443.1 > 135.2
Digoxin	780.9	12.98	2.2	ESI-	779.2 > 85.1
Dihydrocapsaicin	307.4	N/A	4.11	ESI+	308.05 > 137.05
Fructose	180.2	12.2	-1.0	ESI -	556.2 > 120.1
Ibuprofen	206.3	4.91	3.6	ESI-	178.95 > 89.1
Indomethacin	357.8	4.5	3.4	ESI-	205.2 > 161.1
Leu-Enkephalin	555.6	N/A	1.2	ESI+	356 > 312
Nifedipine	346.3	3.9	2.0	ESI+	347.05 > 315
Nonivamide	293.4	N/A	3.82	ESI+	294.2 > 137.15
Omeprazole	345.4	4 & 8.8	0.6	ESI+	346.1 > 198.1
Papaverine	339.4	5.9	3.0	ESI+	339.9 > 202.05
Parathion	291.3	N/A	3.83	ESI+	292.1 > 251
Propranolol	259.3	9.5	3.0	ESI+	260.15 > 56.05
Pyridoxine	169.2	5.6 & 8.6	-0.8	ESI+	170.1 > 134.15
Simvastatine	418.6	N/A	4.7	ESI+	419.2 > 285.2
Temsirolimus	1030.3	N/A	4.4	ESI+	1052.5 > 461.2
Testosterone	288.2	N/A	3.6	ESI+	289.2 > 109.15
Tolbutamide	270.3	5.2	2.2	ESI+	270.95 > 91.15
Vasopressine	1050.2	N/A	N/A	ESI+	542.9 > 328.2
Verapamil	454.6	8.92	4.7	ESI+	455.1 > 165.05
Warfarin	308.3	5.08	3.0	ESI-	307.05 > 161.1
Warfarin	308.3	5.08	3.0	ESI+	309.05 > 163.1

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	Methanol Acetonitrile 2-propanol Acetone Tetrahydrofuran

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) switching time 15 ms

Results

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,
- Bigger 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).

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.

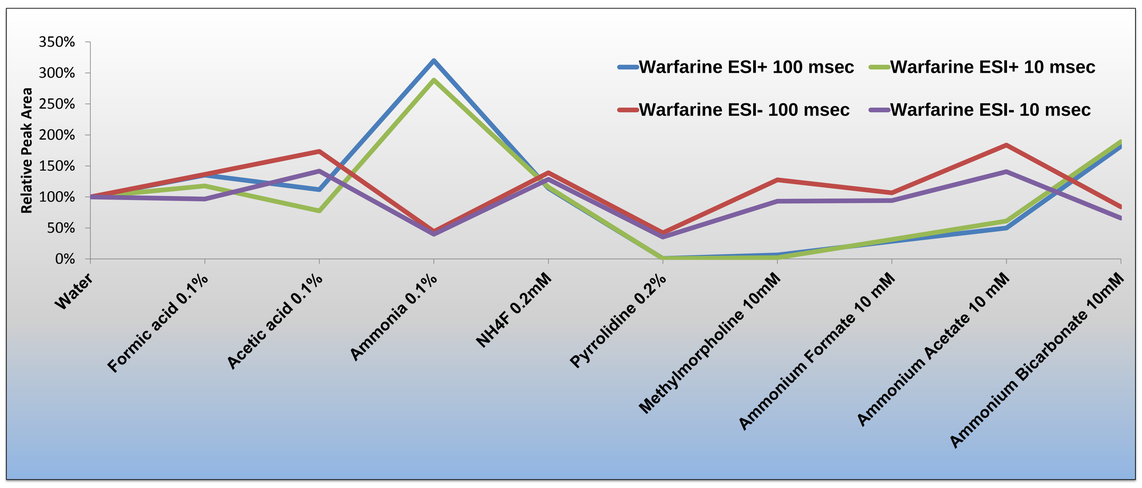

Figure 2: Effect of Dwell time on Warfarine response in various buffer mixtures with methanol.

All compounds mixture

The Table 4 reports best and worst mobile phase for each tested compound during this study. For each compound, the mobile phase mixtures were classified according to the measured peak area. Peak area was used to take noise into account. Figures 3 shows the kind of typical histogram that can be generated to quickly determine the best mixture for a particular compound. These examples clearly emphasize that for some compounds, the solvent choice is very critical for highly sensitive assays.

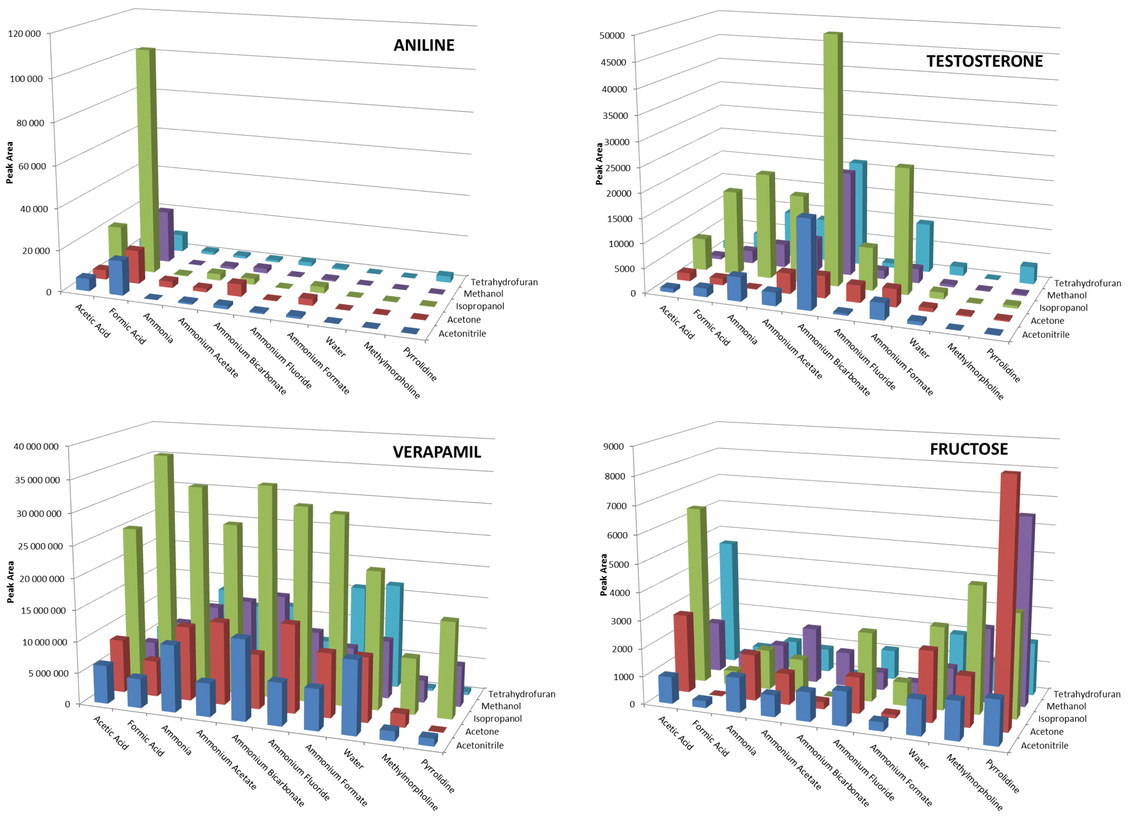

Figure 3: Exemplary histograms

Table 4: Results

Compound	Best mobile phase	Worst mobile phase
ESI NEG		
4-nitrophenol	Isopropanol Acetic Acid	Acetone Ammonium Bicarbonate
Chlorzoxazone	Isopropanol Acetic Acid	Acetone Ammonium Bicarbonate
Difenacoum	Isopropanol Formic acid	Tetrahydrofuran Ammonium Bicarbonate
Digoxin	Isopropanol Pyrrolidine	Any acid or salt mix
Fructose	Acetone Pyrrolidine	Isopropanol Ammonium Bicarbonate
Ibuprofen	Isopropanol Pyrrolidine	Methanol Formic Acid
Indomethacin	Isopropanol Pyrrolidine	Acetonitrile Formic Acid
Warfarin	Isopropanol Formic acid	Acetone Ammonium Bicarbonate
ESI POS		
Acetaminophen	Acetonitrile Ammonium Bicarbonate	Isopropanol Methylmorpholine
Amantadine	Isopropanol Formic acid	Tetrahydrofuran Methylmorpholine
Amodiaquine	Isopropanol Formic acid	Acetone Pyrrolidine
Androstenedione	Tetrahydrofuran Pyrrolidine	Acetone Ammonia
Angiotensine	Methanol Acetic acid	Isopropanol Methylmorpholine
Aniline	Isopropanol Formic acid	Any mixture without Formic Acid
BADGE	Isopropanol Formic acid	Acetone Pyrrolidine
Buspirone	Isopropanol Formic acid	Acetone Pyrrolidine
Caffeine	Acetone Ammonia	Isopropanol Methylmorpholine
DHEA	Methanol / Acetic Acid or Tetrahydrofuran / Pyrrolidine	Tetrahydrofuran Methylmorpholine

Compound	Best mobile phase	Worst mobile phase
ESI POS (continued)		
Capsaicin	Isopropanol Ammonium Bicarbonate	Acetone Pyrrolidine
Cyclosporin A	Isopropanol Ammonium Formate	Methanol Methylmorpholine
Dextrometorphan	Isopropanol Ammonium Acetate	Acetonitrile Pyrrolidine
Dihydrocapsaicin	Isopropanol Ammonium Bicarbonate	Any Methylmorpholine mix
Leu-Enkephalin	Isopropanol Formic acid	Tetrahydrofuran Methylmorpholine
Nifedipine	Isopropanol Ammonium Bicarbonate	Any Methylmorpholine or Pyrrolidine mix
Nonivamide	Isopropanol Ammonium Bicarbonate	Methanol Methylmorpholine
Omeprazole	Isopropanol Ammonia	Acetone Pyrrolidine
Papaverine	Isopropanol Ammonium Bicarbonate	Acetone Pyrrolidine
Parathion	Isopropanol Acetic Acid	Tetrahydrofuran Methylmorpholine
Propranolol	Isopropanol Ammonium Bicarbonate	Acetone Pyrrolidine
Pyridoxine	Isopropanol Ammonium Bicarbonate	Acetone Pyrrolidine
Simvastatine	Isopropanol Ammonium Bicarbonate	Any Methylmorpholine or Pyrrolidine mix
Temsirolimus	Isopropanol Acetic Acid	Acetonitrile Ammonium Formate
Testosterone	Isopropanol Ammonium Bicarbonate	Any Methylmorpholine mix
Tolbutamide	Isopropanol Ammonium Bicarbonate	Acetone Pyrrolidine
Vasopressine	Isopropanol Formic acid	Any Methylmorpholine or Pyrrolidine mix
Verapamil	Isopropanol Formic acid	Acetone Pyrrolidine
Warfarin	Isopropanol Ammonium Bicarbonate	Tetrahydrofuran Methylmorpholine

Conclusion

It is possible to quickly screen solvents, salts 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. Of course, it does not anticipate the peak shapes and separation when using a column.
Popular buffers and solvent (e.g. ammonium acetate and acetonitrile) are not always the best choice for sensitive assays. In negative ESI, it shows that Isopropanol can counteract the well-known deleterious effect of formic acid. In positive ESI, Isopropanol is also a better choice for many compounds in combination with Ammonium Bicarbonate.
This study shows that some solvents and additives not very often used in LC-MS can be very useful to solve some sensitivity challenges. Furthermore, modern UHPLC columns allow the routine use of highly viscous solvents like Isopropanol and/or high pH mobile buffers.

EBF Barcelona 2012