# SELECTION OF NEGATIVE MODE STANDARD IN LC-MS UNDER NEUTRAL CONDITIONS FOR ASSESSING SOLVENT QUALITY

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## INTRODUCTION

Liquid chromatography mass spectrometry (LC-MS) is a widely used technique in pharmaceuticals, proteomics, metabolomics and biotechnology field. With the advancement of instrument sensitivity, demand for interference free mobile phases is increasing. Optimization of the quality of mobile phase solvents can improve the overall performance of LC-MS. The quality of the LC-MS grade solvents should enable the detection and identification of trace level standards without any interference. A number of LC-MS standards are available that ionize easily in positive mode under neutral conditions or by applying additives such as formic acid or acetic acid and using a combination of low pH and optimal ionic strength of the buffer. In contrast, negative mode ionization is rather challenging under neutral conditions.

## MATERIAL AND METHODS

- HPLC-MS: Agilent 1100 HPLC equipped with auto-sampler and diode array detector (DAD) attached to Agilent SL MSD single quadrupole mass spectrometer
- Column: Agilent Zorbax XDB C-18 (150 x 2.1 mm, 3.5 micron), Catalog No: 930990-902
- Flow: 0.3 mL/min
- Injection volume: 5 µL
- Solvents for mobile phase were Optima® LC/MS grade
- Mobile phase A: Water (or 10 mM ammonium acetate in water)
- Mobile phase B: Acetonitrile (ACN) and methanol

### Gradient:

| Time (minute) | Mobile phase A | Mobile phase B |
|---|---|---|
| 0 | 90 | 10 |
| 2 | 90 | 10 |
| 15 | 0 | 100 |
| 25 | 0 | 100 |
| Post time: 10 minutes | | |

- UHPLC: Thermo Scientific Accela UHPLC system comprised of an auto-sampler, photodiode array detector and attached to an LTQ-XL mass spectrometer equipped with an electro-spray ionization interface
- Column: Thermo Scientific Hypersil Gold™ column (50 mm x 2.1 mm x 1.9 micron; part # 26-102-052130)
- Flow: 0.5 mL/min
- Injection volume: 5 µL

### Gradient:

| Time (minute) | Mobile phase A | Mobile phase B |
|---|---|---|
| 0 | 90 | 10 |
| 0.5 | 90 | 10 |
| 2 | 0 | 100 |
| 5 | 0 | 100 |
| Post time: 5 minutes | | |

- Samples were run using the following solvent pairs:
  a. water/ACN
  b. 10 mM ammonium acetate in water/ACN
  c. water/methanol
- Mass spectrometer (single quadrupole) parameters
  - Capillary voltage: 3500 V
  - Capillary temperature: 350 ℃
  - Fragmentor: 70 V
  - Electro spray using negative mode ionization
  - Selected ion monitoring was performed for m/z 138 and m/z 321
- Compounds (purchased from Sigma-Aldrich, St. Louis, MO) used for screening: para-nitrophenol (PNP), chloramphenicol (CHL), mecoprop (MCP), aldicarb, digoxin, naproxen, baicalin, azomethine and quercetin
- Concentration of compounds varied from 10 µg/mL (ppm) to 10 ng/mL (ppb)

### Structure of Compounds



## RESULTS

- Using a water/ACN gradient, selected ion monitoring (SIM) for p-nitrophenol and chloramphenicol showed significant response (Fig. 1).
- Although PNP had a linear response with concentration in LC-UV, the response in mass spec was non-linear; the same trend was observed when PNP was run using AA/ACN gradient and in UHPLC-MS (Figs. 2-4).
- Mecoprop showed linear response both in LC-UV and LC-MS in water/ACN and AA/ACN (Figs. 5 and 6).
- Response of chloramphenicol was observed better in water/ACN compared to AA/ACN and water/methanol (Fig. 7).
- Poor or no ionization was observed for aldicarb, quercetin, azomethine, baicalin, digoxin and naproxen using water/ACN and water/methanol gradient even at 10 µg/mL concentration (data not shown).

### Figure 1. Selected Ion Monitoring for PNP and CHL in Water/ACN



PNP (m/z 138) was observed as m/z 252 (TFA adduct)

### Figure 2. LC-UV and LC-MS Response for PNP in Water/ACN



| PNP (ng/mL) | Peak height (mAU) |
|---|---|
| 0.01 | |
| 0.025 | 0.65 |
| 0.05 | 0.73 |
| 0.1 | 1.5 |
| 0.2 | 4.0 |
| 0.5 | 9.3 |
| 1 | 14.1 |
| 2 | 32.1 |

| PNP (ng/mL) | Peak height (SIM) |
|---|---|
| 0.01 | 1016.8 |
| 0.025 | 2271.2 |
| 0.05 | 5198.8 |
| 0.1 | 9054.4 |
| 0.2 | 14395.1 |
| 0.5 | 18666.4 |
| 1 | 21915.3 |
| 2 | 21924.4 |

### Figure 3. Comparison of Peak Height of PNP in Water/ACN and AA/ACN Gradient



| PNP (ng/mL) | EIC counts - peak height water/ACN | EIC counts - peak height AA/ACN |
|---|---|---|
| 0.01 | 1469.8 | 236.9 |
| 0.02 | 2480.0 | |
| 0.025 | | 444.8 |
| 0.05 | 6176.7 | 1017.9 |
| 0.1 | 11427.7 | 2432.27 |
| 0.2 | 18230.7 | 4019.8 |
| 0.5 | 32442.7 | 7755.2 |
| 1 | 42679.0 | 15960.3 |
| 2 | 50790.3 | 21732.9 |

### Figure 4. UHPLC-MS of PNP in Water/ACN



| PNP (ng/mL) | m/z 138 peak height | m/z 252 peak height | Total of m/z 138 and m/z 252 |
|---|---|---|---|
| 0.01 | 21643 | 8385 | 30028 |
| 0.02 | 46791 | 12227 | 59018 |
| 0.05 | 101872 | 9365 | 111237 |
| 0.1 | 194223 | 19814 | 214037 |
| 0.2 | 292328 | 28251 | 320579 |
| 0.5 | 439106 | 48914 | 488020 |
| 1 | 467128 | 55460 | 522588 |
| 2 | 413924 | 81218 | 495142 |

### Figure 5. LC-UV and LC-MS Response for MCP in Water/ACN



| MCP (ng/mL) | Peak height (mAU) |
|---|---|
| 0.1 | 0.44 |
| 0.2 | 0.82 |
| 0.5 | 2.0 |
| 1 | 3.4 |
| 2 | 6.6 |

| MCP (ng/mL) | Peak height (SIM) |
|---|---|
| 0.05 | 554.05 |
| 0.1 | 1025.23 |
| 0.2 | 2094.84 |
| 0.5 | 4970.21 |
| 1 | 8000.51 |
| 2 | 12138.00 |

### Figure 6. Comparison of Peak Height of MCP in Water/ACN and AA/ACN Gradient



| MCP (ng/mL) | EIC counts - peak height water/ACN | EIC counts - peak height AA/ACN |
|---|---|---|
| 0.1 | 217.5 | |
| 0.2 | 395.3 | |
| 0.5 | | 446.1 |
| 1 | 2118.7 | 758.1 |
| 2 | 4328.7 | 1284.1 |

### Figure 7. LC-MS Response of Chloramphenicol With Different Mobile Phase Gradients



| Chloramphenicol (ng/mL) | Peak height- water/ACN | Peak height- water/MeOH | Peak height- AA/ACN |
|---|---|---|---|
| 0.001 | 86.3 | | |
| 0.005 | 180.1 | | |
| 0.01 | 278.2 | 47.3 | 759.4 |
| 0.02 | 531.9 | 103.2 | 906.3 |
| 0.05 | 1129.6 | 236.2 | 1254.8 |
| 0.1 | 2047.8 | 423.7 | 1774.2 |
| 0.2 | 4020.6 | 971.8 | 2948.4 |
| 0.5 | 9587.3 | 2292.6 | 5981.4 |
| 1 | 18845.8 | 4469.4 | 11359.0 |

## DISCUSSION

- A combination of higher pH and additive (such as ammonia, ammonium formate and ammonium acetate) is commonly used to help ionize compounds in negative ESI-MS although this practice may lead to complexity in determining the actual mass of the analyte. Assessing mobile phase quality is also difficult in the presence of additives. Therefore, it is preferable to use an MS standard that is easily ionized under a neutral condition without any external reagents to assess the solvent quality.
- From our analysis, a significant response was observed from three compounds: p-nitrophenol (PNP), chloramphenicol (CHL) and mecoprop (MCP), and these compounds were detected at parts per billion concentration under neutral conditions in ESI-MS negative mode.
- Selected ion monitoring for the compound of interest was compared with the extracted ion chromatogram from the full scale data.
- The signal intensity under the same analysis condition was observed as PNP>CHL>MCP. The difference in signal intensity may be due to differences of pKa (PNP [pKa 7.16], CHL [pKa 5.5, 7.49, & 11.03] and MCP [pKa 3.78]).
- In water/methanol mobile phase, LC-MS ionization of chloramphenicol was suppressed compared to water/acetonitrile.
- Both PNP and CHL showed adduct formation with trifluoroacetic acid (TFA) anion that could be a carryover contaminant from a previous run. This likely occurs in the gas-phase during evaporation of charged droplets of the mobile phase. The volatile nature of TFA helped increase the local pH of the droplets favoring adduct formation.
- Use of 10 mM ammonium acetate (at neutral pH) in water/acetonitrile mobile phase suppressed the ionization of both PNP and CHL compared to water/acetonitrile mobile phase.
- From these data, chloramphenicol appears to be the best standard for evaluating mobile phase quality in negative ionization mode under neutral conditions without any additives.

## CONCLUSIONS

- A negative mode ESI-MS comparison of standards is performed under neutral conditions to assess the solvent quality for LC-MS analysis.
- Chloramphenicol showed linear response under neutral conditions in water/ACN and water/methanol mobile phases.