

New Solvent Grade Targeted for Trace Analysis by UHPLC-MS

Subhra Bhattacharya,
Deva H. Puranam, and Stephen C. Roemer

Thermo Fisher Scientific – Fisher Chemical,
One Reagent Lane, Fair Lawn, NJ



Fisher Chemical has developed a new solvent grade, UHPLC-MS Optima™, for mobile phases targeting trace analysis by UHPLC-MS. These ultra-pure solvents will provide a very low mass noise level in both positive and negative mode ionization, minimal metal ion content, and very low UHPLC-UV response using photo diode array detection. Fisher Chemical's high purity solvents are specifically qualified for UHPLC-MS and offered in Acetonitrile, Methanol, and Water.

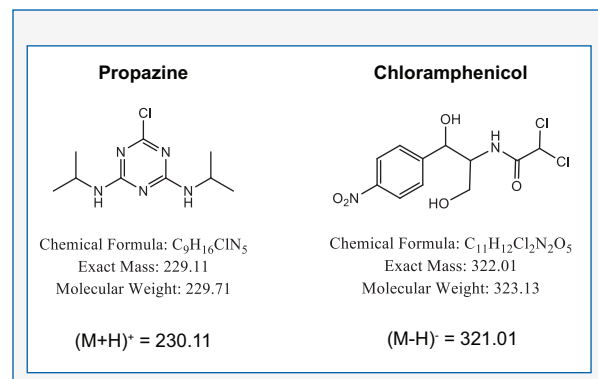
Solvent	Pack size	Packaging	Catalog Number
Acetonitrile	1L	Borosilicate Glass	A956-1
Methanol	1L	Borosilicate Glass	A458-1
Water	1L	Borosilicate Glass	W8-1

Introduction

Ultra-high performance liquid chromatography (UHPLC) is associated with submicron particle size column and high pressure flow resulting in increased resolution and sensitivity for complex sample mixtures and increased speed of analysis. Mass spectrometry (MS) enables the detection and identification of analytes at the parts per trillion level. UHPLC coupled with MS (UHPLC-MS) is a powerful tool in analytical chemistry that requires mobile phase solutions prepared with exceptionally pure solvents permitting trace analysis. UHPLC-MS Optima™, the new Fisher Chemical solvent grade for mobile phases targeting UHPLC-MS will show a very low mass noise level in both positive and negative mode ionization, minimal metal ion content, and very low UHPLC-UV response using photodiode array detection.

Material and Methods

- **Mobile phase:** acetonitrile (ACN), methanol, and water were evaluated; all three solvents are from a new grade, Optima® UHPLC-MS, that will provide very low mass noise level in both positive and negative mode ionization.
- **Instrument:** 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, 1.9 micron), Catalog No: 26-102-052130.
- **Standards:** propazine (SPEX CertiPrep, S-3170, 1000 µg/mL) as the positive mode standard and chloramphenicol (SPEX CertiPrep, S-4032, 1000 µg/mL) as negative mode standard. These standards provided adequate ionization without any additive applied to the mobile phase.
- The mass spectrometer was operated in full scan ESI-MS from 100 to 1500 amu. The collision induced dissociation (CID) mass spectra were obtained with helium as the collision gas after isolation of the particular precursor ion. Other parameters including gas flow and capillary voltage were adjusted as required.
- **HPLC Gradient:**
 - 0 — 0.5 min: 90% water, 10% ACN
 - 0.5 min — 2.0 min: 0% water, 100% ACN
 - 2 min — 5 min: 100% ACN
 - Post run 5.1 min — 10 min: 90% water, 10% ACN
- **Flow rate:** 0.6 mL/min for water/ACN, 0.5 mL/min for water/methanol
- **Injection volume:** 5 µl



Results

- Mobile phase solvent purity was evaluated by linking UHPLC-MS sensitivity to trace analysis of positive and negative mode standards (Figs. 1–10).
- Propazine was used as positive mode standard (Figs. 3–6) and chloramphenicol as negative mode standard (Figs. 7–10) in order to assess interfering baseline peaks in both full scan ESI-MS and CID generated product ions.

Figure 1. Blank Gradient of Water/ACN – PDA, UV and TIC

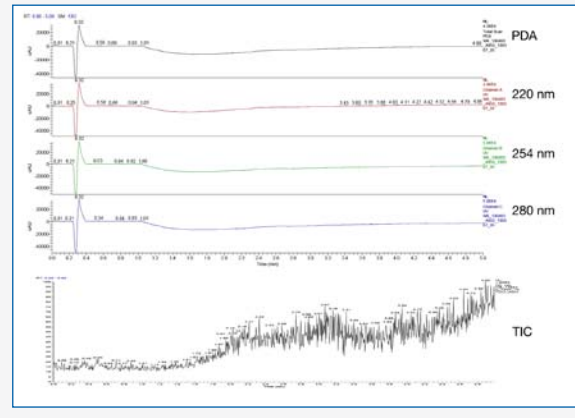


Figure 2. Blank Gradient of Water/Methanol – PDA, UV and TIC

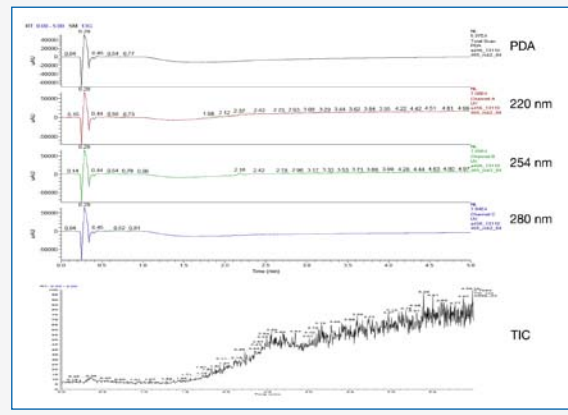


Figure 3. Serial Dilution of Propazine – EIC of m/z 230

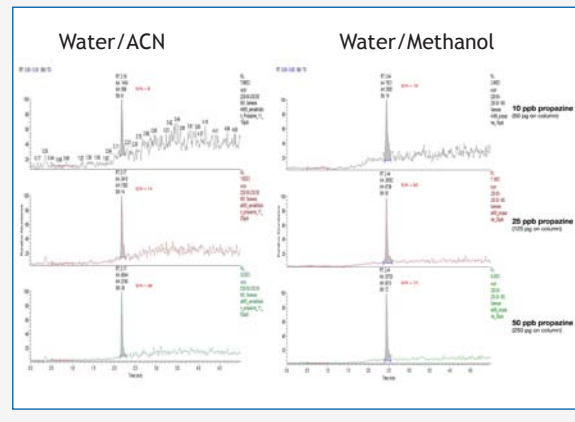


Figure 4. Mass Spectra of m/z 230 Propazine Peak (250 pg)

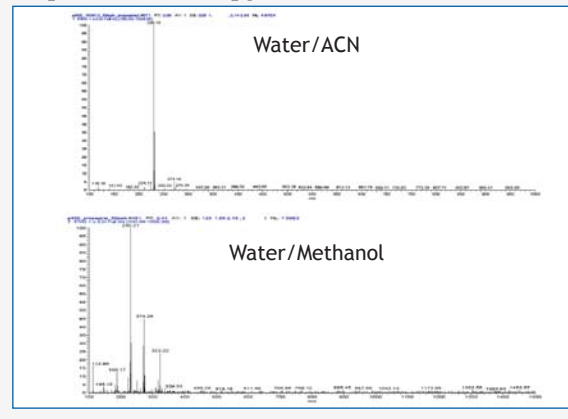
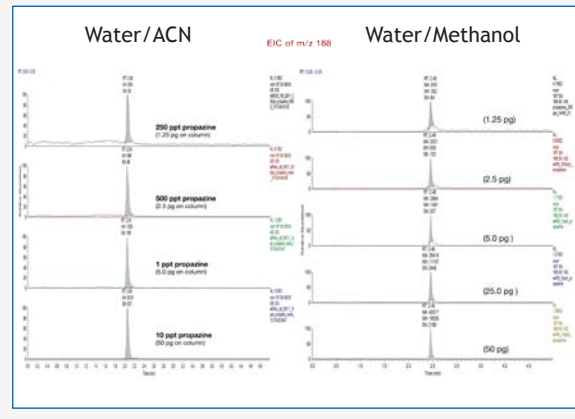


Figure 5. MS/MS of Propazine From Serial Dilution





Discussion

- Blank gradient of water/ACN and water/methanol in PDA, UV and mass spectra is shown in Figs. 1 and 2. No extraneous peaks were observed in either solvent system.
- Trace level of propazine (10 ppb = 10 pg/ μ L) was detected in positive mode ionization both in water/ACN and water/methanol gradient by EIC after full scale data acquisition (100 – 1500 amu). The signal to noise ratio of the peak was observed below the level of quantitation (LOQ) in the ACN gradient. However, a 25 pg/ μ L concentration (25 ppb) of propazine showed S/N >10 in both solvent systems (Fig. 3).
- MS/MS of propazine peak (m/z 230) was accomplished using 250 fg/ μ L (250 ppt) of analyte, and the signal to noise ratio of the m/z 188 product ion was monitored. For both solvent systems, the propazine product ion showed S/N >30 (Figs. 5 and 6) which is consistent with the detection sensitivity of the LTQ-XL ion trap mass spectrometer.
- In negative mode ionization, 50 pg/ μ L (50 ppb) of chloramphenicol showed S/N >10 in the ACN and methanol gradients (Fig. 7). Both solvent systems showed m/z 435 due to TFA (m/z 113) adduct formation coming from the system (Fig. 8).
- MS/MS of 5 pg/ μ L (5 ppb) chloramphenicol generated the product ion of m/z 257 with a S/N >10 (Figs. 9 and 10).

Conclusions

- Using the new Optima® UHPLC-MS mobile phase solvents, trace amounts of propazine and chloramphenicol standards showed significant peak height without interfering background peaks in both full scan ESI-MS and CID generated product ions.
- Monitoring the CID generated product ion peak is common practice for evaluating MS sensitivity, and we have developed a similar approach to assess the purity of mobile phase solvents.
- Propazine (m/z 230) generated the product ion peak of m/z 188 with a signal to noise ratio >30 at 250 ppt using a water/acetonitrile mobile phase.

Figure 6. MS/MS Spectra of Propazine in Two Different Solvent Systems

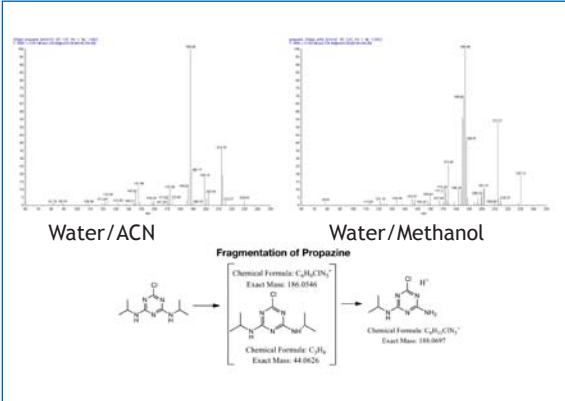


Figure 7. Serial Dilution of Chloramphenicol

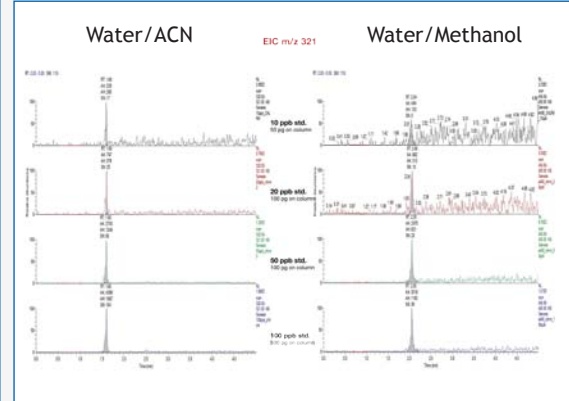


Figure 8. Mass Spectra of Chloramphenicol in Water/ACN and Water/Methanol

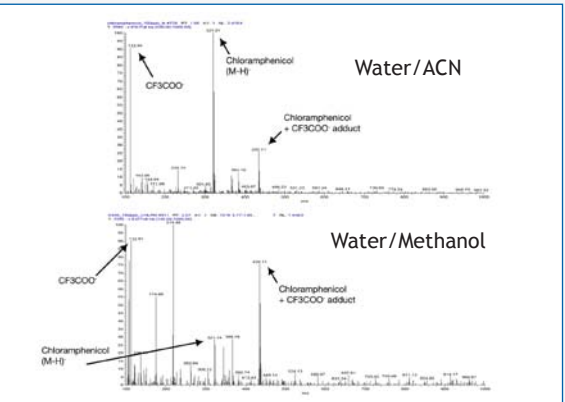


Figure 9. MS/MS of Chloramphenicol in Water/ACN and Water/Methanol Gradient

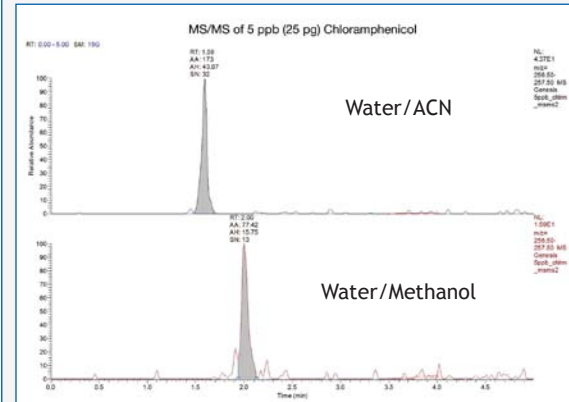
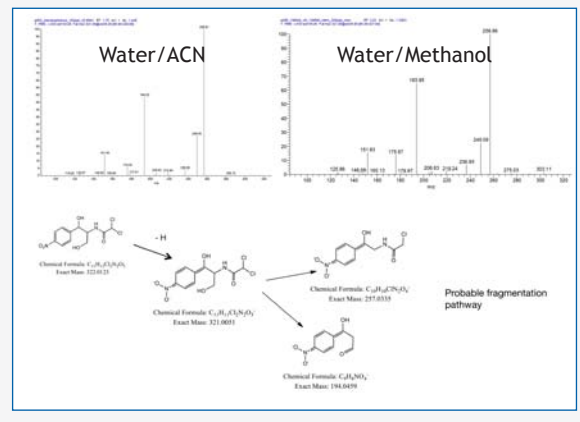


Figure 10. MS/MS Spectra of Chloramphenicol





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Austria

info.austria@thermofisher.com
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Tel: 0800 20 88 40
Fax: 0800 20 66 90

Germany

info.germany@thermofisher.com
www.de.fishersci.com
Tel: 2304 932-5
Fax: 2304 932-950

Portugal

pt.fisher@thermofisher.com
www.pt.fishersci.com
Tel: 21 425 33 50
Fax: 21 425 33 51

Belgium

be.fisher@thermofisher.com
www.be.fishersci.com
Tel: 056 260 260
Fax: 056 260 270

Ireland

fsie.sales@thermofisher.com
www.ie.fishersci.com
Tel: 01 885 5854
Fax: 01 899 1855

Spain

es.fisher@thermofisher.com
www.es.fishersci.com
Tel: 902 239 303
Fax: 902 239 404

Czech Republic

info.cz@thermofisher.com
www.thermofisher.cz
Tel: 466 798 230
Fax: 466 435 008

Italy

it.fisher@thermofisher.com
www.it.fishersci.com
Tel: 02 950 59 478
Fax: 02 950 59 479

Sweden

fisher.se@thermofisher.com
www.se.fishersci.com
Tel: 31 352 32 00
Fax: 31 352 32 50

Denmark

kundeservice@thermofisher.com
www.dk.fishersci.com
Tel: 70 27 99 20
Fax: 70 27 99 29

Lithuania

sales.lt@thermofisher.com
www.lt.fishersci.com
T: +370 5 2444442
F: +370 5 2392756

Switzerland

info.ch@thermofisher.com
www.ch.fishersci.com
Tel: 056 618 41 11
Fax: 056 618 41 41

Finland

fisher.fi@thermofisher.com
www.fi.fishersci.com
Tel: 09-802 76 280
Fax: 09-802 76 235

The Netherlands

nl.info@thermofisher.com
www.nl.fishersci.com
Tel: 020 487 70 00
Fax: 020 487 70 70

United Kingdom

fsuk.sales@thermofisher.com
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France

fr.fisher@thermofisher.com
www.fr.fishersci.com
Tel: 03 88 67 14 14
Fax: 03 88 67 11 68

Norway

fisher.no@thermofisher.com
www.no.fishersci.com
Tel: 22 95 59 59
Fax: 22 95 59 40

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India

lsi_marketing@thermofisher.com
www.fishersci.in
Tel: +91-22-6680 3000
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Korea

sales.fsk@thermofisher.com
www.fishersci.co.kr
Tel: 02-3420-8700
Fax: 02-3420-8710

Malaysia

enquiry.my@thermofisher.com
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Fax: (603) 5121 8899

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