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Chemicals

UHPLC-MS grade solvents for trace level analysis and research methods and assays

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Keywords

UHPLC-MS, UHPLC UV, grade solvents, ultra-pure solvents, trace level analysis, research methods, research assays, acetonitrile, methanol, water.

Summary

Thermo Scientific™ manufactures the solvent grade, UHPLC-MS for use in mobile phases targeting trace level analysis and research studies using UHPLC-MS technology. These ultra-pure solvents 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. These high purity solvents are specifically qualified for UHPLC-MS and offered in acetonitrile, methanol, and water.

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 solvent grade for mobile phases targeting UHPLC-MS 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 UHPLC-MS grade.

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 µm), cat. no. 26102-052130.

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.

Operation

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

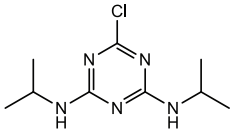
Flow rate

0.6 mL/min for water/ACN, 0.5 mL/min for water/methanol

Injection volume

5 µL

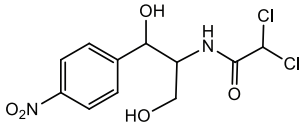
Propazine



Chemical folmula: $C_9H_{16}ClN_5$
Exact mass: 229.11
Molecular weight: 229.71

$(M+H)^+$ = 230.11

Chloramphenicol



Chemical folmula: $C_{11}H_{12}Cl_2N_2O_5$
Exact mass: 322.01
Molecular weight: 323.13

$(M-H)^-$ = 321.01

Results

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

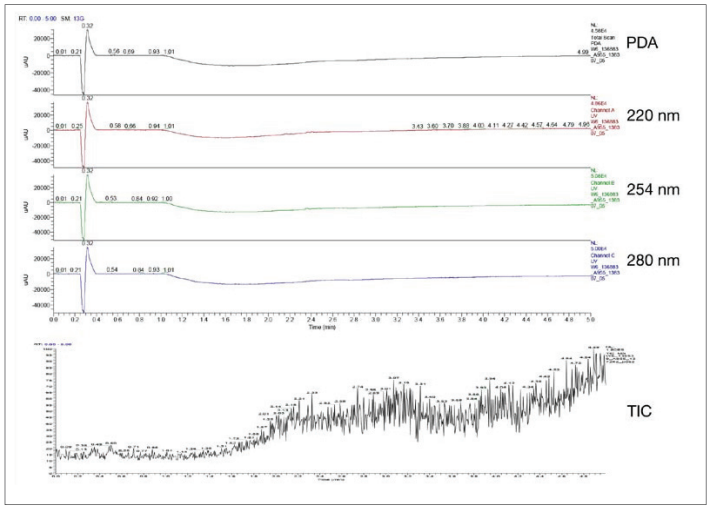


Figure 1. Blank gradient of water/ACN – PDA, UV and TIC

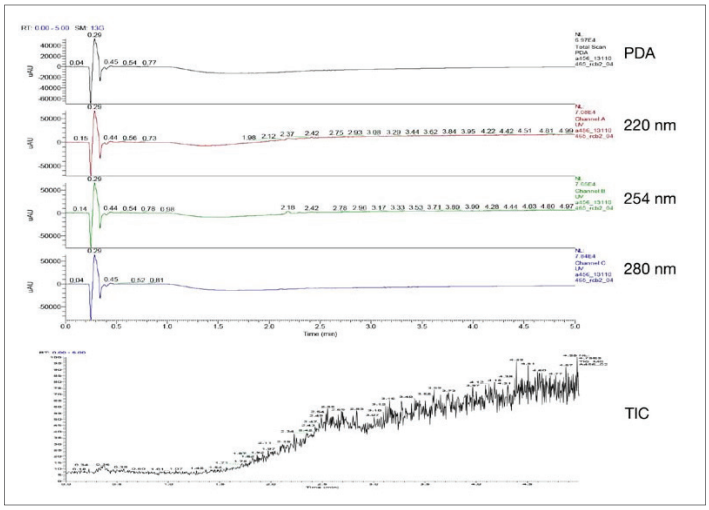


Figure 2. Blank gradient of water/methanol – PDA, UV and TIC

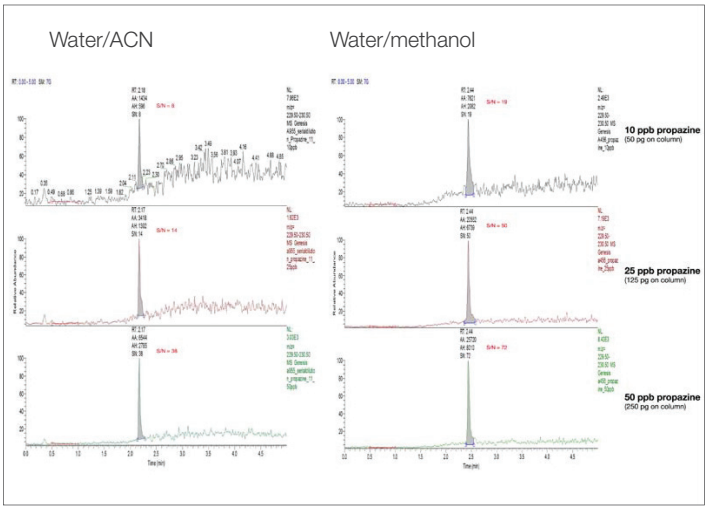


Figure 3. Serial dilution of propazine – EIC of *m/z* 230

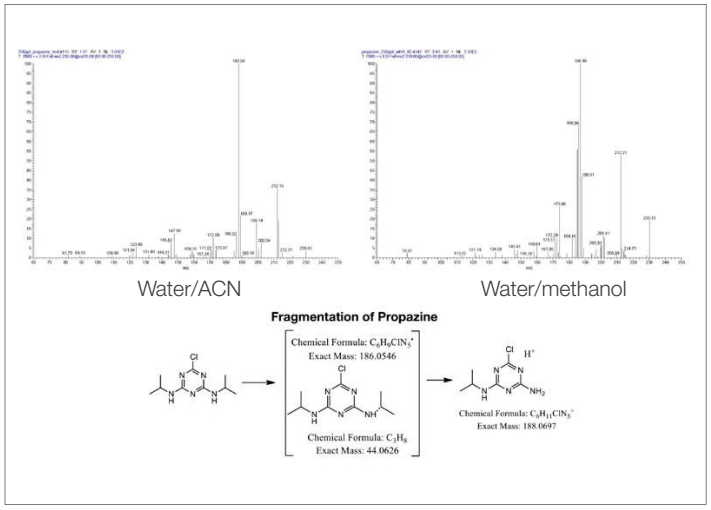


Figure 6. MS/MS spectra of propazine in two different solvent systems

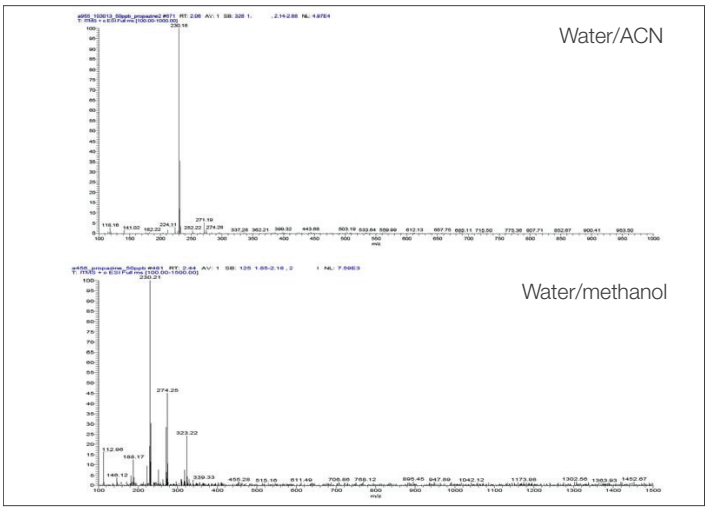


Figure 4. Mass spectra of *m/z* 230 propazine peak (250 pg)

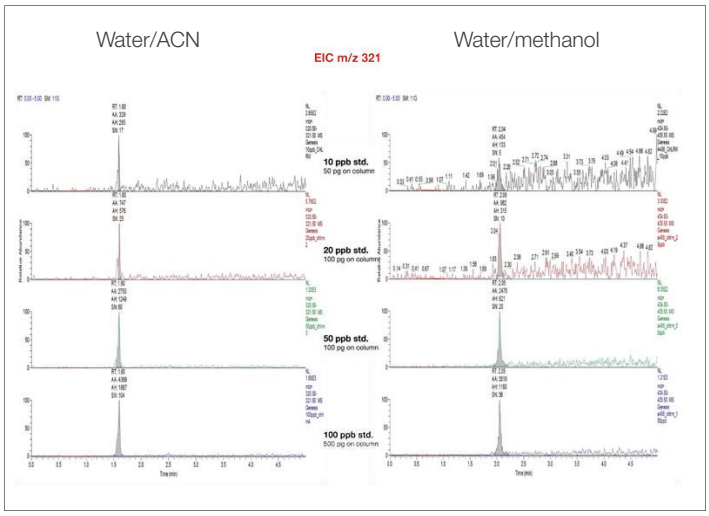


Figure 7. Serial dilution of chloramphenicol

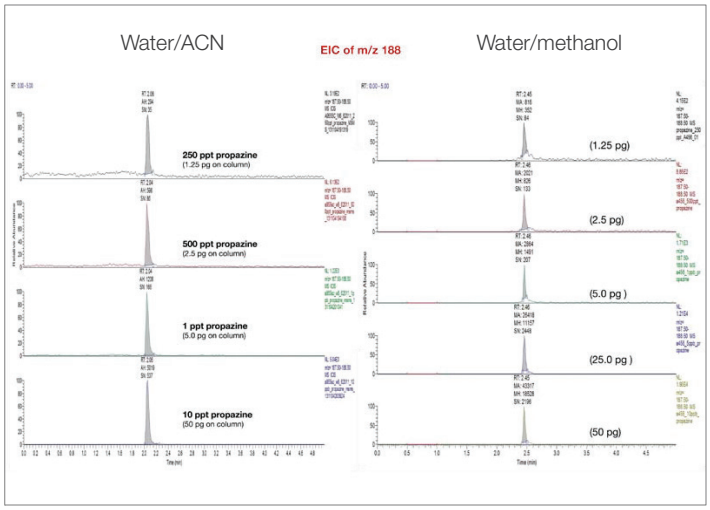


Figure 5. MS/MS of propazine from serial dilution

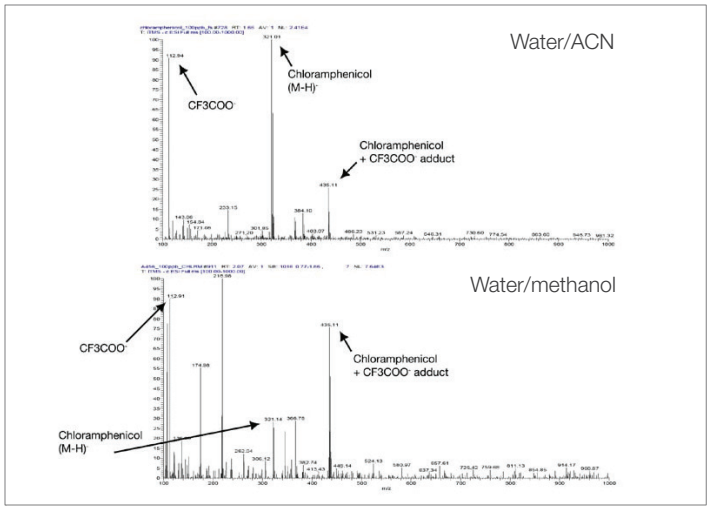


Figure 8. Mass spectra of chloramphenicol in water/ACN and water/methanol

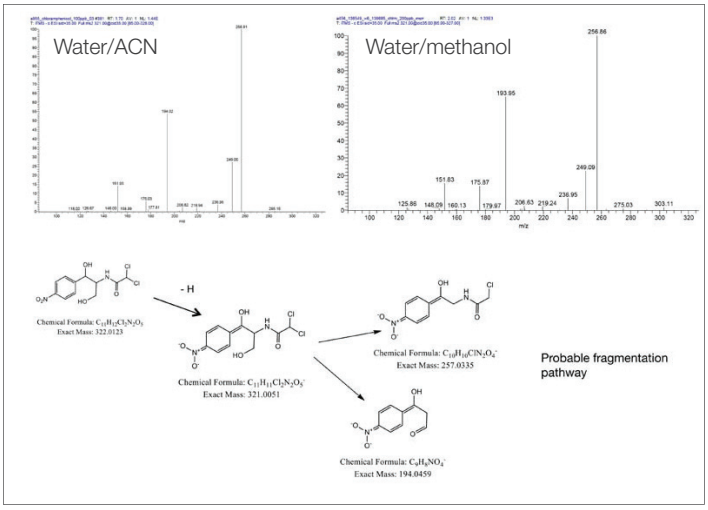


Figure 9. MS/MS of chloramphenicol in water/ACN and water/methanol gradient

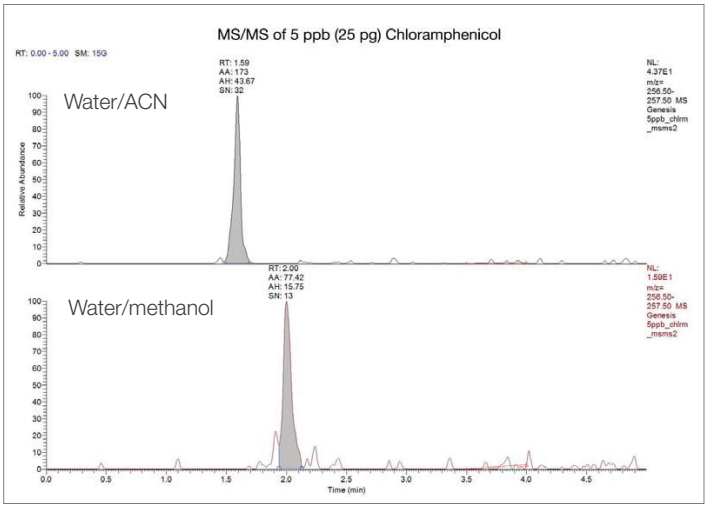


Figure 10. MS/MS Spectra of chloramphenicol

Discussion

- Blank gradient of water/ACN and water/methanol in PDA, UV and mass spectra is shown in Figures 1 and 2. No extraneous peaks were observed in either solvent system.
- Trace level of propazine (10 ppb = 10 pg/µL) 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/µL concentration (25 ppb) of propazine showed S/N >10 in both solvent systems (Figure 3).
- MS/MS of propazine peak (*m/z* 230) was accomplished using 250 fg/µL (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 (Figures 5 and 6) which is consistent with the detection sensitivity of the LTQ-XL ion trap mass spectrometer.
- In negative mode ionization, 50 pg/µL (50 ppb) of chloramphenicol showed S/N >10 in the ACN and methanol gradients (Figure 7). Both solvent systems showed *m/z* 435 due to TFA (m/z 113) adduct formation coming from the system (Figure. 8).
- MS/MS of 5 pg/µl (5 ppb) chloramphenicol generated the product ion of *m/z* 257 with a S/N >10 (Figures 9 and 10).

Conclusions

- Using Thermo Scientific 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.

Ordering information

Solvent	Pack size	Packaging	Cat. no.
Acetonitrile	1 L	Borosilicate glass	**A956-1**
Methanol	1 L	Borosilicate glass	**A458-1**
Water	1 L	Borosilicate glass	**W8-1**

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