

Chemicals

Optimizing mobile phase solvent purity for LC-MS

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Keywords

UHPLC-MS, LC-MS, mobile phase, solvent, purity, LC-UV, acetonitrile, water, TIC, methanol

Introduction

In the life sciences, from biomarker discovery to metabolomics, investigators frequently study molecules requiring analysis in trace amounts (femtomole range). Liquid chromatography mass spectrometry (LC-MS) has gained broad usage in this research because the technique supports qualitative and quantitative applications with low limits of detection. For example, many clinically relevant biomarkers such as parathyroid hormone and prostate specific antigen are present in blood at concentrations of nanograms per milliliter.

Improving sensitivity and mass accuracy in LC-MS is an ongoing process. As instrumentation advances lead to ever-lower analyte detection limits, an underlying issue is the need to reduce noise—the extraneous peaks caused by solvent background, which limits the ultimate performance of LC-MS.

Optimizing the quality of mobile phase solvents can contribute to improving the chromatographic or mass spectroscopic properties of the analyte as well as the overall detection limits of the instrument system (1). Historically, when the LC-MS technique was still in development, HPLC-grade solvents were used to prepare the mobile phase. Years later, some investigators still use HPLC-grade solvents to prepare the composition of the mobile phase for LC-MS applications. However, solvents designated “HPLC grade” do not meet the stringent purity requirements of the mass spectrometry detector and are best used with LC-UV detectors.

In contrast, solvents designated “LC-MS grade” should provide low mass noise level, minimal organic contamination, and minimal metal content to fulfill the high purity need of LC-MS. To address this need, Thermo Fisher Scientific developed the LC-MS solvent grade. These solvents are manufactured using additional purification processes, quality control measures, and packaging innovations in order to meet the required purity level of advanced LC-MS systems.

This paper presents comparative chromatographic data from four different instruments for LC-MS solvents offered by seven vendors.

Data collected examine mass baseline, signal intensity, contamination by PEG and phthalates (plasticizers), and metal ion content which impacts ease of MS interpretation.

Methods

Experiment I was conducted at two Thermo Fisher Scientific sites (Fair Lawn, NJ and Cambridge, MA , USA)

Chromatographic data for the Fisher Scientific™ Optima™ LC-MS grade solvents (acetonitrile and water) were compared with four other solvent brands. Each solvent was analyzed with a gradient as a blank injection and also with an angiotensin standard peptide mixture. The objective was to compare background noise and signal intensity of the peptide standards across each vendor’s solvent system as well to look for typical contamination peaks known to be found in these high purity solvents.

Mobile phase solvents acetonitrile and water were obtained from four vendors, and in the figures/tables these are referred to as vendor J, R, E, and H. These were compared with Optima LC-MS grade acetonitrile (A955) and water (W6). Various LC gradients were utilized.

Experiment II was conducted at three Thermo Fisher Scientific sites (Fair Lawn, NJ, USA; Courtaboeuf, France; and Breda, The Netherlands)

Chromatographic data for Optima LC-MS grade solvents (A456 methanol, W6 water, and A955 acetonitrile) were compared with two other solvent brands (referred to as vendor X and B). Similar to the first experiment, the objective was to compare solvent baseline noise as well as the signal intensity of MS standards across each vendor’s solvent system. Operating conditions for the LC and MS of each instrument system are provided in Table 1.

Table 1. Primary operating conditions of LC and MS in Experiment II

	Courtaboeuf, France	Breda, The Netherlands
MS system	LTQ Orbitrap XL	TSQ Vantage (Triple Quadrupole)
HPLC system	Bypass	Accela UPLC
Type of column	Direct to mass	Thermo Scientific™ Hypersil GOLD™ column, 1.9 μm, 50 × 2.1 mm
Flow rate	0.5 mL/min	0.4 mL/min
Mass range	50 to 800 <i>m/z</i>	Pos-Precursor 230.2; Neg-Precursor 213.1
Gradient (time/min)	A%(Aqu) B%(Solv)	A%(Aqu) B%(Solv)
0	100 0	95 5
2	100 0	10 90
15 (3 -Breda)	0 100	10 90
17 (3.01 - Breda)	0 100	95 5
17.1 (4 - Neth)	100 0	95 5

Results

When selecting solvents from a vendor to use in an LC-MS mobile phase, the chromatographer should consider five fundamental factors related to solvent purity:

1. Level of baseline noise
2. Extent of LC-UV impurities
3. Signal intensity from standard analytes
4. Contamination with phthalates (plasticizers)
5. The metal ion content

Experiment I.

Baseline noise and LC-UV impurities

Many LC-MS systems are equipped with diode array detection (UV/VIS). At 210nm Optima LC-MS grade acetonitrile provides a flat baseline and very low LC-UV noise (Figure 1).

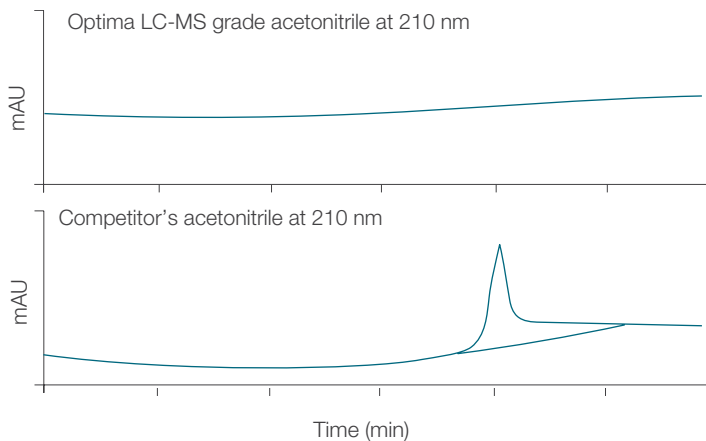


Figure 1. Top panel shows a typical LC-UV baseline for Optima LC-MS grade acetonitrile (A955) compared with a competitor's acetonitrile in the bottom panel. Single peak height approximates ≤ 2 mAU in A955. The competitor's material shows a large impurity peak (60 mAU) at 75 minutes, and its baseline is more "curved" than the baseline for A955.

Figures 2 and 3 show that the Optima LC-MS grade acetonitrile/water mobile phase produces the lowest mass background in positive and negative TIC modes using the single quadrupole LC-MS system. Moreover, analysis by LTQ-FT indicates that regardless of the retention region of the LC-MS gradient, the average TIC intensity is lowest for Optima LC-MS grade acetonitrile/water system (Figure 4 and Table 2). This is especially true for the organic region with Optima LC-MS grade acetonitrile (A955) providing significantly less background compared to other brands.

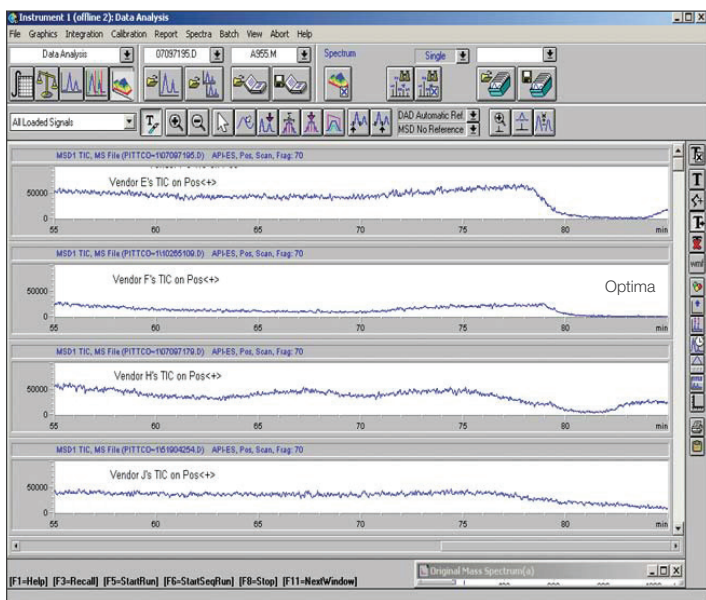


Figure 2. Mass background in positive mode of blank solvent sample using single quadrupole LC-MS observe that the Optima LC-MS grade acetonitrile/water mobile phase consistently produced the lowest background noise in TIC compared to other brands.

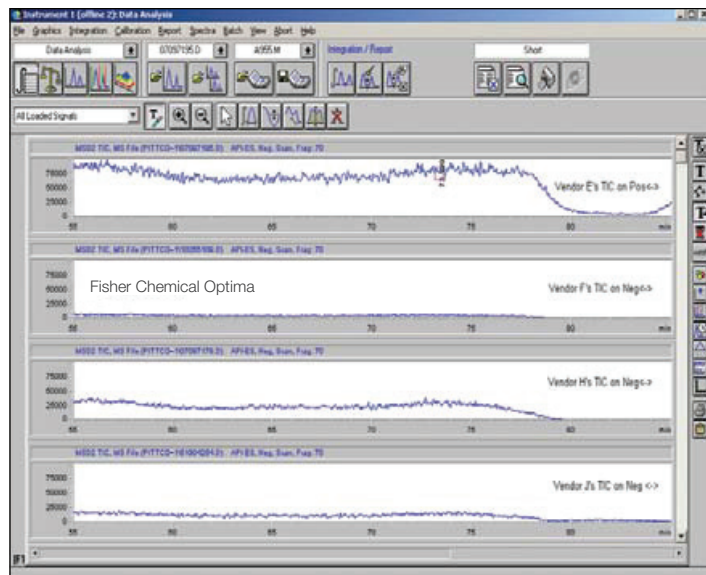


Figure 3. Mass background in negative mode of blank solvent sample using single quadrupole LC-MS. Note that the Optima LC-MS grade solvent system produced the flattest baseline and the lowest background noise in TIC.

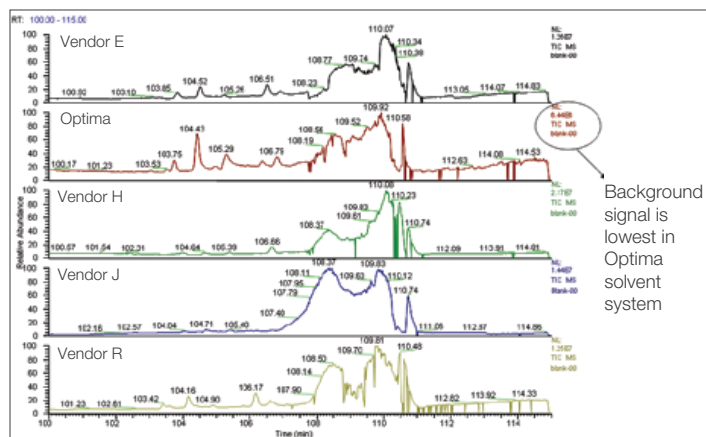


Figure 4. TIC intensity of blank solvent sample (high organic region) using LTQ-FT.

Table 2. Average TIC intensity for blank solvent samples (four replicates) using LTQ-FT system

Vendor	Aqueous	Organic
Vendor J	1.19 e6	1.63 e7
Vendor R	6.98 e5	1.62 e7
Optima	6.08 e5	7.85 e6
Vendor E	6.26 e5	1.43 e7
Vendor H	6.15 e5	1.99 e7

Overall retention time: 0 – 150 min.

Aqueous retention time: 15 – 28 min.

Organic retention time: 100 – 115 min.

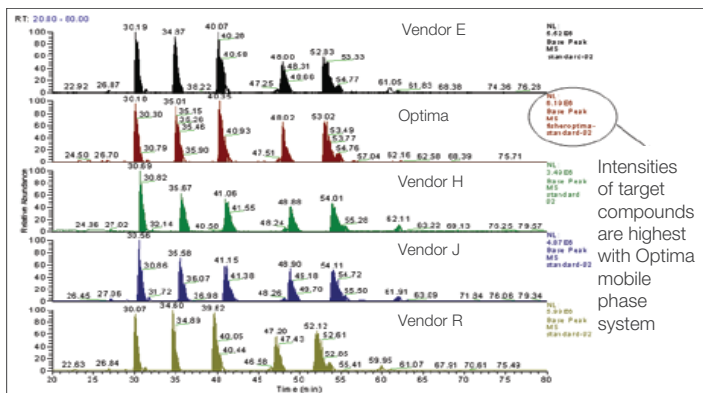


Figure 5. Base peak intensity for 150 fmole angiotensin peptide standard (LTQ-FT).

Signal intensity

According to Dolan², very often LC-MS quantification of an active pharmaceutical ingredient requires precision and accuracy in the 1 – 2% range. For this type of analysis, a large signal intensity of analyte is required. Low background noise contributed by the mobile phase is critical for maximizing signal intensity of target analytes at low concentration. An angiotensin peptide standard was used as a target analyte to compare signal intensities in mobile phase solvents from different vendors. Figure 5 shows that the highest signal intensity for a 150 fmole angiotensin peptide standard was obtained in the LTQFT using the Optima LC-MS grade acetonitrile/water system.

Contamination with phthalates

Phthalates (plasticizers) are contaminants commonly found in mobile phase solvents. Well-known sources of phthalate include lab gloves, plastic bottles and vials, filter paper and even laboratory air containing aerosolized surfactants, fire retardants, and antioxidants. Contamination from common phthalates such as diisooctyl phthalate ($m/z = 391$) and dibutylphthalate ($m/z = 279$) were lowest in the Optima LC-MS grade solvent system (Figures 6, 7). In some cases the Optima LC-MS grade solvents outperformed other vendors' solvents by an order of magnitude across various contaminating peaks. This degree of purity is particularly important as these contaminants take capacity in the instrument's ion traps as well as affecting the success of performing reproducible chromatography over extended periods of analysis.

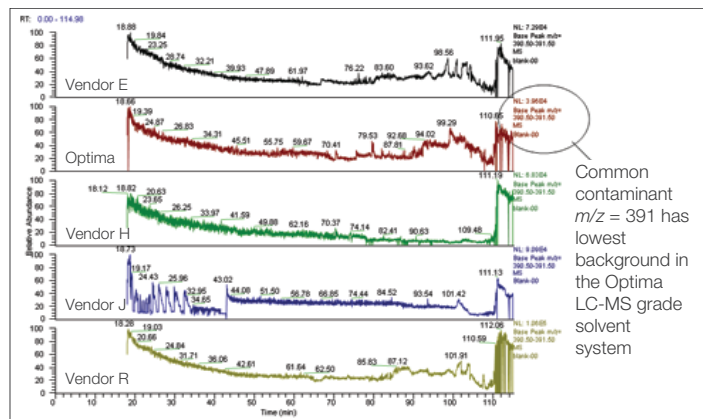


Figure 6. Comparative data from five solvent systems using the LTQ-FT at $m/z = 391$ (M + H)⁺ for diisooctyl phthalate.

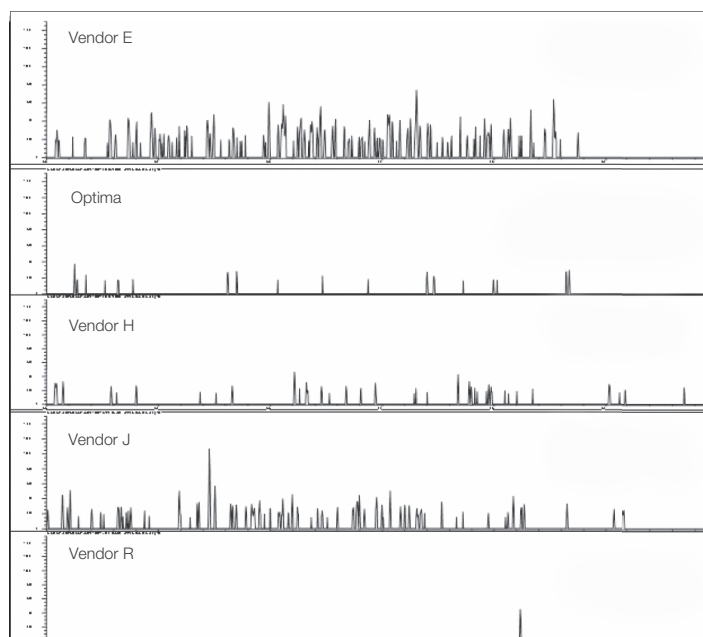


Figure 7. Comparative data from five solvent systems using the single quadrupole LC-MS at $m/z = 391$ (M + H)⁺ for diisooctyl phthalate. Note: Optima LC-MS grade mobile phase has less ion interference compared to other vendors.

Metal ion content

High metal ion content in the mobile phase enables the formation of mass adducts with target analytes. For example, introduction of alkali metals such as sodium and potassium into the experimental system can further complicate interpretation of results, particularly if quantification is an objective of the study. These metals can also join with phthalates having carboxyl and carbonyl ether or ester groups³ to form cluster adducts which create problems with reproducibility of results⁴. Although a variety of approaches exist for interpretation of data collected in the presence of adducts, limitation of their presence with the analyte of interest remains an important factor in method development.

Adducts can form when phthalates and alkali metals are present together. Mass spectra from both instrument systems illustrate adduct formation involving diisooctyl phthalate 413 *m/z* ($M + Na$)⁺ in the various solvent systems (Figures 8, 9). Thermo Fisher Scientific's Fair Lawn, NJ manufacturing site maintains very effective control over the metal ion content during processing and packaging so that Optima LC-MS grade solvents have the lowest metal content in the industry (Table 3).

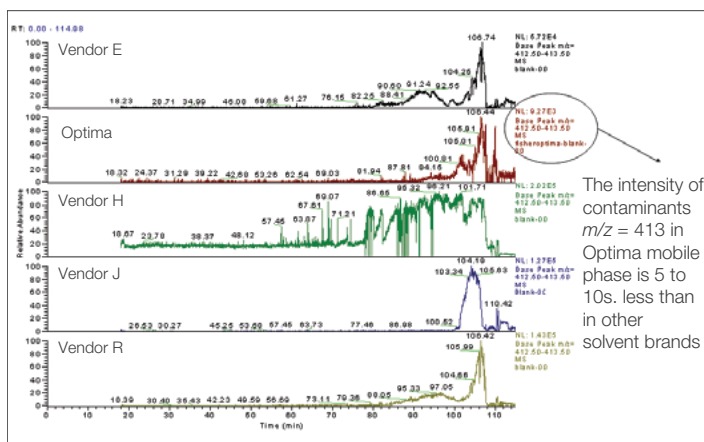


Figure 8. Adduct formation involving diisooctyl phthalate 413 *m/z* ($M + Na$)⁺ in the LQT-FT.

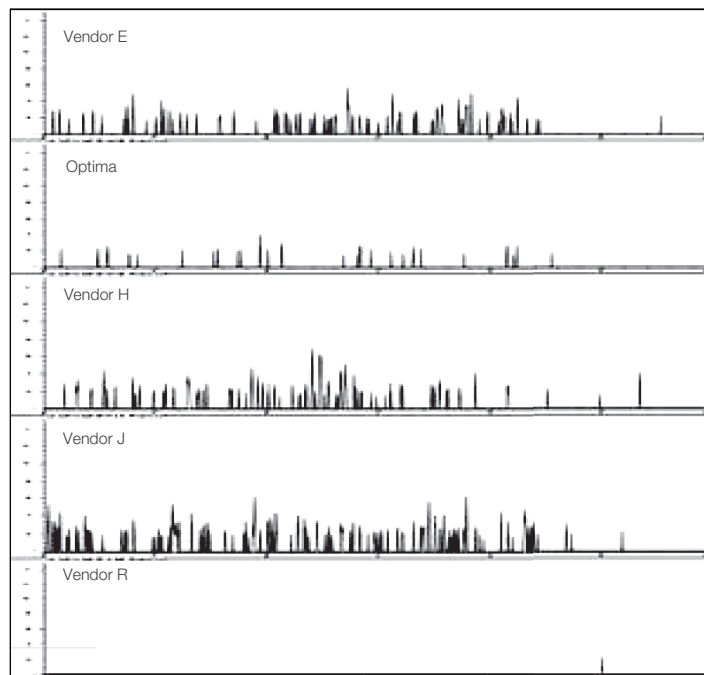


Figure 9. Adduct formation involving diisooctyl phthalate 413 *m/z* ($M + Na$)⁺ in the single quadrupole LC-MS. Observe that the Optima LC-MS grade solvent system has fewer interfering peaks compared with other suppliers.

Table 3. Trace metal ion impurities in Optima LC-MS grade solvents

Specifications (Trace ionic impurities)	A955 Acetonitrile (ppb, max.)	A456 Methanol (ppb, max.)	W6 Water (ppb, max.)
Aluminum (Al)	25	10	10
Barium (Ba)	5	10	10
Cadmium (Cd)	5	10	10
Calcium (Ca)	25	20	20
Chromium (Cr)	5	10	10
Cobalt (Co)	5	10	10
Copper (Cu)	5	10	10
Iron (Fe)	5	10	10
Lead (Pb)	5	10	10
Magnesium (Mg)	10	10	10
Manganese (Mn)	5	10	10
Nickel (Ni)	5	10	10
Potassium (K)	10	10	10
Silver (Ag)	5	10	10
Sodium (Na)	50	50	20
Tin (Sn)	5	10	10
Zinc (Zn)	10	10	10

Experiment II

Baseline noise and LC-UV impurities

At 254 nm Optimac LC-MS grade methanol has a low LC-UV response using diode array detection without any significant impurity peak (Figure 10) which is important for various research and QA/QC applications across multiple market disciplines.

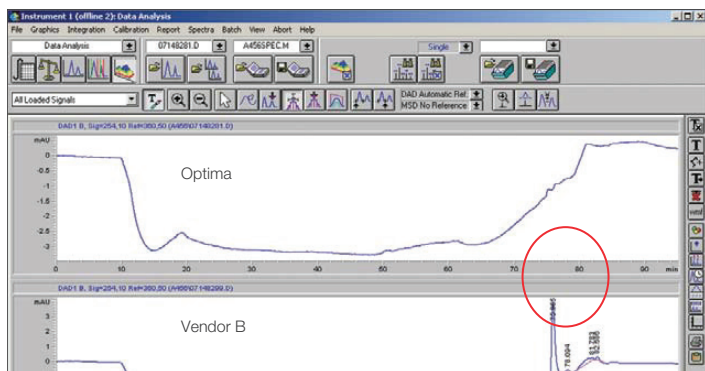


Figure 10. Top panel shows a typical LC-UV baseline at 254 nm for Optima methanol (A456) compared to Vendor B methanol in the bottom panel. A significant impurity peak (4 mAU) is observed at 75 minutes in the Vendor B methanol.

Figures 11 and 12 illustrate that the Optima water/methanol mobile phase produces the lowest mass background in positive TIC mode using the Thermo Scientific LTQ Orbitrap XL system. Moreover, the full MS spectrum from 50 – 800 m/z in positive mode shows fewer background peaks with less intensity for Optima LC-MS grade water/methanol at end of gradient compared to the other solvent brands (Figure 13). Similar results are also observed when LC-MS instruments operated in TIC negative mode revealed the mass baseline (noise level) lowest for the Optima LC-MS grade methanol profile compared to other solvent brands (Figures 14, 15).

TIC/methanol/positive ions

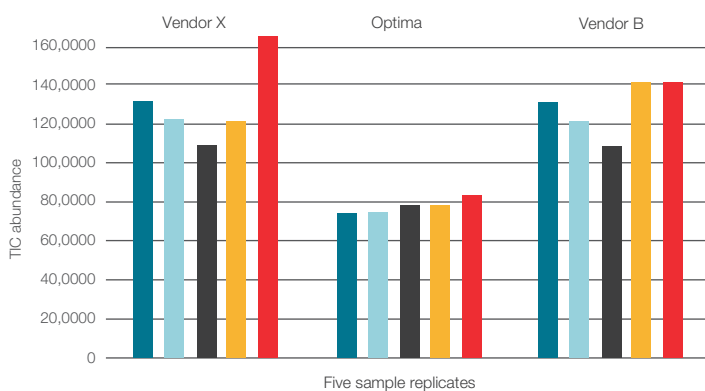
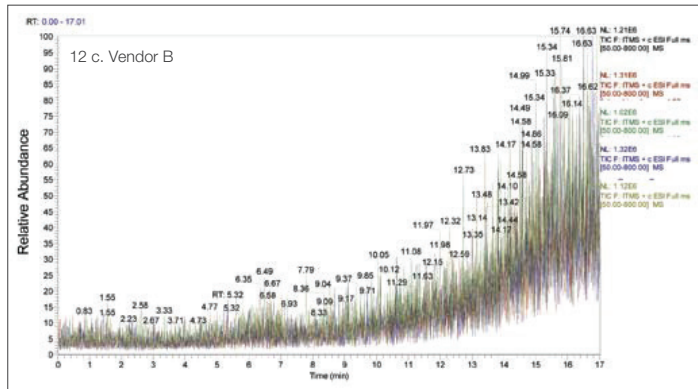
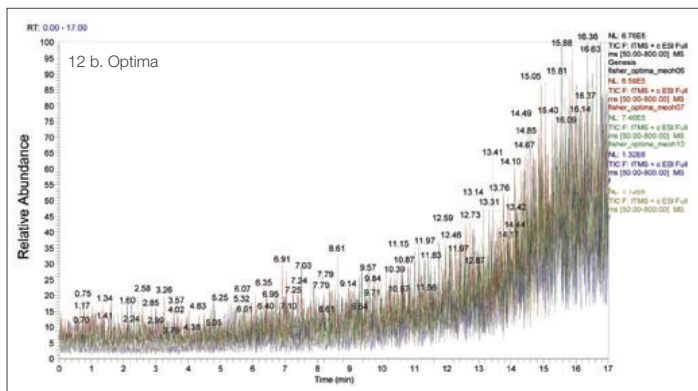
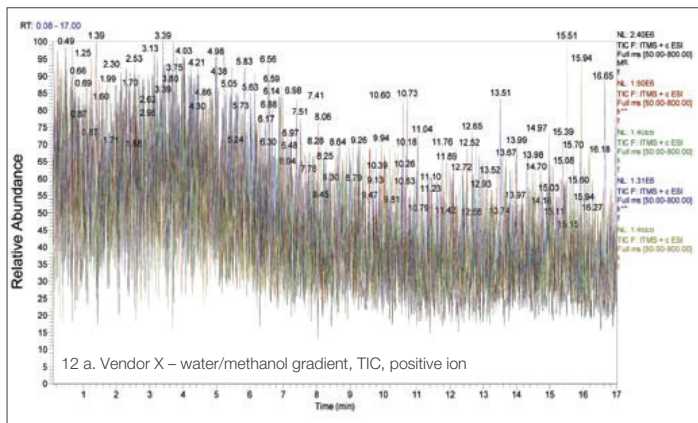
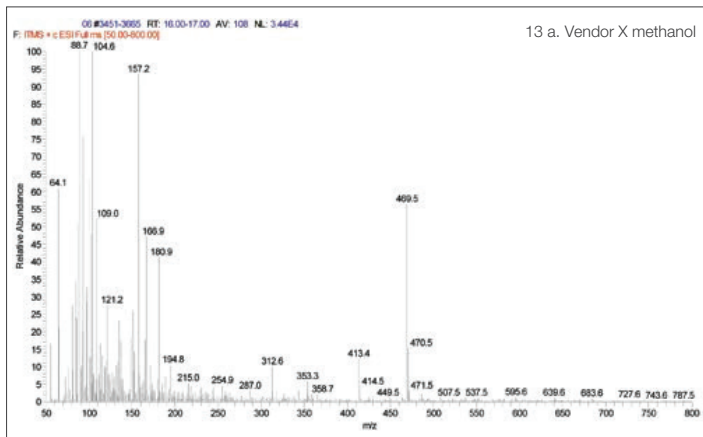


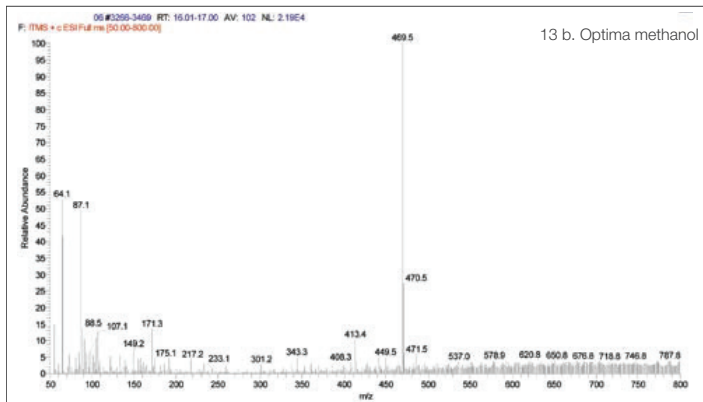
Figure 11. Mass background in positive mode of blank methanol sample using the LTQ Orbitrap XL. Optima LC-MS grade methanol profile consistently produced the lowest background noise in TIC compared to the other vendors.



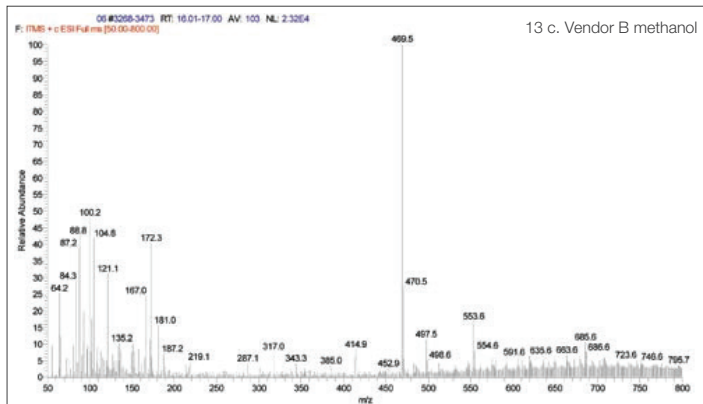
Figures 12 a-c. TIC intensity for five replicates of water/methanol gradient in positive mode using LTQ Orbitrap XL. Methanol data is summarized by vendor in Figure 11. (a) Vendor X, (b) Optima LC-MS grade, and (c) Vendor B.



13 a. Vendor X methanol



13 b. Optima methanol



13 c. Vendor B methanol

Figures 13 a-c. MS spectrum from 50 – 800 m/z in positive mode using LTQ Orbitrap XL. (a) Full MS scan of Vendor X methanol at end of gradient. (b) Full MS scan of Optima LC-MS grade methanol (A456). (c) Full MS scan of Vendor B methanol.

TIC/methanol/negative ions

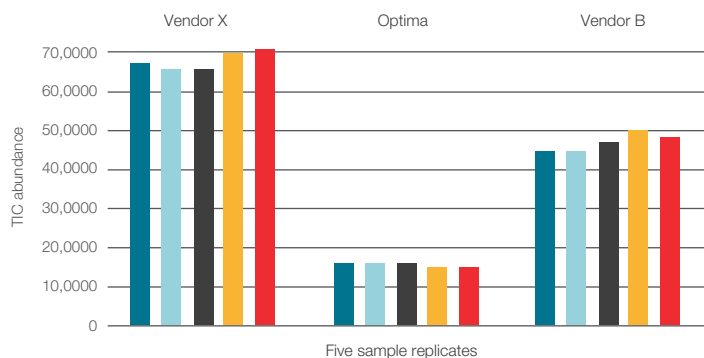


Figure 14. Mass background in negative mode of blank methanol sample using the LTQ Orbitrap XL. Optima methanol profile consistently produced the lowest background noise in TIC compared to the other vendors.

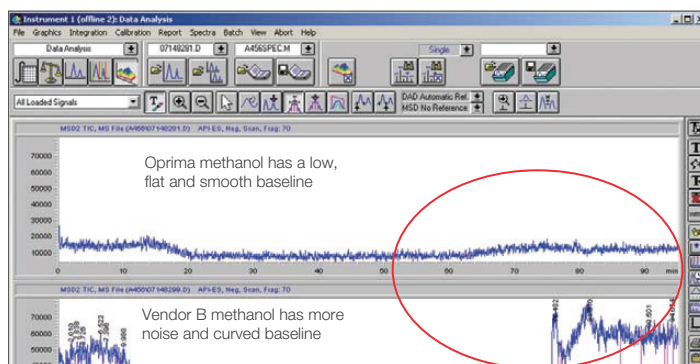


Figure 15. Mass background in negative mode of blank methanol sample using single quadrupole LC-MS. Top panel shows a typical Optima LC-MS grade methanol (A456) profile displaying low noise and a flat baseline in TIC compared to Vendor B which showed more noise and a curved baseline.

On the other hand, for water/acetonitrile gradients analyzed in TIC negative and positive mode with LTQ Orbitrap, the mass background for water and acetonitrile was similar to Vendor B but significantly lower than Vendor X (data not shown). Nevertheless, the mass spectrum of acetonitrile background in positive mode is about 5x lower for the Optima LC-MS grade solvent compared to Vendor B using the single quadrupole LC-MS (Figure 16)

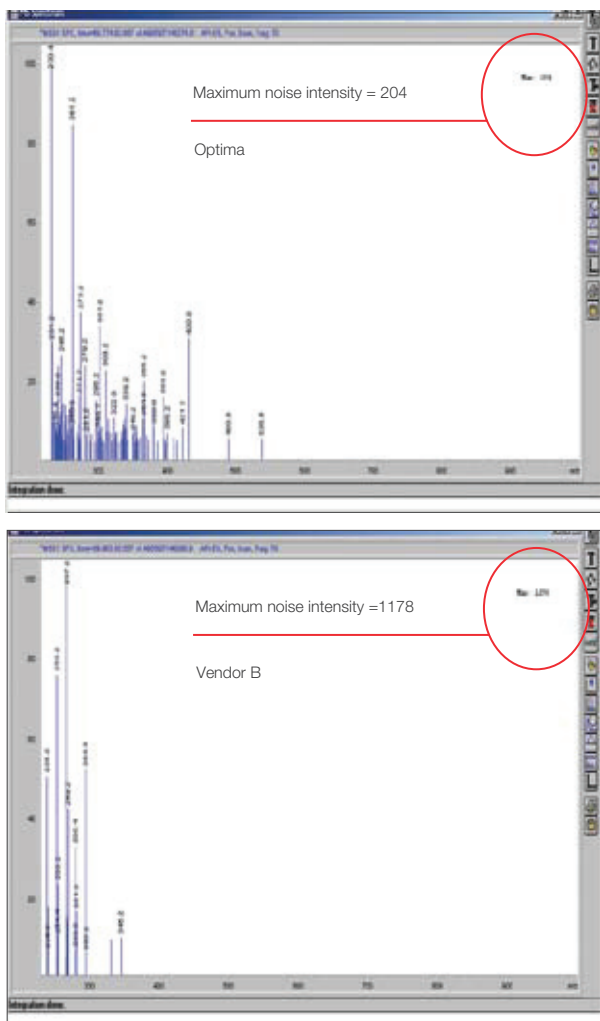


Figure 16. MS spectrum of acetonitrile background from 200 – 1000 *m/z* in positive mode using single quadrupole LC-MS. Note: maximum noise intensity for Optima LC-MS grade acetonitrile is 5× lower than Vendor B acetonitrile.

Signal intensity

In Experiment I, the signal intensity of target compounds was highest with Optima LC-MS grade acetonitrile/water system (Figure 5). Similarly, signal intensity for two different analytes was evaluated in an acetonitrile/water system using the TSQ Vantage MS with the precursor conditions listed in Table 4. For propazine (CAS # 139-40-2) in the positive mode, a 0.05 ng/mL (217 pM) sample showed higher background for Vendor B acetonitrile/water than for the Optima LC-MS grade solvent pair. Therefore, the signal-to-noise is somewhat better for the Optima LC-MS grade solvents (Figure 17). However, a 0.05 ng/mL (232 pM) sample of mecoprop (CAS # 7085-19-0) in the negative mode yielded similar baseline noise for both solvent systems (Figure 18).

Table 4. Precursor conditions for target compounds propazine and mecoprop analyzed with TSQ Vantage MS

Compound	Polarity	Precursor mass	S-lens voltage (V)	Product mass	Collision energy (V)
Propazine	+	230.2	69	146.0	23
		230.2	69	188.1	16
Mecoprop	-	213.1	40	141.3	15

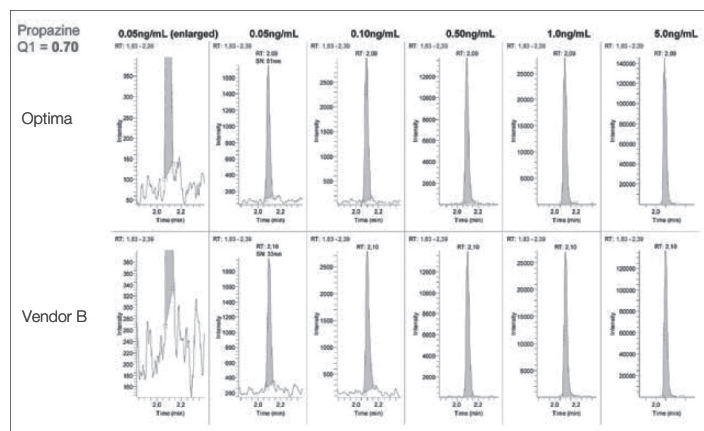


Figure 17. Signal intensity for propazine compound in positive mode using TSQ Vantage MS. Background noise is higher for Vendor B acetonitrile/water mobile phase than for the Optima LC-MS grade solvent pair. Therefore, S/N is somewhat better for the Optima LC-MS grade solvent system.

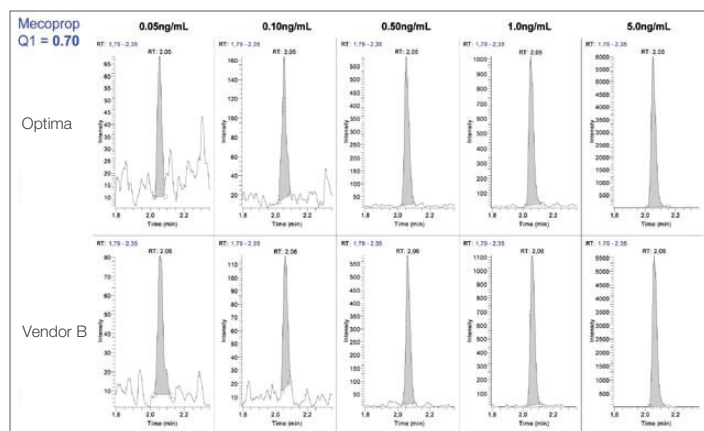


Figure 18. Signal intensity for Mecoprop compound in negative mode using TSQ Vantage MS. Background noise is nearly equal for both Optima LC-MS grade and Vendor B acetonitrile/water mobile phases.

Contamination with PEG

Polyethylene glycol (PEG) is a synthetic polymer produced in a range of molecular weights. It is a contaminant commonly found in LC-MS mobile phase solvents. Well-known sources of PEG include skin creams and shampoos, toothpaste, Thermo Scientific Triton X-100, and glassware detergents. PEG contamination was 2-3x lower in the Optima LC-MS grade solvent system as compared to other solvent brands (Figure 19).

PEG contamination in water, acetonitrile and methanol

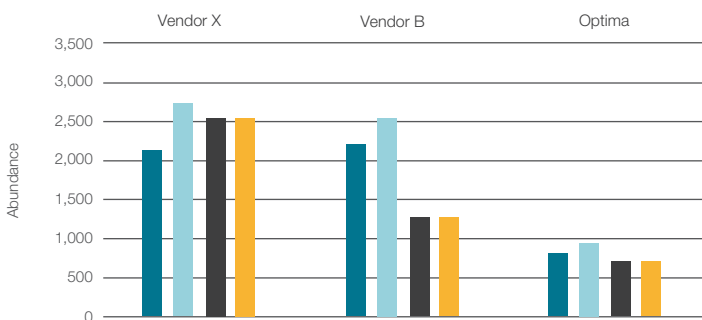


Figure 19. PEG contamination lowest in Optima LC-MS grade mobile phase solvents (water, acetonitrile, and methanol) as determined by LTQ Orbitrap XL.

Conclusions

Purity is a crucial consideration when selecting appropriate solvents for use in the LC-MS mobile phase. Seven commercially available solvent brands were compared using four different LC-MS systems with the following results:

1. Optima LC-MS grade solvents have fewer peaks compared to other vendors' LC-MS solvents, especially within the high organic portions of the gradient. Also, the mass baseline (noise level) is very low for both positive and negative modes in TIC.
2. Optima LC-MS grade solvents are not only low in mass background, they also have very low LC-UV response using diode array detection.
3. Signal intensity for standard peptide peaks was highest using Optima LC-MS grade mobile phase solvents compared to other commercial brands.
4. Contamination from PEG and various phthalate peaks was prevalent in greater quantities in other vendors' LC-MS solvents than with Optima LC-MS grade solvents.
5. Optima LC-MS grade solvents provide exceptionally low metal ion content, which makes MS interpretation easier. Thermo Fisher Scientific uses proprietary manufacturing and packaging techniques at its Fair Lawn, NJ facility to prepare these solvents with minimal metal ion contamination.

References

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- ⁴W. Lambert. T+K (2004) 71(2): 64.

Cross-references to competitor products

Table 5. Cross-reference

Acetonitrile	1 L	2.5 L	4 L
Thermo Scientific	A955-1	A955-212	A955-4
EMD	AX0156-6	–	AX0156-1
Merck	1000291000	1000292500	–
J.T. Baker	9829-2	–	9829-3
Sigma-Aldrich	34967-1L	34967-2.5L	34967-4x4L
Biosolve	12041	–	–
Methanol	1 L	2.5 L	4 L
Thermo Scientific	A456-1	A456-212	A456-4
EMD	MX0486-6	–	MX0496-6
Merck	1060351000	1060352500	–
J.T. Baker	9830-2	–	9830-3
Sigma-Aldrich	34966-1L	34966-2.5L	34966-4x4L
Biosolve	136841	–	–
2-propanol	1 L	2.5 L	4 L
Thermo Scientific	A461-1	A461-212	A461-4
J.T. Baker	9627-2	–	9627-3
Sigma-Aldrich	34965-1L	34965-2.5L	–
Biosolve	162641	–	162641
Water	1 L	2.5 L	4 L
Thermo Scientific	W6-1	W6-212	W6-4
EMD	WX0001-6	–	WX0001-1
J.T. Baker	9831-2	–	9831-3
Sigma-Aldrich	39253-1L	–	39253-4x4L
Biosolve	232141	–	–



Specification

Table 6. Thermo Scientific LC-MS

Assay (by GC), min	99.9%
Optical Abs, wave length, nm	au max
280	0.005
254	0.005
230	0.01
225	0.015
220	0.015
215	0.025
210	0.03
205	0.04
200	0.05
195	0.15
190	1.00
LC-MS gradient suitability, nm	Single max peak (au)
254	0.0005
210	0.002
LC/MS at positive mode max	50 ppb reserpine
LC/MS at negativemode max	50 ppb aldicarb
Water (KF) %	0.01
Residue after evaporation, ppm max	0.8
Trace ionic impurities	ppb, max
Aluminum (Al)	25
Barium (Ba)	5
Cadmium (Cd)	5
Calcium (Ca)	25
Chromium (Cr)	5
Cobalt (Co)	5
Copper (Cu)	5
Iron (Fe)	5
Lead (Pb)	5
Manganese (Mn)	5
Magnesium (Mg)	10
Nickel (Ni)	5
Potassium (K)	10
Silver (Ag)	5
Sodium (Na)	50
Tin (Sn)	5
Zinc (Zn)	10
Titrateable acid, mEQ/g	0.008
Titrateable base, mEQ/g	0.0006

Specification (continued)

Table 7. Thermo Scientific LC-MS grade ethanol

Assay (by GC), min	99.9%
Optical Abs, wave length, nm	au max
280	0.005
260	0.005
254	0.01
230	0.1
220	0.2
214	0.4
210	0.5
LC-MS gradient suitability, nm	Single max peak (au)
254	0.001
220	0.005
LC/MS at positive mode max	50 ppb reserpine
LC/MS at negativemode max	50 ppb aldicarb
Water (KF) %	0.02
Residue after evaporation, ppm max	1
Trace ionic impurities	ppb, max
Aluminum (Al)	10
Barium (Ba)	10
Cadmium (Cd)	10
Calcium (Ca)	20
Chromium (Cr)	10
Cobalt (Co)	10
Copper (Cu)	10
Iron (Fe)	10
Lead (Pb)	10
Manganese (Mn)	10
Magnesium (Mg)	10
Nickel (Ni)	10
Potassium (K)	10
Silver (Ag)	10
Sodium (Na)	50
Tin (Sn)	10
Zinc (Zn)	10
Titratable acid, mEQ/g	0.0003
Titratable base, mEQ/g	0.0002



Table 8. Thermo Scientific LC-MS grade 2-propanol

Assay (by GC), min	99.9%
Color (APHA), max	5
Optical Abs, wave length, nm	au max
254	0.005
230	0.05
220	0.1
210	0.4
LC-MS gradient suitability, nm	Pass test
Water (KF) %	0.05
Residue after evaporation, ppm max	1
Titratable acid or base meq/g	0.0001
Trace ionic impurities	ppb, max
Aluminum (Al)	10
Calcium (Ca)	10
Copper (Cu)	5
Iron (Fe)	5
Lead (Pb)	5
Manganese (Mn)	5
Magnesium (Mg)	5
Nickel (Ni)	5
Potassium (K)	10
Silver (Ag)	5
Sodium (Na)	50
Zinc (Zn)	10



Specification (continued)

Table 9. Thermo Scientific LC-MS grade water, W6

Assay (by GC), min	99.9%
Optical Abs, wave length, nm	au max
280	0.005
260	0.005
254	0.005
240	0.01
230	0.01
220	0.01
210	0.01
LC-MS gradient suitability, nm	Single max peak (au)
254	0.0005
210	0.005
LC/MS at positive mode max	50 ppb reserpine
LC/MS at negativemode max	50 ppb aldcarb
Water (KF) %	0.02
Residue after evaporation, ppm max	1
Protease	Not detected

Assay (by GC), min	99.9%
Trace ionic impurities	ppb, max
Aluminum (Al)	10
Barium (Ba)	10
Cadmium (Cd)	10
Calcium (Ca)	20
Chromium (Cr)	10
Cobalt (Co)	10
Copper (Cu)	10
Iron (Fe)	10
Lead (Pb)	10
Manganese (Mn)	10
Magnesium (Mg)	10
Nickel (Ni)	10
Potassium (K)	10
Silver (Ag)	10
Sodium (Na)	20
Tin (Sn)	10
Zinc (Zn)	10
Total halogens (as chloride)	Not detected



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