brighter
LC-MS

LC-MS Grade Solvents and Reagents
for accurate, brilliant results

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LiChrosolv®
Aceton
hypergrad

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1.00029.1000   05.10.18   1 l

LiChrosolv®
Acetonitril
hypergrade für LC-MS

Merck
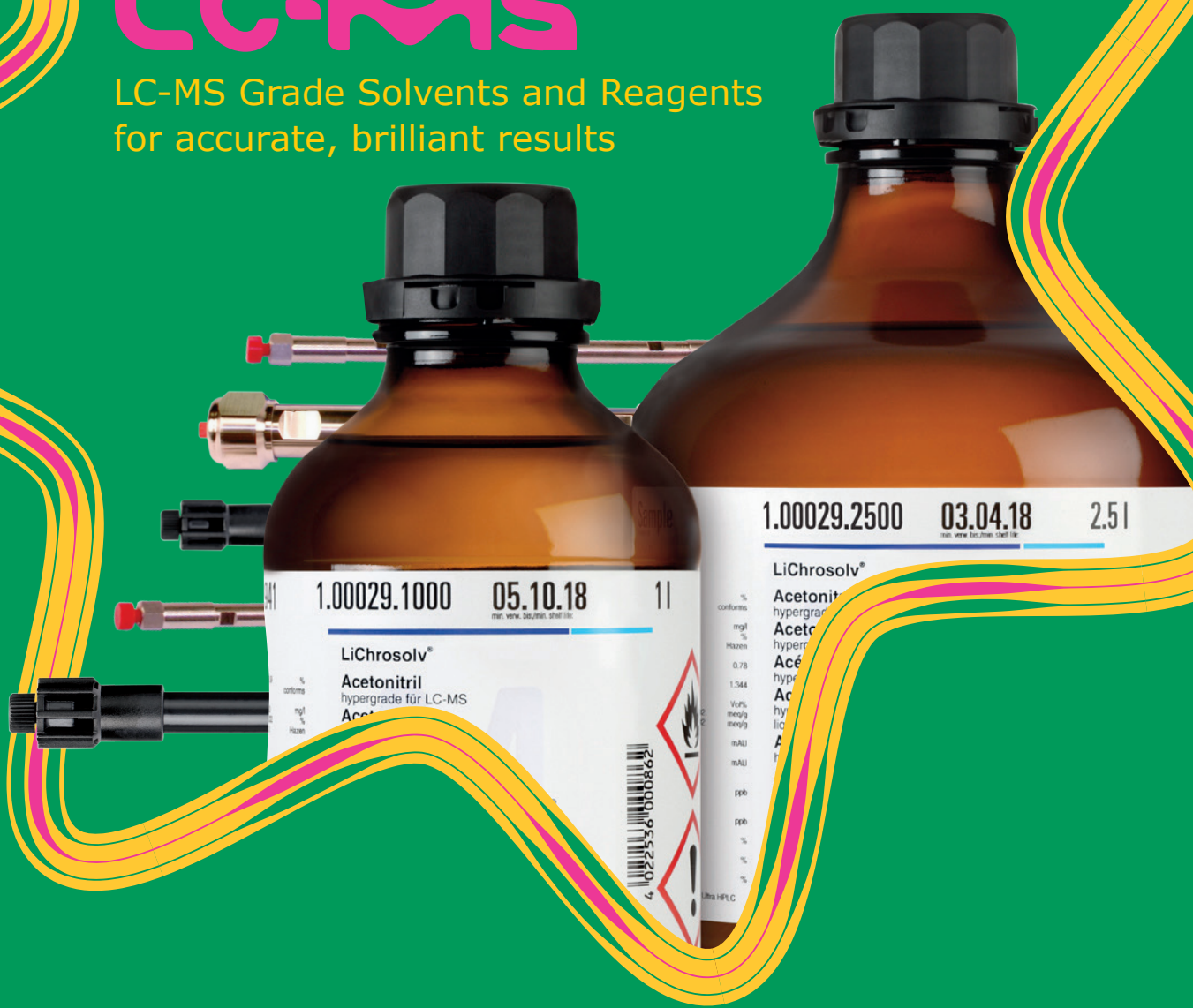
LC-MS: Raising the bar on purity

Liquid chromatography - mass spectrometry (LC-MS) is fast becoming a routine fixture in today's well-equipped analytical laboratory. Along with the increased use of LC-MS comes instrumental, chemical and database methods aimed at increasing the sensitivity, specificity and speed of analysis of this invaluable technique. New ion sources, high-resolution LC systems and rapid mass spectrometers with enhanced ion optics and detectors have lowered the limits of detection, but have raised the bar on the purity expectations of reagents used for sample preparation, mobile phases, and as additives. Some notable examples of how the purity and composition of the chemicals used in LC-MS that affect the analysis include:

- Polymers—including biopolymers such as proteins and DNA—form adducts with inorganic salts, leading to complex mass spectra and a broad distribution of multiply-charged sodium, potassium and chloride adducts.

- Salts can suppress ionization in ESI sources, even with small molecules.

- Reagents, solvents and devices used in sample preparation along with additives always present a risk of contamination.

Some particular compound classes that can be problematic are alkali ions, plasticizers and surfactants, as they are widespread and interfere strongly with LC-MS by forming adducts and causing higher background noise as well as leading to signal suppression. Because of the integral part that chemistry plays in a successful LC-MS analysis, we have developed and introduced a broad portfolio solvents, additives and reagents which are designed specifically to meet the requirements of high purity and consistency. This brochure contains a compilation of articles on LC-MS additives and the advantages of high purity solvents for both small and large molecule analysis.

For an overview on our HPLC/UHPLC column offer, please visit
SigmaAldrich/HPLC

Table of Contents

Solvents and Blends for LC-MS

Superior resolution and sensitivity

Why use LC-MS grade solvents?

- No ghost peaks
- Full reproducibility
- Extends the lifetime of your columns
- Optimized and tested for LC-MS applications
- Minimal background ion suppression
- Global availability

Why Pre-Blended?

- Reduces the risk of contamination
- Saves time
- No cleaning of glassware or filtration
- Less exposure to hazardous chemicals

As LC-MS is a highly sensitive analytical technique, impurities in your solvents can have an impact on the accuracy and reproducibility of your analytical results. Have confidence in your analysis by using our high purity solvents and blends designed to meet the demanding requirements of LC-MS applications, ensuring baseline stability, lowest impurity levels and, in addition, high UV transmittance.

Developed specifically for LC-MS, we have introduced a range of high purity solvents pre-blended with acetic acid, formic acid or trifluoroacetic acid, to provide convenient ready-to-use mobile phases for accurate LC-MS. We have also extended our product range to add four new LC-MS grade solvents including ethyl acetate, hexane, heptane and isopropanol.

This complete product portfolio sets the standard for accurate, reproducible and high-resolution analytical separations for superior performance and sensitivity.

For more information visit
SigmaAldrich.com/lcms-solvents

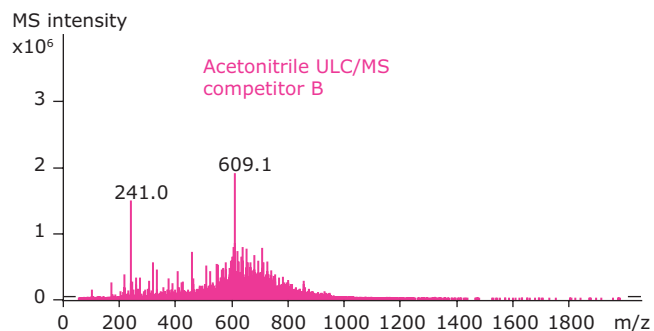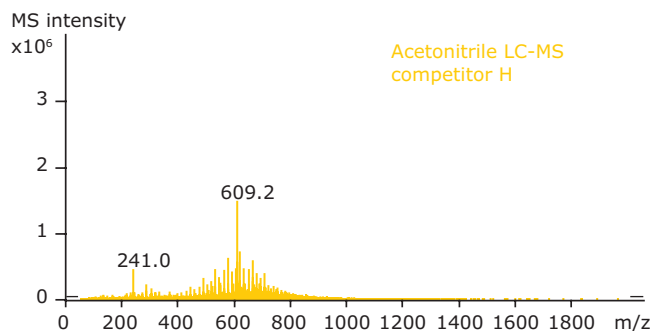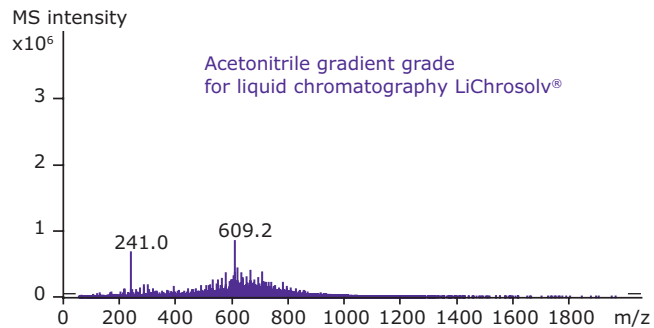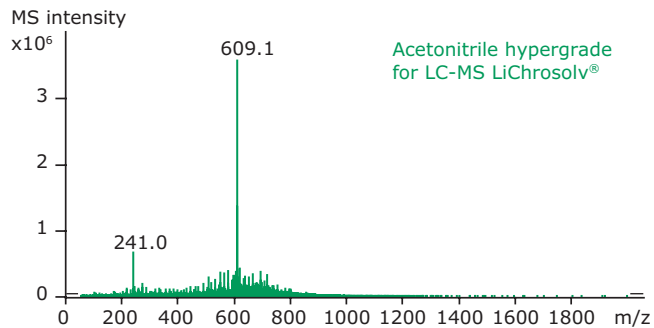| Product No.	Name	Description	Package Size
159004.2500 159004.4000	Acetonitrile + 0.1% Acetic acid (v/v)	Hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL*
159002.1000 159002.2500 159002.4000	Acetonitrile + 0.1% Formic acid (v/v)	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL
159014.2500 159014.4000	Acetonitrile + 0.1% Trifluoroacetic acid (v/v)	Hypergrade for LC-MS LiChrosolv®	2.5 L GL 4 L GL*
159007.2500 159007.4000	Water + 0.1% Acetic acid (v/v)	Water for Chromatography (LC-MS grade) LiChrosolv®	2.5 L GL 4 L GL*
159013.2500 159013.4000	Water + 0.1% Formic acid (v/v)	Water for Chromatography (LC-MS grade) LiChrosolv®	2.5 L GL 4 L GL*
480112.2500 480112.4000	Water + 0.1% Trifluoroacetic acid (v/v)	Water for Chromatography (LC-MS grade) LiChrosolv®	2.5 L GL 4 L GL*
100029.1000 100029.2500 100029.9010 100029.9030	Acetonitrile	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 10 L ST** 30 L ST**
106035.1000 106035.2500	Methanol	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL
115333.1000 115333.2500 115333.4000 115333.9010 115333.9030	Water	Water for Chromatography (LC-MS grade) LiChrosolv®	1 L GL 2.5 L GL 4 L GL* 10 L ST** 30 L ST**
103649.1000 103649.2500	Ethyl acetate	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL
103701.1000 103701.2500	Hexane	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL
103654.1000 103654.2500	Heptane	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL
102781.1000 102781.2500 102781.4000	2-Propanol	Hypergrade for LC-MS LiChrosolv®	1 L GL 2.5 L GL 4 L GL*

* special treated amber glass bottle
** returnable
All solvents are filtered through 0.2 µm. | GL = glass bottle | ST = stainless steel returnable barrel only in EU

For Dispensing tools and couplings of
solvents to LC-MS, visit
SigmaAldrich.com/safety-accessories

Why your choice of solvent matters

MS intensity x10⁶ — Acetonitrile hypergrade for LC-MS LiChrosolv®

Peaks: 609.1, 241.0

MS intensity x10⁶ — Acetonitrile gradient grade for liquid chromatography LiChrosolv®

Peaks: 241.0, 609.2

MS intensity x10⁶ — Acetonitrile LC-MS competitor H

Peaks: 609.2, 241.0

MS intensity x10⁶ — Acetonitrile ULC/MS competitor B

Peaks: 241.0, 609.1

MS conditions	
System	Bruker Esquire 3000+ ion trap MS
Detection	Pos. ESI-MS, m/z range 50 – 2000
Flow rate	0.2 mL/min via syringe pump
Temperature	25°C
Sample	Reserpine (m/z 609.1), internal standard (m/z 241.0)

Mass spectra displaying the results of reserpine comparing different acetonitrile qualities from Merck and two alternative competitors.

The mass spectra of these four different acetonitrile grades clearly shows the variation in the intensity of the reserpine signal ([M+H]+ = 609), as well as the extent of the background signals. The differences in the intensity of the reserpine signal are caused by ion suppression. This effect occurs due to interfering trace contaminants that can be present in acetonitrile, which can be avoided using the correct high grade solvent for this purpose.

LC-MS Reagents and Additives

Features:

- LC-MS application tested for consistent quality according to the reserpine test

- Optimized to improve ionization and resolution

- Extremely low levels of inorganic and organic impurities

- Manufactured specifically for accurate and fast LC-MS

- Highest quality acids, bases & salts - specified in the certificate of analysis

Introduction

It is common practice in LC-MS to add certain reagents to the mobile phase, or to introduce them post-column prior to the interface to influence analyte ionization. Most often the goal is for an improvement in the analyte signal. In addition, some additives may be used to suppress unwanted signals, or selectively enhance the signal of particular compounds in a mixture. For example, glycosidic species in a mixture of peptides.

To help you obtain the highest quality analysis, we offer a wide range of high purity mobile phase additives for LC-MS applications. The LC-MS portfolio includes the most commonly used acids, bases and volatile salts of high purity tested for LC-MS applications.

Impurities, such as alkali ions, plasticizers or surfactants, that can be commonly found in lower-grade solvents are particularly problematic as they interfere strongly with LC-MS, resulting in higher background noise and formation of adducts. Only ultrapure reagents enable high signal-to-noise ratios, which results in the highest and most reliable performance for small and large molecule applications.

Reserpine test

All of our LC-MS solvents and reagents are specified using the standard reserpine test. Reserpine (608.68) is used as the reference substance to quantify possible impurities in the LiChropur® LC-MS reagents. It is performed by diluting 2.5% (v/v) acid, base or 2.5% (w/v) salt in 50/50 (v/v) acetonitrile/water. Every lot produced is analyzed via flow injection analysis mass spectrometry (FIA-MS). The dissolved reagent and the appropriate reserpine reference solutions are introduced into the MS ion source syringe pumps. The total ion chromatogram (TIC) is accumulated during three minutes. The relative intensities of the detected masses are compared with the reserpine signal.

For electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive mode, the specified amount of reserpine is 2 ppb for acids and bases, and 20 ppb for salts. In the negative mode, the specified amount of reserpine is 20 ppb for both.

Typical solvent QC mass spectrum, ESI positive (flow injection analysis)
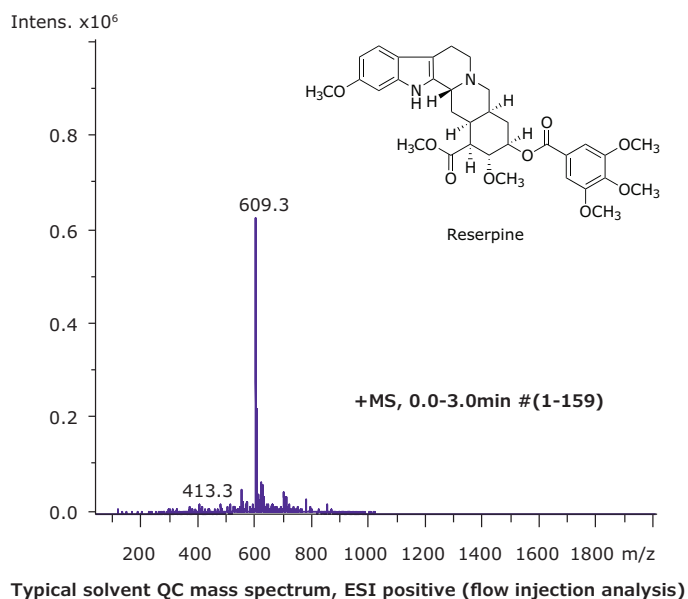
Acid additives

Volatile, low molecular weight organic acids such as formic and acetic acid improve ionization and resolution of a wide range of molecules. Addition of organic acids to the mobile phase can help to overcome the ionization-suppressing effect of trifluoroacetic acid (TFA) present in the mobile phases used for the analysis of proteins and peptides.

Neutral salts

Neutral volatile salts, such as ammonium acetate or ammonium formate are typically used as buffer compounds to control the analytes (and phases) ionization state, which has a strong influence on the LC-MS separation and performance.

Sodium adduct formation

Alkali adducts diminish instrument sensitivity. When adduct formation tendency is strong, often the addition of defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

Cat. No.	Substance	Description	Package Size
5.33001.0050	Acetic acid	100% for LC-MS LiChropur®	50 mL
5.33002.0050	Formic acid	98-100% for LC-MS LiChropur®	50 mL
5.33003.0050	Ammonia solution	25% for LC-MS LiChropur®	50 mL
5.33004.0050	Ammonium acetate	for LC-MS LiChropur®	50 g
5.33005.0050	Ammonium hydrogen carbonate	for LC-MS LiChropur®	50 g

Extensive QC testing ensuring highest specification

Residue on ignition (evaporation residue) tests show the low content of insoluble matter in the reagent. This provides confidence that your eluents have the low particle content needed for accurate LC-MS measurement.

Sodium and Potassium ions are particularly likely to form adducts with the analyte molecules. This leads to complex mass spectra leading to time-consuming data evaluation. The content of trace metals is in the low ppb range for LiChropur® LC-MS reagents to minimizing the risk of adduct formation in the ion source for cleaner results.

Our LiChropur® LC-MS reagents are stored in borosilicate bottles to prevent leaching of alkali ions out of the glass. The content of the potentially complex forming ions aluminum, copper and iron is also specified.

Full specification can be found in the certificate of analysis for each of our LC-MS grade products.

Specification (Acids/Bases)	
Assay (acidimetric)	≥ 98,0%
Colour	≤ 10 Hazen
Residue on ignition	≤ 2 ppm
Al	≤ 5.0 ppb
Ca	≤ 10.0 ppb
Cu	≤ 1.0 ppb
Fe	≤ 5.0 ppb
K	≤ 5.0 ppb
Mg	≤ 2.0 ppb
Na	≤ 5.0 ppb
NH_4^+	≤ 10 ppm
LC-MS Suitability ESI Positive (Reserpine Test)	≤ 2 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine
LC-MS Suitability ESI Negative (Reserpine Test)	≤ 20 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine

For more information HPLC buffers, visit
SigmaAldrich.com/lcms-reagents

Chemical Derivatization Reagents for LC-MS

Modern mass spectrometry techniques such as APCI or ESI are highly successful in providing valuable structural information, and allow the detection of very low analyte concentrations in various sample matrices. For certain samples e.g. non-polar compounds, and in research areas, such as clinical metabolomics and forensics analytics, there are many cases where such methods can be insufficiently sensitive.

Derivatization reactions in mass spectrometry are used to improve ionization efficiency [1-4]. The derivatization reagents have functional groups possessing high proton (cation) affinity that stabilize a positive charge. Of similar importance when derivatizing is the improvement of qualitative analysis by modifying fragmentation behavior to form unique product ions and the shifting. Finally, derivatization can enhance precise quantitative analysis for profiling of relatively small analyte molecules, particularly in metabolomics.

References

1. Zaikin V, Halket J, 2009. A handbook of derivatives for mass spectrometry. Chichester: IM Publications LLP,

2. Santa T. 2013. Derivatization in liquid chromatography for mass spectrometric detection *Drug Discov. Ther. 7*:9-17

3. Santa T. 2011. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry. *Biomed. Chromatogr. 25*:1-10

4. Santa T, Al-Dirbashi OY, Fukushima T. 2007. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry for biomedical analysis. *Drug Discov. Ther. 1*:108-118.

For more information, visit
SigmaAldrich.com/derivatization

| Cat. No.	Derivatization Reagent	Analyte Functional Group	Typical Application
69706	6-Bromo-3-pyridinylboronic acid	1,2-Dihydroxy	Brassinosteroids
05689	Diethyl ethoxymethylenemalonate	Amine	Amino acids
29208	(N-Succinimidyloxycarbonylmethyl) tris(2,4,6-trimethoxyphenyl)phosphonium bromide	Amine	Protein sequence analysis
61224	N-Succinimidyl 4-(dimethylamino)benzoate	Amine	Glycerophosphoethanolamine lipids
73177	1-Fluoro-2,4-dinitrobenzene	Amine	Prim./sec. aliphatic amines
94076	{1-[2-(Diethylamino)ethoxy]-2-isothiocyanatoethyl}benzene	Amine	—
59934	2,5-Dioxopyrrolidin-1-yl N-tri(pyrrolidino) phosphoranylideneaminocarbamate	Amine	Amino acids
73103	Dibenzyl ethoxymethylenemalonate	Amine	Amino acids
03334	Dansylhydrazine	Carbonyl	—
06963	4-(Diethylamino)benzhydrazide	Carbonyl	—
08843	2-Hydrazinopyridine	Carbonyl	Steroids
4465962	Amplifex Keto Reagent Kit	Carbonyl	—
5037804	Amplifex Diene Reagent Kit	Diene	—
59799	4-(Diethylaminomethyl)benzhydrazide	Carbonyl	—
65562	2-Picolylamine	Carbonyl	Steroids
89397	Girard's reagent T	Carbonyl	Nucleosides
92989	4-(Dimethylamino)benzohydrazide	Carbonyl	—
93742	Pentafluorophenylhydrazine	Carbonyl	Oligosaccharides
75821	1,2-Benzo-3,4-dihydrocarbazole-9-ethyl-p-toluenesulfonate	Carboxylic acid	Fatty/bile acids
79291	4-[2-(N,N-Dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole	Carboxylic acid	Fatty acids
42579	4-Phenyl-1,2,4-triazoline-3,5-dione	Diene	Vitamin D
97622	2-Mercaptoethanol	Double bond	Microcystins
00721	4-(Dimethyl-d_6-amino)benzoyl chloride	Hydroxy	Deuterium mass shift
03641	Dansyl chloride	Hydroxy	—
05022	N,N-Dimethylglycine	Hydroxy	Cholesterol
06696	3-Amino-9-ethylcarbazole	Hydroxy	Sugars
67954	4-(Dimethylamino)benzoyl chloride	Hydroxy	17β-Estradiol
72702	3,5-Dinitrobenzoyl chloride	Hydroxy	Tetrahydrocorticosterones
41368	p-Toluenesulfonyl isocyanate	Hydroxy	Steroids

Olmesartan medoxomil

New LC-MS/MS method for Impurity Profiling

Here we present a new method similar to the USP40-NF35 monograph but using a Purospher® STAR RP-8 endcapped (2 μm) 100x2.1 HPLC column for the analysis of Olmesartan Medoxomil RS and its related compound A (Olmesartan medoxomil RC A = 1-{[2'-(1H-Tetrazol-5-yl)biphenyl-4-yl]methyl}-4,4-dimethyl-2-propyl-1*H*-furo[3,4-d]imidazol-6(4*H*)-one). The new procedure is both MS and UV compatible, and meeting all system suitability requirements.

Column	Purospher® STAR RP-8 endcapped (2 μm) 100x2.1 mm (**1.50653.0001**)
Injection	0.3 μL
Detection	ESI-(+)-MS (m/z 100-800) Nebu.405 psi, Dry Gas 12L/min, Dry Temp. 365°C, Scan mode -normal
Flow Rate	210 μL/min

Mobile Phase

Buffer: 15 mM ammonium acetate pH 3.4

Solution A: Acetonitrile/buffer 4:1 (v:v)

Solution B: Acetonitrile/buffer 1:3 (v:v)

Mobile phase: Mix solutions A+B 1:3 (v:v)

Mobile Phase

Solution A Solution B Acetonitrile/Buffer 1/3 (v/v)

Buffer: 0.015 M ammonium acetate, pH adjusted to 3.4 with glacial acetic acid Gradient

Temperature	40°C
Diluent	Acetonitrile
SST for Impurity	0.01 mg/mL each of Olmesartan medoxomil RS and related compound A in Acetonitrile

Chromatographic Data: (SST solution)

Compound	Retention Time (min)	Molecular Weight	m/z
Olmesartan medoxomil RC A	4.8	428	429
Olmesartan medoxomil RS	6.0	558	559
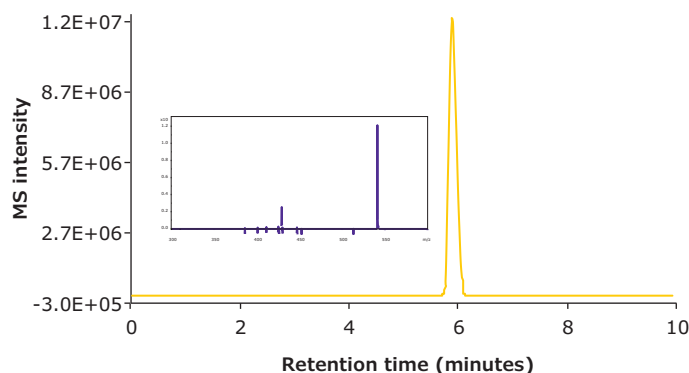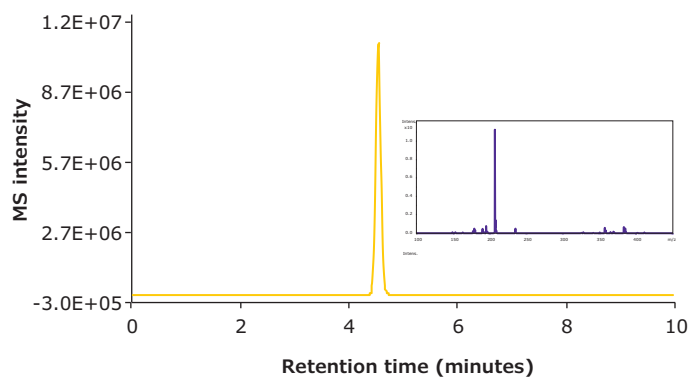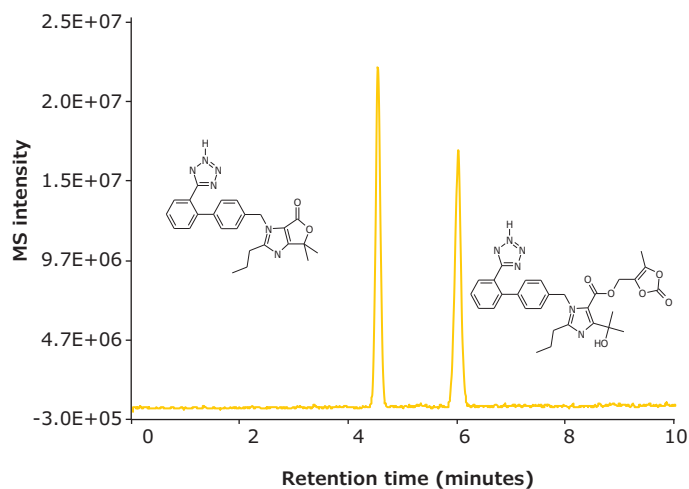
Specificity

Determined by injection of system suitability test (SST) solution and determination of the retention time and relative retention time for Olmesartan medoxomil RC A and Olmesartan medoxomil RS using a Purospher® STAR RP-8 endcapped (2 µm) 100x2.1 mm column.
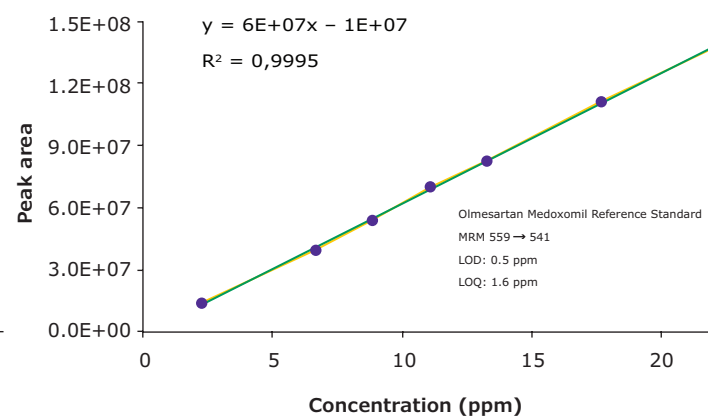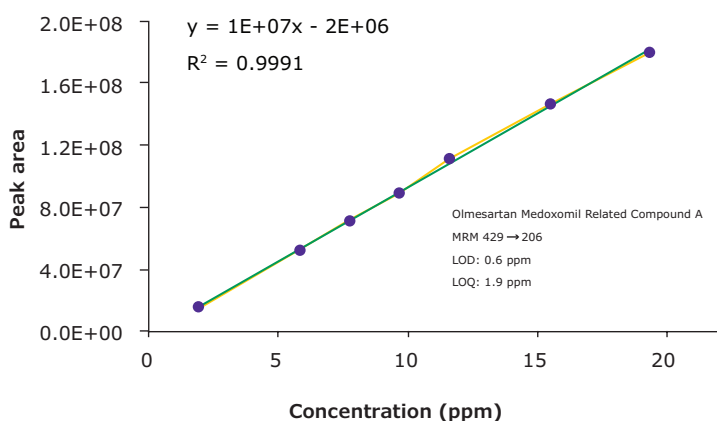
Compound	Retention Time (min)	Tailing factor	Resolution
Olmesartan medoxomil RC A	4.8	1.1	—
Olmesartan medoxomil RS	15.3	1.1	>>5

Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Determined by injecting six concentration levels from 2-22 ppm of Olmesartan medoxomil Related Compound A and six concentration levels ranging from 2-19 ppm of Olmesartan medoxomil RS.

Olmesartan medoxomil RC A		Olmesartan medoxomil RS	
ppm	Counts	ppm	Counts
2.2	13616131	1.9	14727570
6.6	39221377	5.8	52720141
8.8	54139425	7.7	71536517
11.0	69691922	9.7	88994869
13.2	82201653	11.6	110832984
17.7	111025202	15.5	146305786
22.1	137770528	19.3	179311621

	Olmesartan medoxomil RC A	Olmesartan medoxomil RS
	Counts	Counts
STEYEX	1004073	1876144
SLOPE	6308188	9530333
LOD	0.5	0.6
LOQ	1.6	1.9

$y = 1E+07x - 2E+06$
$R^2 = 0.9991$

Olmesartan Medoxomil Related Compound A
MRM 429 → 206
LOD: 0.6 ppm
LOQ: 1.9 ppm

$y = 6E+07x - 1E+07$
$R^2 = 0,9995$

Olmesartan Medoxomil Reference Standard
MRM 559 → 541
LOD: 0.5 ppm
LOQ: 1.6 ppm

For an overview on our HPLC/UHPLC column offer, please visit
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