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Pharmaceutical Impurity Analysis Overview

A Primer

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1. Pharmaceutical Impurity Analysis – Overview and Regulatory Situation

Pharmaceutical impurities are the unwanted chemicals that remain with active pharmaceutical ingredients (APIs) or drug product formulations. The impurities observed in drug substances may arise during synthesis, or may be derived from sources such as starting materials, intermediates, reagents, solvents, catalysts, and reaction by-products. During drug product development, impurities may:

- Be formed as a result of the inherent instability of drug substances
- Be due to incompatibility with added excipients, or
- Appear as the result of interactions with packaging materials and container closure systems (CCSs)

The amount of various impurities found in drug substances determines the ultimate safety of the final pharmaceutical product. Therefore, the identification, quantitation, qualification, and control of impurities are a critical part of the drug development process.

Various regulatory authorities focus on the control of impurities:

- The International Council for Harmonisation (ICH)
- The United States Food and Drug Administration (USFDA)
- The European Medicines Agency (EMA)
- The Canadian Drug and Health Agency
- The Japanese Pharmaceutical and Medical Devices Agency (PMDA)
- The Australian Department of Health and Ageing Therapeutic Goods

In addition, several official compendia, such as the British Pharmacopoeia (BP), the United States Pharmacopeia (USP), the Japanese Pharmacopoeia (JP), the European Pharmacopoeia (EP), and the Pharmacopoeia of the People's Republic of China (ChP) are incorporating limits that restrict the impurity levels present in APIs and drug formulations. The regulations are based on exposure limits, so the level of contaminants must be controlled in the final dosage form, as administered. In practice, this means that drug manufacturers must perform a risk assessment that takes into consideration sources of contamination post-manufacturing—such as packaging, transportation, and CCS, as well as from raw materials and manufacturing processes.

1.1 The Three Major Categories of Pharmaceutical Impurities

According to ICH guidelines, impurities related to drug substances can be classified into three main categories: organic impurities, inorganic impurities, and residual solvents.

Organic Impurities

Organic impurities can arise in APIs or drug product formulations during the manufacturing process or during the storage of drug substances. They may be known, unknown, volatile, or nonvolatile compounds with sources including starting materials, intermediates, unintended byproducts, and degradation products. They may also arise from racemization— the contamination of one enantiomeric form with another. In all cases, they can result in undesired biological activity or toxicity.

Recently, genotoxic pharmaceutical impurities have received considerable attention from regulatory bodies and pharmaceutical manufacturers. In general, genotoxic impurities include DNA-reactive substances that have the potential for direct DNA damage. Potential genotoxic impurities include process impurities or degradants present at trace levels, which are generated during drug manufacturing and storage. Per FDA and EMA guidelines, potential genotoxic impurities are to be controlled at levels much lower than typical impurities. The recommended acceptable thresholds for genotoxic impurities in pharmaceuticals can be found in the guideline documents published by the USFDA and EMA (See the selected list of key publications provided at the end of this section). The ICH M7 guidance on genotoxic impurities is under preparation with the working title *M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*.

Inorganic (Elemental) Impurities

Elemental impurities are unwanted elements that occur in drug formulations. They can arise from active pharmaceutical ingredients, raw materials, synthetic additives, excipients, catalysts, and production processes and equipment used during manufacturing. Regulatory bodies require that drug manufacturers perform a risk assessment to ensure that such impurities are monitored and controlled in final drug products. Manufacturers must also assess potential sources of contamination from packaging, container closure, and drug delivery systems, and these must be identified and quantified using an Extractables and Leachables (E&L) assessment. The levels of the four most toxic elemental impurities: As, Cd, Hg, and Pb, must be measured in all drug products. Another group of elements may be added during production; these impurities must be monitored in final drug products. In addition to the raw materials, other potential sources of inorganic impurities include manufacturing process reagents such as ligands, catalysts (for example, platinum group elements (PGE)), metals derived from other stages of production (for example, process water and stainless steel reactor vessels), charcoal, and elements derived from materials used in filtration.

The United States Pharmacopeia (USP) procedure for testing elemental impurities (general chapter USP<232>) has been harmonized with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline Q3D. The new procedures replace existing colorimetric and wet chemicals tests, such as USP Heavy Metals Limit Test (USP<231>) and EP Heavy Metals chapter 2.4.8, which are widely acknowledged to be inadequate in terms of scope, accuracy, sensitivity, and specificity. USP has also introduced the related elemental impurity method <2232>, which applies only to dietary supplements.

USP<232> and ICH Q3D define permitted daily exposure (PDE) limits for the 24 elemental impurities listed in Table 1. The elements are divided into classes according to their toxicity and likely presence in drug products. The class determines which elemental impurities must be included in the risk assessment for each drug product, depending on its route of administration. The method validation procedures that should be used for system suitability qualification of any instrumentation used for the analysis of elemental impurities in pharmaceutical materials are defined in USP general chapter <233> and ICH Q2(R1). Validation of analytical instruments used for USP and ICH procedures is performance-based to ensure that the analysis is specific, accurate, and precise, offering a significant improvement over the previous wet chemical tests. The elemental impurity methods recognize that the toxicity of some elements varies depending on the chemical form of the element. As a result, the limits for As and Hg are based on the inorganic forms of these two elements. The elemental impurity methods allow for separation of the chemical forms of these elements, for example, using HPLC coupled to ICP-MS, to ensure that the more toxic forms of the elements meet the PDE limit, even if the total level exceeds the PDE.

Table 1. The permitted daily exposure (PDE) limits for elemental impurities in drug products, according to their route of administration. Elements shaded in yellow should be considered in product risk assessment. All elements listed should be included in risk assessment if naturally present, or if intentionally or unintentionally added.

ICH/USP Class	Element	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalational PDE (µg/day)
Class 1	Cadmium (Cd)	5	2	2
	Lead (Pb)	5	5	5
	Arsenic (As) (inorganic)	15	15	2
	Mercury (Hg) (inorganic)	30	3	1
Class 2A	Cobalt (Co)	50	5	3
	Vanadium (V)	100	10	1
	Nickel (Ni)	200	20	5
Class 2B	Thallium (Tl)	8	8	8
	Gold (Au)	100	100	1
	Palladium (Pd)	100	10	1
	Iridium (Ir)	100	10	1
	Osmium (Os)	100	10	1
	Rhodium (Rh)	100	10	1
	Ruthenium (Ru)	100	10	1
	Selenium (Se)	150	80	130
	Silver (Ag)	150	10	7
	Platinum (Pt)	100	10	1
Class 3	Lithium (Li)	550	250	25
	Antimony (Sb)	1,200	90	20
	Barium (Ba)	1,400	700	300
	Molybdenum (Mo)	3,000	1500	10
	Copper (Cu)	3,000	300	30
	Tin (Sn)	6,000	600	60
	Chromium (Cr)	11,000	1,100	3

Residual Solvents

Residual solvents are the volatile organic chemicals used during the manufacturing process, or generated during drug production. Several organic solvents used in the synthesis of pharmaceutical products are known to have toxic or environmentally hazardous properties, and their complete removal can be very difficult. The final purification step in most pharmaceutical drug substance processes involves a crystallization step that can lead to the entrapment of solvent, which can act as a residual impurity, or cause potential degradation of the drug. Residual solvent levels are controlled by the ICH, USP, and EP.

Residual solvents are categorized into three classes with their limits in pharmaceutical products set by ICH guidelines Q3C. Class 1 solvents, including benzene, carbon tetrachloride, 1,1-dichloroethane, 1,2-dichloroethylene, and 1,1,1-trichloroethane, should be avoided. Class 2 solvents, such as methanol, pyridine, toluene, N,N-dimethylformamide, and acetonitrile have permitted daily exposure limits (PDEs). Table 2 presents a few examples of common organic solvents that are found as volatile impurities and have their limits set by ICH guidelines. Class 3 solvents, such as acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate should be limited by GMP or other quality-based requirements.

Table 2. ICH limits for a selected list of common organic solvents found as volatile impurities.

Volatile organic impurity	Limit (ppm)	PDE (mg/day)
Acetonitrile	410	4.1
1,4-Dioxane	380	3.8
Chloroform	60	0.6
Methylene chloride	600	6.0
Pyridine	200	2.0
1,1,2-Trichloroethane	80	0.8

USP <467> 2009 General Chapter contains a more comprehensive method for residual solvent analysis, similar to the ICH guidelines developed in 1997. This document presents a limit test for Class 1 and Class 2 solvents, while Class 2C solvents are usually determined by nonheadspace methods due to their higher boiling points. The limits of detection (LODs) recommended for Class 3 solvents are up to 5,000 ppm. When the levels of residual solvents exceed USP or ICH limits, quantitation is required.

1.2 Selected Publications and Guidelines for the Control of Pharmaceutical Impurities

Key topics	Title
Guidelines for the control of impurities	International Conference on Harmonisation (ICH) Q3A (R2) Impurities in New Drug Substances, 25 October 2006
	Guidance for Industry Q3A Impurities in New Drug Substances, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), June 2008
	EMA/CPMP/ICH/2737/99 Note for guidance on impurities testing: impurities in new drug substances; ICH Topic Q3A (R2) Impurities in new Drug Substances (October 2006)
	ICH Q3B (R2) Impurities in New Drug Substances, 2 June 2006
	Guidance for Industry Q3B(R2), Impurities in New Drug Products, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), July 2006
	EMA/CPMP/ICH/2738/99 Note for guidance on impurities in new drug products; ICH Topic Q3B (R2) Impurities in New Drug Products, June 2006
Specific guidelines for the control of genotoxic impurities	Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended approaches; US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); Silver Spring, MD, USA, December 2008
	EMA/CHMP/SWP/431994/2007 Rev. 3, Questions and answers on the guideline on the limits of genotoxic impurities, adopted September 23, 2010
	Guideline on the Limits of Genotoxic Impurities, CPMP/SWP/5199/02, EMEA/CHMP/QWP/2513442006; Committee for Medicinal products (CHMP), European Medicines Agency (EMEA); London 28 June 2006
	Pharmeuropa, Vol 20, No. 3, July 2008, Potential Genotoxic Impurities and European Pharmacopoeia monographs on Substances for Human Use
	EMA/CHMP/ICH/83812/2013 Committee for Human Medicinal Products ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk Step 4, 25 August 2015
	M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER), May 2015
	International Conference on Harmonisation (ICH) Harmonized Guideline M7 (R1): assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (current step 4 version dated 31 March 2017)

Note: Regulatory limits for impurities mentioned in this document are given as examples and may not provide the complete information needed. For complete, current regulatory information and the latest updates, please check the websites of the various regulatory authorities.

Guidelines relevant to analytical methods for the control of genotoxic impurities	International Conference on Harmonisation (ICH) Harmonized Tripartite Guideline Q8(R2), Pharmaceutical development, August 2009
	International Conference on Harmonisation (ICH) Harmonized Tripartite Guideline Q9. 2005. Quality Risk Management, November 2009
	Guidance for Industry Q8(R2) Pharmaceutical Development U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), November 2009
	Guidance for Industry Q9 Quality Risk Management U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), June 2006
	EMA/CHMP/ICH/167068/2004 Committee for Human Medicinal Products ICH guideline Q8 (R2) on pharmaceutical development Step 5 (22 June 2017)
	EMA/CHMP/ICH/24235/2006 Committee for Human Medicinal Products ICH guideline Q9 on quality risk management Step 5 (September 2015)
	International Conference on Harmonisation ICH S2 (R1): Harmonized Tripartite Guideline: guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use; (current step 4 version 9 November 2011)
	Guidance for Industry S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), June 2012
	EMA/CHMP/ICH/126642/2008 ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use Step 5 (June 2012)
Guidelines for the control of elemental impurities	International Conference on Harmonisation (ICH) Harmonized Guideline for elemental impurities Q3D (Current Step 4 version dated 16 December 2014)
	Q3D Elemental Impurities Guidance for Industry, U. S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), (September 2015)
	EMA/CHMP/ICH/353369/2013 Committee for Human Medicinal Products, ICH guideline Q3D on elemental impurities Step 5 (25 July 2016)
	Elemental Impurities in Drug Products Guidance for Industry Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), June 2016 (this document contains guidance on both ICH Q3D and the latest version of USP <232/233>)
	USP Chapter <232> Elemental Impurities- Limits, Pharmacopeial Forum, 42(2), March–April 2016
	USP Chapter <233> Elemental Impurities- Procedures, USP 38–NF 33, Second Supplement
Guidelines for the control of residual solvents ICHQ3C	International Conference on Harmonisation (ICH) Harmonized Guideline Impurities: guideline for residual solvents Q3C(R6) (Current Step 4 version dated October 20, 2016)
	EMA/CHMP/ICH/82260/2006 Committee for Human Medicinal Products, ICH guideline Q3C (R6) on impurities: guideline for residual solvents Step 5 (6 December 2016)
	FDA Guidance on ICH Q3C Maintenance Procedures for the Guidance for Industry Q3C Impurities: Residual Solvents (2017); https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm125820.htm

Note: This list is a limited selection of key, recent regulatory publications. For complete, current regulatory information and the latest updates, please check the websites of the various regulatory authorities.

2. Analytical Technologies for Impurity Profiling in Pharmaceutical Development

Overview

An impurity profile is a description of the identified and unidentified impurities present in a drug substance (Source: Guidance for Industry, Q3A Impurities in New Drug Substances). Impurity profiling processes usually begin with the detection of impurities, followed by their isolation and characterization. For all three types of impurities, it is critical to develop a robust method during process development that can eventually be validated and transferred for use in QA/QC. Developing reliable methods for impurities regulated at very low levels, such as genotoxic impurities, adds further challenges to this process.

To better detect, identify, quantify, and characterize the impurities present in drug substances and products, pharmaceutical scientists rely on robust analytical tools with high sensitivity and specificity. Major analytical tools for impurity analysis include spectroscopy, chromatography, mass spectrometry, and various combinations of these, that is, tandem techniques. The appropriate technique is selected based on the nature of the impurity and the level of information required from the analysis. There are various complex analytical problems in pharmaceutical development that require the use of more than one analytical technique for their solution. Analytical techniques such as LC/UV, LC/MS, GC/MS, CE/MS, and LC/UV provide the orthogonal detection and complementary information that can address these challenges in a time-efficient manner. As a result, they play a vital role in impurity profiling of pharmaceuticals from identification to the final structure elucidation of unknown impurities.

Table 3 summarizes some of the techniques used in impurity analysis. The following section provides further details on key single and tandem techniques for impurity profiling.

Table 3. Impurity analysis techniques.

Type of impurity	Technologies
Organic impurities	FTIR, Preparative LC, LC/UV, LC/MS (SQ, Q-TOF, and QQQ), CE, and SFC
Inorganic/elemental impurities	ICP-OES and ICP-MS
Residual solvents	GC and GC/MS

See the following sections for definitions of abbreviations.

2.1 Preparative Liquid Chromatography (LC)

Agilent 1260 Infinity II Preparative LC/MSD System

Liquid Chromatography and Ultraviolet Detection (LC/UV)

Agilent InfinityLab LC Series instruments and columns

Since impurities in drug substances are usually present at very low quantities, detailed analysis is only possible after isolation of the impurities. This is a major challenge in pharmaceutical laboratories. Preparative LC helps isolate impurities (usually from impurity-enriched analytes, such as the solution remaining from the crystallization of APIs) in sufficient quantities to carry out structural analysis, using techniques such as FTIR, NMR, LC/MS, or GC/MS.

Several impurity analysis methods found in pharmaceutical quality control (QC) laboratories use high-performance liquid chromatography (HPLC) coupled with UV detection (HPLC/UV methods). UV detection helps identify impurities or degradants in drug substances based on absorption maxima. This technique is one of the most important and versatile analytical methods available for impurity profiling due to its selectivity (that is, the ability to quantitatively determine many of the individual components present in a sample using a single analytical procedure), especially for routine analysis where standards are available. Stationary phase systems are available that operate in several modes, such as ion pairing, increased hydrophobic interactions, and variable pH, allowing a variety of samples to be analyzed concurrently based upon their unique properties. High resolution is particularly helpful when using LC/UV analysis for impurity detection, because all impurities can be identified with less chance of error.

Agilent InfinityLab solutions for analytical LC include highly efficient HPLC and UHPLC systems that feature the latest technology, while maintaining full compatibility with legacy LC instrumentation. The Agilent 1220 Infinity II LC delivers high-quality results for an affordable price. The Agilent 1260 Infinity II LC is the flexible instrument choice for operational efficiency, with the 1260 Infinity II Prime LC bringing more operational convenience. The Agilent 1290 Infinity II LC embodies the next generation of liquid chromatography with ultrahigh performance for superior outcomes. The 1260 Infinity II Prime LC is the most capable and convenient LC system within the 1260 Infinity II LC portfolio, with a power range up to 800 bar and up to 5 mL/min. Equipped with proven 1290 Infinity II LC technology, it offers the highest ease-of-use and functionality for your everyday work.

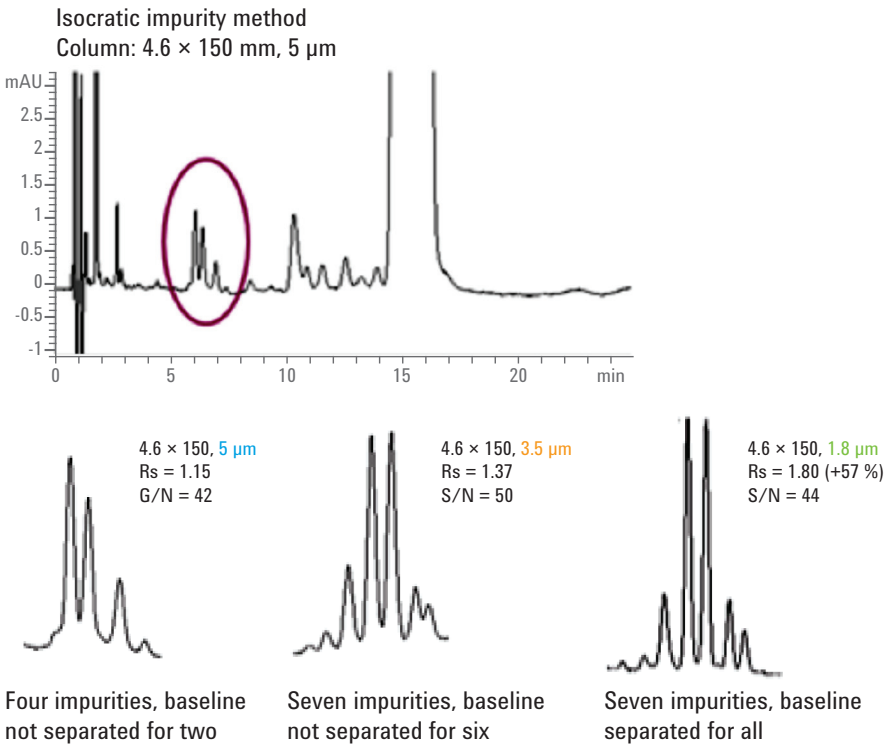
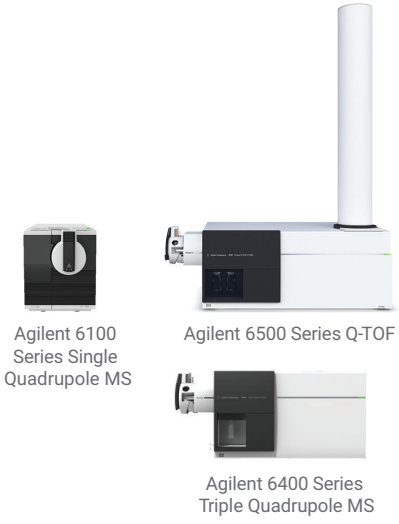


Figure 1. These data demonstrate the value of UHPLC systems, such as the Agilent 1290/1260/1220 Infinity Series systems, for impurity analysis. When combined with Agilent 1.8 µm RRHD columns, it is possible to identify all seven impurities with good baseline separation for accurate quantification. *Agilent Technologies, unpublished data.*

Liquid Chromatography and Mass Spectrometry (LC/MS)



Agilent mass spectrometers

LC/MS is a highly sensitive and specific analytical tool that is routinely used in pharmaceutical development to detect, identify, and quantify product impurities. A detection limit of a few hundred ppm is readily achievable, ensuring the identification of impurities present at concentrations greater than 0.1 %. Mass spectrometry-based methods generally provide additional sensitivity and specificity compared to techniques such as UV alone. While single quadrupole mass spectrometers are well suited to the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass spectrometers provide high-resolution accurate mass information that enables the unambiguous identification of unknown trace impurities. This makes them particularly useful for genotoxic impurity analysis. MS-based methods are often selected for the impurity profiling of APIs during process development. UV-based methods are generally used to test for genotoxic impurities in QC laboratories at manufacturing sites.

Triple quadrupole (QQQ) LC/MS/MS systems have become a standard platform for the quantitative analysis of organic impurities in pharmaceutical analytical laboratories. Combining multiple reaction monitoring (MRM) with a triple quadrupole tandem mass spectrometer, such as the Agilent 6400 Series Triple Quadrupole, enables very high sensitivity for multi-analyte quantitative assays. MRM assays are particularly useful for bioanalytical studies of compounds present in complex mixtures and biological matrices such as plasma and urine.

2.2
Gas Chromatography (GC)

Agilent 7890A combined with an Agilent 5975C GC/MS system with an Agilent 7697A Headspace sampler

Agilent Intuvo 9000 GC

Agilent Intuvo GC columns

In combination with flame ionization detection (FID), GC is the standard choice for the analysis of volatile organic impurities, such as residual solvents. The gas chromatography headspace method is used worldwide for residual solvent analysis in quality control laboratories because it closely follows ICH Q3C guidelines. Sample preparation and introduction is through a static headspace, which facilitates the selective introduction of volatile solvents without contamination by mostly nonvolatile drug substances or drug products. Therefore, the use of an FID detector helps preferentially identify and quantify residual solvents. The combination of gas chromatography and mass spectroscopy (GC/MS) can be used for confirmation and identification purposes, highlighting the flexibility of this technology.

2.3
Capillary Electrophoresis (CE)

Agilent 7100 CE

The determination of drug-related impurities is one application of CE in pharmaceutical analysis because it achieves high separation efficiencies compared to other chromatographic techniques. CE can be used when HPLC techniques are not able to adequately measure impurities, especially very polar compounds. A reporting threshold of 0.05 % is widely accepted as a minimum requirement for a related impurities determination method, and this can be achieved using CE. In addition, CE is very useful for the separation of closely related compounds such as diastereomers and enantiomers. An example of the value of CE in impurities analysis is demonstrated using heparin (a polymeric anticoagulant). In this case, standard LC failed to distinguish drug lots associated with adverse events, while CE was easily able to identify an unknown impurity (Figure 2). As a result, the use of CE helped to solve this analytical challenge.

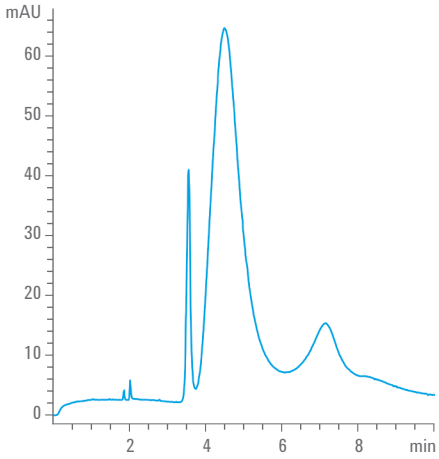


Figure 2. Capillary electrophoresis of heparin and related impurities using highly concentrated buffers in a 25-µm bubble cell capillary. See Agilent publication 5990-3517EN.

2.4 Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS)

Harmonized USP and ICH elemental impurities methods (ICH Q3D/Q2(R1) and USP<232>/<233>) require that an appropriate, specific, quantitative analytical procedure is used to determine the level of elemental impurities. USP<233> specifies that an instrument-based method is used, and the reference methods use ICP-MS or ICP-OES. With both methods, sample analysis can be accomplished in three ways:

- Directly (unsolvated)
- Following sample preparation by solubilization in an aqueous or organic solvent, or
- After acid digestion using a closed-vessel microwave system

ICP-OES

Agilent 5110 ICP-OES

Agilent 7800 ICP-MS

ICP-OES provides parts per billion (ppb) detection limits for most regulated elements in pharmaceutical products, easily meeting the specified limits for oral dosage forms in cases where direct sample analysis or small dilution factors are appropriate. It also provides extended dynamic range, robust plasma, and one-step measurement of major, minor, and trace elements. Therefore, ICP-OES addresses the needs of a wide range of users, including those seeking a cost-effective solution for the direct analysis of elemental impurities in bulk raw materials and oral dosage pharmaceutical products.

ICP-MS is a highly sensitive and specific technique that delivers reliable trace-level analysis of all 24 elements, whose limits are defined in ICH Q3D and USP<232>. The low (typically ng/L (ppt)) LODs of ICP-MS ensure that all regulated elements are measured accurately at the lower limits required in parenteral and inhalational medicines, as well as in drug substances or drug products for oral administration. All required analytes are easily determined, at or below regulated levels, even when large sample dilutions are used during sample preparation, for example when a very small sample amount is available. ICP-MS can also be connected to chromatographic separation techniques, typically HPLC, providing separation (or speciation) of the different chemical forms of the elements. The Agilent 7800 and 7900 quadrupole ICP-MS instruments provide effective control of interferences, tolerance of high and variable sample matrices including organic solvents, and wide dynamic range, to allow major and trace elements to be measured accurately in a single run. Agilent ICP-MS systems include a

Preset Method and Setup Wizard, system optimization functions, and a Standard Operating Procedure to assist with rapid implementation and validation for routine pharmaceutical analysis.

ICP-QQQ

Agilent 8900 ICP-QQQ

While ICP-MS and ICP-OES are perfectly suited to routine analysis of elemental impurities in drug products and components, some method development applications can benefit from the flexibility and high performance of triple quadrupole ICP-MS (ICP-QQQ). Agilent ICP-QQQ instruments offer improved limits of detection compared to quadrupole ICP-MS, especially for difficult elements such as S, P, and Si, together with superior accuracy at trace levels, particularly in complex sample matrices.

2.5 Supercritical Fluid Chromatography (SFC)

Agilent 1260 Infinity II SFC System

SFC, which uses supercritical CO_2 as the mobile phase, is a chromatography separation technique that can be used for impurity detection because it offers HPLC-level sensitivity with reduced organic solvent usage (Figure 3). SFC also offers the advantage of chiral impurity analysis, and may aid in the determination of enantiomeric excess at very low impurity levels (Figure 4).

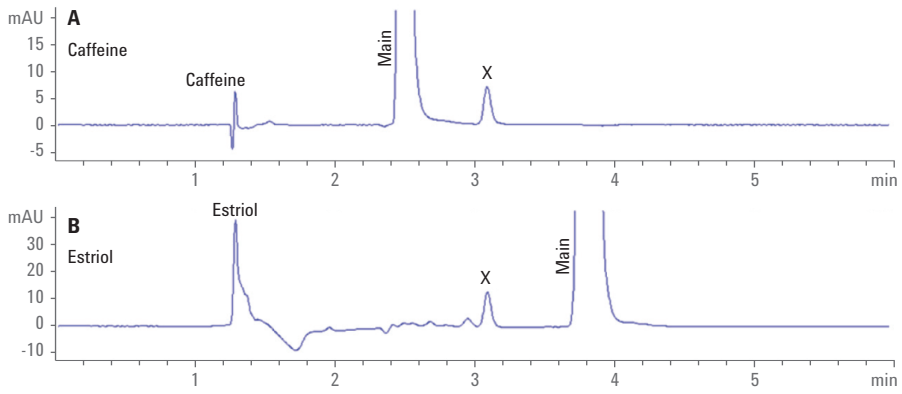


Figure 3. Isocratic separation of the impurity (0.05 % w/w level) from the main component, caffeine (A), and estriol (B); the S/N for the impurity at the 0.01 % level is well above 2–3, which is usually the LOD. See Agilent publication 5990-6413EN.

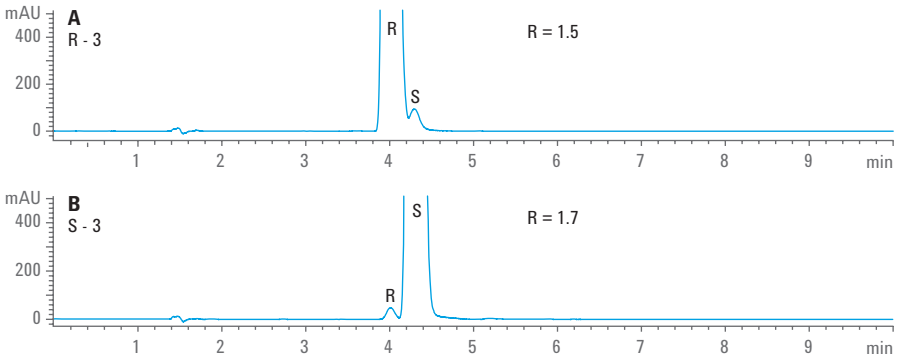
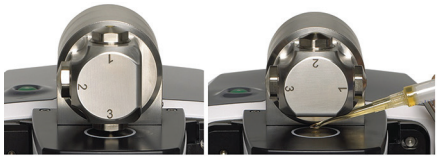


Figure 4. Determination of enantiomeric excess at impurity levels below 0.05 % using SFC. Chromatograms of R-1,1'-bi-2-napththol (A) and S-1,1'-bi-2-naphthaol (B) at 5,000 ppm. See Agilent publication 5990-5969EN.

2.6
Fourier Transform Infrared Spectroscopy (FTIR)

Agilent Cary 630 FTIR



Agilent DialPath acessory

FTIR is very useful for identifying and confirming the structure of an impurity or degradant because every compound has a unique spectral fingerprint. Especially useful in analyzing organic compounds, FTIR spectra are determined by molecular structure and the functional groups present. Conversely, functional group analysis can help identify the structure and measure the concentration of the compound under investigation. Changes in structure can be correlated by comparing the FTIR library spectrum of a pure reference material to that of the impurity or degradant.

The Agilent Cary 630 FTIR provides a powerful combination of precision and compliance, making it one of the best FTIR systems for both qualitative and quantitative analysis in pharmaceutical laboratories. A complete family of accessories provides analyses for liquid and solid samples using the 630 FTIR. Liquid sampling, using the patented Dialpath, simplifies finding the right path length and optimum measurement conditions to facilitate sampling and reduce the risk of error. Measuring contaminants, such as ethylene glycol and diethylene glycol in glycerol, is quick and easy with the Cary 630 FTIR. MicroLab Pharma software streamlines the process to meet regulatory requirement 21 CFR Part 11, with method-driven software that alerts users when the impurity level is outside specification range (Figure 5).

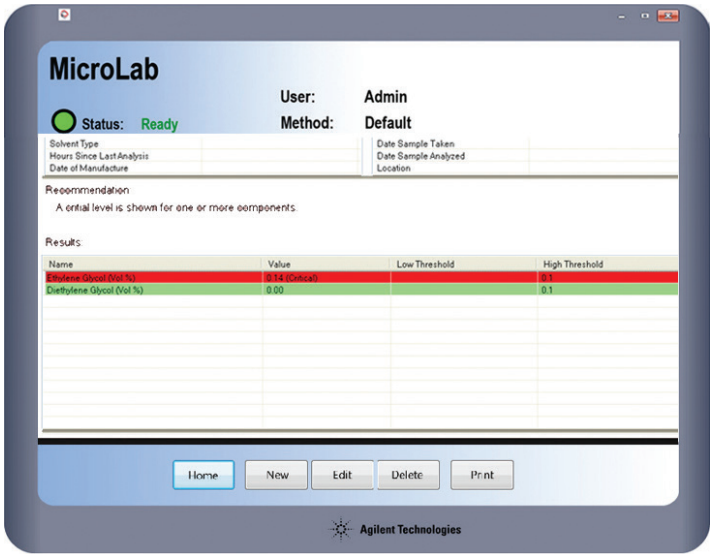


Figure 5. Agilent MicroLab software displays analysis results for the level of ethylene glycol, an impurity in glycerol. The red color band shows that the level of impurity is outside specification range. See Agilent publication 5990-7880EN.

3. A Selection Of Agilent Application Solutions For The Three Major Types Of Impurities

Overview

This section contains examples of Agilent applications that have been developed to meet the challenges encountered when analyzing: the qualitative and quantitative analysis of trace level organic impurities, the determination of elemental impurities, and the analysis of residual solvents according to USP procedures.

3.1 Analysis of Organic Impurities

- Achieve precision, linearity, sensitivity, and speed in impurity analysis with the Agilent 1200 Infinity Series HPLC/UV systems
- Improve profiling productivity for the identification of trace-level impurities using Agilent LC/Q-TOF instruments
- Quantitative analysis of genotoxic impurities in APIs using Agilent LC/QQQ instruments

3.2 Analysis of Inorganic Impurities

- Determination of elemental impurities in pharmaceutical ingredients according to USP procedures by Agilent ICP-OES and ICP-MS-based methods

3.3 Analysis of Residual Solvents

- Faster analysis and enhanced sensitivity in residual solvent analysis as per USP <467> procedures using Agilent GC instruments

3.1 Analysis of Organic Impurities

Achieve selectivity, sensitivity, and precision for impurity analysis with Agilent 1200 Infinity Series LC systems

Agilent 1200 Infinity Series LC/UV systems are an ideal solution for impurity analysis in pharmaceutical quality control laboratories seeking to achieve the precision, linearity, sensitivity, and speed required to meet regulatory standards for impurity analysis. Figure 7 illustrates the analysis of a fixed-dose combination drug and its impurities using the Agilent 1290 Infinity II High Dynamic Range (HDR) Diode Array Detector (DAD) Solution. This approach enables the simultaneous detection and quantitation of the major drug component as well as the minor components and impurities in a single LC analysis. This analysis was completed in less than 7 minutes, and provided an improved LOD of ~0.1 ng, compared to an LOD of ~1 ng using the conventional DAD. Excellent precision of peak areas was also achieved, with RSDs <0.1 % for the drug components (vitamin C, paracetamol, caffeine, and chloramine) and between 1 and 3 % for the four impurities. This compared to RSDs <2 % for the drug components and 5–15 % for the impurities, using the conventional DAD.

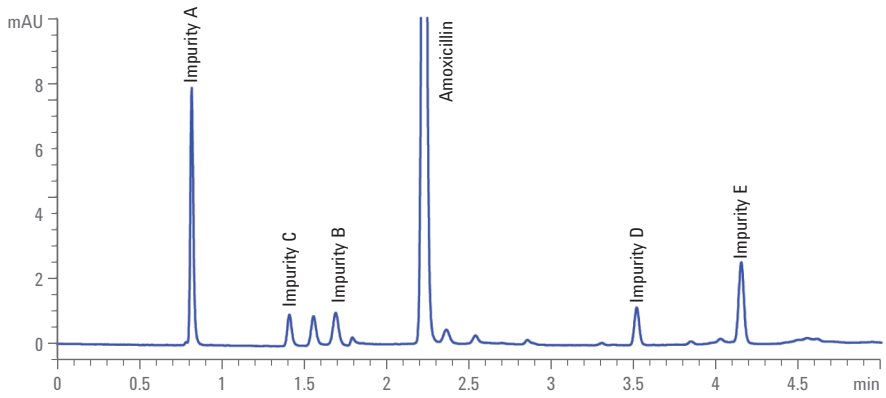


Figure 6. Analysis of amoxicillin and five impurities using the Agilent 1220 Infinity LC System and a gradient method in combination with UV detection, an Agilent ZORBAX SB-Aq column, and Agilent ChemStation software. See Agilent publication 5990-6093EN.

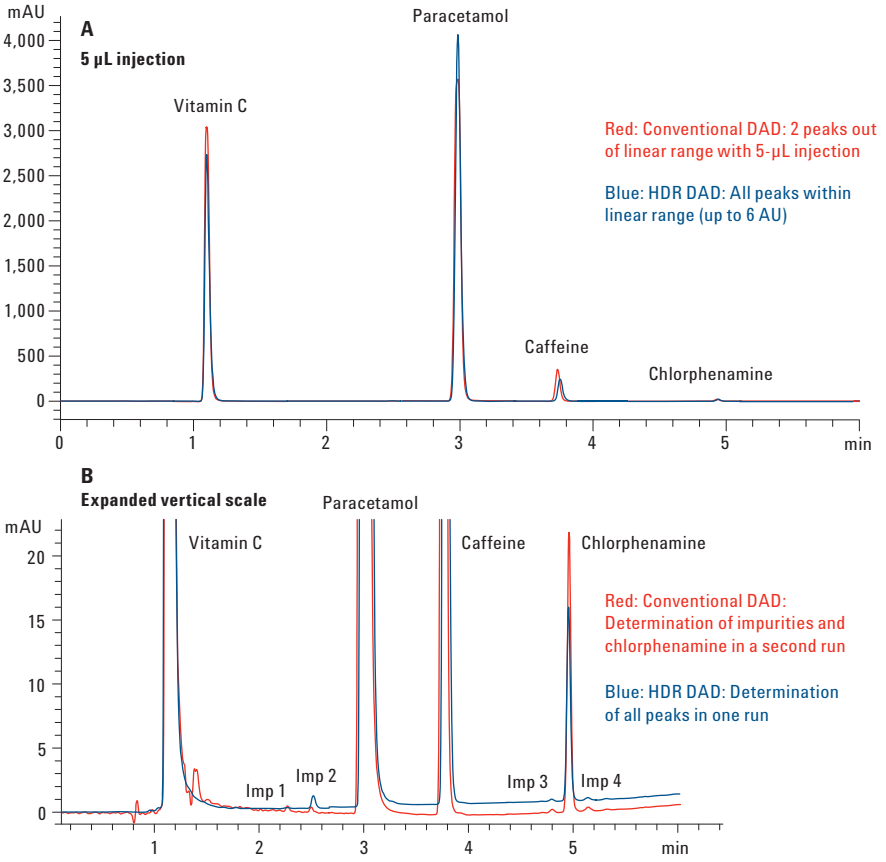


Figure 7. Overlay showing a 5 µL injection using a conventional DAD signal and HDR DAD signals (A) with the vertical scale also expanded (B) to show the four impurities. Using the conventional DAD (red trace, top panel), the vitamin C and paracetamol peaks are out of range, and cannot be quantitated. See Agilent publication 5991-3874EN.

Agilent UHPLC/UV systems help achieve higher sensitivity, faster sample throughput, and significant cost savings for impurity profiling. Since the 1290 Infinity II LC system can be operated at a pressure of up to 1,300 bar, significantly faster methods can be developed using a very sensitive DAD for profiling impurities in a highly productive manner. The result is a significant reduction in the cost-per-analysis.

The 1290 Infinity II HDR-DAD Impurity Analyzer Solution, the Agilent dedicated system for impurity analysis of well characterized drugs, provides simultaneous detection and quantitation capability, with up to 30x higher dynamic range, and 30x higher sensitivity than conventional UV detection, with UHPLC speed and resolution.

The Agilent 1290 Infinity II Method Development tool for UHPLC and HPLC is ideally suited for testing experimental chromatography conditions, such as determining the ideal combination of stationary and mobile phases. Evaluation of stationary and mobile phases is a simple, automated task, particularly when short run times are used.

In addition to its impressive analytical capabilities, the 1290 Infinity II LC optimizes the performance of any chromatographic analysis. When there is a need to transfer methods between different LC systems, the Agilent Intelligent System Emulation Technology (ISET) rapidly mirrors the performance of other (U)HPLC systems—including those from other manufacturers.

The analyst is no longer restricted to limitations of column dimension, particle type, mobile or stationary phase, flow rate, or pressure. The 1290 Infinity II LC system with ISET (Figure 8) can switch seamlessly between HPLC and UHPLC systems with different designs, all with one mouse click.

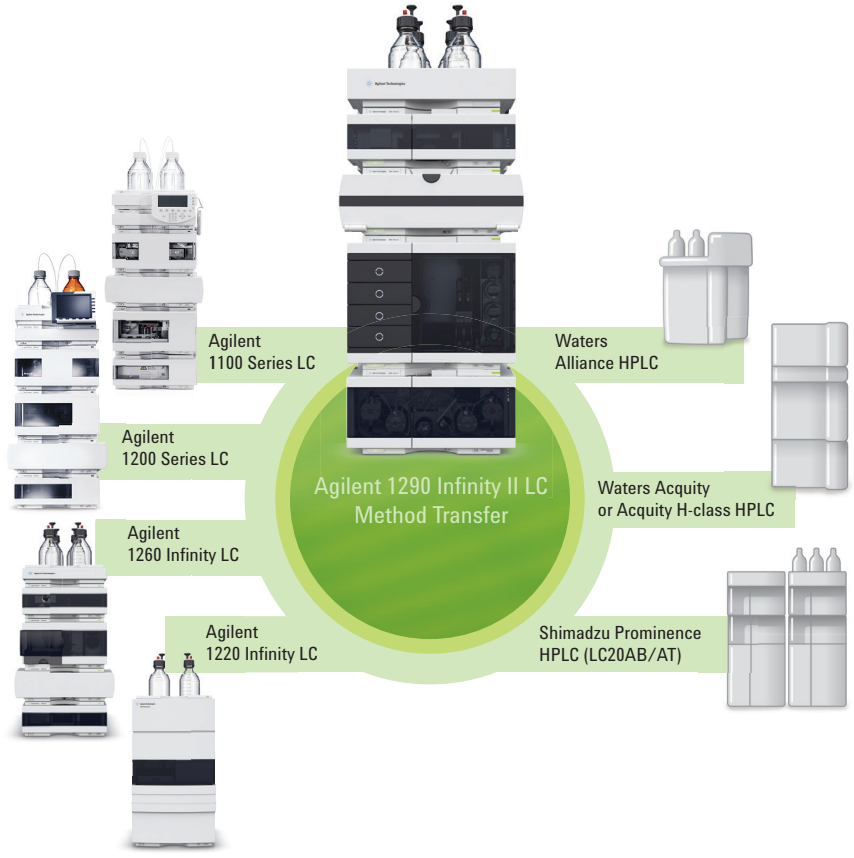


Figure 8. The Agilent ISET system can be used to efficiently transfer methods from a range of systems to the final QC environment. See Agilent publication 5991-4605EN.

The same chromatographic results obtained with the original method can be obtained simply by emulating the LC system used to develop that method (Figure 9). Legacy methods can continue to be used, while gaining the advantages of modern UHPLC systems, including higher sensitivity and accuracy and less carryover, without the need to retain the outdated legacy LC systems. The analyst can have full confidence that the new method will perform as intended.

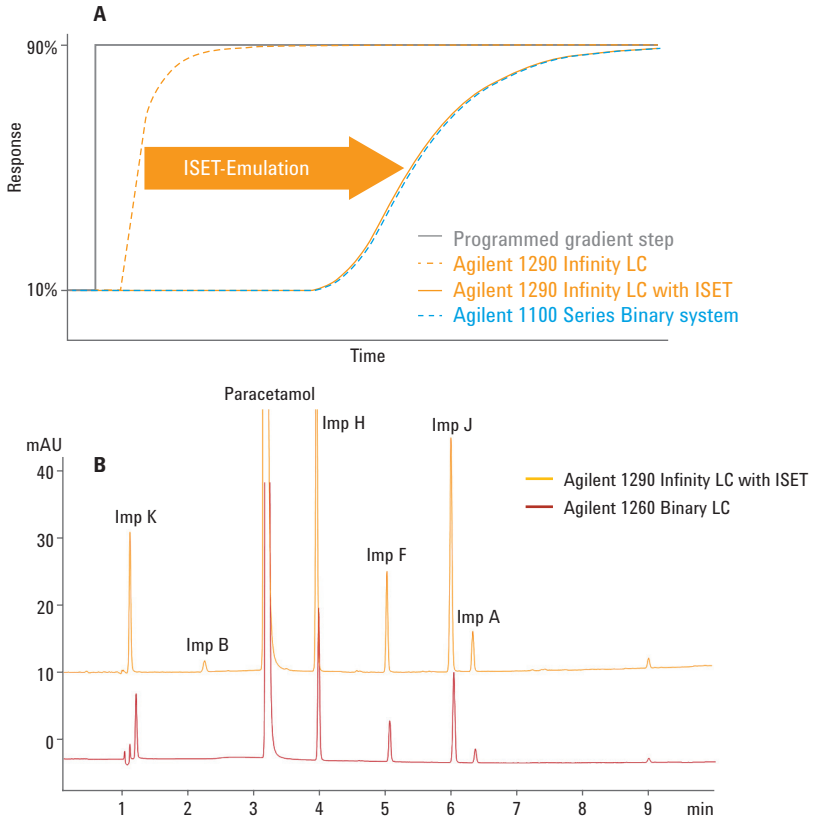


Figure 9. Using ISET to emulate a gradient program on the Agilent 1290 Infinity II LC that was originally developed using the Agilent 1100 Series (A). B shows a comparison of chromatograms between a gradient program developed on the Agilent 1260 Infinity LC system and the emulated program run on the 1290 Infinity II LC system using ISET. Note the consistency in elution patterns, as well as the increased sensitivity of the 1290 Infinity II LC system.

Figure 9 demonstrates the advantage of using the ISET seamless method transfer for impurity analysis. With ISET technology simulating delay volume and mixing performance, the 1290 II Infinity LC provides the same gradients as the Agilent 1100 series binary LC system. The resolution and retention time are the same, without needing to change system conditions and methods.

In addition to LC systems, columns can significantly impact the results achieved for organic impurity profiling. For example, laboratories performing compendia analyses with conventional, long, 5-µm porous LC columns can benefit from the increased speed, resolution, and sensitivity that superficially porous Agilent InfinityLab Poroshell 120 columns provide.

Agilent InfinityLab Poroshell 120 columns

Since USP and EP guidelines allow for isocratic methods and flexibility in reducing column length and particle size, transferring methods to shorter 2.7-µm InfinityLab Poroshell 120 columns can save significant time, while maintaining chromatographic separation. The result is higher throughput and greater productivity using Agilent InfinityLab Poroshell 120 columns, compared to conventional 5 µm columns (Figure 10).

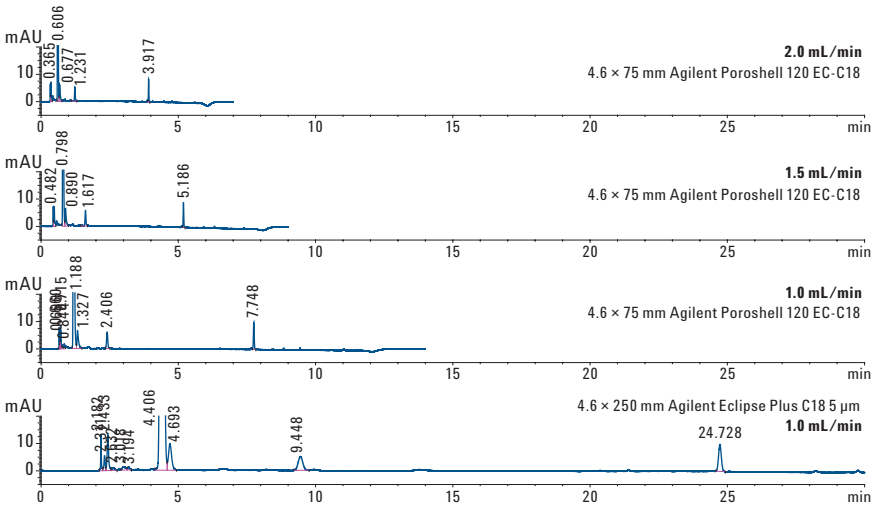


Figure 10. Rapid analysis of cefepime and related impurities using Agilent ZORBAX Eclipse Plus (5 µm) and Agilent InfinityLab Poroshell 120 EC-C18 (2.7 µm) columns. See Agilent publication 5990-7492EN.

Software can also assist in several key tasks required for impurity profiling. For example, Agilent OpenLAB ECM guides the chemist through the complete workflow and documents all data in a central and secure repository that meets regulatory standards.

Agilent OpenLAB Chromatography Data Software (CDS) offers built-in peak purity evaluations (Figure 11), and enables the generation of a final impurity profile report. By comparing UV spectra from the upslope, apex, and downslope, impurities present at less than 0.5 % can be identified. To achieve reliable high-quality data, this can and should be done as a matter of routine. Custom calculation functionality in the analytical software helps calculate the total level of impurities for a complete run, and includes a PASS/FAIL notification according to user-definable limits, depending on the toxicity class of the impurities.

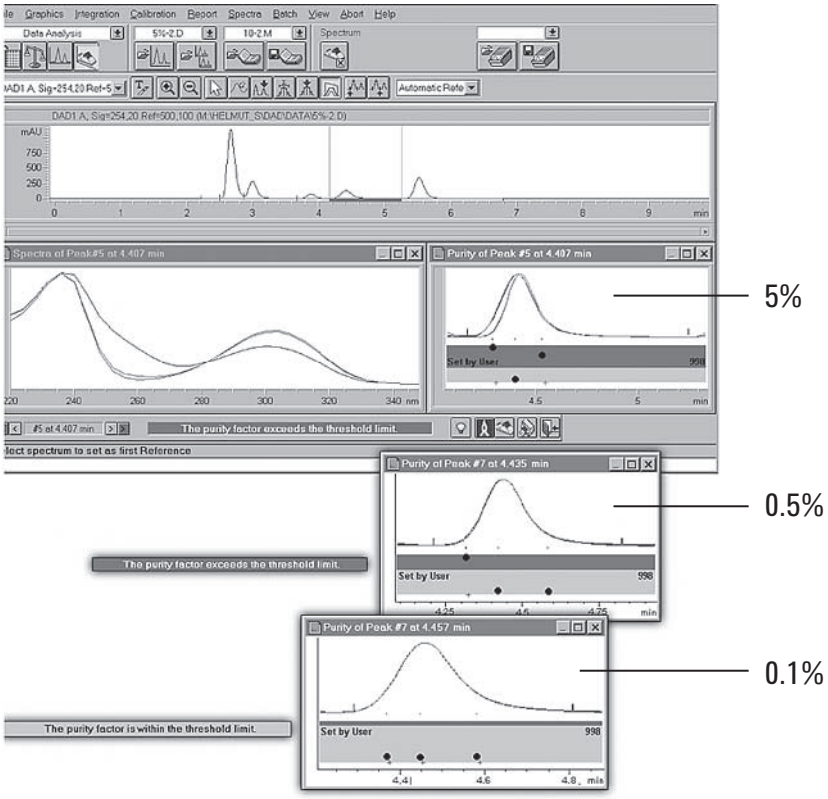


Figure 11. Agilent ChemStation peak purity software can be used to determine impurities present at less than 0.5 %, based on spectral differences. See Agilent publication 5988-8647EN.

LC/MS for the Rigorous Analysis of Organic Impurities in Pharmaceuticals

As the method of choice, combining the separation power of liquid chromatography with the selectivity, analytical sensitivity, and accuracy of quantitative mass spectrometry meets the laboratory need for organic impurity analysis. The LC/MS system chosen for a particular impurity analysis application must satisfy key criteria.

Generally, impurities present at 0.05 % or greater (relative to the API) must be reported, and impurities present at 0.1 % must be identified. The pharmacological and toxicological properties of impurities at levels of 0.15 % or greater must be understood. To meet these requirements, the separation of impurities by LC must be both efficient and compatible with MS analysis, which provides the required specificity and analytical sensitivity. The final step requires effective data analysis to identify and accurately quantitate impurities.

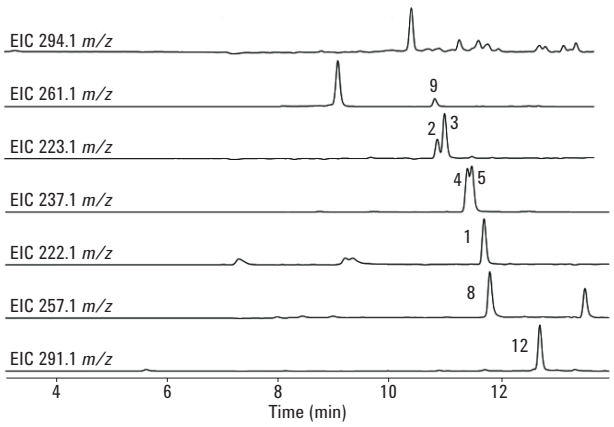
Agilent provides comprehensive analysis solutions that successfully address these challenges, from high-resolution chromatographic separation to heart-cutting 2D-LC, without requiring complex method transfer for MS compatibility, and proficient MS data analysis.

Cost-Effective, High-Performance Impurity Analysis Using Single Quadrupole LC/MS Instruments

Agilent InfinityLab LC/MSD

For impurities analysis, the Agilent MSD Series Single Quadrupole LC/MS systems deliver superior MS data quality in an easy-to-use, compact, and high-performance package. With configurations to fit all budgets, the industry's most popular single quad LC/MS complements a laboratory's existing LC detectors, and integrates seamlessly with Agilent ChemStation LC control.

The 6100 Series Single Quadrupole LC/MS systems provide complementary information to diode array analysis, which can aid in impurity detection and identification (Figure 12). For example, the LC/MSD takes full advantage of the higher throughput of faster UHPLC chromatography.



Recovery	
Compound	%
1	92.5
2	39.2
3	94.7
4	78.2
5	105.9
8	86.5
9	124.4
12	57.8
13	92.9

Figure 12. LC/MS chromatogram for the analysis of vitamin C spiked 1 ppm with potentially genotoxic impurities (PGIs). The PGIs were analyzed after derivatization. See G. Vanhoenacker, *et al. J. Chromatogr. A* **2009**, *1216*, 3563-3570.

Even if the UHPLC peak width is as narrow as 1 second, ultra-fast ion polarity switching delivers both positive and negative spectra, providing maximum information from a single injection. Productivity is further enhanced by an injection-to-injection cycle time of less than 10 ms, for analysis of more samples per hour. MS data can also be converted to MassHunter data format for further qualitative and quantitative analysis.

Sensitivity and Reliability for Impurities Analysis in APIs Using Agilent LC/Triple Quad Instruments

Agilent 6470 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology

Agilent 6400 Series Triple Quadrupole LC/MS systems deliver industry-leading sensitivity, high efficiency, and value for applications such as trace quantitation of genotoxic impurities in APIs and botanical medicines. Maximized ionization efficiency and transmission across a broad mass range ensure low LODs and limits of quantitation (LOQs) for the widest range of sample types, with sensitivity as low as the femtogram level.

An innovative collision cell design eliminates cross-talk, and enables fast multiple reaction monitoring (MRM), even with ion dwell times as short as 1 ms, for higher efficiency. Innovative dynamic MRM (dMRM) allows up to 4,000 MRMs to be analyzed automatically in a single time period. An Agilent MassHunter Optimizer automatically optimizes MS parameters such as collision voltages and collision energies for each compound, providing further efficiency improvements.

Figure 13 demonstrates an MRM-based quantitation of nine arylamine and aminopyridine potential genotoxic impurities (PGIs) at trace levels (well below 1 ppm relative to the API) using a 1290 Infinity LC coupled to an Agilent 6400 Series Triple Quadrupole LC/MS. Detection limits for these nine PGIs were below 20 ppb relative to the API, using MS/MS (Agilent publication 5990-5732EN). Selectivity in the presence of related impurities was ensured using specific quantifiers and qualifiers for each PGI. All nine PGIs were well separated in less than 9 minutes using an Agilent ZORBAX Eclipse Plus C18 RRHD column (2.1 × 150 mm, 1.8 µm). Analysis time can further be reduced to 3 minutes using a ZORBAX Eclipse Plus C18 RRHD, 50 mm column. The recoveries calculated by comparison with a standard solution of the PGIs provided accuracy levels of 70–130 %, which are typical limits in pharmaceutical trace analysis procedures (for example, limit tests).

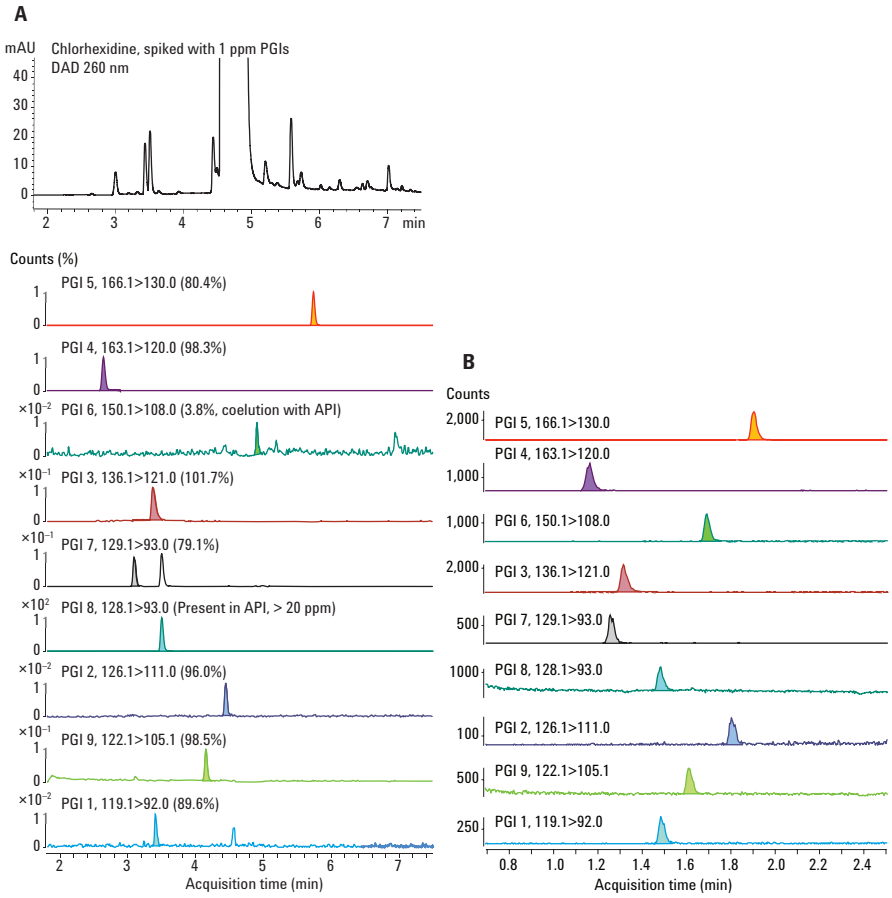


Figure 13. DAD result and quantifier MRM transitions for the analysis of a chlorohexidine sample spiked with PGIs. A comparison is shown between results achieved with 150 mm (A) and 50 mm (B) Agilent ZORBAX Eclipse Plus C18 RRHD (2.1 mm id, 1.8 µm) columns. Transitions and calculated recoveries are indicated. See Agilent publication 5990-5732EN.

Improve Profiling for the Identification of Trace Level Impurities Using Agilent LC/TOF and LC/Q-TOF Instruments

Agilent 6500 Series Q-TOF System

For confirming synthetic pharmaceutical compounds and identifying impurities by their accurate mass, the Agilent 6200 Series Accurate Mass Time-of-Flight (TOF) LC/MS systems deliver the highest speed and performance in a compact benchtop instrument. Sub-ppm mass accuracy improves confidence in results, and reduces false positives. A mass resolving power of 20,000 distinguishes target compounds from interferences. Up to five orders of in-spectrum dynamic range enables simultaneous detection and quantitation of compounds at both high and low concentrations. Low picogram on-column sensitivity enables the detection and quantitation of impurities at extremely low concentrations.

When structure analysis is needed to identify unknown API impurities, Agilent 6500 Series Q-TOF LC/MS systems provide an unmatched combination of mass accuracy, mass resolution, sensitivity, dynamic range, and speed to ensure accurate identification and quantitation. The quadrupole provides collision-induced dissociation (CID) to generate fragments that are used to identify the structures of sample components (MS/MS).

The sub-ppm mass accuracy of the 6500 Q-TOF dramatically reduces uncertainty, and improves database search scores and molecular formula generation of unknowns, while minimizing false positives. The high resolving power of up to 45,000 dramatically enhances the reliability of results for all components of interest in complex samples, and superior MS/MS sensitivity helps identify component peaks at the femtogram level.

With unmatched long-term reliability, high resolution, and mass accuracy, the 6500 Q-TOF system is the perfect instrument for structural analysis of pharmaceutical impurities. A closely eluting impurity in duloxetine was identified as a methyl derivative of the drug using this Q-TOF, the Agilent 1200 Series Infinity LC configured for standard heart-cutting, and Agilent MassHunter software tools (Figure 14). The 2D-LC/MS approach is discussed in detail in the following section.

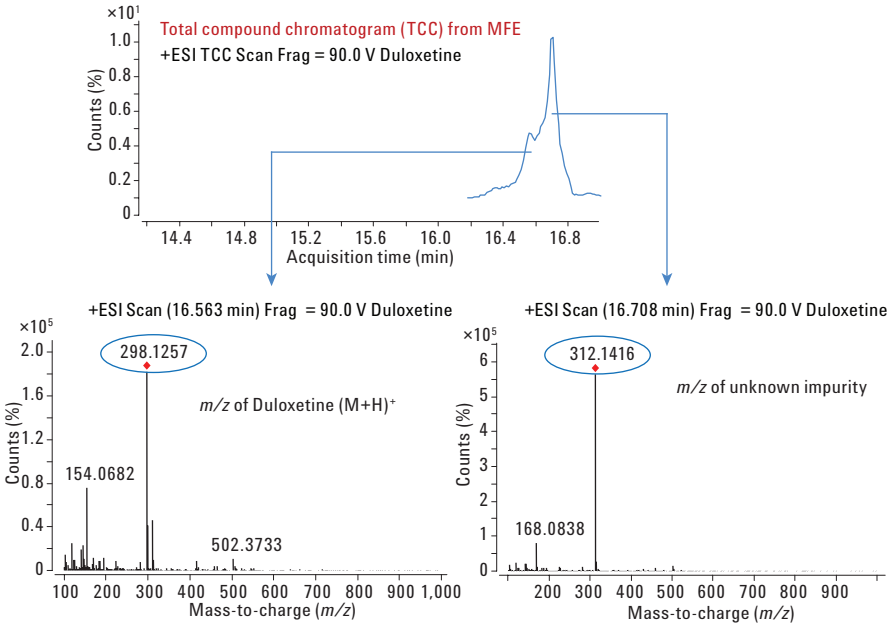


Figure 14. Mass spectra of the API duloxetine (298.1257) and its impurity (312.1416), which was identified as a methyl derivative of duloxetine. See Agilent publication 5991-1873EN.

Optimize Method Transfer for MS Compatibility Using 2D-LC/MS

To obtain good chromatographic separation, an HPLC method often requires the use of a large amount of buffering salts such as phosphates, EDTA, TFA, sodium sulfate, triethanolamine, and ion-pair reagents, which are not compatible with MS analysis. Replacing them with MS-compatible reagents may result in a series of problems, including changes in the retention time of impurities and the elution order of peaks. This method change complicates impurity separation and identification. Agilent has a unique and innovative solution for this challenge.

Agilent has developed a 2D-LC methodology based on valve switching, which changes the MS-incompatible mobile phase in the original LC separation to an MS-compatible mobile phase in the second dimension. The retention behavior of each chromatographic peak is preserved, since the original chromatographic conditions are unchanged. Each target component separated in the first dimension is transferred to the second-dimension column for subsequent MS analysis. This approach enables the user to apply MS analysis to LC methods with MS-incompatible mobile phases, without the need to alter the initial LC methods. Identification of impurities can be completed rapidly and efficiently using this technology, in combination with an Agilent impurity identification data processing workflow.

The 1290 Infinity II LC equipped for standard heart-cutting 2D-LC has been proven to be suitable for mobile phase systems with inorganic salts (phosphates) and systems with organic ion-pair modifiers (for example, sodium dodecyl sulfate and tetrabutylammonium hydroxide). In addition, users can extend the application scope of this method based on the specific needs of the experiment, such as replacement of the mobile phase in an analysis method and the reduction of matrix suppression. Finally, the modular design of Agilent LC systems provides the flexibility to configure systems according to the performance requirements for chromatographic separation. Legacy Agilent LC/MS systems can be upgraded to 2D-LC/MS configurations simply by adding or removing modules, enabling the best use of existing instruments. The 1290 Infinity LC equipped for standard heart-cutting 2D-LC has been used for impurity analysis of several APIs. Figure 15 shows the results of the analysis of one of these, ceritinib. The original chromatographic separation conditions used a mobile phase A that contained a tetrabutylammonium hydroxide buffer, which is incompatible with electrospray MS analysis. After separation of the API from an impurity using these conditions and a DAD detector (Figure 15A, upper trace), the impurity peak was heart-cut and sent to a second C18 column that used a water and methanol gradient. The effluent from this column was directed to an Agilent 6540 Q-TOF MS. Figure 15A shows the extracted ion current (EIC) trace of this component, and Figure 15B shows its mass spectrum. These data were analyzed using MassHunter software, and the Molecular Formula Generator (page 29) determined the formula for the impurity, $C_{13}H_{15}ClN_3O_3S$ (Figure 15C).

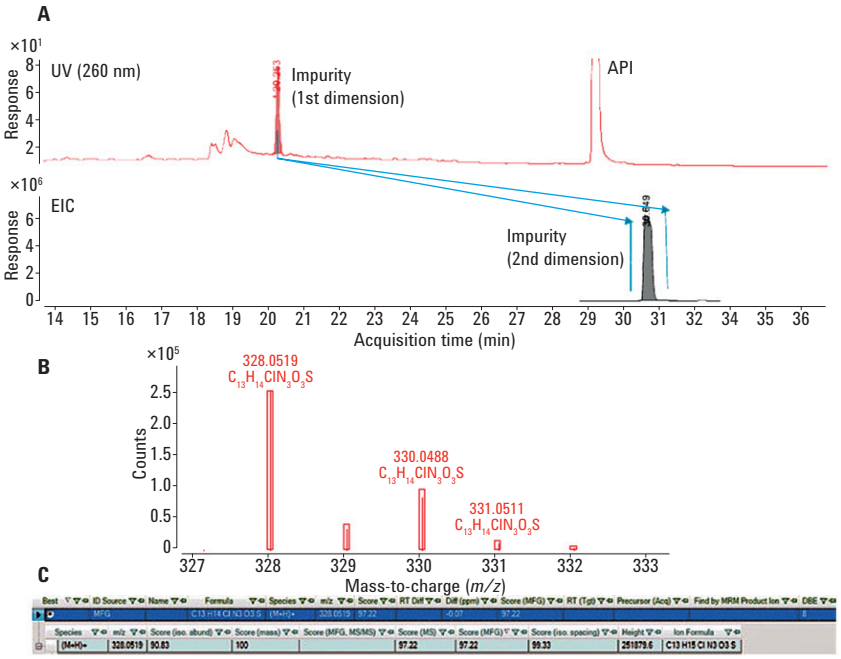


Figure 15. Impurity analysis of ceritinib, showing the UV trace of the first-dimension separation (A, upper trace) and the EIC of the second-dimension chromatography of the impurity (A, lower trace), as well as the molecular ion portion of the mass spectrum for the impurity (B), and the molecular formula determined for the impurity using the MS data and Agilent MassHunter software tools (C).

This approach preserved the elution sequence from the original separation, while providing essential MS information that was used to identify the impurity. The same approach has been used to identify a closely eluting, low abundance impurity of duloxetine (Application Note 5991-1873EN, page 27). Other APIs whose impurities have been characterized using this approach include leucovorin, guanosine 5'-triphosphate trisodium salt, daptomycin, gadobenic acid, betahistine, fluticasone furoate, amoxicillin, phospholipids, and insulin.

MS Data Analysis is Essential to Accurate Impurity Detection and Identification

The amount of MS data collected, particularly for high-resolution MS analysis, can be very large. This requires the use of very efficient software processing tools for structure analysis using MS/MS data and comparison with reference library spectra.

Agilent MassHunter software tools and Personal Compound Database Libraries (PCDLs) provide a highly efficient approach for compound identification using MS data. Failure to detect impurities may lead to potential drug safety issues. Therefore, it is critical to detect as many impurities as possible within a sample. This may not be achieved by a simple method of peak finding, since a single peak may contain multiple components, even with the best mass resolution. However, proprietary Agilent MassHunter software contains tools for MS data analysis that provide accurate and rapid impurity identity and structure elucidation. The Molecular Feature Extractor (MFE) tool for high-resolution MS data finds unknown impurities (Figure 16). A molecular feature is a discrete molecular entity defined by the combination of LC retention time (RT), mass (including isotopic information), and signal response. MFE is an essential analytical tool for extracting valuable information on putative impurities from large sets of raw LC/MS data.

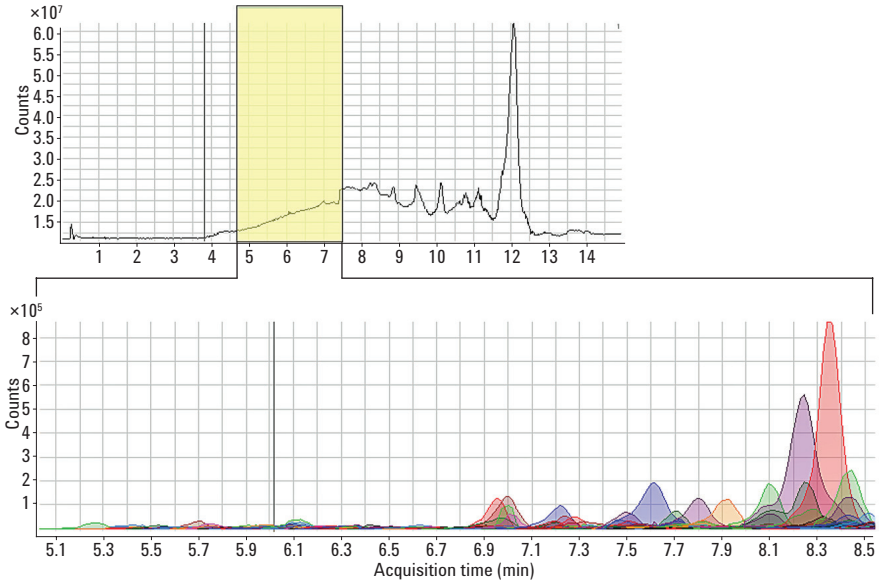


Figure 16. The Molecular Feature Extractor (MFE) in Agilent MassHunter software can define multiple separate molecular entities from a region of the chromatograph that does not show discrete separation.

For known impurities, Find By Formula (FBF) and Molecular Formula Generator (MFG) are the most direct and effective MassHunter tools for impurity characterization. These software tools search and extract molecular entities directly from the raw MS data using the characteristics of the known impurities. The software provides accurate results based on the combination of formula, mass spectrum, and isotopic abundance and spacing matching (Figure 17).

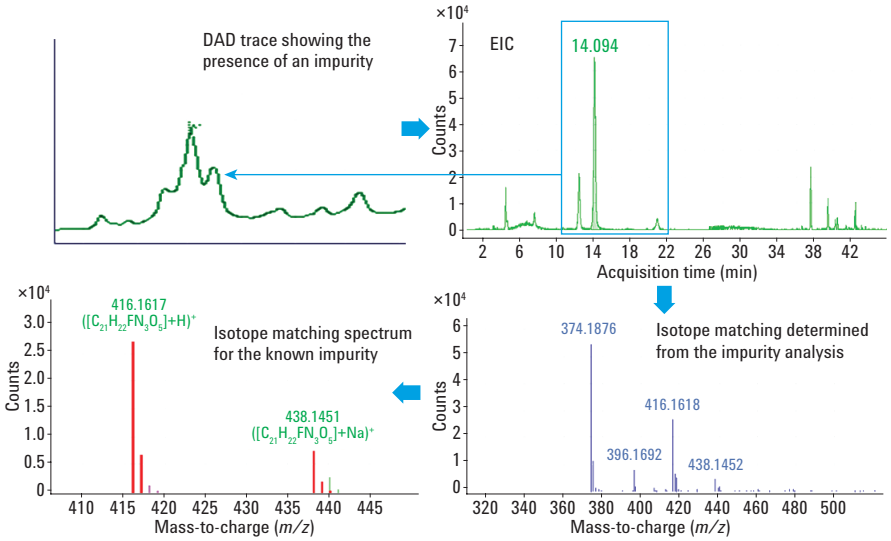


Figure 17. Identification of a known impurity in an API using the FBF and MFG Agilent MassHunter software tools. The MS EIC is determined for the impurity peak and an isotope spectrum is produced from the ESI scan. This is then compared to the isotope spectrum for the known impurity to confirm its presence.

The Agilent Personal Compound Database & Library (PCDL) is essential for efficient impurity analysis. Users can easily import compound information into a PCDL to create a highly customized project database. A PCDL can include the compound name, structure, formula, accurate mass, CAS, and IUPAC identifiers. It can be used to perform combined retention time and accurate mass matching for greater confidence in compound identification.

In addition, MS/MS library searching with a PCDL provides a highly specific means of compound identification. High-resolution MS spectra of a reference compound obtained at different collision energies can be stored for comparison with fragment ions obtained in the analysis of an impurity. A similarity search for MS/MS spectra of compounds in the database can aid structure confirmation, which is very useful for the analysis of both process and degradation impurities.

Soft ionization techniques, in particular electrospray ionization (ESI), generate quasi-molecular ions. These mass spectra may lack significant structurally diagnostic fragmentation. To obtain such information, the techniques of CID and MS/MS can be used. However, analyses of the same compound with different instruments may generate different MS/MS spectra due to the selection of collision gas or adjustment of collision energy. Therefore, several standard mass spectral libraries are commercially available that contain spectra acquired with a range of conditions.

Project-specific ESI-MS/MS PCDLs can be constructed to fill this void. MassHunter Molecular Structure Correlator (MSC) is then used to help confirm the structure of unknown impurities based on the accurate mass and elemental composition of MS/MS fragment ions (Figure 18). MSC also supports database search functions (for example, ChemSpider) to help identify unknown impurities in various ways, including similarity searches.

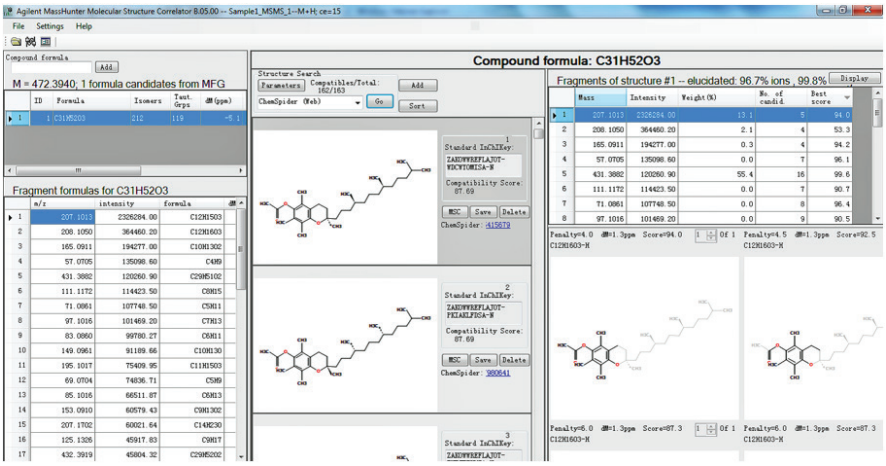


Figure 18. Use of the MSC structural analysis tool in Agilent MassHunter software.

For repeated data analysis of the same APIs or impurities, the MassHunter data analysis workflow can be automated, from the process of molecular feature extraction to structure analysis and setup, to start as soon as the data are acquired. The result is a streamlined analysis process that significantly improves analytical efficiency.

Agilent Organic Impurity Profiling Resources

Publication number	Title
5991-3874EN	Single-Run Assay and Impurity Testing of Fixed-Dose Combination Drugs Using the Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Solution
5991-5743EN	Impurity Testing of Fixed-Dose Combination Drugs Using the Agilent 1290 Infinity II HDR-DAD Impurity Analyzer Solution
5991-4605EN	Agilent 1290 Infinity II LC: More Efficiency More Free Time
5990-7492EN	Fast analysis of cefepime and related impurities on Agilent Poroshell 120 EC-C18
5990-9715EN	Method development on the Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET) with Subsequent Transfer to an Agilent 1100 Series LC: Analysis of an Analgesic Drug
5989-7925EN	Success Story at AstraZeneca: Direct analysis by LC/MS speeds up determination of potential genotoxins in pharmaceutical drug candidates
5990-4460EN	Quantification of genotoxic “Impurity D” in atenolol by LC/ESI/MS/MS using the Agilent 1200 Series RRLC and Agilent 6410B Triple Quadrupole LC/MS
5990-5732EN	Analysis of potential genotoxic arylamine and aminopyridine impurities in active pharmaceutical ingredients by UHPLC and UHPLC-MS/MS using the Agilent 1290 Infinity LC system and the Agilent 6460A Triple Quadrupole MS system
5991-3175CHCN	Replacement of MS incompatible mobile phase in impurity analysis of amoxicillin achieved by 2D-LC Heart Cutting
5991-1873EN	Heart-cut 2D-LC/MS approach for pharmaceutical impurity identification using an Agilent 6540 Q-TOF LC/MS System
5991-5643EN	Application of multiple heart-cutting 2D-LC in method development for impurity analysis: The Agilent 1290 Infinity 2D-LC Solution
5991-1375EN	Pharmaceutical Impurity Identification and profiling using Agilent Q-TOF LC/MS combined with advanced MassHunter Data Processing Software
5991-5564CHCN	Application of Agilent 2D-LC/MS Heart-cutting technology in pharmaceutical impurity identification
5991-2796EN	Pharmaceutical applications compendium purity and impurity analysis
5991-0090EN	Pharmaceutical impurity analysis solutions
5991-1876CHCN	Selection guide to purification, purity analysis, and impurity analysis solutions for pharmaceutical development
5990-9380EN	Purification, purity analysis, and impurity analysis solutions for pharmaceutical research and development
5989-8529EN	Detection and identification of impurities in pharmaceutical drugs: Computer-assisted extraction, profiling and analysis of Q-TOF data for determination of impurities using Agilent MassHunter software
5991-1876EN	Genotoxic Impurities in pharmaceutical products, regulations and analysis primer

3.2 Analysis Of Inorganic Impurities

Determination of elemental impurities in pharmaceutical ingredients according to ICH and USP methods, using Agilent ICP-OES and ICP-MS instruments

The methodology for the preparation and analysis of pharmaceutical samples is described in ICH Q3D and USP General Chapters <232> and <233>. To replace the previous, outdated heavy metals limit test (USP<231>), these recently updated procedures provide an opportunity for pharmaceutical laboratories to benefit from simplified methodology and modern instrumentation. The new methods require laboratories to use accurate, quantitative analytical methods such as ICP-OES or ICP-MS to determine all target elements in the USP<233> and ICH/Q3D documents pertaining to elemental impurities. ICP techniques provide an ideal combination of fast multi-element analysis, good matrix tolerance, wide linear dynamic range, and high sample throughput. The robust plasma system on the Agilent 5110 ICP-OES can handle the most challenging samples, such as undiluted organic solvents and concentrated salt solutions. Detection limits meet the regulatory requirements for bulk raw materials and pharmaceutical products intended for oral administration. The 5110 ICP-OES provides a cost-effective solution for fast, accurate analysis where samples do not require a large dilution factor during preparation or digestion (See Figure 19).

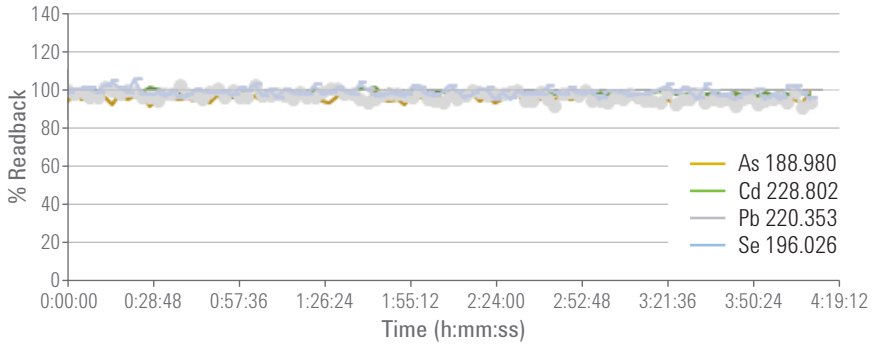


Figure 19. The robust vertical plasma system of the Agilent 5110 ICP-OES ensures the stable analysis of difficult samples, such as the 250 ppb spike in 25 % NaCl solution shown here. *Agilent Technologies, unpublished results.*

The Agilent 7800 and 7900 quadrupole ICP-MS systems can determine all regulated elements at low levels in a wide range of pharmaceutical materials. This is beneficial for laboratories that measure elemental impurities in parenteral or inhalational drug products and ingredients (where the regulated limits are much lower), as well as in oral dosage forms (see Agilent publications 5991-8333EN and 5991-7674EN). Simple method development and routine operation are provided by the built-in USP/ICH preset method for elemental impurities, which uses a single set of consistent helium (He) cell mode conditions for all analytes and samples. As required in USP<233>, unequivocal identification and verification of analyte results is provided by secondary (qualifier) isotopes measured in He mode.

Low limits of detection (LODs) are particularly important for critical, potentially toxic trace elements (notably As, Cd, Hg, and Pb, which are defined as Class 1 impurities) and must be measured in all drug products, according to ICH Q3D and USP<232>. Figure 20 shows calibrations for these elements, together with Pd and Pt, which are representative members of the platinum group elements (PGEs) that must be monitored when added as catalysts, per ICH Q3D and USP<232>. Note that all of the elements were measured in the same mode (He cell gas), illustrating the simplicity of method setup, which is a highly valued characteristic of Agilent quadrupole ICP-MS systems.

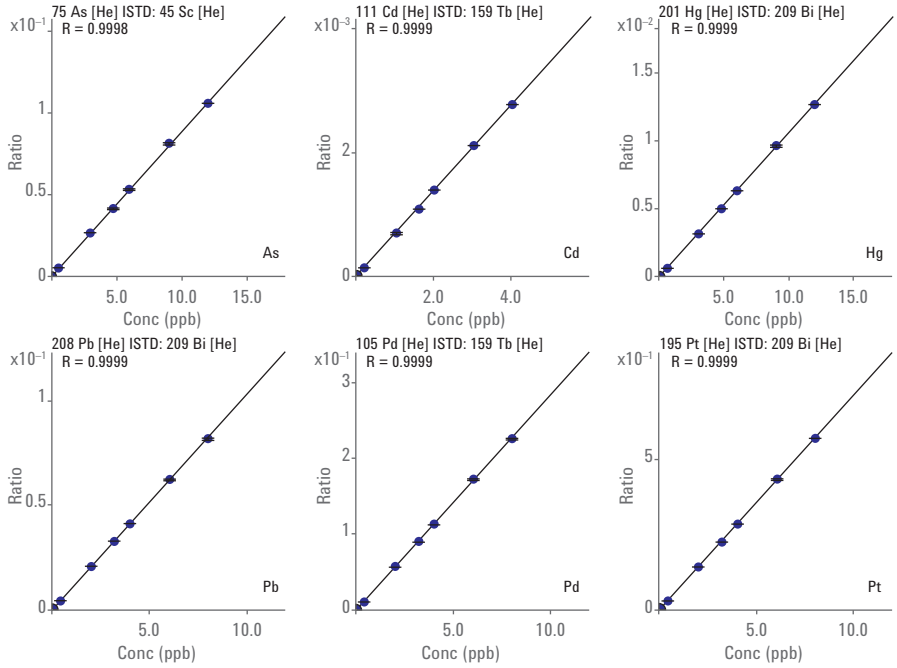


Figure 20. Agilent 7800 ICP-MS calibration curves for As, Cd, Hg, Pb, Pd, and Pt in He mode, demonstrating LODs of 1 ng/L or below, and good sensitivity and linearity for all elements including Hg, Pd, and Pt, which require stabilization in HCl. See Agilent publication 5991-8335EN.

System performance validation of the 7800 and 7900 ICP-MS systems delivers data that are easily within method requirements for accuracy, stability, and spike recovery, with detection limits that are all several orders of magnitude lower than the levels at which the trace elements are currently controlled. This provides the reassurance that the 7800 and 7900 quadrupole ICP-MS systems are able to meet regulatory requirements for all pharmaceutical materials regulated under ICH/USP methods, even if control limits are reduced in the future.

The 7800 and 7900 quadrupole ICP-MS systems also provide full mass spectrum screening capability, are tolerant of all commonly used organic solvents, and can be linked to a chromatography system to provide integrated separation and analysis of the different forms of As and Hg, if required to confirm compliance with the limits for the inorganic forms of these elements defined in ICH Q3D and USP<232>.

Agilent Elemental Impurity Analysis Publications

Publication number	Title
5991-8140EN	Agilent Resources for Workflow Compliance—Measuring elemental impurities in pharmaceutical materials
5991-8335EN	Validating performance of an Agilent 7800 ICPMS for USP <232>/<233> & ICH Q3D/Q2(R1): Elemental Impurity Analysis in Pharmaceuticals
5991-7674EN	Determining Elemental Impurities in Pharmaceutical Ingredients using USP/ICH Methodology and ICPMS
5991-8337EN	Elemental Impurities in Aspirin; Validating USP <233> and ICH Q3D using the 5110 ICP-OES
5991-8149EN	USP <232>/<233> and ICH Q3D Elemental Impurities Analysis: Agilent's ICPMS solution
5991-8150EN	USP <232>/<233> and ICH Q3D Elemental Impurities Analysis: Agilent's ICP-OES solution
5991-2002EN	Support for 21 CFR Part 11 and Annex 11 Compliance: SDA module for Agilent ICPMS MassHunter software
5991-8143EN	Support for 21 CFR Part 11 and Annex 11 Compliance: SDA module for Agilent ICP Expert software
5991-2593EN	Meeting regulatory compliance guidelines with Agilent ICPMS MassHunter and OpenLAB Server
5991-1925EN	Meeting regulatory compliance guidelines with Agilent ICPMS MassHunter and OpenLAB ECM

3.3 Residual Solvent Analysis

Faster analysis and enhanced sensitivity in residual solvent analysis as per USP <467> procedures using Agilent GC instruments

Quality assurance laboratories routinely use United States Pharmacopeia (USP) method <467> for residual solvent analysis. The Agilent 7697A Headspace Sampler coupled to an Agilent Intuvo GC or 7890 GC offers a very efficient solution for the analysis of USP<467> Class 1 and Class 2 residual solvents at their limit concentrations in aqueous solutions.

USP<467> specifies three procedures for Class 1 and Class 2 residual solvents:

- **Procedure A:** Identification and limit test
- **Procedure B:** Confirmatory test (if solvent is above limit)
- **Procedure C:** Quantitative test

Procedure A uses G43 phase Agilent 624 columns (VF-624ms or DB-624), and Procedure B uses a G16 phase (HP-INNOWax) column. In general, analytes that coelute in one of these phases do not coelute in the other.

Figures 18 and 19 demonstrate that the Agilent 7697A Headspace sampler is capable of outstanding repeatability for the analysis of residual solvents. Repeatability is better than 2.5 % relative standard deviation (RSD) for Class 1, Class 2A, and Class 2B solvents.

An inert sample path, thermal zones with set point stability of better than ±0.1 °C, and EPC-controlled vial sampling using absolute pressure, all contribute to system performance. Carryover is essentially nonexistent in all configurations. User-programmable flow rates and times, needle/loop purges, and vent line purges are effectively used to clean the system between runs. Laboratories should perform system suitability studies and validate their proposed methods according to USP or ICH guidelines.

For new drug development and quality control, a dual-channel configuration using both FID and a mass selective detector (MSD) is a powerful tool for residual solvent determinations, especially when unknown identification or confirmation is needed. This system is particularly well suited for the development of generic methods that do not need to follow USP<467> guidelines. MSD analysis also helps avoid ambiguity, as over 60 solvents are currently used in pharmaceutical manufacturing. When unknown peaks or solvents are present, this system may be the best solution for confirmation and quantitation.

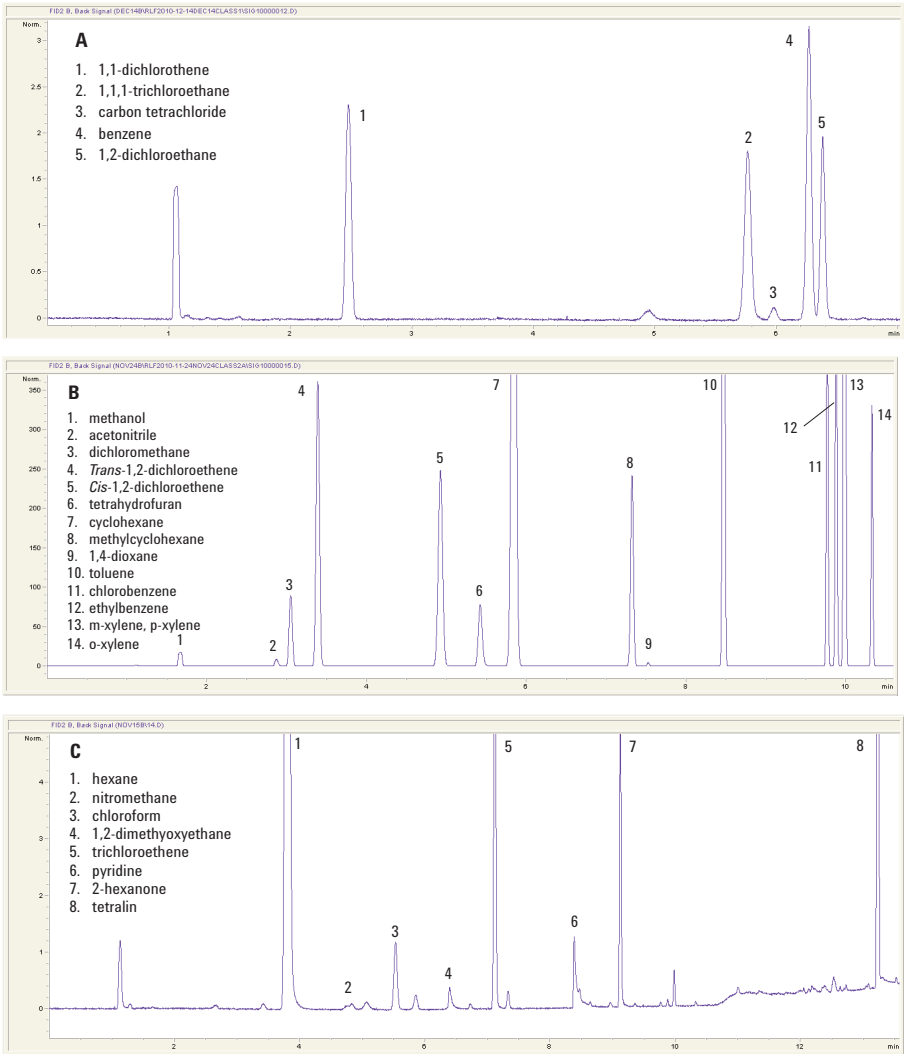


Figure 21. Class 1 (A), Class 2A (B), and Class 2B (C) solvents at USP<467> limit concentrations. See Agilent publication 5990-7625EN.

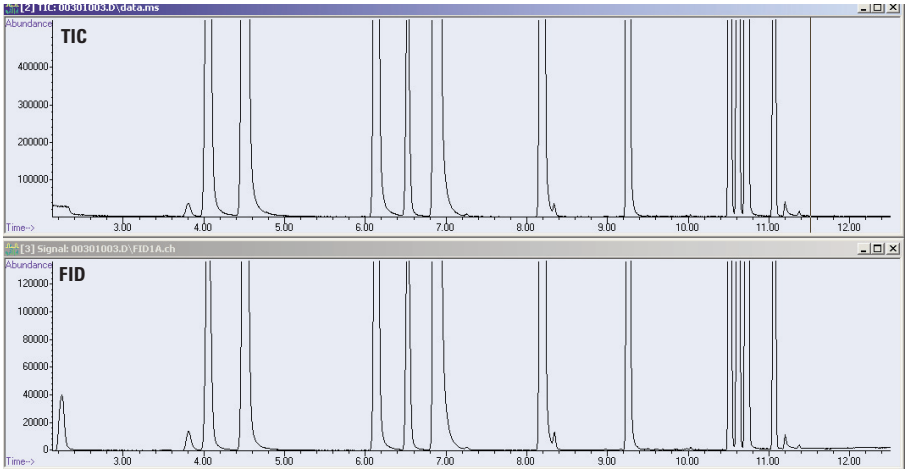


Figure 22. Class 2A solvents at limit concentrations with FID-MSD. See Agilent publication 5990-7625EN.

Agilent Residual Solvent Analysis Publications

Publication number	Title
5990-7625EN	Analysis of USP <467> residual solvents with improved repeatability using the Agilent 7697A Headspace Sampler
5989-8085EN	Simultaneous dual capillary column headspace GC with flame ionization confirmation and quantification according to USP <467>
5989-9726EN	A generic method for the analysis of residual solvents in Pharmaceuticals using static headspace GC-FID/MS
5990-5094EN	Fast analysis of USP <467> residual solvents using the Agilent 7890A and low thermal mass (LTM) system
5989-6079EN	Improved retention time, area repeatability and sensitivity for analysis of residual solvents
5991-4418EN	Flier Streamline your USP <467> residual solvent detection

4. Appendix

4.1 Agilent Solutions for Pharmaceutical Impurities Analysis

Agilent leads the industry with a wide range of instrumentation, LC and GC column choices, and software and informatics solutions for impurities analysis.

Instrumentation

Category of impurity	Application	Instrumentation
Organic impurities	Impurity detection and rapid method scouting/development	Agilent 1200 Infinity Series LC + Diode-array Detector SL
	Detection of impurities not easily separated by HPLC (for example, highly polar compounds)	Agilent 7100 CE System
	Detection of chiral impurities	Agilent 1260 Infinity Analytical SFC System
	Isolation of impurities	Agilent 1260 Infinity Preparative-scale Purification System
	Identification of impurity structure	Agilent 600-IR series FTIR + Agilent 1200 Infinity Series LC + Agilent 6100 Series Single Quadrupole or 6200 Series Accurate-Mass TOF or 6500 Series Q-TOF LC/MS Systems (for trace level genotoxic impurities)
	Quantitation of genotoxic impurities	Agilent 1200 Infinity Series LC + Agilent 6400 Series Triple Quadrupole LC/MS Systems
Inorganic impurities	Analysis of elemental impurities in pharmaceutical ingredients—basic requirements of USP that do not necessitate the lowest detection limits	Agilent 5110 ICP-OES
	Analysis of all 24 regulated elements in all types of pharmaceutical materials. Accurate analysis at and below the regulated levels in the new USP and ICH methods, even when large sample dilutions are required	Agilent 7800 and 7900 Series ICP-MS; Agilent 8900 ICP-QQQ (suitable for advanced materials and research
	Speciation of certain regulated elements (As and Hg)	Agilent 1200 Infinity Series LC + Agilent 7700 Series ICP-MS
Residual solvents	Analysis per USP <467> procedures	Agilent 7890A GC or Agilent Intuvo 9000 GC + Agilent 7967A Headspace sampler
	Analysis involving unknown peaks/solvents	Agilent 7890A GC or Agilent Intuvo 9000 GC + Agilent 5975C GC/MS system + Agilent 7697A Headspace sampler

4.2 Columns and Supplies

To ensure maximum instrument performance and reproducible results, Agilent offers a comprehensive portfolio of GC and LC columns, and supplies for chromatography, spectrometry, and spectroscopy, all meeting ISO 9001 standards.

Agilent leads the LC industry with Agilent InfinityLab, an optimized portfolio of LC instruments, columns, and supplies that work together seamlessly for maximum efficiency and performance, regardless of application area. InfinityLab Poroshell 120 columns provide high-efficiency separations with superior peak shape and resolution. A wide range of stationary phases across three particle sizes (1.9 µm, 2.7 µm, and 4 µm) supports the pharmaceutical lifecycle with selectivity and scalability across laboratory development settings. Agilent also has the broadest portfolio of GC columns available, including innovative options such as our ultra-inert GC columns.

The Agilent comprehensive portfolio of supplies includes vials, syringes, gas management, flowmeters, leak detectors, fittings, tools, and standards, all engineered or selected by our instrument design teams, manufactured to our demanding specifications, and tested under a variety of conditions.

4.3 Software and Informatics

The Agilent industry-leading software and informatics portfolio is continuously expanding to cover a broader range of analytical workstations, data and laboratory management solutions, and applications to satisfy the growing needs of the life sciences and chemical industries. Agilent software solutions are integrated to address the complete lifecycle of scientific data, including experimental design, data acquisition, knowledge management, and analysis in an open system architecture. The Agilent OpenLAB Software Suite includes several chromatography data systems and content management solutions to fit the needs of any laboratory.

4.4 Laboratory Compliance and Compliance Consulting

Data integrity is of increasing concern for laboratories in all industries. This focus extends into how instrument performance is evaluated, documented, and defended during laboratory audits. Agilent ACE is the software Agilent engineers use to test and document the performance of laboratory instruments, irrespective of manufacturer. It can provide Analytical Instrument Qualification (AIQ) services aligned to the 2017 version of USP<1058> for pharmaceutical laboratories, and Functional Verification (FV) services designed to help laboratories comply with ISO 17025 accreditation requirements. Agilent also offers custom validation services, such as computer system validation, audits and assessments, customer procedure writing, and many more. With a proven track record of hundreds of thousands of qualifications to date, you can rely on Agilent to safely deliver your qualification in a data integrity-ensured way.

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agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
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