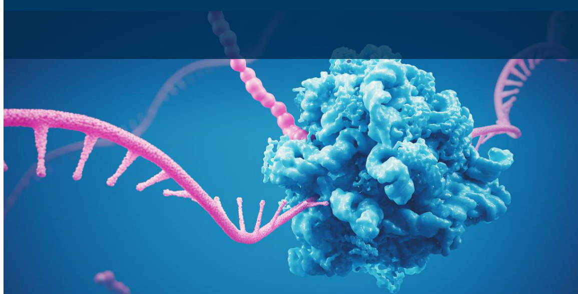
Oligo Workflow Resource Guide

End-To-End Spectroscopy-Based Workflow Solutions for Oligonucleotide Analysis

From research discovery to production QA/QC

Agilent
Trusted Answers



Synthetic oligonucleotides are widely used in research and genetic testing. This class of short nucleic acid polymers includes small interfering RNA, antisense oligonucleotides, aptamers, and CRISPR guides. The popularity of these macromolecules means that there is a growing need for robust analytical methods and easy-to-use data analysis workflows to characterize them. Common attributes of interest include the mass, sequence, purity, and relative quantity of specific impurities. In addition, the raw material (or starting material) and final product identification, (trace) elemental impurities analysis, and residual solvent analysis are equally important in sample validation and quality control (QC) under FDA guidelines.

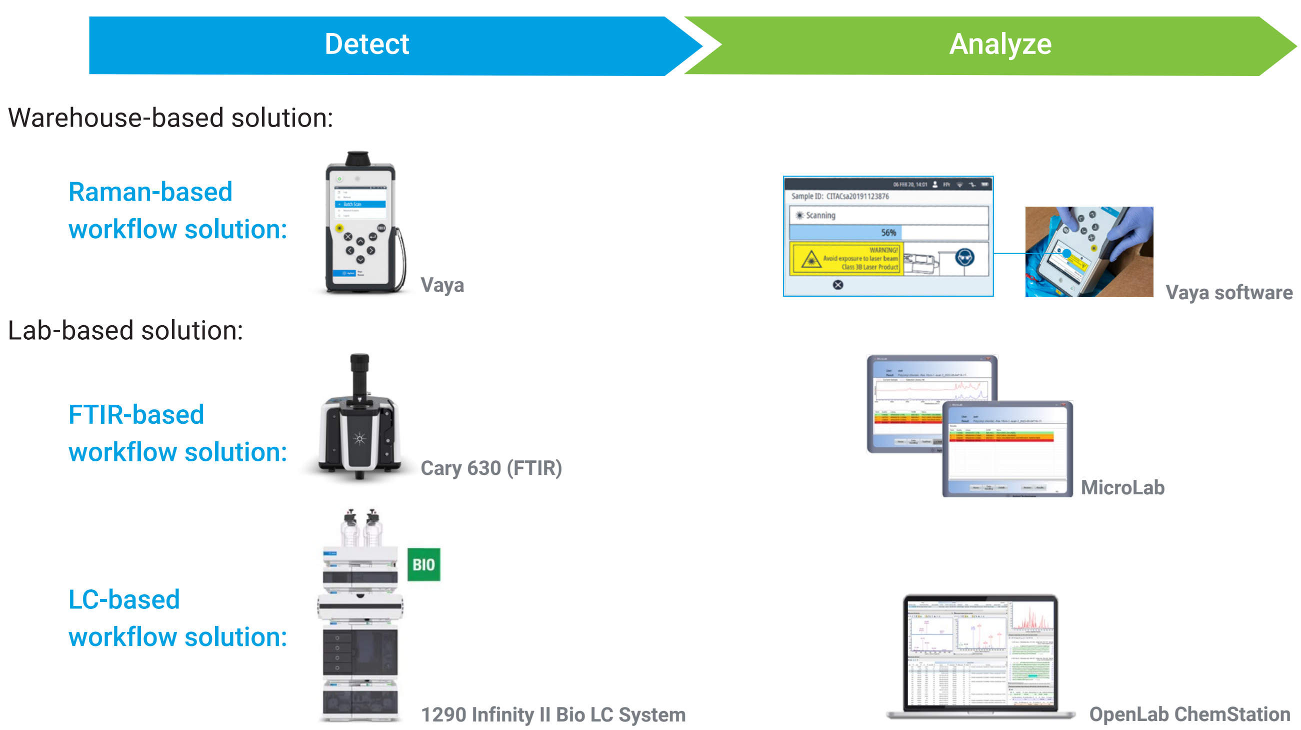
Raw material identification by Raman, FTIR, and LC/UV analysis (warehouse or lab-based solution)

Raw material or starting material identification is a critical quality assurance or safety control analysis that is widely performed in many applications, especially for pharmaceutical drug products. Confirming the identity of received starting materials helps to establish quality as well as prevent contaminated, counterfeit, and incorrectly labeled materials before proceeding into production.

Agilent has developed several workflow solutions for raw material identification (Figure 1). They are: 1) a warehouse-based solution using the Agilent Vaya Raman raw material identity verification system; and 2) lab-based solutions using either the Agilent Cary 630 FTIR or the Agilent 1290 Infinity II Bio LC.

Vaya, a handheld Raman spectrometer, is an effective solution for the identification or differentiation of biopharmaceutical materials (such as phosphoramidites used in the synthesis of oligonucleotides) through transparent and opaque containers in a cGMP manufacturing environment.

FTIR is also widely used for material identification studies. The Cary 630 FTIR spectrometer, along with Agilent MicroLab software, can be used to perform quick, easy, and reliable raw material identification of different sample types. The optional MicroLab Pharma software provides secure data storage and user/access privilege management for users operating in GMP environments. Agilent has also developed an HPLC-based workflow solution utilizing the 1290 Infinity II Bio LC and Agilent OpenLab ChemStation software for excellent sample separation and sensitive detection for raw or starting material identification.



Workflow 1: Raw material ID: warehouse-based (Raman spectroscopy) or lab-based (FTIR or HPLC) solutions.

Finished product identification by UV-Vis analysis (with the Cary 3500 UV-Vis)

UV-Vis spectrophotometers have been used extensively for nucleic acid quantification and QC. The concentration of nucleic acids can be easily estimated by measuring the UV absorbance at 260 nm and the established absorption coefficient. UV-Vis spectrophotometry is also used to measure the melting temperature (Tm) of double-stranded nucleic acids, which gives an accurate indication of the base composition in the sample.

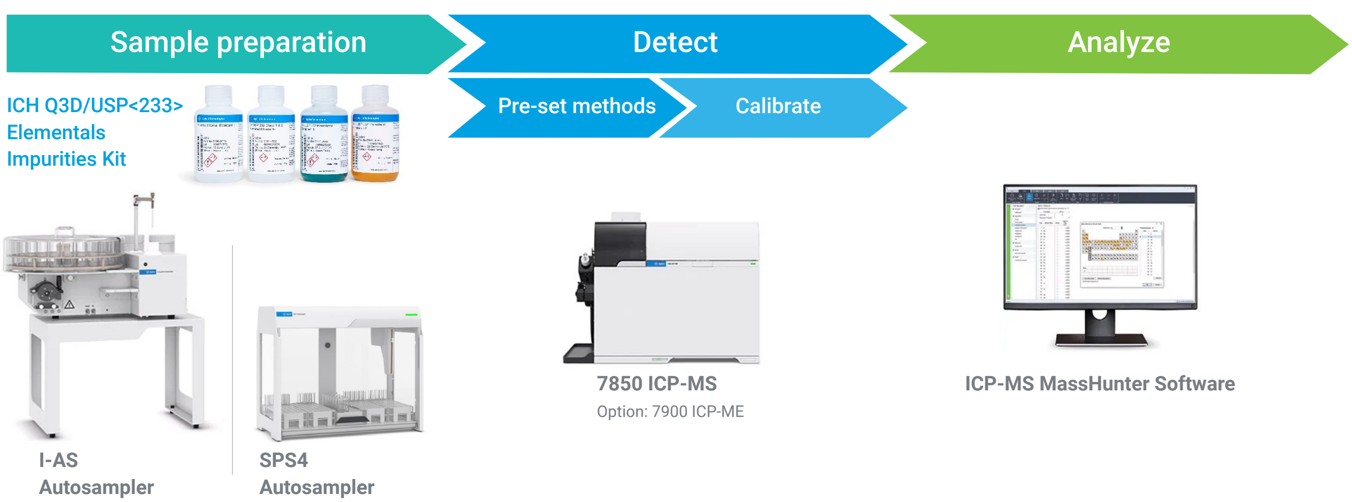
The Agilent Cary 3500 Multizone UV-Vis spectrophotometer uses integrated in-cuvette temperature probes to accurately control the temperature of the solutions during thermal melt experiments at a very fast temperature ramp rate. The Agilent Cary UV Workstation software includes built-in capabilities for DNA melting temperature calculations (smoothing and derivative) that quickly provide actionable results (Figure 2).

Workflow 2: Fnished product Identification by UV-Vis analysis (with the Agilent Cary 3500 UV-Vis).

Trace elemental impurity analysis by ICP-MS

Trace elemental impurities in pharmaceutical drug products may be toxic, affect drug stability or shelf life, and may cause unwanted side effects. Therefore, the current USP and ICH chapters require more elements to be monitored at lower levels than previously, and recommend modern instrumental analytical procedures to determine the concentration of elemental impurities. Manufacturers must be able to demonstrate that their biological drugs comply with the limits for the listed elements in their final drug formulation.

Figure 3 presents an Agilent workflow for trace elemental impurity analysis by ICP-MS. The Agilent 7850 ICP-MS proved to be ideal for the determination of trace elemental impurities in pharmaceutical ingredients. The 7850 ICP-MS produced excellent results in terms of sensitivity, stability, robustness, recovery, and detection limits for all the required elements.

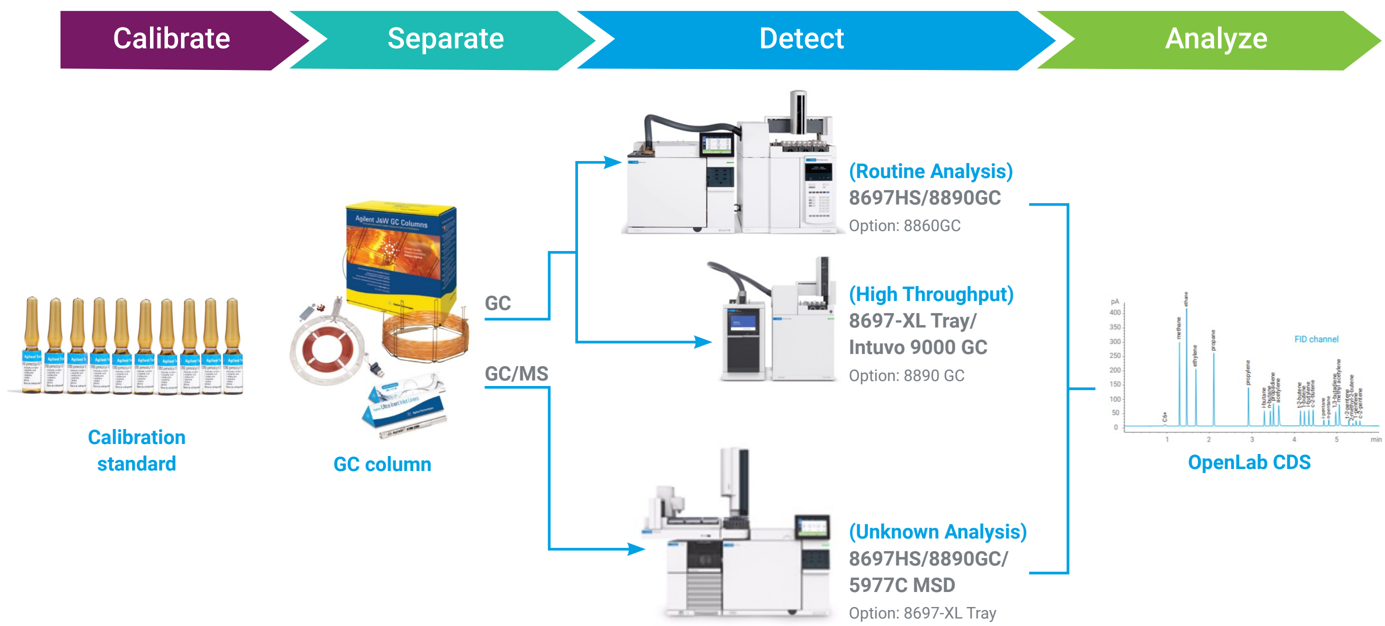


Workflow 3: Trace elemental impurity analysis by ICP-MS.

Residual solvent analysis by GC or GC/MS

Similarly, residual solvents in pharmaceutical drug products do not enhance the product's efficacy and must be removed as much as possible to meet product specification and good manufacturing practices.

Agilent offers the most comprehensive portfolio of flexible and reliable solutions for residual solvent analysis by gas chromatography (GC) or GC and mass spectrometry (GC/MS). No matter what kind of residual solvent analysis (routine, high-throughput, or unknown) you are working on, the Agilent 8890 GC system, equipped with an Agilent 8697 headspace sampler and inert tee, provides an excellent method for separating, identifying, and quantifying all the relevant residual solvents outlined by USP <467>.



Workflow 4: Illustrates an Agilent workflow for residual solvent analysis by GC or GC/MS.

References

Raw material identification by Raman, FTIR, and HPLC analysis

1) Neo, A.; Welsby, C. Rapid Testing of Solvents Through Amber Bottles Using an Agilent Vaya Handheld Raman Spectrometer. Agilent Technologies application note, publication number **5994-5929EN**, 2023.
2) Pulliere, F.; Welsby, C. Identification of Commercially Available Oligonucleotide Starting Materials Directly Through Containers. Agilent Technologies application note, publication number **5994-4239EN**, 2021.
3) Pulliere, F.; Welsby, C. Differentiating Biopharmaceutical Raw Materials Using Spatially Offset Raman Spectroscopy. Agilent Technologies application note, publication number **5994-3534EN**, 2021.
4) Pulliere, F.; Welsby, C. Rapid Identification of Polysorbates 20 and 80 Directly Through Amber Bottles. Agilent Technologies application note, publication number **5994-3459EN**, 2021.
5) Pulliere, F. Rapid Identification of Raw Materials Inside Packaging. Agilent Technologies application note, publication number **5994-2936EN**, 2023.
6) Interview: Evaluating an Innovative Analytical ID Testing Strategy for Oligonucleotides. Agilent Technologies application note, publication number **5994-5144EN**, 2022.
7) **Video**: Run Your UV-Vis Thermal Melt Assay in Less Than 10 Minutes.
8) Alwan, W.; Zieschang, F. The Agilent Cary 630 FTIR Spectrometer for Material Identification Applications. Agilent Technologies application note, publication number **5994-4992EN**, 2022.

Finished product Identification by UV-Vis analysis (with the Cary 3500 UV-Vis)

1) Best Practice for Nucleic Acid Thermal Stability Measurements Using the Cary 3500 UV-Vis Spectrophotometer. Agilent Technologies white paper, publication number **5994-4028EN**, 2022.
2) Alwan, W.; Rault, M. Fast Determination of Thermal Melt Temperature of Double-Stranded Nucleic Acids by UV-Vis Spectroscopy. Agilent Technologies white paper, publication number **5994-0384EN**, 2022.
3) **Video**: Run Your UV-Vis Thermal Melt Assay in Less Than 10 Minutes. ()
4) Data Integrity Options for GMP Facilities for the Agilent Cary 3500 UV-Vis Spectrophotometer. Agilent Technologies flyer, publication number **5994-0740EN**, 2022.

Trace elemental impurity analysis by ICP-MS

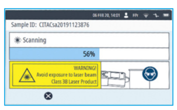
1) Analysis of Elemental Impurities in Synthetic Oligonucleotides by ICP-MS. Agilent Technologies application note, publication number **5994-6470EN**, 2023.
2) Data Integrity Options for GxP facilities: For Agilent ICP-MS and ICP-QQQ spectrometers. Agilent Technologies flyer, publication number **5994-4746EN**, 2022.
3) Sanderson, J.; Whitecotton, L. Analysis of Artificial Tear Eye Drops for Elemental Impurities. Agilent Technologies application note, publication number **5994-1561EN**, 2022.
4) Whitecotton, L.; McCurdy, E.; Jones, C.; Liba, A. Validating performance of an Agilent ICP-MS for USP <232>/<233> & ICH Q3D(R2)/Q2(R1). Agilent Technologies application note, publication number **5991-8335EN**, 2022.
5) USP <232>/<233> and ICH Q3D Elemental Impurities Analysis: The Agilent ICP-MS Solution. Agilent Technologies white paper, publication number **5991-8149EN**, 2021.
6) Measuring Elemental Impurities in Pharmaceutical Materials. Agilent Technologies brochure, publication number **5991-8140EN**, 2022.
7) Dhuria, R. S.; Jain, V.; Kapadnis, G.; Vyas, S. Determining Elemental Impurities in Pharmaceutical Ingredients using ICP-MS. Agilent Technologies application note, publication number **5991-7674EN**, 2021.
8) Jing, M.; Ni, Y.; Wang, Y.; Zhang, Z. Determination of Chromium in Gelatin Capsules Using ICP-MS. Agilent Technologies application note, publication number **5991-1531EN**, 2021.

Residual solvent analysis by GC or GC/MS

1) Sanderson, J. Residual Solvent Analysis in Hemp Consumer Products Using Headspace Gas Chromatography and Mass Spectrometry. Agilent Technologies application note, publication number **5994-5237EN**, 2022.
2) Zhang, Y.; Na, S. Analysis of USP <467> Residual Solvents of Class 1, Class 2, and Class 3 Using the Agilent 8890 GC/ FID /5977B MSD System. Agilent Technologies application note, publication number **5994-1488EN**, 2019.
3) Wieder, L.; Pan, J.; Veeneman, R. Analysis of USP Method <467> Residual Solvents on the Agilent 8890 GC System. Agilent Technologies application note, publication number **5994-0442EN**, 2019.
4) Residual Solvents Analysis Using an Agilent Intuvo 9000 GC System. Agilent Technologies application note, publication number **5991-9029EN**, 2018.

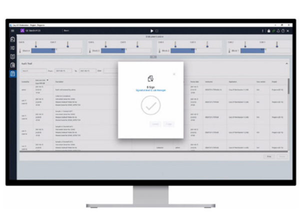
Workflow 1

Raw/starting material identification by Raman, FTIR and LC/UV analysis (Warehouse and lab-based solutions)

Bundle	**Vaya** (Warehouse based)	**FTIR** (Lab-based)	**LC/UV** (Lab-based)
Instrument	 **Vaya** (G6915A)	 **Cary 630 FTIR Spectrometer** G8043AA or G8044AA (with PC) Recommended: #200 and #320 PIKE-162-5450	 **1290 Infinity II Bio LC** G7131A or G7132A, G7137A, and G7116B, G7114B (VWD) or G7117B (DAD)
Software	 **Vaya software**	 **MicroLab** MicroLab Pharma Software G4984AA	 **OpenLab ChemStation** M8301AA M8510AA (Instrument Driver for Agilent LC) M8360AA - 3D UV (PDA) Add-on (optional)

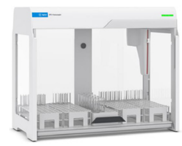
Workflow 2

Finished product identification by UV-Vis (Cary 3500) analysis

Bundle	UV-Vis	
Instrument		**Cary 3500 Multicell UV-Vis Spectrophotometer** G9874A with G9874A#001 Option: G9874A #002: Purge kit
Software		**Cary UV Workstation Software** G5194AA: Cary UV Workstation Plus or G5195AA: Cary UV Workstation Plus with PC or G6894AA: Cary UV Networked Workstation SW without PC or G6896AA: Cary UV Networked Workstation SW with PC

Workflow 3

Trace elemental impurity analysis by ICP-MS

Bundle	ICP-MS			
Sample prep		Agilent Integrated Autosampler (I-AS) (G3160C)		SPS4 Autosampler (G8415A)
Standard	USP standard (ICH Q3D and USP 232) solutions kit (5190-9771) (5 standards: 5190-9766, 5190-9767, 5190-9768, 5190-9769, 5190-9770)			
Instrument		7850 ICP-MS (G8422AA) Option: 7900 ICP-MS (G8403AA)		
Software		Agilent ICP-MS MassHunter Software G7201D		

Workflow 4A, 4B, 4C

Residual solvent analysis by GC or GC/MS

Bundle	4A – GC (Routine)	4B – GC (HT Analysis)	4C – GC/MS (Unknown)
Calibrate	USP 467 Class 1: p/n 5190-0490 USP 467 Class 2A: p/n 5190-0492 USP 467 Class 2C: p/n 5190-0493		
GC column	Headspace injection: Agilent DB-624 60 m × 0.25 mm, 1.4 µm (p/n: 122-1364) Liquid injection: Agilent DB-wax UI 30 m × 0.25 mm, 0.25 µm (p/n: 122-7032UI)		
Instrument	 8697 Headspace Sampler/8890 GC System 8697HS (G4511A) 8890GC (G3540A) Option: 8860GC (G2790A)	 **8697-XL Tray** (G4512A) Intuvo 9000 GC (G3950A) Option: 8890 GC (G3540A)	 **8697HS/8890GC/** 5977C GC/MSD **8697HS** (G4511A) **8890GC** (G3540A) Option: 8697-XL Tray (G4512A)/8890GC/5977C MSD **5977C GC/MSD Inert Plus system (G7077CA)**
Software	 OpenLab CDS Workstation Plus (M8410AA) Or: OpenLab CDS AIC (M8420AA) OpenLab CDS Instrument Connection (M8431AA)		

Learn more:
www.agilent.com/oligonucleotides

Buy online:
www.agilent.com/chem/store

Get answers to your technical questions and access resources in the Agilent Community:
community.agilent.com

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

DE71227509

Published in the USA, October 19, 2023
5994-6549EN

Agilent
Trusted Answers