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Dear Reader,

Over the past several years, biologics have continued to make up an increasing proportion of biopharmaceutical sales and pipeline candidates. Biologics now account for more than half of the top selling drugs by revenue. In fact, 2018 saw a record 17 biologics license application (BLA) approvals and the 5-year annual average has more than doubled over the last decade. This trend is expected to continue for the foreseeable future with many different modalities of biologics in preclinical and clinical development. Currently, most global pharmaceutical companies' pipelines consist of 20-40% biologics; the tipping point to a majority biologics pipeline is expected this decade. But relative to synthetic small molecule therapeutics, the manufacturing process for biologics is far more complicated and requires the use of more intense characterization, from even the earliest stages of development through production and QA/QC to ensure safety and effectiveness. While liquid chromatography/mass spectrometry (LC/MS) has always had an important role in small molecule pre-clinical animal studies and in human clinical studies, expanded use is realized both upstream into target identification and downstream into manufacturing and QC release testing with biologics.

Just some of the characterization of biologics being performed in the LC-MS lab include: intact mass, glycan analysis, peptide mapping, impurity and degradant identity, and bioanalytical assays for DMPK purposes. Beyond the capabilities of the mass spectrometer, consumables suppliers are now introducing novel sample preparation tools as well as isotopically labeled and certified reference standards for the biologics market. Specifically, for preclinical analysis of monoclonal antibodies, the introduction of immunoaffinity capture and release sample preparation kits are becoming popular. Furthermore, chromatography solvents and columns are tailored to meet the needs of large biologics. This trend includes large pore size (1000 A) chromatography columns, smaller particles and smaller internal diameter columns all designed to couple biologics separations with mass spectrometers. Improvements include improved peak shape and faster analysis. Ultra-pure LC-MS solvents have been introduced to minimize unwanted fragmentation or ionization.

My expectation is that as biologics continue towards being a majority modality in the pharmaceutical pipeline, new and unique LC-MS tools will become available for use from discovery through QC.

Sincerely yours,

Wayne K. Way

Wayne Way
Pharma QC Strategic Marketing Mgr

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BIOshell™ IgG 1000 Å C4 UHPLC Column for Improved mAb Separations

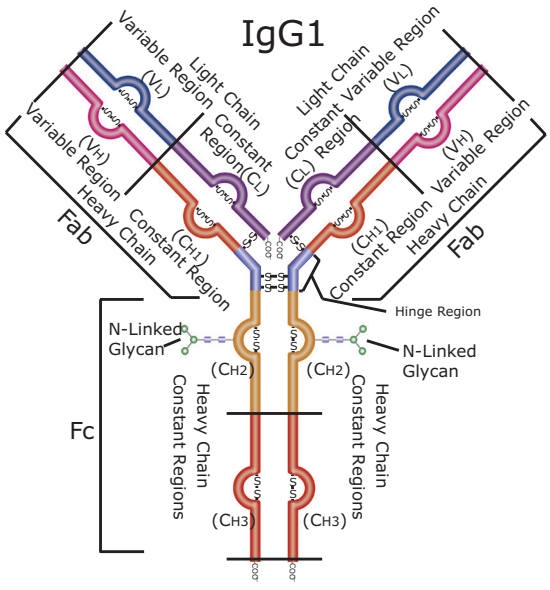
Cory E. Muraco, Senior R&D Scientist, Technology and Workflow R&D, Analytix@merckgroup.com

Introduction

Monoclonal antibodies (mAbs) are widely manufactured by many biopharmaceutical companies to treat a myriad of diseases ranging from Alzheimer's disease, Parkinson's disease, ulcerative colitis, and many types of cancers. Most recombinant therapeutic mAbs belong to the human immunoglobulin G (IgG) category among the immunoglobulin superfamily. A schematic of an IgG antibody is depicted in **Figure 1**.

A general IgG antibody is composed of two light chains (LC) that are tethered to two heavy chains (HC) through disulfide bonds. In addition, due to the fact that the LC and HC are composed of amino acids with reactive side chains, IgG's can be post-translationally modified through phosphorylation, methylation, oxidation, and nitrosylation, among other modifications. These modifications may change the binding affinity of the mAb so that it binds either the wrong antigen, does not bind any antigen, or associates with the wrong cell surface receptor. Biopharmaceutical companies need to develop rigorous methods to assess lot-to-lot reproducibility of their candidate biologic drug, and the above mentioned modifications are known as Critical Quality Attributes (CQAs) that both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) monitor. Due to these stringent requirements from regulatory bodies, much research has been pursued in the past 20 years to develop accurate, robust, and high-throughput methods to assess biopharmaceutical purity and structure.

Figure 1. Graphical Depiction of a Human IgG1 Antibody. Note the Structural Complexity of the Different Domains of the Antibody[1]



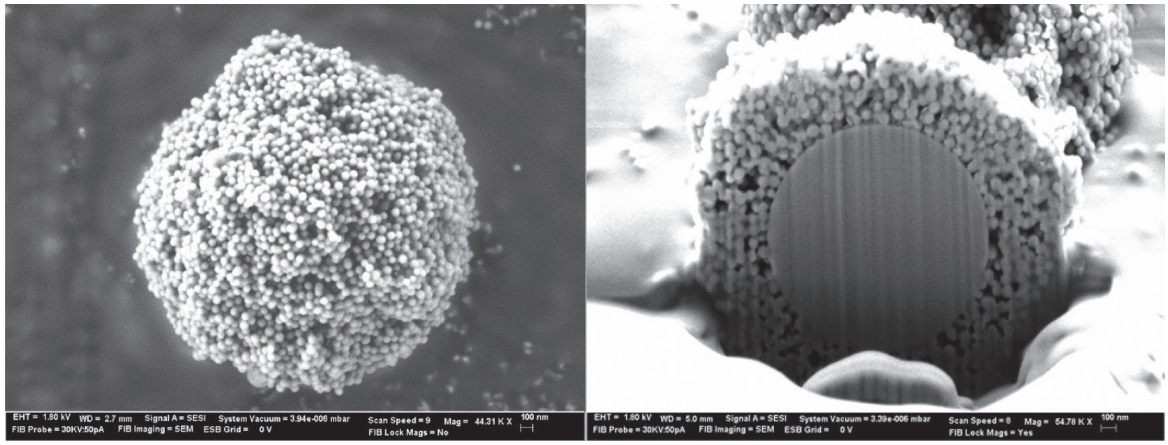
Ultra-high performance liquid chromatography (UHPLC) has emerged as a promising technique to characterize these biomacromolecules. Most biopharmaceutical R&D laboratories, as well as quality control (QC) laboratories, have ready access to this type of instrumentation. Due to the lower system dispersion, lower dead volume, and higher upper pressure limit of these instruments, biopharmaceutical companies have been able to develop methods that not only probe the finest structural details of a candidate drug, but have enabled QC labs to assay hundreds of sample in a single day.

Besides advances in UHPLC instrumentation, there have been many advances in the field of HPLC column and stationary phase development. Two main types of particle morphology are prevalent in the industry today: fully porous particles (FPPs) and superficially porous particles (SPPs, also called core-shell or Fused-Core™ particles). To take advantage of the low dispersion of UHPLC instrumentation, columns with sub-2 µm FPPs with pore sizes of 300 Å have been used to accommodate for the larger hydrodynamic radii of biomacromolecules. These columns have been the industry standard since the mid 2000's. However, these columns suffer limitations when analyzing larger or more complex proteins like mAbs and antibody-drug conjugates (ADCs). The relatively small pore size, in addition to a totally porous architecture, restricts the free diffusion of large molecules through the particle. This architecture concomitantly results in an increase in the mass transfer term of the van Deemter equation, leading to peak tailing, loss of resolution, and low recovery.

In recent years, the use of columns packed with SPPs has been in vogue, especially in the area of biologic characterization. Historically, Horvath and Kirkland pioneered the concept and initial synthetic techniques for producing SPPs in the late 1960's to early 1970's.[2,3] The past 40 years have seen a resurrection and renaissance of these particles' use in UHPLC, and now, advanced versions of these particles are available for several different application areas. These applications include small molecule pharmaceutical separations, pesticide analysis, glycan analysis, chiral separations,

(continued on next page)

Figure 2. SEM Image of the BIOshell™ IgG 1000 Å C4 SPP Particle. Left Panel Shows the Porous Outer Layer (shell) of the Particle. Right Panel is a Cutaway of the Particle, Revealing the Solid, Non-porous Silica Core. Data Courtesy of Advanced Materials Technology (AMT)



and large molecule separations. **Figure 2** shows a scanning electron microscopy (SEM) image of an SPP. Note the presence of the solid silica core in the SPP.

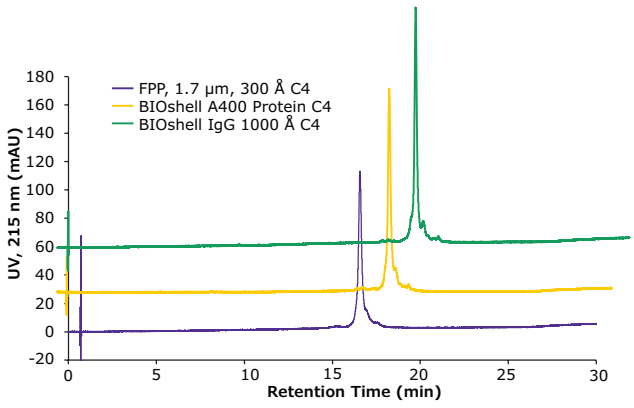
The current BIOshell™ line of columns utilizes SPP technology to provide an alternative to sub-2 µm FPPs for biomolecule separations. Recently, a new addition to the BIOshell™ line of columns has been introduced: the BIOshell™ IgG 1000 Å C4 column. This column is packed with 2.7 µm SPPs that are composed of a 0.5 µm shell thickness and a 1.7 µm solid silica core. The particle is also composed of 1000 Å pores, permitting the unrestricted analysis of mAbs, ADCs, and other, much larger, biomacromolecules. Advantages over columns packed with FPPs are numerous: the SPP shows significant advantage in mass transfer, leading to less band spreading; columns packed with SPPs are more uniformly packed than columns composed of FPPs, leading to a lower Eddy dispersion (A term) in the van Deemter equation; and larger particle sized SPPs have efficiencies similar to or better than sub-2 µm FPPs, leading to the ability of the analyst to run at higher flow rates with less risk of on-column frictional heating due to elevated column backpressure. The remainder of this article will highlight, in more detail, advantages of the BIOshell™ IgG 1000 Å C4 column as compared to commercial FPP columns for large molecule separations.

Efficiency Advantage of the BIOshell™ IgG 1000 Å C4 Column

As noted in the previous paragraph, the efficiency gain observed when comparing SPP and FPP particle architecture is due mostly to the short diffusion path within the SPP architecture, thus enhancing mass transfer. In addition, the BIOshell™ IgG column has 1000 Å pores, therefore minimizing any secondary size-exclusion effects that could lead to band broadening and loss of resolution. These concepts are illustrated in **Figure 3** in which the SigmaMAb monoclonal antibody

Figure 3. Comparisons of Chromatographic Results of the Analysis of SigmaMAb, a Recombinant IgG1 Antibody Standard

Conditions	
Column:	As indicated
Mobile Phase:	[A] 70:30 water (0.1% TFA): acetonitrile (0.1% TFA)
	[B] 50:50 water (0.1% TFA): acetonitrile (0.1% TFA)
Gradient:	0% B to 50% B in 25 min
	50% B to 100% B in 5 min
Flow Rate:	0.4 mL/min
Column Temp.:	75 °C
Detector:	UV, 215 nm
Injection:	1.0 µL
Sample:	SigmaMAb (**MSQC4**), 1 g/L, water (0.05% TFA)



standard is assayed on three columns: BIOshell™ IgG 1000 Å C4, BIOshell™ A400 Protein C4, and a 1.7 µm, 300 Å FPP packed column. Note the lower peak width, at half height, of the main antibody peak as well as improved resolution of minor variants surrounding the main antibody peak, observed on the BIOshell™ IgG column.

Figure 4. Comparison of peak widths, at half height, for a series of five monoclonal antibodies

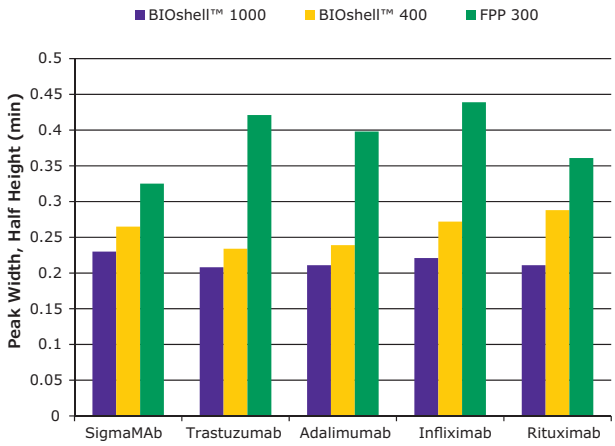


Figure 5. SigmaMAb Peak Volume Versus Flow Rate. Note How the Two Columns Packed With SPPs Have Shallow Slopes Compared to the FPP Column. Conditions Were the Same as Outlined in **Figure 3**, Except in each Case, Gradient Volume Was Held at 10 mL. Peak Volume Was Calculated as Peak Width at Half Height Multiplied by Flow Rate

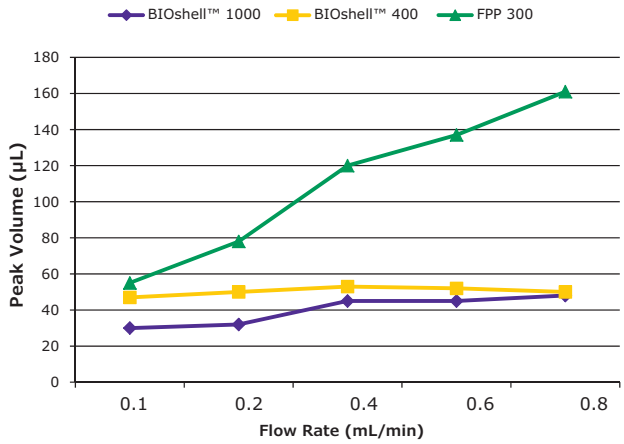
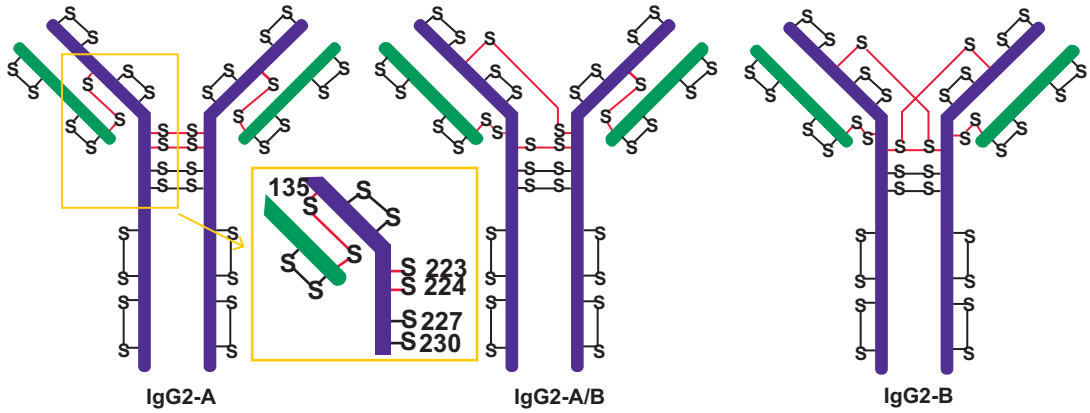


Figure 6. Major Disulfide Isoforms of IgG2 Antibodies. The Inset in the Yellow Square Shows the Amino Acid Numbers of the Cysteines in the Hinge Region. The "S" with the Red Line Connecting it Denotes Cysteines Active in Disulfide Bond Scrambling. Adapted from Reference 4



In order to ensure that this trend observed in **Figure 3** was translatable to any mAb, a series of mAbs was assayed on the same columns, using the same chromatographic method, and the peak width at half height, a measure of efficiency, was compared. **Figure 4** summarizes those results. None of the mAbs assayed generated peak widths, at half height, greater than 0.23 min on the BIOshell™ IgG 1000 Å C4 column whereas this value was consistently higher on the other two columns.

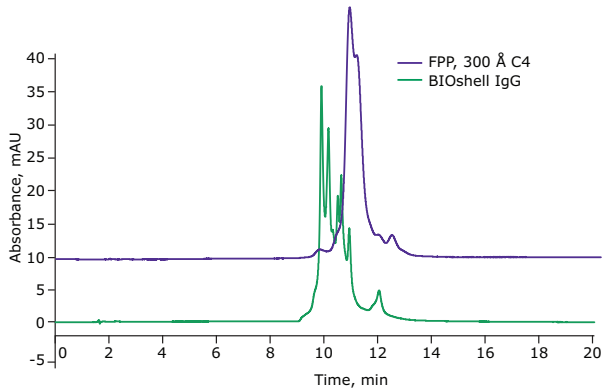
Reduced Mass Transfer Effects from the BIOshell™ IgG Column

One method to gauge the efficiency of a column operating at high flow rates is to examine the peak volume of an analyte at varying flow rates. For SPPs, the mass transfer term of the van Deemter equation is relatively unaffected by flow rate. Thus, theoretically, peak volume should show relatively little change with increasing flow rate but should change (i.e., increase) for analytes assayed with FPP-packed columns. Using SigmaMAb as the analytical probe, this investigation was conducted using the BIOshell™ IgG 1000 Å C4 column, BIOshell™ A400 Protein C4, and the FPP, C4 column used previously. **Figure 5** summarizes the results. The two BIOshell™ columns, as expected, show little change in peak volume with increasing flow rate while the FPP 300 column shows a steep increase due to the effect on the C term in the van Deemter equation.

(continued on next page)

Figure 7. Analysis of Denosumab, an IgG2 Antibody, by RPC

Conditions:	
Column:	As indicated
Mobile Phase:	[A] 88:10:2 water (0.1% DFA): acetonitrile (0.1% DFA): n-propanol (0.1% DFA)
	[B] 10: 20:70 water (0.1% DFA): acetonitrile (0.1% DFA): n-propanol (0.1% DFA)
Gradient:	14% B to 24% B in 20 min
Flow Rate:	0.2 mL/min
Column Temp.:	80 °C
Detector:	UV, 280 nm
Injection:	2.0 µL
Sample:	Denosumab, 2.0 mg/mL, water (0.1% DFA)



Resolving Cysteine Variants of IgG2 Antibodies with the BIOshell™ IgG Column

Because of their reduced effector function activity, IgG2 antibodies are becoming the favored format for some protein therapeutics. All IgG2 biologics, however, are composed of different ratios of IgG2 isoforms that only differ by the pattern of disulfide bonding in the hinge region. **Figure 6** displays some of the possible isoforms of an IgG2 antibody.[4]

Because some of these isoforms may have immunogenic effects on a patient, and to ensure lot-to-lot reproducibility of a biologic, a method is required to resolve these different variants. Recently, an analytical reversed-phase chromatography (RPC) method was developed to resolve these different variants on the BIOshell™ IgG 1000 Å C4 column. **Figure 7** compares the chromatographic results of using a column packed with FPP versus using the BIOshell™ IgG column in resolving different disulfide bond isoforms of denosumab. Notice the drastically improved resolution of disulfide bond isoforms using the BIOshell™ IgG column over the column packed with FPPs. Using this RPC method with the BIOshell™ IgG column, in combination with techniques such as redox amplification, thiol tagging, and mass spectrometry, it would be possible to identify and confirm the peaks in **Figure 7**.

Conclusion:

As the market for new drugs is slowly overtaken by biologics, the challenges in determining the purity of a new drug will be daunting. New column technology, like the BIOshell™ IgG 1000 Å C4 column, will help scientists develop new methodologies to resolve these challenges. The BIOshell™ IgG column, incorporating SPPs, allows for high speed, high efficiency separations without a drastic increase in backpressure. As regulatory agencies require biopharmaceutical companies to add more CQAs in the monitoring of new drugs, the BIOshell™ IgG column can provide a valuable addition to the analyst's tool box.

References

1. C. E. Muraco, *LC/GC North America*, **35**, 734-745 (2017)
2. C. G. Horvath, B. A. Preiss, S. R. Lipsky, *Anal. Chem.*, **39**, 1422-1428 (1967)
3. J. J. Kirland, *Anal. Chem.* **41**, 218-220 (1969)
4. L. M. Jones, W. Cui, H. Zhang, M. L. Gross, *J. Am. Soc. Mass Spectrom.* **24**, 835-842 (2013)

Featured Products

Description	Cat.No.
BIOshell™ IgG 1000 Å C4, 10 cm x 2.1 mm I.D., 2.7 µm	**63288-U**
BIOshell™ A400 Protein C4, 10 cm x 2.1 mm I.D., 3.4 µm	**66825-U**
EXP® Pre-Column Filter	**51163-U**
EXP® Pre-Column Filter Cartridges	**51164-U**
SILu™Lite SigmaMAb Universal Antibody Standard human	**MSQC4**
Water for chromatography (LC-MS Grade) LiChrosolv®	**115333**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**100029**
Trifluoroacetic acid, ≥99%, purified by redistillation, for protein sequencing*	**299537**

*Product available in North America

Related Products

BIOshell™ lgG, C4 2.7 µm Guard Cartridge 5mm x 2.1mm, Pk.3	**63291-U**
Guard Cartridge Holder, for use with Ascentis® Express & BIOshell™ Guard Columns, pk of 1	**53500-U**
Trifluoroacetic acid for protein sequence analysis	**1.08178**

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Analysis of a Bispecific Monoclonal Antibody Using Size Exclusion Chromatography and Mass Spectrometry

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Cory E. Muraco, Senior R&D Scientist, Technology and Workflow R&D, Analytix@merckgroup.com

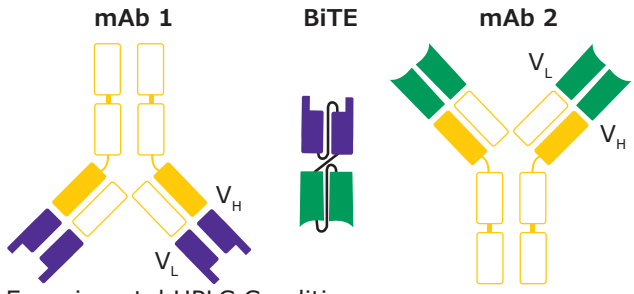
Introduction

More potent formats of monoclonal antibodies (mAbs), such as bispecific antibodies (bsAbs), are on the rise in the area of biotherapeutics. bsAbs recognize two different epitopes. This dual specificity increases the potency of these molecules compared to mAbs and expands the range of possible applications. bsAbs can be used to redirect T cells to tumor cells, block two different signaling pathways simultaneously, dually target different disease mediators, and deliver payloads to targeted sites. At this time, more than 50 bsAb products are currently undergoing clinical evaluation.[1]

Characterization of bsAbs is essential to ensuring product safety and efficacy. Size Exclusion Chromatography (SEC) coupled with Mass Spectrometry (MS) is increasingly being used to identify the accurate molecular mass of biomolecules, including bsAbs. SEC-MS, however, requires the use of mobile phases that do not contain high concentrations of non-volatile salts and the use of columns that do not exhibit column bleed, both of which will interfere with the MS signal response.

In this application, a Bispecific T cell Engager (BiTE®) consisting of two single-chain variable fragments (scFvs) recombinantly linked by a nonimmunogenic five-amino-acid chain (**Figure 1**) was analyzed by SEC-MS using a TSKgel® UP-SW3000, 2 µm column.

Figure 1. Formation of Bispecific T Cell Engager (BiTE)



Experimental HPLC Conditions

Column:	TSKgel® UP-SW3000, 30 cm x 4.6 mm, 2 µm
HPLC Instrument:	Nexera® XR UHPLC system
MS Instrument:	Q Exactive™ Plus*
Mobile phase:	20 mmol/L ammonium acetate, 10 mmol/L ammonium bicarbonate; pH 7.2
Gradient:	Isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	30 ºC
Injection vol.:	5.0 µL
Samples:	BiTE, 0.3 mg/mL (Creative Biolabs) parent mAb shown, 0.5 mg/mL (Creative Biolabs)
Ionization mode:	Electrospray ionization, positive mode
MS mode:	Scanning, m/z 800-6000

*SEC-MS analysis was performed by the Wistar Proteomics and Metabolomics Facility (*Philadelphia, PA*)

Results and Discussion

The ~55 kDa BiTE and ~150 kDa parent mAbs were injected separately onto a TSKgel UP-SW3000 column coupled to a Q Exactive Plus mass spectrometer for molar mass determination. **Figure 2** shows the (a) total ion chromatogram, (b) mass spectrum, and (c) deconvoluted mass spectrum of the BiTE. A main peak can be seen at m/z 54,143; adjacent peaks at m/z 54,181, 54,219 and 54,086 correspond to different salt adducts.

Figure 3 shows the (a) total ion chromatogram, (b) mass spectrum, and (c) deconvoluted mass spectrum of one of the parent mAbs. A main peak can be seen at m/z 149,264; adjacent peaks at m/z 149,426, and 149,592 correspond to different glycoforms. Similar results (not shown) were obtained for the other parent mAb.

These results demonstrate accurate molar mass determination for the BiTE and both parent mAbs utilizing a 20 mmol/L ammonium acetate, 10 mmol/L ammonium bicarbonate (pH 7.2) mobile phase with SEC-MS compatibility.

Prior to analysis, a blank injection was run in order to assess column bleed. **Figure 4a** shows the total ion chromatogram of a blank injection that was run on a new TSKgel UP-SW3000 column. MS data indicates that there is no column bleed from the TSKgel UP-SW3000 column prior to sample injection. Additionally, a blank injection was run between each of the sample injections in order to monitor sample carryover. **Figure 4b** shows the total ion chromatogram of a blank injection run between the BiTE and parent mAb. No evidence of carryover can be seen in the run after sample injection. The lack of column bleed and carryover indicate that the TSKgel UP-SW3000 column is suitable for use with MS.

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Figure 2. SEC-MS Analysis of the BiTE. Accurate Molar Mass of the BiTE Was Calculated to be 54.1 kDa via SEC-MS

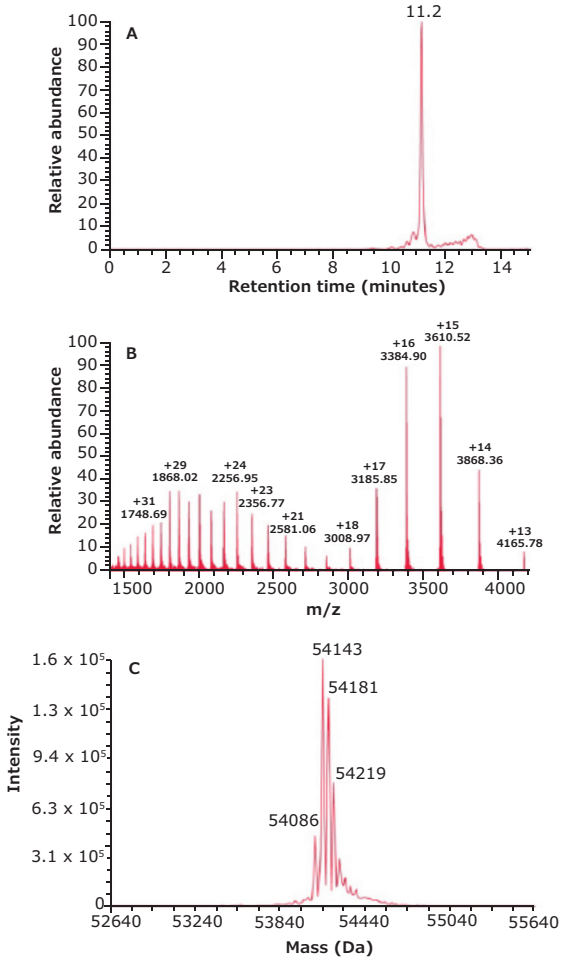
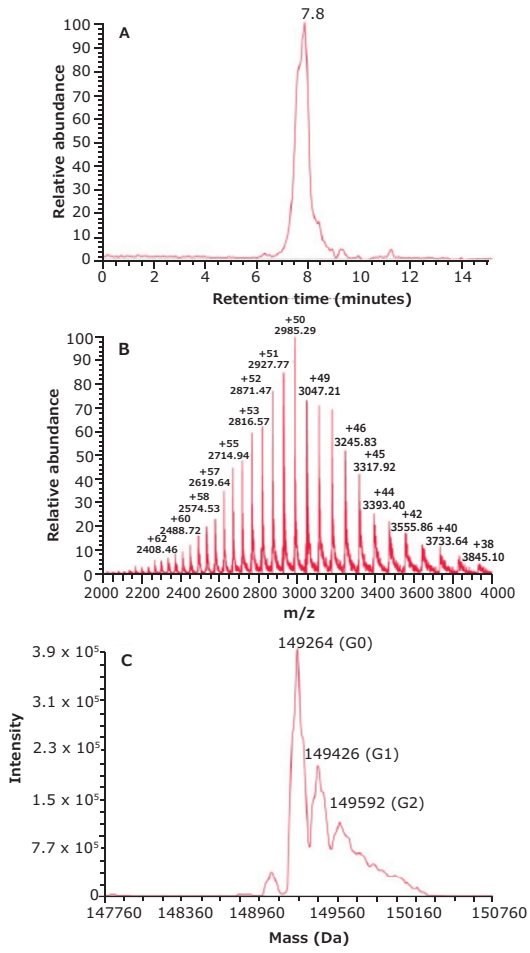


Figure 3. SEC-MS Analysis of Parent mAb. Accurate Molar Mass of the Parent mAb Was Calculated to be 149.3 kDa via SEC-MS



Conclusion

The TSKgel UP-SW3000, 2 µm SEC column can be used as a platform method for bispecific antibody accurate mass determination using SEC-MS. A MS compatible mobile phase under non-denaturing conditions was successfully used with the TSKgel UP-SW3000 column. No signs of column bleed or sample carryover, which may interfere with MS signal response, were noted with the TSKgel UP-SW3000 column.

Reference

1. Trivedi, A.; Stienen, S.; Zhu, M.; Li, H.; Yuraszeck, T.; Gibbs, J.; Heath, T.; Loberg, R.; Kasichayanula, S. *Clin. Transl. Sci.*, 10, 147-162 (2017).

Featured Products

Description	Cat.No.
TSKgel® UP SW3000, 2 µm, 4.6 mm ID × 30 cm	**80023448**
Related products	
Ammonium acetate for LC-MS LiChropur®	**5.33004**
Ammonium hydrogen carbonate for LC-MS LiChropur®	**5.33005**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**

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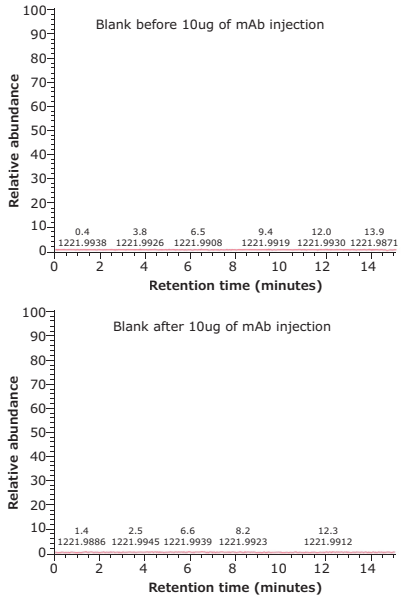
Figure 4. Column Bleed and Carryover Analysis. No Column Bleed or Carryover Was Observed via MS Total Ion Chromatogram

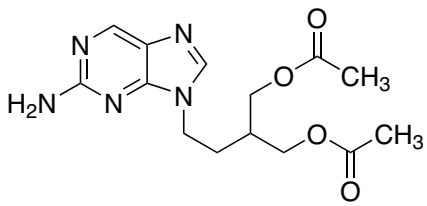


Famciclovir Tablets USP Monograph Method Using Purospher™ STAR RP-8 Endcapped HPLC Column and UV Detection

Sonal Shinde, Application Specialist, Analytix@merckgroup.com

Famciclovir is an antiviral drug indicated for the treatment of herpes zoster, herpes simplex virus 2 (genital herpes), herpes labialis (cold sores), etc. It is a guanosine analogue, a prodrug form of penciclovir, and marketed by Novartis under the trade name Famvir. Generics are produced by TEVA and Mylan, among others.

Purospher™ STAR RP-8 endcapped HPLC columns can be used to monitor organic impurities in tablet formulations following the new USP monograph for Famciclovir Tablets. The method suitability requirements are defined by the relative standard deviation (NMT 5.0% for Famciclovir standard solution) and the chromatographic resolution between propionyl famciclovir and 6-chloro famciclovir (NLT 1.2 using the system suitability solution). The method acceptance criteria is defined by the relative retention times for Famciclovir related compound A, Famciclovir related compound B, Famciclovir, 6-Chloro famciclovir, and Propionyl famciclovir and are about 0.2, 0.5, 1.0, 1.32, and 1.35, respectively. This application note illustrates with required analytical data that the method meets USP41-NF36 guidelines.



Experimental Conditions	
Column:	Purospher™ STAR RP-8 endcapped (5 µm) 250x4.6 mm (**1.51454**)
Mobile phase:	[A] 2.72 g/L of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 4.0 ±0.05. [B] Acetonitrile
Gradient:	See table
Flow rate:	1 mL/min
Pressure drop:	132-147 bar (1914-2131 psi)
Detection:	UV @ 220 nm (analytical flow cell; 10 µL)
Temperatures:	Column: 50 °C; Autosampler: 8 °C
Injection volume:	20 µL
Samples:	
Standard solution:	1 µg/mL of USP Famciclovir RS in Mobile Phase A (**Figure 1**)
SST solution:	0.5 mg/mL of USP Famciclovir System Suitability Mixture RS in Mobile Phase A (**Figure 2**)
Test solution:	Nominally 1 mg/mL of Famciclovir in Mobile Phase A prepared as follows. Transfer an amount equivalent to 250 mg of Famciclovir, from not less than (NLT) 10 finely powdered tablets, to a 250-mL volumetric flask. Add about 125 mL of mobile phase A and sonicate for 30 min with intermittent shaking. Dilute with mobile phase A to volume. (**Figure 3**)
Other samples in monograph method (not shown here)	
Peak ID solution	4 µg/mL of USP Famciclovir Related Compound A RS and 10 µg/mL of USP Famciclovir Related Compound B RS in Mobile Phase A

Gradient		
Time (min)	**A (%)**	**B (%)**
0	95	5
50	75	25
60	75	25
65	95	5
75	95	5

Figure 1. Chromatographic Data – Blank & Standard Solution

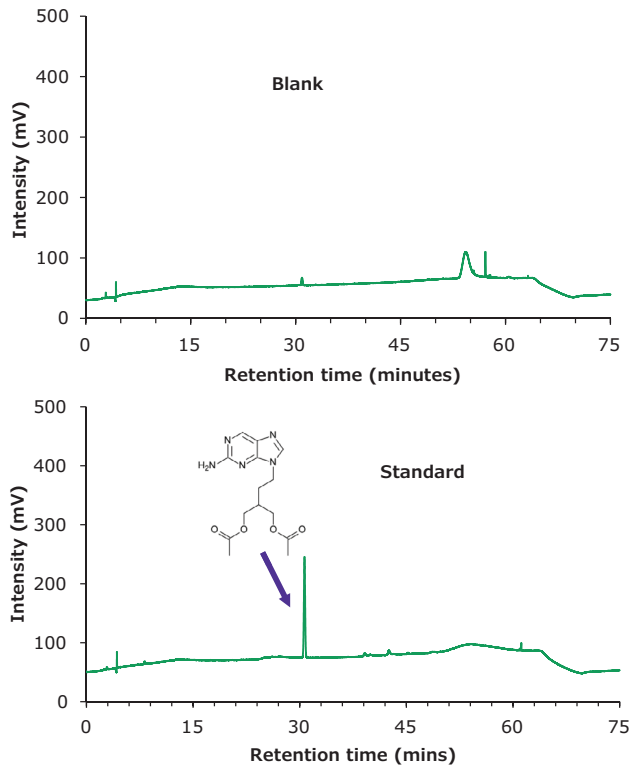


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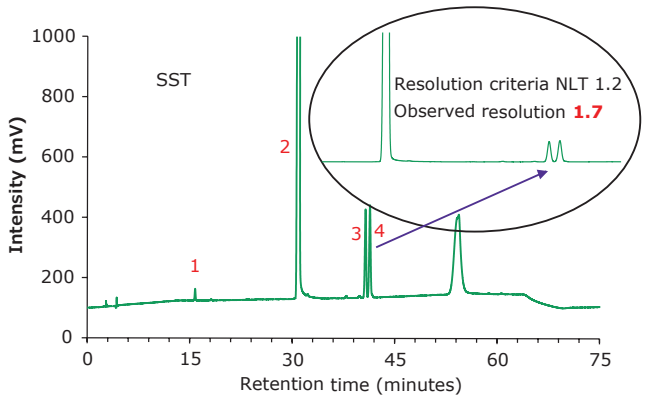
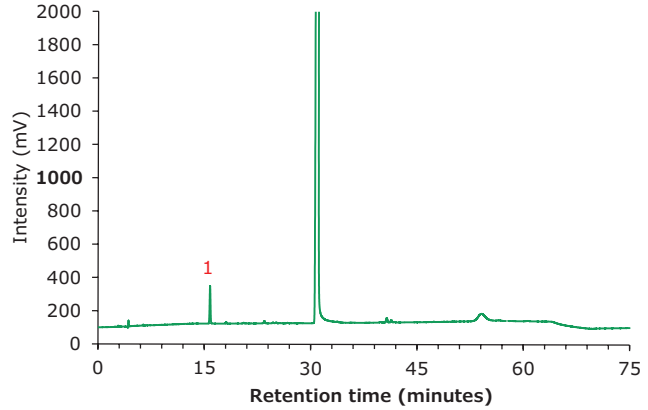
Figure 2. Chromatographic Data - System Suitability Test (SST) Solution



Peaks	Compound	Retention Time (min)	RRT	Resolution	Tailing Factor
1	Famciclovir Related compound B	15.8	0.51	-	0.99
2	Famciclovir	30.8	1.00	47.9	1.02
3	6-Chloro famciclovir	40.7	1.32	25.7	0.98
4	Propionyl famciclovir	41.4	1.34	1.7	0.98

Figure 3. Chromatographic Data - Test Solution



Peaks	Compound	Retention Time (min)	RRT	Resolution	Tailing Factor
1	Famciclovir Related compound B	15.8	0.51	0	0.99
2	Famciclovir	30.8	1.00	47.5	1.02
3	6-Chloro famciclovir	40.7	1.32	8.9	0.98
4	Propionyl famciclovir	41.4	1.34	7.7	0.98

Validation and Verification

1. Specificity: Inject solution and determine the retention time of desired analyte in the presence of other components such as impurities and excipients.

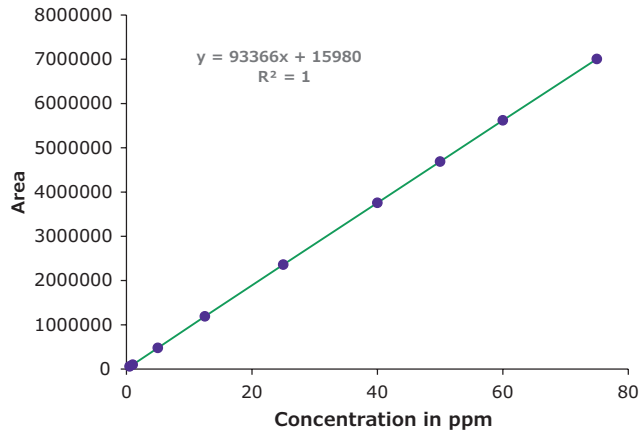
	Compound	RT (min)
1	Famciclovir Related compound B	**15.8**
2	Famciclovir	**30.9**
3	6-Chloro famciclovir	**40.7**
4	Propionyl famciclovir	**41.3**

2. Standard Repeatability (1 ppm)

Sample	Area Units
STD 1	**214,771**
STD 2	**213,539**
STD 3	**214,102**
STD 4	**214,935**
STD 5	**214,216**
Mean	**214,313**
Standard Deviation	**559**
RSD (%)	**0.3**

3. Linearity, LOD & LOQ

Famciclovir Concentration (µg/mL)	Area Units
0.5	**59,951**
1	**98,532**
5	**478,367**
12.5	**1,190,770**
25	**2,360,140**
40	**3,757,032**
50	**4,687,957**
60	**5,618,958**
75	**7,007,616**
LOD (ppm)	**0.3**
LOQ (ppm)	**0.9**



Featured Products

Description	Cat. No.
Purospher™ STAR RP-8 endcapped (5µm) Hibar® 250-4.6 HPLC column, 250 x 4.6 mm	**1.51454**
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur.	**1.00030**
ortho-Phosphoric acid 85 % for analysis EMSURE® ACS,ISO,Reag. Ph Eur	**1.00573**
Potassium dihydrogen phosphate for analysis EMSURE® ISO	**1.04873**
Reference Materials	
Famciclovir United States Pharmacopeia (USP) Reference Standard	**1269152**
Famciclovir Related Compound A United States Pharmacopeia (USP) Reference Standard	**1269174**
Famciclovir Related Compound B United States Pharmacopeia (USP) Reference Standard	**1269185**
Famciclovir System Suitability Mixture United States Pharmacopeia (USP) Reference Standard	**1269163**

For our complete listing of pharma reference materials including primary (compendial) standards visit us at **SigmaAldrich.com/standards-pharma**

Improve the Efficiency of Your Impurity Testing

Impurity Solution Mixtures for use in Pharmaceutical Analysis

Nick Hauser, Product Manager Reference Materials, Analytix@merckgroup.com

To ensure the safety of pharmaceutical products, active pharmaceutical ingredients (APIs) need to be tested thoroughly. The accurate detection and control of impurities in drug substances and products is an essential element of ICH and GMP requirements. Reference Materials for these impurities are often hard to find or very expensive. In pharmaceutical quality control (QC) laboratories, APIs and related substances are weighed daily or weekly to make multi-analyte stock or working level solutions. This cumbersome approach introduces the potential for inconsistencies and error, especially with difficult to handle materials.

Our new product range of pharmaceutical impurity solutions saves both cost and labor by providing all regulated impurities in one mix. These impurity solutions are manufactured as Certified Reference Materials (CRMs) according to ISO 17034 and ISO/IEC 17025 double accreditation. Accelerated stability studies are conducted under sub-freezer, freezer, refrigerated, room temperature and 40 °C conditions over several weeks to determine any degradation or interaction of the individual analytes. If necessary, individual impurities will be removed from the mixtures and manufactured as single component solutions using conditions that will fortify stability. These single component solutions are manufactured at convenient concentrations to easily be combined with the existing mixtures with minimal sample handling. Also, storage in amber ampoules under argon protects the materials from air, light, and changes in concentration.

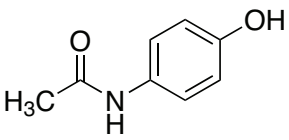
Another unique aspect of these solutions is that the individual neat materials that are used as starting materials are Secondary Pharmaceutical Reference Standards. These Secondary Standards are manufactured as Certified Reference Materials and made traceable to a corresponding Primary Compendial Standard. These products are designed to be used as stocks and/or working level solutions.

Table 1 shows the composition of the combination of Acetaminophen impurity mix **A-134**, 4′-Acetoxyacetanilide (Acetaminophen RC A) solution **A-135**, 4-Aminophenol (Acetaminophen RC K) solution **A-136**, and 4-Chloroacetanilide (Acetaminophen RC J) solution **C-166** comprising the components required for analysis according to the monograph methods. See compound structures in **Figure 1**.

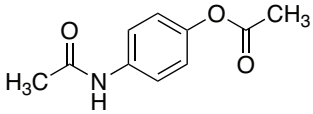
Table 1. Acetaminophen Impurity Portfolio (Combining Cat. Nos. **A-134**, **A-135**, and **A-136**).

Component	USP Related Compound (RC)	EP Impurity
Acetaminophen	-	-
4-Acetoxyacetanilde	A	H
N-(4-Hydroxy phenyl)propanamide	B	B
2-Acetamidophenol	C	A
Acetanilide	D	D
4-Nitrophenol	F	F
4-Chloroacetanilide	J	J
4-Aminophenol	-	K

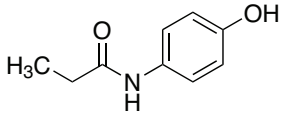
Figure 1. Chemical Structures of the Acetaminophen Impurities



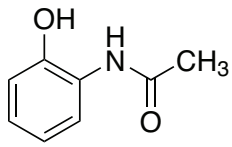
Acetaminophen



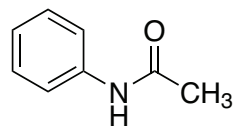
4'-Acetoxyacetanilide



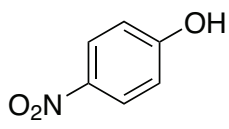
N-(4-Hydroxyphenyl)propanamide



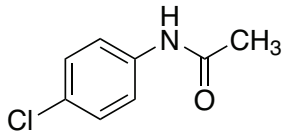
2-Acetamidophenol



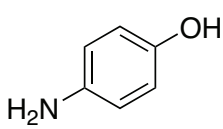
Acetanilide



4-Nitrophenol



4'-Chloroacetanilide



4-Aminophenol

(continued on next page)

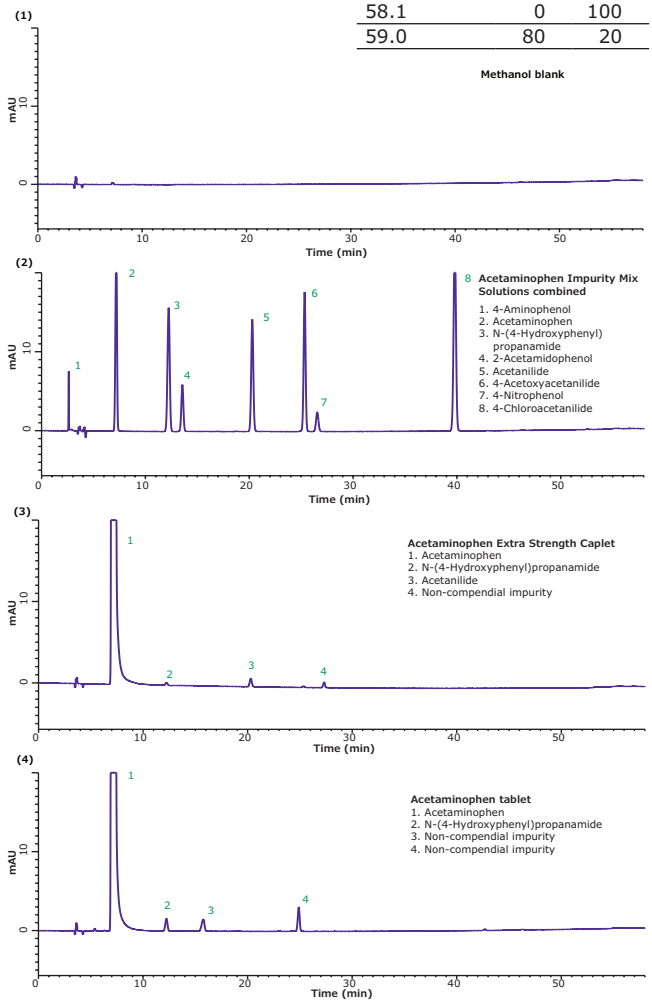
Figure 2 shows an example chromatogram of the Acetaminophen and related compound solutions (structures of components shown in **Figure 1**) combined as one sample solution solution, as well as two different tablet types (extra and regular strength) being analysed.

Figure 2. HPLC-UV Analysis of a Blank (1), the Combined Acetaminophen Impurities (Cat.Nos. A 134, A 135, A 136) (2), an Extra Strength over the Counter Tablet (3), and a Regular Strength over the Counter Tablet (4)

Column:	Discovery® C8, 25 cm x 4.6 mm I.D., 5 µm (59354-U)
Mobile Phase:	[A]: Methanol, Water, glacial acetic acid (50:950:1) [B]: Methanol, Water, glacial acetic acid (500:500:1)
Flow Rate:	0.9 mL/min
Column Temp.:	40 °C
Detector:	UV, 254 nm
Injection:	5 µL
Sample:	Blank – methanol. Mixture of Acetaminophen impurity mix (**A-134**), 4′-Acetoxyacetanilide (Acetaminophen RC A) solution (**A-135**), and 4-Aminophenol (Acetaminophen RC K) solution (**A-136**), 20.8 µg/mL each individual compound in methanol. Extra and regular strength tablets 25mg/mL in methanol.

Gradient

Time (min)	%A	%B
0.0	80	20
8.0	80	20
53.0	0	100
58.1	0	100
59.0	80	20



Pharmaceutical Impurity CRM Mixes and Solutions Currently Available* (each pack size 1 mL)

Description	Conc. (µg/mL)	Cat. No.
Acetaminophen (Paracetamol) Impurity Mix	25	**A-134**
4′-Acetoxyacetanilide (Acetaminophen RCA)	250	**A-135**
4-Aminophenol (Acetaminophen RCK)	250	**A-136**
4-Chloroacetanilide (Acetaminophen RCJ)	250	**C-166**
Aspirin Impurity Mix	100	**A-143**
Acetylsalicylic Anhydride (Aspirin Impurity F)	100	**A-155**
Bupropion Impurity Mix	100	**B-082**
Bupropion System Suitability Mix	12-20	**B-069**
Caffeine Impurity Mix	0.4-2.0	**C-175**
6-Amino-1,3-dimethyl-5-(formylamino) uracil (Caffeine Impurity B)	40	**C-194**
Caffeine Impurity E Nitrate	100	**C-195**
Chlorpheniramine Impurity Mix	50	**C-193**
Chlorpheniramine Impurity C	50	**C-197**
Fluconazole Impurity Mix	10	**F-051**
Furosemide Impurity Mix	5	**F-052**
Fluticasone Impurity Mix	20	**F-061**
Guaifenesin Impurity Mix	500-1000	**G-022**
Ibuprofen Impurity Solution	25	**I-036**
(2RS)-2-(4-butylphenyl)propanoic acid (Ibuprofen Impurity B)	100	**I-037**
Lidocaine Impurity Mix	20-200	**L-048**
Metformin Impurity Mix	50	**M-195**
Cyanoguanidine (Metformin Related Compound A)	50	**M-196**
Nevirapine Impurity Mix	15-30	**N-116**
Omeprazole Impurity Mix	60	**O-048**
Parabens Impurity Mix	50	**P-125**
Pyridoxine Impurity Mix	50	**P-126**
Pramipexole Organic Impurity System Suitability Mix	15-37.5	**P-141**
Pramipexole Chiral System Suitability Solution	10	**P-140**
Riboflavin Impurity Mix	90	**R-029**
Salicylic Acid Impurity Mix	10-500	**S-100**
Thiamine Impurity Mix	100	**T-134**

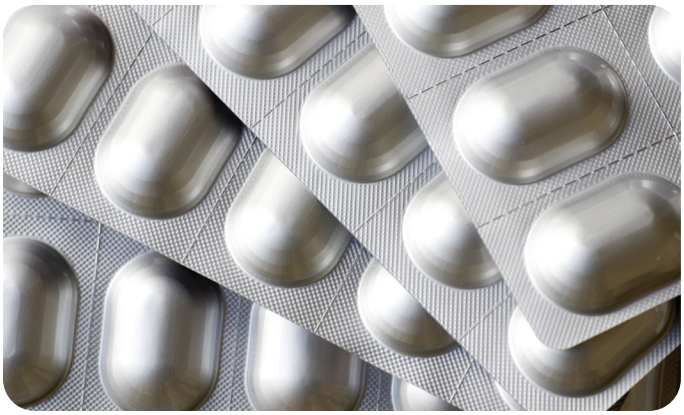
*The content of a mix (compound listing) and the CRM's solvent can be seen on the product detail page on our website.

A complete overview of all our available reference materials for pharmaceutical impurities (neats and solutions) listed by API can be found at **SigmaAldrich.com/pharma-impurity-mixture**

To see all our reference materials for Pharmacopoeias, please visit **SigmaAldrich.com/standards-pharma**

New Reference Materials for Extractables and Leachables Testing

Matthias Nold, Product Manager Reference Materials, Analytix@merckgroup.com



Extractables and Leachables (E&L) are chemical compounds with the potential to migrate into pharmaceutical or clinical products from packaging materials, tubing, or medical devices, resulting in patient exposure.

Manufacturers of pharmaceutical products and medical devices are obligated to perform extensive E&L studies to identify compounds that might leach into the product and, if necessary, assess the toxicity of these chemicals.

Since it is never entirely predictable which migrants may occur, it is crucial that no analytes are overlooked. Depending on the nature of the packaging material, the product, and the applied conditions, new unexpected or unknown compounds can be found. Therefore, there is no finite list of analytes to be tested for. However, there are certain monomers or additives that are very often detected in extractables and leachables studies.

To facilitate identification and quantification of extractables and leachables, we offer a comprehensive portfolio of reference materials. A list of more than 100 certified reference materials and analytical standards for commonly found extractables and leachables can be found at **SigmaAldrich.com/extractablesandleachables**

Recently we added a series of sixteen new reference materials to this range, which are listed below. These new products are provided with a NIST SRM traceable value determined by quantitative NMR (qNMR) in accordance with ISO/IEC 17025. The values are given with an uncertainty which takes into account the stability and homogeneity of the material. We have also developed certified reference material (CRM) mixes for LC and GC which cover a broad range of compound classes that are most commonly found in extractables and leachables studies. Please regularly check our website for the most recent product additions.

New Reference Materials for Extractables and Leachables Testing (Pack Size 100 mg)

Description	Synonyms/Abbreviations	CAS	Cat. No.
Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate)	Irganox 1010 (Ir 1010)	6683-19-8	**96656**
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	Irganox 1076 (Ir1076)	2082-79-3	**00318**
2-(2-Hydroxy-5-methylphenyl)benzotriazol	Dometrizol (Dome)/Tinuvin P	2440-22-4	**96697**
ε-Caprolactam	CAP	105-60-2	**01483**
Dibenzylamine	DBA	103-49-1	**95728**
2-Mercaptobenzothiazole	2-MBT	149-30-4	**96051**
Bis[4-(glycidyloxy)phenyl]methane, mixture of isomers	Bisphenol F diglycidylether (BPFE)	2095-03-6	**96142**
Bis(4-chlorophenyl) sulfone	CPS	80-07-9	**96153**
3,5-Di-tert-butyl-4-hydroxybenzyl alcohol	2,6-Di-tert-butyl-4-hydroxymethyl-phenol (DBOHP)	88-26-6	**96857**
1,3-Di-tert-butylbenzene	DBB	1014-60-4	**96659**
Oleamide	Ole	301-02-0	**96709**
cis-13-Docosenoamide	Erucamide (Eruca)	112-84-5	**01374**
Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate	Irganox 3114 (Ir3114)	27676-62-6	**96737**
Tris(2,4-di-tert-butylphenyl)phosphate	Irgafos 168-oxide	95906-11-9	**96839**
2,4-Di-tert-butylphenol		96-76-4	**00437**
2,6-Di-tert-butylphenol		128-39-2	**96852**

For a complete overview on our reference materials for pharma applications visit us at **SigmaAldrich.com/standards** and see our Pharma Industry Segment specific page

Certified Reference Materials in Solution for Quantitative NMR (qNMR)

New product line for our qNMR standards range improves user convenience

Alex Rück, Christine Hellriegel, Romana Rigger, Markus Obkircher
Matthias Nold, Product Manager Reference Materials, Analytix@merckgroup.com

In recent years, quantitative NMR (qNMR) has become widely accepted as a very efficient and precise method for the quantification of organic compounds and is increasingly used in the pharmaceutical and chemical industry. Its main advantage, when compared to most other analytical techniques, is that the signal integrals are independent of the chemical structure of the compound. This opens the possibility of quantitatively comparing different compounds and thus getting quantitative values that are traceable to internationally recognized primary standards such as those from NIST or NMIJ.

With our new qNMR standard solutions we offer increased convenience to users by providing the qNMR standards already dissolved in a deuterated solvent at a certain concentration. They can be used for internal as well as for external qNMR experiments. In the case of external calibration (e.g., PULCON), no exact weighing will be required.

Our site in Buchs, Switzerland, holds an ISO/IEC 17025 and ISO 17034 (formerly ISO Guide 34) double accreditation as a manufacturer of certified reference materials for quantitative NMR since 2009. This enables us to develop our TraceCERT® product line currently consisting of 300 traceable organic CRMs covering a wide range of analytes such as pesticides, PAHs, phthalates, amino acids, natural products and many more (**SigmaAldrich.com/organiccrm**). The internal standards we use for the certification of these products are also available in our catalog. This range of calibration CRMs for qNMR includes more than 20 traceable CRMs designed for use as internal standards for ^{1}H, ^{31}P and ^{19}F qNMR.[1]

These products have all been provided in neat form to offer maximum flexibility. However, the procedure still requires some critical steps to be performed by the user. The CRM needs to be weighed and dissolved in a deuterated solvent, either directly (for external calibration) or measured together with the analyte (for internal calibration). To avoid this handling step and to add convenience for the end user, we are now launching a series of calibration solutions providing some of the most commonly used calibration standards for ^{1}H quantitative NMR as well as one for ^{31}P quantitative NMR dissolved in a deuterated solvent. The certified value is given as mass fraction (mg/g), allowing a gravimetric sample preparation, which gives the most accurate results. A certified value for the density is also provided, to enable volumetric sampling if desired.

The products are manufactured under ISO/IEC 17025 and ISO 17034 accreditation and are provided in quantities of 1 mL in sealed brown glass ampoules.

New ^{1}H qNMR Calibration Solutions

Cat. No.	Package Size	Compound	Solvent	Concentration	Chemical Shifts	Nucleus
39387	1 mL	Dimethyl terephthalate solution	DMSO-d_6	5 mg/g	8.1ppm, 3.9ppm	^{1}H
39751	1 mL	Dimethyl terephthalate solution	$CDCl_3$	5 mg/g	8.13ppm, 3.97ppm	^{1}H
76201	1 mL	Fumaric acid solution	D_2O	1 mg/g	6.5ppm	^{1}H
39606	1 mL	Calcium formate solution	D_2O	5 mg/g	8.1ppm	^{1}H
16736	1 mL	Dimethyl sulfone solution	DMSO-d_6	5 mg/g	2.98ppm	^{1}H
39457	1 mL	Benzoic acid solution	DMSO-d_6	5 mg/g	7.5ppm, 7.6ppm, 7.9ppm	^{1}H
79251	1 mL	Phosphonoacetic acid solution	D_2O	5 mg/g	15.7ppm	^{31}P

Find the entire range of qNMR standards at

SigmaAldrich.com/qnmr

References

1. Analytix 2, 2015, page 4 (SigmaAldrich.com/Analytix)

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Elemental Impurities

Certified Reference Materials for ICH Q3D, USP<232> & <2232> and Ph.Eur. 5.20

Ingrid Hayenga, Product Manager Reference Materials, Analytix@merckgroup.com

Metallic contamination in drug products, referred to as elemental impurities, may arise from several sources. They may be added intentionally in synthesis, or may be present as contaminants, (e.g., through interactions with processing equipment or by being present in components of the drug product) and are consequently detectable in the drug product. Since elemental impurities pose a risk to patient health due to toxicological effects, element impurity levels should be controlled within acceptable limits in the drug product.[1]

Evolution of ICH Q3D guidelines

In 2009 the International Conference on Harmonization (ICH) proposed the development of a new harmonized guideline to provide a global policy for limiting metal impurities in drug products and ingredients. This approach should provide clear regulatory guidance on specification limits for elemental impurities worldwide and logically should have an impact on the work of the national regulatory bodies in having transparent and comparable results.

In a step 4 version of its "Guidelines for Element Impurities" document, the ICH categorized the various elemental impurities in four different classifications which were intended to facilitate decisions during the risk assessment process:

Class 1: impurities are significantly toxic to humans and have limited or no use in the manufacture of pharmaceuticals. They can be found as impurities from commonly used materials (e.g., mined excipients). All four elements require evaluation during the risk assessment across all potential sources of elemental impurities and routes of administration.

The class 1 elements are: As, Cd, Hg, Pb.

Class 2: impurities are generally considered route-dependent human toxicants. These impurities are further divided into two sub-classes, 2A and 2B, based on their relative likelihood of occurrence in the drug product.

- Class 2A elements have relatively high probability of occurrence in the drug product and thus require risk assessment across all potential sources of elemental impurities and routes of administration (as indicated). The class 2A elements are: Co, Ni and V.
- Class 2B elements have a reduced probability of occurrence in the drug product related to their low abundance and low potential to be co-isolated with other materials. As a result, they may be excluded from the risk assessment unless they are intentionally added during the manufacture of drug substances, excipients or other components of the drug product.

Class 2B elements are: Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl.

Class 3: includes elements which have relatively low toxicity at oral administration but may require a risk assessment if applied via inhalation or parenteral routes.

Class 3 elements are: Ba, Cr, Cu, Li, Mo, Sb and Sn.

Other elements: There are some elemental impurities for which Permitted Daily Exposures (PDEs) have not been established due to their low toxicities and/or differences in regional regulations. If they are present in a drug product, they are addressed by other guidelines and/or regional regulations.

These elements are: Al, B, Ca, Fe, K, Mg, Mn, Na, W and Zn.

Evaluation of USP and EP

Up to 2010, the USP and EP proof of heavy metal contamination in drugs was obtained via a colorimetric analytical method based on the precipitation of a metal sulfide in a sample and comparing it to a lead standard (USP <231> and Ph.Eur. 2.4.8).

Based on the Guideline for Elemental Impurities (Q3D) which was published by the International Conference on Harmonization (ICH) in 2010, the USP proposed three new General Chapters covering impurity limits, analytical procedures in pharmaceutical products and raw materials, and elemental contaminants in dietary supplements.

- Chapter USP <232>, Ph.Eur. 5.20: Elemental Impurities in Pharmaceutical Products - Limits
- Chapter USP <233>: Elemental Impurities in Pharmaceutical Products – Procedures
- Chapter USP<2232>: Elemental Contaminants in Dietary Supplements

In January 2015, the USP established January 1, 2018 as the new date of applicability for General Chapters <232>, <233> and <2232>. The implementation should align with limits and timelines set down by other pharmaceutical and medical agencies such as the ICH Q3D Step 4 Guidelines for Elemental Impurities announced on December 16, 2014.

The Pharmacopoeia Europe announced in July 2014 their strategy regarding elemental impurities and the implementation of the ICH Q3D. Nearly one year later, in April 2015, they published their policy on elemental impurities and timelines for revision of general and individual texts. In August of the same year, clarification was given for products outside the scope of ICH Q3D.

The implementation of the guideline compliances should start in June 2016 for products with new marketing authorization, either containing new active substances or already approved substances.

Marketed products, including new mutual recognition applications of already approved substances, should comply with the Guideline from December 2017.

The implementation of the General Test 5.20 and the General Method 2.4.20 replaced the EMA guideline on metal catalysts and metal reagents by the principles of the ICH. The publication was done in the Ph.Eur. Suppl. 9.3 (implementation date January 1, 2018), having no test for elemental impurities in the individual monographs except for substances of natural origin. Given the intrinsic nature of elemental impurities in these substances, they are among the major potential sources of elemental contamination in medicinal products. The Ph.Eur. Commission has also specifically recommended keeping the different tests for elements for which no PDE limits have been established, i.e., those identified as “other elements” in the ICH Q3D guideline in individual monographs.[2]

Analytical methods

Concerning new analytical methods, ICH Q3D does not include any recommendation on instrumental methods but the following analytical procedures are suggested in USP<233> dependent on the expected concentration of the elemental impurity in the product or component:

- Parts-per-million (ppm) concentrations ICP-OES or atomic absorption
- Parts-per-billion (ppb) concentrations ICP-MS

ICH Q3D limits for elemental impurities

For a total of 24 elements, toxicity limits are specified and defined as maximum PDE levels in mg/day for the four major drug delivery categories. **Table 1** lists the PDE values in µg/day, valid for drug products with an intake of ≤10 g/day.

Table 1. Permitted Daily Exposure (PDE) for Elemental Impurities

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
As	1	15	15	2
Cd	1	5	2	2
Hg	1	30	3	1
Pb	1	5	5	5
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rb	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

Table 2 lists the elements to be considered in the risk assessment

For the new adapted USP <232> and Ph.Eur.Suppl. 9.3 chapters, we offer three *Trace*CERT® element mixes with element ratio corresponding to the oral concentrations of the ICH Q3D guideline, mix I covers class 1, 2A and some of 2B elements; mix II covers the remaining 2B class elements; mix III covers all class 3 elements.

A second series of three mixes covers the parenteral concentration ratios.

All products with their element respective concentrations (mg/L) are listed in **Table 3**.

Table 4 lists the features of the *Trace*CERT® Certified Reference Material (CRM) solutions.

(continued on next page)

Table 2. Elements to be Considered in the Risk Assessment

Element	Class	If Intentionally Added (all routes)	If not intentionally added		
			Oral	Parenteral	Inhalation
As	1	Yes	Yes	Yes	Yes
Cd	1	Yes	Yes	Yes	Yes
Hg	1	Yes	Yes	Yes	Yes
Pb	1	Yes	Yes	Yes	Yes
Co	2A	Yes	Yes	Yes	Yes
V	2A	Yes	Yes	Yes	Yes
Ni	2A	Yes	Yes	Yes	Yes
Tl	2B	Yes	No	No	No
Au	2B	Yes	No	No	No
Pd	2B	Yes	No	No	No
Ir	2B	Yes	No	No	No
Os	2B	Yes	No	No	No
Rb	2B	Yes	No	No	No
Ru	2B	Yes	No	No	No
Se	2B	Yes	No	No	No
Ag	2B	Yes	No	No	No
Pt	2B	Yes	No	No	No
Li	3	Yes	No	No	No
Sb	3	Yes	No	No	No
Ba	3	Yes	No	No	No
Mo	3	Yes	No	No	No
Cu	3	Yes	No	No	No
Sn	3	Yes	No	No	No
Cr	3	Yes	No	No	No

Table 4. Features of the TraceCERT® CRMs

*Trace*CERT® Solutions
Unique level of accuracy and lot-specific value
Produced according to ISO Guide 34 and analyzed in our ISO/IEC 17025 accredited lab; traceable to at least two independent references (NIST, BAM or SI unit kg)
Sophisticated packaging and comprehensive documentation including proper uncertainty calculation, expiry date and storage information
Packaged in opaque and gas-tight aluminum foil bags for extended stability. Certificates are included and list up to 70 trace impurities for the *Trace*CERT® products.
250 mL package size*

For more information and to view sample certificates, please visit
SigmaAldrich.com/inorganiccrm

References:

1. ICH Q3D limits from Step 4 version, December 16, 2014 Option 1
2. Thermo Fischer, the Medicine Maker, Edition 4 – August 2016100

Table 3. Suitable Multi-Element CRM Solutions According to ICH Q3D

Element	Class	*Trace*CERT® Elemental Impurities Mix according to ICH Q3D oral			*Trace*CERT® Elemental Impurities Mix according to ICH Q3D parenteral		
		Standard 1	Standard 2	Standard 3	Standard 1	Standard 2	Standard 3
		Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.
		19041	73108	69729	89118	89922	07368
		In 12% HNO_3	In 10% HCl	In 5% HNO_3 & HF<0.5%	In 12% HNO_3	In 10% HCl	In 5% HNO_3 & <0.5% HF
Ag	2B	150 mg/L			10 mg/L		
As	1	15 mg/L			15 mg/L		
Au	2B		100 mg/L			100 mg/L	
Ba	3			140 mg/L			70 mg/L
Cd	1	5 mg/L			2 mg/L		
Co	2A	50 mg/L			5 mg/L		
Cr	3			1100 mg/L			110 mg/L
Cu	3			300 mg/L			30 mg/L
Hg	1	30 mg/L			3 mg/L		
Ir	2B		100 mg/L			10 mg/L	
Li	3			55 mg/L			25 mg/L
Mo	3			300 mg/L			150 mg/L
Ni	2A	200 mg/L			20 mg/L		
Os	2B		100 mg/L			10 mg/L	
Pb	1	5 mg/L			5 mg/L		
Pd	2B		100 mg/L			10 mg/L	
Pt	2B		100 mg/L			10 mg/L	
Rh	2B		100 mg/L			10 mg/L	
Ru	2B		100 mg/L			10 mg/L	

Titration Goes Digital

3S Reagents - Titration Made Safer and More Reliable

Bettina Straub-Jubb, Product Manager Titration, Analytix@merckgroup.com

3S afe mart ecure

3S Reagents - Safe, Smart, Secure

For volumetric titration with OMNIS titration platform by Metrohm

Closed end-to-end solution for chemical consumables: 3S Adapter Technology connects reagents to instrument with automated data transfer via RFID technology

Benefits

- Ready-to-use volumetric solutions sealed with 3S cap
- Unique opening mechanism with originality seal
- RFID chip on the cap with complete information about reagent in the bottle
- Absorber cartridge integrated in the cap
- Aspiration tube already integrated in the bottle

Safe bottle opening

Enhanced Safety - Minimized risk of contamination through unique opening mechanism

Smart data transfer

Enhanced ease of use - Automated communication between reagent and instrument software

Secure data transfer

Enhanced quality - Data transfer via RFID technology eliminates transcription errors

Description	Pack size	Cat. No.
Cerium(IV) sulfate solution 0.1 mol/L for 3S adapter technology	1 L	**1.50693.1000**
Hydrochloric acid solution 0.1 mol/L for 3S adapter technology	1 L	**1.50677.1000**
Hydrochloric acid solution 1 mol/L for 3S adapter technology	1 L	**1.50696.1000**
Iodine solution 0.05 mol/L for 3S adapter technology	1 L	**1.50690.1000**
Perchloric acid solution 0.1 mol/L in glacial acetic acid for 3S adapter technology	1 L	**1.50695.1000**
Silver nitrate solution 0.1 mol/L for 3S adapter technology	1 L	**1.50689.1000**
Sodium hydroxide solution 0.1 mol/L for 3S adapter technology	1 L	**1.50705.1000**
Sodium hydroxide solution 1 mol/L for 3S adapter technology	1 L	**1.50706.1000**
Sodium thiosulfate solution 0.1 mol/L for 3S adapter technology	1 L	**1.50691.1000**
Sulfuric acid solution 0.25 mol/L for 3S adapter technology	1 L	**1.50692.1000**
Titriplex III solution 0.1 mol/L for 3S adapter technology	1 L	**1.50711.1000**

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Titration Goes Digital

SmartChemicals Improve Titration Data Integrity & Efficiency

Bettina Straub-Jubb, Product Manager Titration, Analytix@merckgroup.com

Titration Goes Digital - a joint project

In a joint project, METTLER TOLEDO, Switzerland, known as a manufacturer of high-end titration instruments, and Merck KGaA, Darmstadt, Germany, known as a producer of highest-quality analytical reagents and certified reference materials, have together developed this new titration technology to improve data integrity. With combined expert knowledge, we created a user-friendly and easy to use tool for transferring data wirelessly from the reagents to the titrator to open a new page of titration.

Introduction

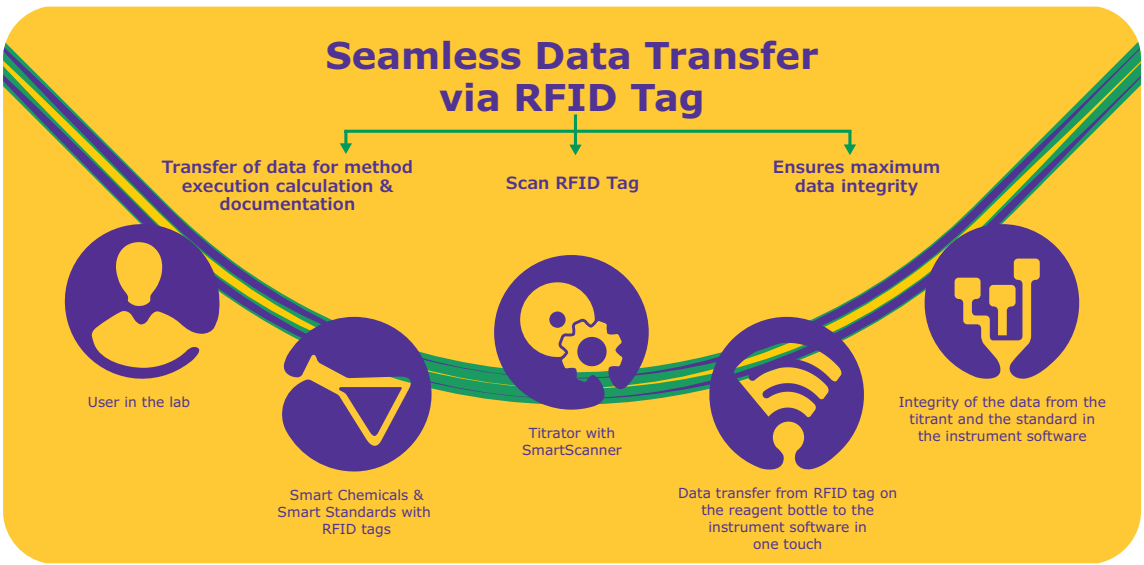
This new technology using SmartChemicals in your titration process can secure your data transfer. Additionally, it saves time by eliminating manual data transmission.

Certificates of analysis often need to be downloaded and data subsequently copied manually to the titrator software for the correct calculation of the results and for documentation. This process is time consuming and creates sources of errors - such as typing mistakes.

The use of SmartChemicals eliminates these time-consuming steps and the potential for human errors by transferring all data wirelessly and instantly to the titrator software (see below). Volumetric solutions, Karl Fischer titrants and all standards are embedded with an RFID (Radio Frequency Identification) tag bearing all relevant information from the Certificate of Analysis.

Table 1 lists the information stored on the RFID tag for SmartTitrants and SmartStandards. Just a brief touch of the titrator's SmartReader on the SmartChemical tag on the bottle (**Figure 1**) conveys all relevant data to the titrator software, saving time, reducing errors and ensuring maximum data integrity.

Secure data integrity in the titration process was never so easy



(continued on next page)

RFID tag on the SmartChemical label contains all relevant data

All data needed for execution of the method, result calculation and documentation, such as product name, catalog number, lot/batch number, concentration/ purity, date of release, shelf life/expiry date, are stored on the RFID tag on the SmartChemical label (**Figure 1**) for seamless transfer to the titrator software. For standards, additional data is transferred, including molecular weight, supplier/producer name, uncertainty and compliance according to Pharmacopeias or ISO 17034 (**Table 1**).

Once the information is transferred from the RFID to the titrator, it is stored in the titration software and displayed on the touchscreen (**Figure 2 & 3**).

Figure 1. Titrator's RFID Reader and Example Label of a SmartChemical (RFID Tag Marked)

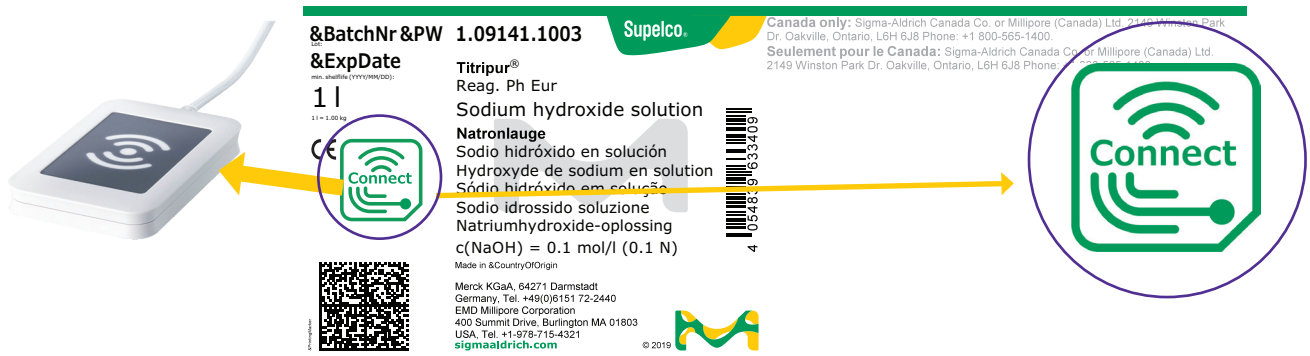


Figure 2. Information on SmartTitrant as Displayed in the Titrator Software

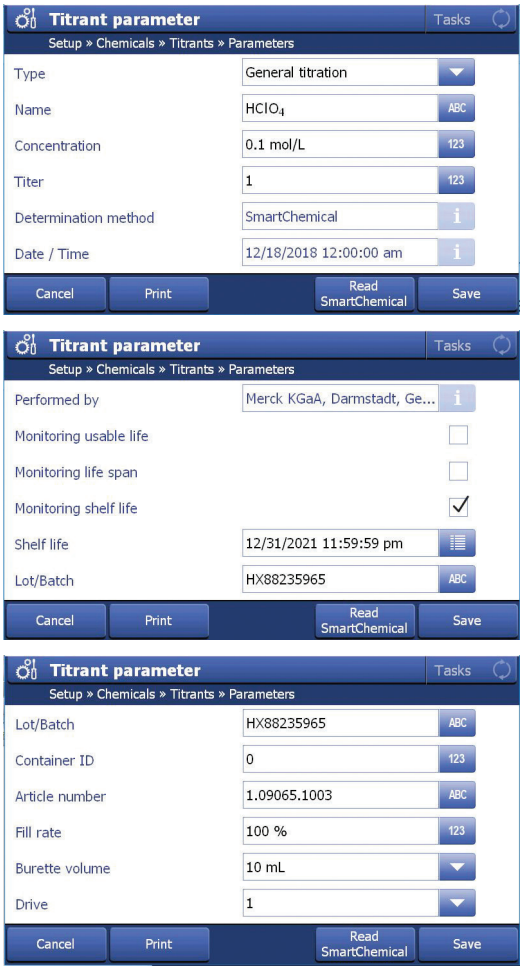
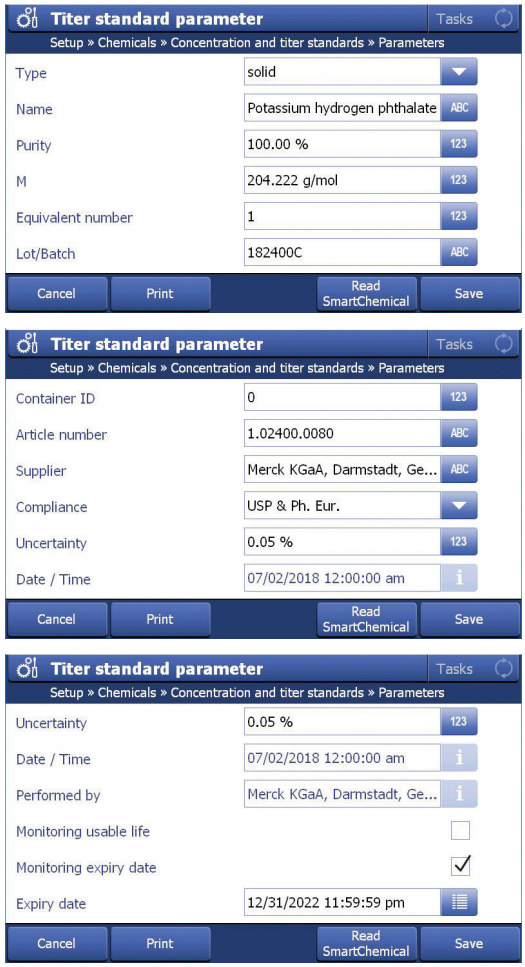


Figure 3. Information on SmartStandards as Displayed in the Titrator Software



Previously opened chemicals are also recognized, and the initial opening date is stored on the RFID tag and is shown on the instrument's touchscreen. Expired chemicals are automatically blocked from use preventing an incompliance of the measurement. In addition, user-specific guidelines regarding use period after opening can be set up and monitored by entering an individual life span into the software.

Table 1. RFID Data Content

SmartTitrant	SmartStandard
Name	Name
Concentration	Concentration/Purity
Article number	Article number
Lot/Batch no.	Lot/Batch no.
Shelf life	Expiry date
Initial opening date	Initial opening date
Date of release (Date / Time)	Date of release (Date / Time)
	Molecular weight
	Compliance
	Uncertainty

SmartChemicals are compatible with the METTLER TOLEDO Instruments:

- Excellence Titrator models T5, T7, T9
- Compact Titrator models G10S, G20S, V30S, V20S, V10S.

Conclusion

SmartChemicals with the RFID tag on the label provide

- Secure data transfer – ensures complete and correct reagent data
- Ease of use – intuitive operation and convenient data transfer in one touch
- Extended quality management – titer determination, shelf life, compliance data, initial opening date
- Improved efficiency – fast data transfer - saves time - no manual writing and no need to employ the four-eyes principle

Featured Products

Description	Cat. No.*
Titripur® Volumetric Solutions	
Perchloric acid in acetic acid 0.1 mol/L, 1 L	**1.09065.1003**
Potassium hydroxide solution in ethanol 0.5 mol/L, 1 L	**1.09114.1003**
Potassium hydroxide solution in ethanol 0.1 mol/L, 1 L	**1.09115.1003**
Hydrochloric acid solution 0.1 mol/L, 1 L	**1.09060.1003**
Sodium hydroxide solution 0.1 mol/L, 1 L	**1.09141.1003**
Sodium thiosulfate solution 0.1 mol/L, 1 L	**1.09147.1003**
Sodium thiosulfate solution 0.1 mol/L , 4 L Titripac®	**1.09147.4003**
Certipur® Volumetric Standards (CRMs)	
Potassium hydrogen phthalate, Certified Reference Material, 80 g	**1.02400.0083**
Benzoic acid, Certified Reference Material, 60 g	**1.02401.0063**
TRIS(hydroxymethyl)aminomethane, Certified Reference Material, 80 g	**1.02408.0083**
Zinc, Certified Reference Material, 100 g	**1.02409.0103**
Aquastar® Karl Fischer Titrants	
CombiTitrant 5, Karl Fischer one component reagent 5 mg H_2O/mL, 1 L	**1.88005.1003**
Titrant 5, Karl Fischer two component reagent 5 mg H_2O/mL, 1 L	**1.88010.1003**
Titrant 5, Karl Fischer two component reagent 5 mg H_2O/mL (for North America only), 1 L	**1.88010.1043**
CombiTitrant 2, Karl Fischer one component reagent 2 mgH_2O/mL, 1 L	**1.88002.1003**
CombiTitrant 2, Karl Fischer one component reagents 2 mg H_2O/mL, 1 L (for North America only)	**1.88002.1043**
Aquastar® Water Standards (CRMs)	
Water Standard 1% in ampoules, Certified Reference Material, 10x8 mL	**1.88052.0013**
Water Standard 1% in ampoules (for North America only), Certified Reference Material, 10x8 mL	**1.88052.0313**
Water Standard 0.1 % in ampoules, Certified Reference Materials, 10x8 mL	**1.88051.0013**
Water Standard 0.1 % in ampoules (for North America only), Certified Reference Material, 10x8 mL	**1.88051.0313**

*The catalog numbers for our SmartChemicals are the same as the existing products, only the last digit has been changed from a 0 to a 3 e.g. 1090651000 becomes 1090651003 for the SmartChemical with an RFID tag.

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