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Analytix Reporter

Titration 4.0

SmartChemicals Improve Data Integrity & Efficiency

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Analytical Products

ISSUE 6 | 2019

Analytix Reporter

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Dear Reader,

In the fast-paced world of routine lab testing, increasing pressure is placed on chemists and technicians to analyze more samples in less time, and often with fewer personnel. Analytical processes and methods therefore need to be streamlined to improve efficiency. Easy, secure and reliable transfer of the data, required for method execution and documentation, is becoming more important, also to prevent human errors. Digitalization and connectivity are the key phrases often used to describe the demand for fast and seamless access to the required data, and the transfer of data should be simple and smart.

At Merck, we are addressing these challenges in multiple ways. On a broader scale, we implemented a 2D matrix barcode for our chemicals and reagents, allowing users to scan a barcode to retrieve product data from the Certificate of Analysis, along with up-to-date product safety information from the SDS, using a smart phone app or a barcode reader, for example. For more details, visit **SigmaAldrich.com/2DBarcode**.

Chemicals with smart labels take us a step further to streamlining the analytical process in the lab by transferring reagent and reference standard data directly to your analytical instrument. We have introduced titration reagents and reference materials with an RFID tag that carries all the data needed to perform your analyses, such as product/catalog number, lot/batch number, concentration and shelf life. The tag is readable via an RFID reader on the instrument and the data is transferred wirelessly to the instrument's software. Truly "Titration Goes Digital". This new technology prevents transcription errors for more efficient, faster and reliable analytical results and records.

In 2018, we introduced our "3S" reagents for volumetric titration (Safe, Smart, Secure) with a special cap containing an RFID chip for data transfer to the Metrohm Omnis systems (**SigmaAldrich.com/3S**). This year we released the new line of "SmartChemicals" encompassing reagents and reference materials for the METTLER TOLEDO titrators with an RFID tag on the label (**SigmaAldrich.com/SmartChemicals**).

We will continue to extend these product lines to make your daily analytical work safer, easier and more efficient, as well as providing support to ensure you achieve the most accurate results.

We hope you find our lead article on this interesting.

Sincerely yours,

Andrea Ziegler

Andrea Ziegler
Head of Instrumental Analysis Workflows

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Titration 4.0

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

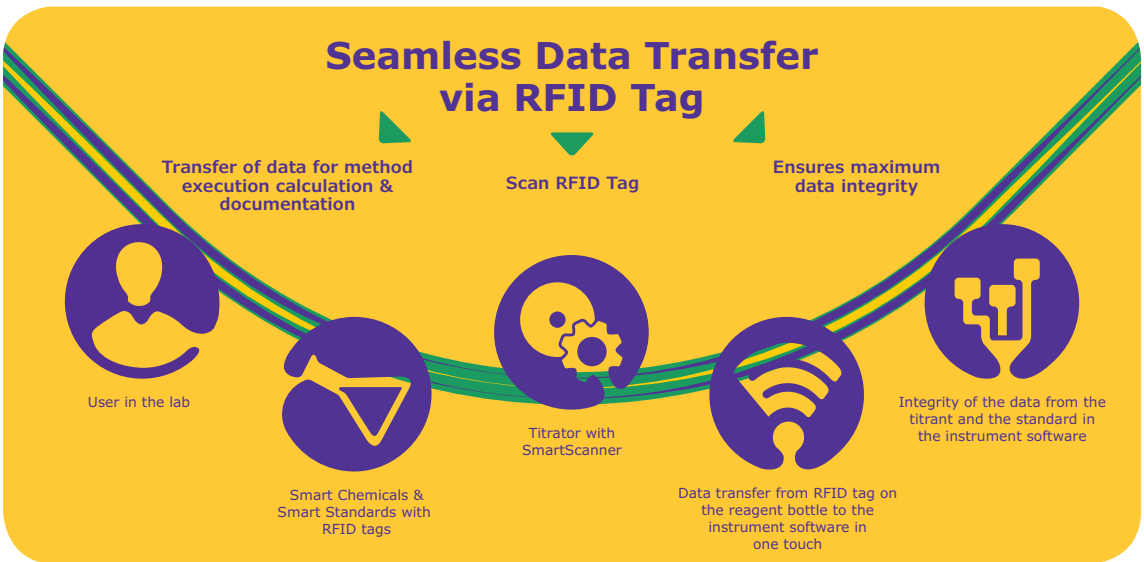
This new technology using SmartChemicals in your titration process can secure your data transfer. Additionally, it saves time by eliminating manual data transmission.

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



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).

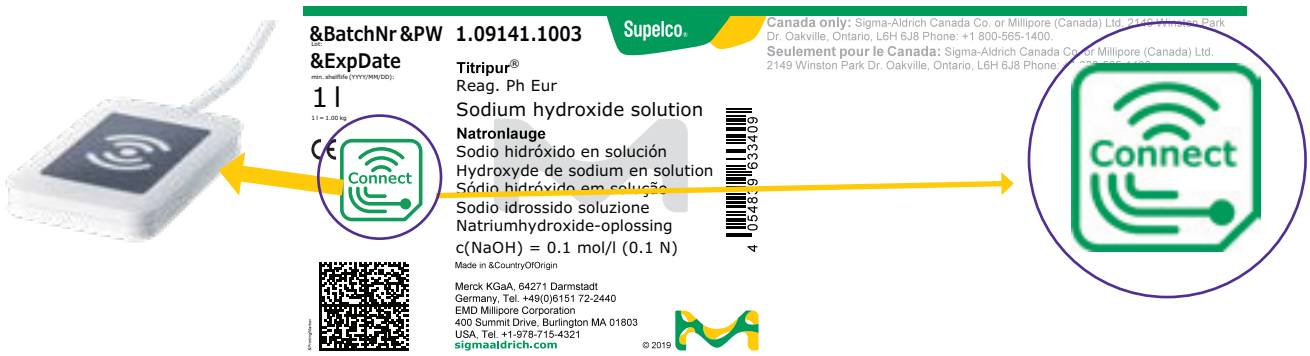


Figure 2. Information on SmartTitrant as displayed in the titrator software

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.

More products will follow in 2019. For an up-to-date listing, visit us at
SigmaAldrich.com/SmartChemicals

For our complete portfolio of titration products visit us at
SigmaAldrich.com/Titration

SCIENCE & TECHNOLOGY INNOVATIONS

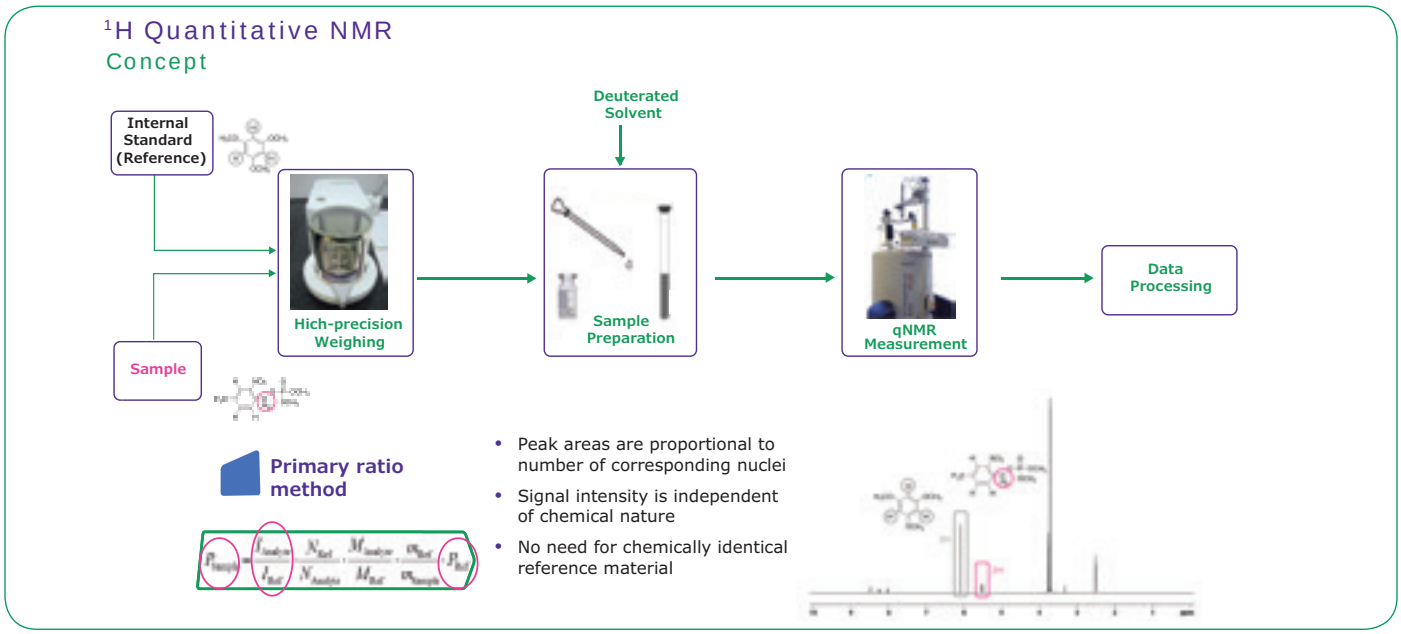
Quality Assurance for qNMR Supported by Reference Materials and Proficiency Testing Schemes

Participate in a Proficiency Testing Quick Turn Study to Show Your Competence in qNMR Measurements

Jennifer Duhon, Product Manager Proficiency Testing,
Kathrin Breitruck, Senior R&D Scientist, Analytix@merckgroup.com

Quantitative NMR (qNMR) spectroscopy has evolved to become one of the most important tools for content determination of organic substances and for the quantitative evaluation of impurities in various industries such as chemistry, food and pharmacy. In NMR spectra, signal integrals are independent of the compound's chemical nature. This opens the possibility to compare different compounds quantitatively by mixing an analyte with an internal standard (e.g. a CRM or a primary standard from a National Metrology Institute, NMI), and dissolve them in deuterated solvent to conduct a quantitative NMR experiment. The laboratory then gets a quantitative result that is traceable to internationally recognized primary material, e.g. from NIST or NMIJ. Furthermore, typical measurement uncertainties which can be achieved are very small, for example in the range of 0.2% (k=2) for pure substances.

While there is a comprehensive portfolio of certified reference materials (CRMs) available for qNMR calibration (see **SigmaAldrich.com/qnmr**) up to now, there have only been limited possibilities for laboratories to participate in proficiency testing (PT) schemes. We therefore developed a PT material for performance evaluation with ≥90% content of the main component. This covers a typical range in the content determination of pure neat materials. It is set up as a quick-turn study material, which means that it can be ordered any time. The laboratory does not have to wait for a Proficiency Testing campaign to start. The analytical result can be submitted online by the user, and the PT result will be provided shortly thereafter. The material is available worldwide to ensure a better comparability of all the labs performing qNMR is made possible.

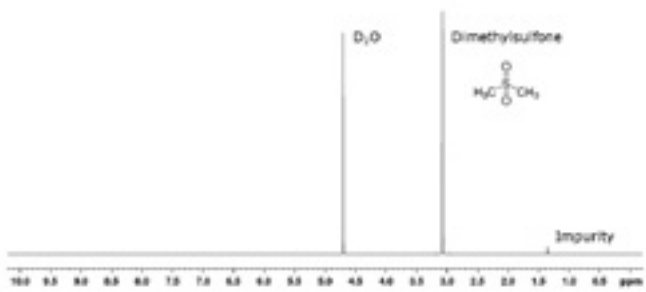


Our Laramie, WY USA site holds an ISO/IEC 17043, ISO/IEC 17025 and ISO 17034 (formerly ISO Guide 34) triple accreditation as a proficiency testing provider, testing laboratory, and manufacturer of certified reference materials. In addition, several other sites within Merck KGaA, Darmstadt, Germany hold the double accreditation ISO/IEC 17025 and ISO 17034 and can therefore produce Certified Reference Materials of the highest quality, which can also be employed in proficiency testing schemes. In addition to the comprehensive portfolio of certified reference materials, a large offering for proficiency testing can be found online on **SigmaAldrich.com/pt**

Process of this qNMR PT study:

1. Place your order for Cat.No. **PE5000**-100MG at **SigmaAldrich.com**.
2. If this is your first PT order with us, you will be contacted by a customer service representative to help you get an account set up on our online PT Portal (**Merck-pt.com**).
3. You will receive your PT sample and a reporting packet that gives sample preparation and reporting instructions.
4. The sample contains Dimethylsulfone ($C_2H_6O_2S$, molecular mass 94.13 g/mol) as main component, and the user has to determine the mass fraction value by quantitative ^1H NMR.
5. You will have 45 days to complete your analysis and submit your results to the PT Portal.
6. Your qNMR evaluation report will then be emailed directly to you containing information on the performance of your analytical quality for qNMR testing.

^1H-NMR spectrum of **PE5000** (BCBX6075) in D_2O, measured on a 600 MHz NMR spectrometer



For any questions, please contact:

PTService@merckgroup.com

Featured Products

Description	Cat. No.
Dimethyl Sulfone – qNMR proficiency testing material, 100mg	**PE5000**

For an overview on our complete offer for

- proficiency testing, visit **SigmaAldrich.com/pt**
- qNMR reference materials, visit **SigmaAldrich.com/qnmr**

What is Proficiency Testing?

Proficiency Testing (PT) or Interlaboratory Comparison (ILC) is the name defined by the International Standards Organization (ISO) for a procedure that is used as a tool for external quality assessment. In simple terms, Proficiency Testing comprises a sample sent to a group of laboratories for measurement. The labs know what might be in the sample, but they don't know exactly the content or the concentration. Their results are compared with a known reference value or against the mean value of the study. A performance score is assigned to each laboratory to show how closely their result matches with the target value.

The most common use of proficiency testing is to demonstrate to a regulatory or accreditation body that a laboratory is capable and competent to perform a specific analytical test or to apply a specific technology. This is demanded by the ISO/IEC 17025:2017 as a part of the program to ensure the validity of results. Another and sometimes overlooked benefit of proficiency testing is its use as a critical tool for quality assurance and continuous improvement since it can provide a true picture of a laboratory's testing quality over time.

PHARMA & BIOPHARMA

Fast Analysis of Monoclonal Antibodies Using Silica Monoliths Designed for Bioanalysis

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Benjamin Peters, Lab Head Instrumental Analytics R&D, Analytix@merckgroup.com

Introduction

In the last two decades, the pharmaceutical market has changed dramatically from small molecules to protein-based drugs and antibodies, which have a higher potential for targeting extraneous substances.[1] This is supported by the fact that six of the top ten best-selling pharmaceuticals are based on monoclonal antibodies (mAbs). In comparison to chemical entities, the analysis of mAbs is complex and requires several separation techniques, e.g., affinity chromatography, reversed phase chromatography, size exclusion chromatography and ion-exchange chromatography. In particular, the strong growth in development of biosimilars due to expiring patents of those blockbusters, led to increased requirements concerning column performance in HPLC.[2]

The analysis of biomolecules using conventional HPLC columns is usually associated with limited accessibility of surface for larger molecules, slow diffusion leading to extremely broad peaks with severe tailing, and possible conformational changes during elution. One of the reasons for those observations is the mesopore size ranging from 8-15 nm (80-150 Å). The separation quality could be improved by using column materials with wider mesopores of ca. 30 nm (300 Å) or larger.[3-5]

During product development, process monitoring, and quality control testing of mAbs, fast and precise analytical separations are necessary. The most important technique for determination of mAb titer is affinity chromatography using protein A. Protein A, a cell wall protein from *Staphylococcus aureus*, was the first isolated protein with a high affinity to the Fc region of immunoglobulin G (IgG).[6] Its high selectivity and high resistance to temperature, pH and tryptic cleavage make protein A a powerful tool in antibody purification.[7] Today, the use of protein A as a ligand on chromatographic media is one the most commonly employed methods for antibody capture during the purification process. In comparison to process protein A media, only a few vendors provide analytical protein A columns for process monitoring and quality control. Most of these columns are packed with particles limited in column backpressure, chromatographic performance, and application for matrix rich samples, such as harvested cell culture fluids. As a consequence, sample preparation is more complex and the limited column backpressure results in long analysis time due to lower flow rates.

Silica monoliths, which consist of one continuous piece of silica, are an alternative to fully porous or superficially porous particles, and are preferred for the application and fast separation of matrix-rich ("dirty") samples due to their bimodal pore structure consisting of large through pores (macropores) and mesopores.[8, 9] They are prepared according to a sol-gel process leading to a bimodal pore structure where both macropore and mesopore formation can be controlled individually.[9, 10] The design of larger macropores offers good flow characteristics resulting in much lower column backpressure as compared to columns packed with particles, and maintaining column performance even at higher flow rates.[11-13] Due to the hydrodynamic volume of mAbs (mostly 5-6 nm), the alteration of mesopore development up to a minimum of 30 nm (300 Å) is necessary to allow them to enter the pores for improved chromatographic separation and to prevent size exclusion effects. Recently, the development of silica monoliths containing larger mesopores, and their application for the analysis of biomolecules, has been reported,[14] leading to the now commercially available Chromolith® WP 300 column family.

Another sector in chromatography gaining more and more interest is the coupling of desired ligands for specific interactions with certain analytes, e.g., antibodies, aptamers or enzymes.[15-20] The majority of described protein immobilizations were performed on polymeric particles or monoliths and silica particles. In the last decade, silica-based monoliths were also used for the immobilization of several ligands to enable their use in the applications of affinity chromatography, for chiral separations, or as on-column bioreactors.[21-26] The development of wide pore silica monoliths enhanced the column properties for the immobilization and analysis of larger molecules. In this study, recombinant

staphylococcal protein A (rSPA) was immobilized onto a silica monolith containing larger mesopores suitable for the separation of mAbs. The analysis performed on the monolithic column was characterized by different parameters including detector response linearity, reproducibility and long-term stability.

Experimental

Chemicals

See the reagents & chemicals listing at the end of this article. In addition, native recombinant staphylococcal protein A (rSPA) ligand was purchased from Repligen (Waltham, MA, USA), pure cetuximab stock solution was a research sample from Merck KGaA (Darmstadt, Germany), and gammanorm IgG was obtained from Octapharma (Heidelberg, Germany).

Epoxy-modified wide pore silica monolith (25 x 4.6 mm) columns were prepared as research samples at Merck KGaA, Darmstadt, Germany (now available as Chromolith® WP 300 Epoxy).

Apparatus

All modification and cleaning steps necessary for the immobilization of rSPA ligand on epoxy-modified columns were performed using a Merck-Hitachi L-6200 HPLC Pump (Darmstadt, Germany). The following chromatographic studies were performed on an Ultimate 3000 HPLC system consisting of LPG-3400RS HPLC pump, WPS-3000TRS autosampler, TCC-3000RS column oven compartment, and VWD-3000 UV detector from Dionex (Germering, Germany).

Preparation of rSPA silica monoliths

For this work a research prototype of Chromolith® WP 300 Epoxy was used, prepared following published processes.[27, 28, 22]

The immobilization of rSPA on wide pore epoxy-modified monoliths was done according to a dynamic process circulating ligand solution through the column. The rSPA ligand solution was dissolved in 6 mL immobilization buffer (50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0) resulting in a rSPA concentration of 2 mg/mL. Before immobilization, columns were equilibrated with 50 mL immobilization buffer. The protein solution was circulated at a flow rate of 0.2 mL/min for 4 hours. Finally, the rSPA silica monolith was washed with 50 mL of 100 mM sodium phosphate buffer pH 7.4 and remaining epoxy functions were hydrolyzed with 150 mM phosphoric acid pH 1.5.

Results and Discussion

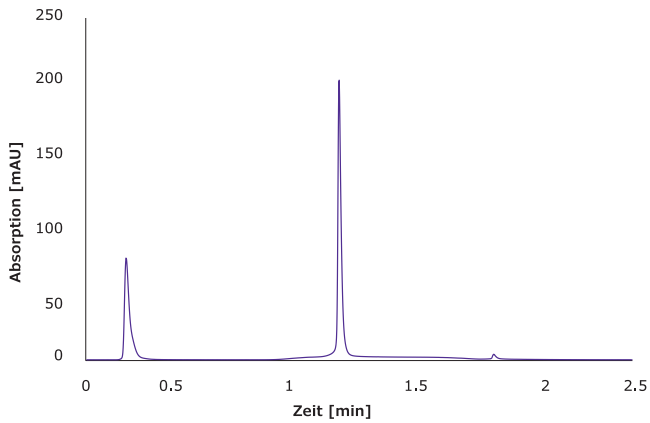
General column evaluation

Before the immobilization process, the silica monoliths were characterized by mercury intrusion porosimetry and nitrogen adsorption/desorption (BET). They consisted of macropores of 1.83 µm and mesopores of 28.6 nm (286 Å), resulting in a total surface area of 120 m^2/g silica. The final column possessed a dynamic binding capacity of 2.75 (±0.11) mg for monoclonal IgG.

The rSPA modified silica monoliths were used for separation of monoclonal antibodies from their related impurities. The separation of cetuximab from BSA impurity was performed in just over one minute and a typical chromatogram is shown in **Figure 1**. The unbound BSA eluted first followed by cetuximab, which was released due to the pH shift to pH 2.5. The rSPA silica monoliths provided sharp peaks with peak widths <0.040 minutes in a short run time which was supported by the hydrophilic column surface reducing undesirable backbone interactions with the analytes.

Figure 1. Separation of cetuximab (1 mg/mL) and BSA (1 mg/mL) by immobilized rSPA silica monolith.

Column:	Immobilized rSPA silica monolith 300 Å, 25 x 4.6mm		
Mobile Phase:	[A] 100 mM sodium phosphate pH 7.4 [B] 100 mM sodium phosphate pH 2.5		
Gradient:	**Time (min)**	**% A**	**% B**
	0.25	100	0
	0.25-0.26	0	100
	1.00	0	100
	1.00-1.01	100	0
	1.01-2.50	100	0
Flow Rate:	2.0 mL/min		
Column Temp.:	25°C		
Detector:	UV, 280 nm		
Injection:	10 µL		



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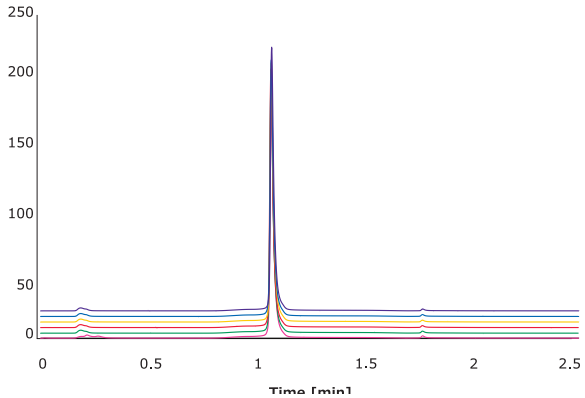
Reproducibility

The cetuximab sample was injected 50 times and all chromatographic parameters were evaluated, especially with respect to their relative standard deviation (RSD). An overlay of all 50 chromatograms is shown in **Figure 2** indicating a robust and reproducible column performance. The RSD of the retention time of eluted cetuximab was <0.1% with the peak width being equal for every run. The peak area RSD was 0.4%, supporting the previous data and assumptions of a robust column modification and reproducible column performance.

Figure 2. Analysis of 50 injections of cetuximab (1 mg/mL) on immobilized rSPA silica monolith. Only the first and every 10th chromatogram are shown. For chromatographic conditions, see **Figure 1**.



Furthermore, different batches were synthesized under exactly the same conditions and six columns of each batch were immobilized with rSPA ligand, respectively. Summaries of chromatographic data of each batch are shown in **Table 1**. The data revealed only slight differences between columns from the same batch as well as minor differences between different batches. Those results demonstrate that the immobilization process via epoxide functions is very robust and reproducible.

Table 1. Chromatographic data of separated IgG by immobilized rSPA silica monolith from different batches. For chromatographic conditions, see **Figure 1**.

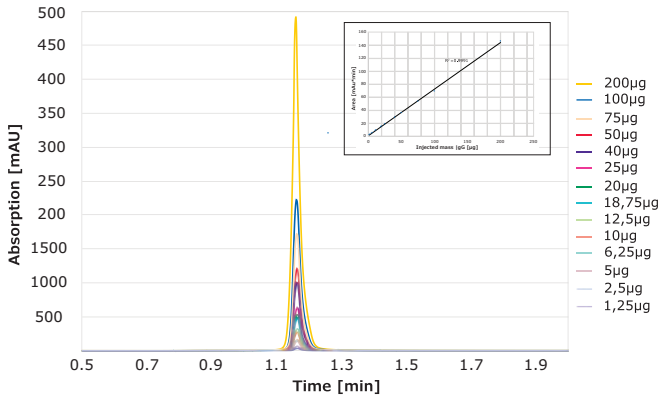
Batch No.	Retention time [min]	Peak Symmetry (USP)	Peak Width (10 %) [min]	Column back pressure [bar]
1 (n=6)	1.15 ±0.01	1.57 ±0.15	0.037 ±0.003	10 ±0
2 (n=6)	1.16 ±0.00	1.53 ±0.12	0.035 ±0.003	10 ±0
3 (n=6)	1.15 ±0.00	1.52 ±0.06	0.035 ±0.003	10 ±0
Total (n=18)	**1.15 ±0.00**	**1.54 ±0.11**	**0.036 ±0.003**	**10 ±0**

Column linear range

For titer determination, the linear range of the analytical column is a key property for its application. In antibody production, the bioreactors used contain antibody concentrations between 0.5-7 g/L, which is the minimum requirement for an analytical protein A column. The rSPA modified monolith was evaluated by analyzing different concentrations of pure cetuximab ranging from 0.0125 to 10 mg/mL. The fifteen calibration standards used covered a broad analytical range from 1.25-200 µg of injected cetuximab onto the immobilized rSPA silica monolith. The retention time of eluted cetuximab concentrations varied by only 0.1% and was confirmed by an overlay of all calibration chromatograms as shown in **Figure 3**. The correlation coefficient was higher than 0.999, supporting the hypothesis of a high column capacity.

Figure 3. Calibration curve of Cetuximab on rSPA silica monolith ranging from 1.25 µg to 200 µg.

Chromatographic conditions, same as in **Figure 1**, except injection volume: 20 µL.



Flow rate

Since monolithic silica columns exhibit a low column backpressure due to their high flow through pores, the separation capability of immobilized rSPA silica monoliths was evaluated at higher flow rates. The comparison of column performance was done with pure cetuximab spiked with BSA. It was expected that retention time and peak width of eluted cetuximab would decrease with increasing flow rate.

In **Table 2**, key data is shown for a flow rate comparison. It was evident that cetuximab column binding was not affected by flow rate and the relative peak area of eluted cetuximab was constant. Additionally, column backpressure increased linearly (R2 = 0.998) enabling high flow rates and even shorter run times with constant column performance and without any loss in binding efficiency. The high-speed separation of monoclonal antibodies at high flow rates is enabled by the high mass transfer properties of

Table 2. Chromatographic data of separated IgG (1 mg/mL) and BSA (1 mg/mL) by immobilized rSPA silica monolith at different flow rates. For chromatographic conditions, see **Figure 1**; same gradient was used as shown in **Figure 1** and was adjusted based on flow rate used; flow rate: 1.0-5.0 mL/min

Flow rate [mL/min]	Retention time [min]	Peak Width (10%) [min]	BSA area	IgG area	Column back pressure [bar]
1.0	2.31	0.058	39%	61%	3
2.0	1.16	0.032	39%	61%	6
3.0	0.77	0.024	39%	61%	10
4.0	0.58	0.021	39%	61%	13
5.0	0.47	0.020	39%	61%	17

the macropores. The flow rate data demonstrated the significant time savings and high separation efficiencies obtained by using silica monoliths.

Stability

The examination of column long-term stability was performed with IgG as a control. The stability of the monolithic structure, column modification and protein A linkage were extensively investigated by more than 5,000 runs, including 10,000 pH shifts which corresponded to more than 53,000 CV applied to the column.

The results of IgG control samples are shown in **Figure 4**. Immobilized rSPA silica monolith provided constant analysis of IgG during the complete stability test - remaining retention time, peak area, and peak width were nearly unchanged. The RSD of IgG retention time was smaller than 0.5% whereas for the peak area of eluted IgG, the RSD was below 1.1%, indicating no loss of binding capacity.

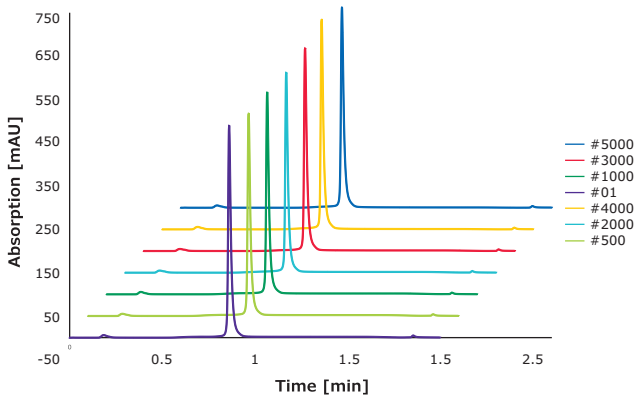
Conclusions

Recombinant protein A was covalently attached to the surface of silica monoliths with larger mesopores suited for biomolecules. The immobilized rSPA column was successfully utilized in the separation of monoclonal antibodies by affinity chromatography. The applied method for immobilization yielded a high dynamic binding capacity, leading to a broad range of applicable antibody concentrations. Furthermore, the stability test revealed a strong linkage between silica monolith and rSPA ligand due to the constant analysis of monoclonal antibodies. In conclusion, silica monoliths with bimodal pore structure immobilized with rSPA ligand are ideally suited for the chromatographic separation of monoclonal antibodies.

Figure 4. Stability test of immobilized rSPA silica monolith against 10,000 pH shifts using gammanorm IgG (1 mg/mL) as control for column performance. Chromatographic conditions, same as **Figure 1** except for gradient.

Gradient Conditions

Mobile Phase:	[A] 100 mM sodium phosphate pH 7.4 [B] 100 mM sodium phosphate pH 2.5		
Gradient:	**Time (min)**	**% A**	**% B**
	0.05	100	0
	0.05-0.06	0	100
	1.10	0	100
	1.10-1.15	100	0
	1.15-2.00	100	0



References

1. G. Walsh, Biopharmaceuticals: An overview, in: G. Walsh, B. Murphy (Eds.), Biopharmaceuticals: An Industrial Perspective, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1-34 (1999).
2. Bell, D. LC GC N Am 34 (4) (2016) 242-252.
3. Fekete, S.; Veuthey, J.L.; Guillarme, D. J. Pharm. Biomed. Anal. 69 (2012) 9-27.
4. Guillarme, D.; Ruta, J.; Rudaz, S.; Veuthey,J.L. Anal. Bioanal. Chem. 397 (3) (2010) 1069-1089.
5. Vuignier, K.; Fekete, S.; Carrupt, P.A.; Veuthey, J.L.; Guillarme, D. J. Sep. Sci. 36 (2013) 2231-2243.
6. Forsgren, A.; Sjöquist, J. J. Immunol 97 (1966) 822-827.
7. Gorbatiuk, O.B.; Bahmachuk, A.O.; Dubey, L.V.; Usenko, M.O.; Irodov, D.M.; Okunev, O.V.; Kostenko, O.M.; Rachkov, A.E.; Kordium, V.A. Biopolym Cell 31(2) (2015) 115-122.
8. Unger, K.K.; N. Tanaka, E. Machtejevas. Monolithic Silicas in Separation Science. Wiley-VCH Verlag GMBH, Weinheim, Germany, 2011.
9. Cabrera, K. LC GC N Am 30 (4) (2012) 30-35.
10. Altmaier, S.; Cabrera, K. J. Sep. Sci, 31 (2008) 2551-2559.
11. Cabrera, K.;Lubda, D.;Eggenweiler, H.M.; Minakuchi, H.; Nakanishi, K. J High Resolut Chromatogr 23 (1) (2000) 93-99.
12. Nakanishi, K.; Ishizuka, N.; Minakuchi, H.;Hirao, K.;Tanaka, N. Colloids Surf A Physicochem Eng Asp 187-188 (2001) 273-279
13. Tanaka, N.; Nagayama, H.; Kobayashi, H.; Ikegami, T.; Hosoya, K.; Ishizuka, N.; Minakuchi, H.; Nakanishi, K.;Cabrera, K.; Lubda, D. J High Resolut Chromatogr 23 (1),(2000) 111-116.
14. Cabrera, K.; Kupfer, T.; Jung, G.; Knoell, P.; Peters, B. The Column 10 (22) (2014) 40-43.
15. Spross, J.; Sinz, A. J. Sep. Sci. 34 (2011) 1958-1973.
16. Pfaunmiller, E.; Paulemond,M.; Dupper, C.; Hage, D. Anal. Bioanal. Chem 405 (2013) 2133-2145.
17. Moser, A.; Hage, D. Bioanalysis 2 (4) (2010) 769-790.

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18. Michaud, M.; Jourdan, E.; Villet, A.; Ravel, A.; Grosset, C.; Peyrin, E.J. Am. Chem. Soc. 125 (28) (2003) 8672-8679.
19. Han, B.; Zhao, C.; Yin, J.; Wang, H. J. Chrom B 903 (2012) 112-117.
20. Girelli, A.; Mattei, E. J. Chrom. B 819 (1) (2005) 3-16.
21. Lubda, D.; Cabrera, K.; Nakanishi, K.; Lindner, W. Anal Bioanal Chem 377 (2003) 892-901.
22. (22) Calleri, E.; Massolini, G.; Lubda, D.;Temporini, C.; Loiodice, F.;Caccialanza, G. J Chrom A 1031 (2004) 93-100.
23. Calleri, E.; Temporini, C.; Perani, E.; Stella, C.; Rudaz, S.; Lubda, D.;Mellerio, G.; Veuthey, J.L.; Caccialanza, G.; Massolini,G. J Chrom A 1045 (2004) 99-109
24. Temporini, C.; Calleri, E.; Campèse, D.; Cabrera, K.; Félix, G.; Massolini, G. J. Sep. Sci. 30 (2007) 3069-3076.
25. Mallik, R.; Hage, D.S. J Pharm Biomed Anal 46 (2008) 820-830.
26. Yoo, M.J.; Hage, D.S. J. Sep. Sci 32 (2009) 2776-2785.
27. Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka,N. Anal. Chem. 68 (1996) 3498-3501.
28. Minakuchi, H.; Nakanishi, K.; Soga, N.; Ishizuka, N.; Tanaka, N. J. Chromatogr. 797 (1998) 121-131.

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Description	Cat. No.
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Chromolith® WP 300 Protein A 25-4.6 (25 x 4.6 mm)	**1.52258**
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Sodium hydroxide solution 50 % for analysis EMSURE®	**1.58793**
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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.

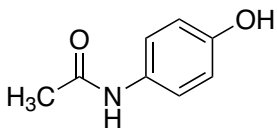
Another unique aspect of these solutions is that the individual neat materials that are used as starting materials are Secondary Pharmaceutical Reference Standards. These Secondary Standards are manufactured as Certified Reference Materials and made traceable to a corresponding Primary Compendial Standard. 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**.

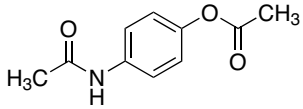
Table 1. Acetaminophen impurity portfolio (combining Cat. Nos. **A-134**, **A-135**, and **A-136**).

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



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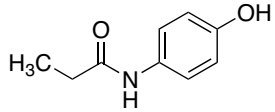
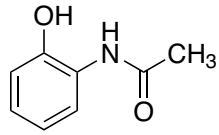
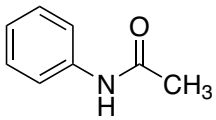


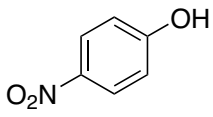
N-(4-Hydroxyphenyl)propanamide



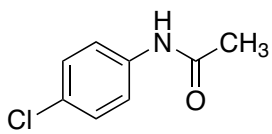
2-Acetamidophenol



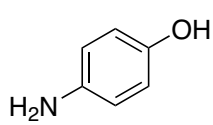
Acetanilide



4-Nitrophenol



4'-Chloroacetanilide



4-Aminophenol

(continued on next page)

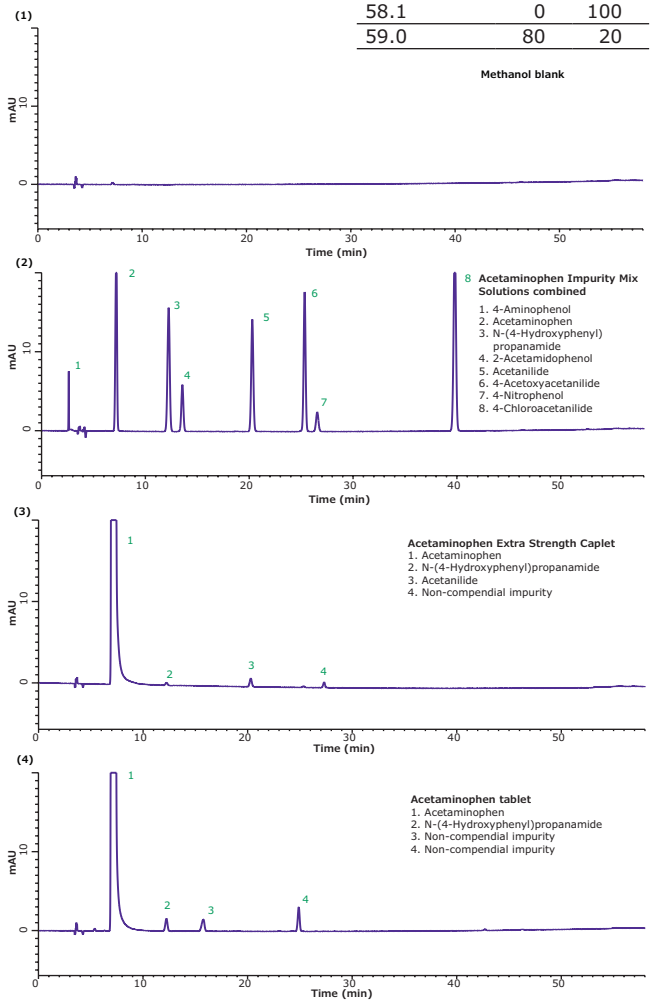
Figure 2 shows an example chromatogram of the Acetaminophen and related compound solutions (structures of components shown in **Figure 1**) combined as one sample solution solution, as well as two different tablet types (extra and regular strength) being analysed.

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.

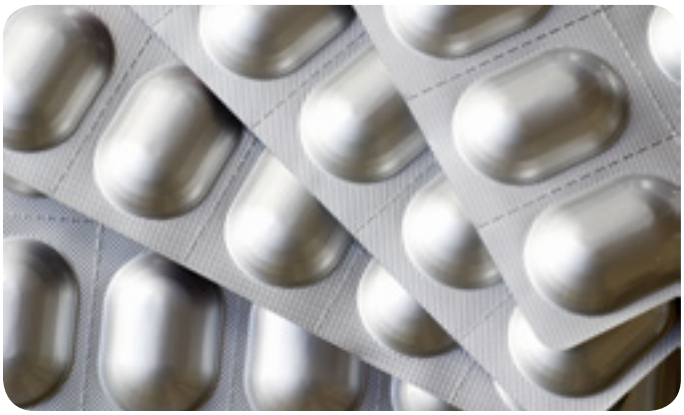
A complete overview of all our available reference materials for pharmaceutical impurities (neats and solutions) listed by API can be found at **SigmaAldrich.com/pharma-impurity-mixture**

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

PHARMA & BIOPHARMA

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 are also developing 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

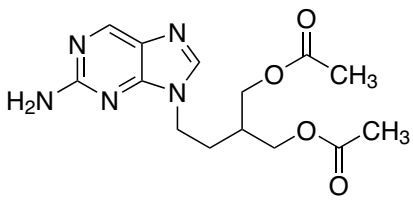
PHARMA & BIOPHARMA

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

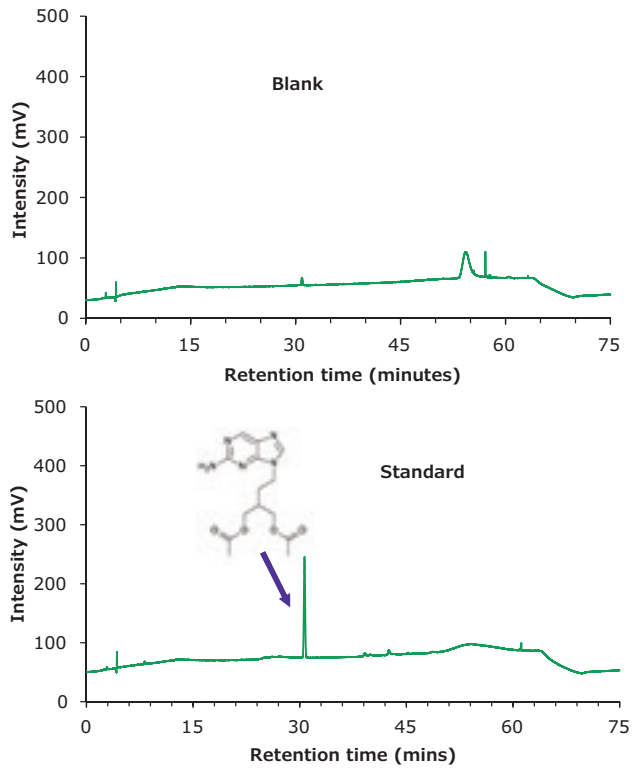
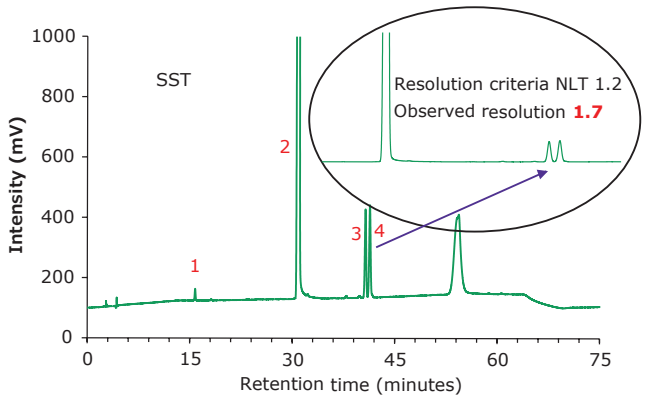
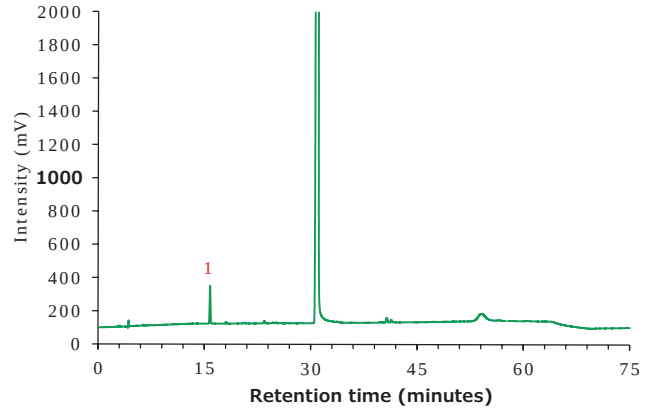


Figure 2. Chromatographic data - System Suitability Test (SST) solution



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.

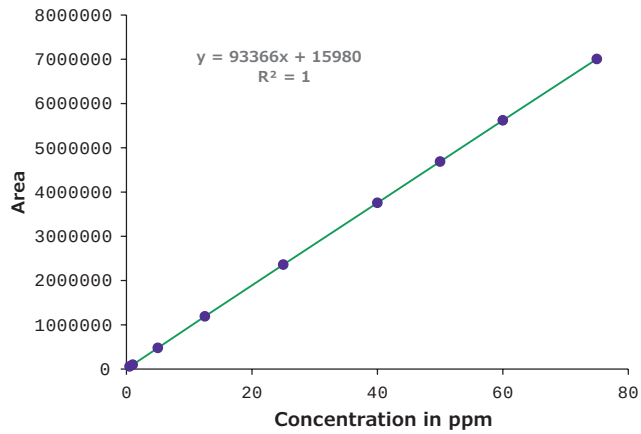
	Compound	RT (min)
1	Famciclovir Related compound B	**15.8**
2	Famciclovir	**30.9**
3	6-Chloro famciclovir	**40.7**
4	Propionyl famciclovir	**41.3**

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**

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FOOD & BEVERAGE

Analysis of Pesticide Residues in Ginger Powder Using QuEChERS Extraction and Cleanup with a Novel Dual Layer SPE Cartridge

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Introduction

Ginger comes from the rhizome of the plant *Zingiber officinale*. It has been used for thousands of years as both a spice for cooking and a treatment for ailments such as nausea, gastrointestinal (GI) irritation, inflammations and cold & flu symptoms. In addition, recent studies indicate that it may be useful in pain reduction and supporting cardiovascular health.[1] Ginger is native to southeast Asia, however it is also grown in various countries in the western hemisphere. The world production of ginger was approximately 2.1 million metric tons in 2013, with about half coming from China and India.[2] After harvesting, ginger root is first washed and then boiled in a process known as "killing", which stops enzymatic activity. It is then dried and subjected to further processing such as grinding for powdered ginger, or solvent extraction and distillation for production of ginger oil and oleoresin.[3] Many pesticides used on the plant can be carried through these processes and end up in the final product. Since dried ginger is used for both cooking and as a dietary supplement, there is a risk for exposure to pesticides as a result of more frequent consumption. Thus, pesticide residue analysis of ginger is required by many countries. For example, Canada has maximum residue limits for a variety of pesticides in ginger root ranging from 0.01 to 0.15 ppm.[4]

The odor and pungency of ginger is due to the presence of terpenes, gingerols and shogaols.[5] These compounds contribute to the highly complex matrix of ginger, which subsequently presents a special challenge in low level analyses of contaminants. The analysis of pesticide residues in ginger can be achieved using the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) approach for extraction. Following extraction, a cleanup step is essential to produce a sample that is amenable to both LC and GC chromatographic analysis. A common approach, included in the QuEChERS methodology is to use dispersive solid phase extraction (dSPE) with loose sorbents. However, dry commodities such as ginger powder often produce too much background for effective cleanup using dSPE. A traditional solid phase extraction (SPE) cartridge has more cleanup capacity than dSPE, and is recommended for these types of samples. In this application, a new dual-layer SPE cartridge, the Supelclean™ Ultra 2400, was used in the cleanup of QuEChERS extracts of ginger powder prior to analysis of pesticide residues by both LC-MS/MS and GC-MS/MS. This cartridge contains a blend of primary-secondary amine (PSA), C18, and graphitized carbon in the top layer and Z-Sep sorbent in the bottom layer. The cartridge contains sorbents traditionally used for cleanup (PSA and C18) as well as two novel materials; Graphsphere™ 2031 and Z-Sep. Graphsphere™ 2031 is a specially engineered carbon with a lower surface area than standard graphitized carbon black (GCB). The lower surface area provides a balance between removal of pigments with planar structures such as chlorophyll, and weaker retention of planar pesticides. The Z-Sep in the bottom layer is zirconia coated silica which can retain carotenoids as well as some fatty constituents. Together these two layers of sorbents provide more rigorous cleanup than dSPE. In addition, compared to current dual layer SPE cartridges containing GCB and PSA or aminopropyl silica, the Ultra 2400 is much smaller, but still provides sufficient sample cleanup with much less solvent consumption. The use of Graphsphere™ 2031 carbon in the Ultra 2400 offers an advantage over traditional GCB containing cartridges in the form of improved recoveries of planar pesticides without the use of toluene in the elution solvent.

In this study, ginger powder was spiked at 10 ng/g with a variety of pesticides, and extracted using a standard QuEChERS approach. The extract was then cleaned for LC-MS/MS and GC-MS/MS analyses using the 3 mL Supelclean™ Ultra 2400 SPE cartridge.

Experimental

Dry ginger powder was obtained from a local grocery store, and spiked at 10 ng/g with the pesticides listed in **Tables 1 and 2**. Samples were extracted as described in **Figure 1** and subjected to separate cleanups for LC and GC as described in **Figure 2**. Spiked replicates of ginger extract (n=3) and blanks were subjected to cleanup using the described procedures. Samples were analyzed by external standard analysis against matrix-matched calibration curves using the HPLC and GC conditions shown in **Tables 3 and 4**. The MRMs used for quantitation are indicated in **Tables 1 and 2**.

Figure 1. QuEChERS extraction procedure used for ginger powder

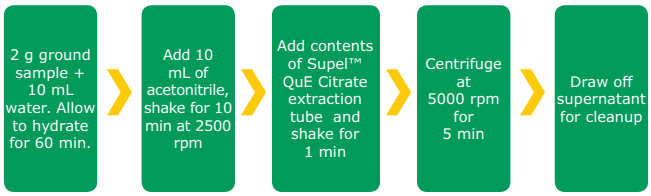

Figure 2. Cleanups used for QuEChERS extracts of ginger powder using 3 mL Supelclean™ Ultra 2400 cartridge

Table 1. Pesticides analyzed in dry ginger powder by LC-MS/MS

Compound	MRM	Frag (V)	CE (V)	Cell Acc (V)
Acephate	184/143	70	0	5
Acetamiprid	223.1/126	80	27	2
Boscalid	343/307.1	145	16	6
Carbendazim	192.1/160.1	105	16	2
Chlorbufam	224/172.02	120	5	3
Cycluron	199.2/72	120	20	2
Diflubenzuron	311/158	80	8	2
Fenoxanil	329.08/189	80	30	3
Fosthiazate	284/61	90	60	2
Methabenzthiazuron	222.1/165.1	90	12	2
Methamidophos	142/125	85	10	2
Methomyl	163.1/106	50	4	2
Monocrotophos	224.1/193	65	0	5
Nitralin	346.11/304	100	10	3
Oxamyl	237.1/72	60	12	2
Pirimicarb	239.15/72.1	100	20	2
Procymidon	301/284*	70	8	2
Propaquizafop	444.12/100.1	125	16	2
Tetraconazole	372/159	130	36	2
Uniconazole-P	292.1/125	135	40	2

*ammonium adduct

Table 2. Pesticides analyzed in dry ginger powder by GC-MS/MS

Compound	MRM	CE
Alachlor	188/160	10
Aldrin	263/193	35
Azinphos-methyl	160/132	0
γ-BHC	217/181	5
Chloropyrifos	197/169	15
Chloropyrifos-Methyl	125/47	15
Cypermethrins	163/127	5
4,4'-DDT	237/165	20
Diazinon	137/84	10
Dichlorvos	185/93	10
Dimethoate	87/46	15
Disulfoton	88/59	15
Endosulfan β	207/172	15
Endosulfan-α	195/160	5
Ethion	153/97	10
Fenitrothion	277/260	10
Heptachlor	274/239	15
Hexachlorobenzene	284/249	20
Iprodione I	187/124	25
Iprodione II	244/187	5
Malathion	173/99	15
Metalaxyl	220/192	5
Methoxychlor	227/169	30
Mevinphos	127/95	15
Parathion-Methyl	125/47	10
Permethrins	183/168	10
Phenthoate	274/125	15
Phorate	121/47	30
Phosalone	182/111	15
Pirimiphos-methyl	290/125	20
Profenophos	208/63	30
Quintozene	295/237	20
Vinclozolin	187/124	20

Table 3. LC-MS/MS analysis conditions

column:	Ascentis® Express C18, 10 cm × 2.1 mm I.D., 2 µm
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in water; [B] 5 mM ammonium formate, 0.1% formic acid in methanol
gradient:	95% A, 5% B held for 1 min; to 50% B in 3 min; to 100% B in 8 min; held at 100% B for 1 min, to 95% A, 5% B in 0.5 min, held 1.5 min.
flow rate:	0.4 mL/min
column temp.:	30 °C
detector:	MS, ESI(+), MRM (see **Table 1**)
injection:	5 µL

Table 4. GC-MS/MS analysis conditions

column:	SLB®-5ms, 20 m × 0.18 mm I.D., 0.18 µm
oven:	45 °C (3 min), 8 °C/min to 325 °C (2 min)
inj. temp:	250 °C
carrier gas:	helium, 1.2 mL/min, constant flow
detector:	MS, MRM (see **Table 2**)
MSD interface:	325 °C
injection:	1 µL pulsed splitless (40 until 0.5 min, splitter on at 0.5 min)
liner:	4 mm I.D. FocusLiner™ with taper

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Table 5. Average recoveries and %RSD values for pesticides from ginger powder; QuEChERS extraction followed by cleanup with Supelclean™ Ultra 2400 SPE

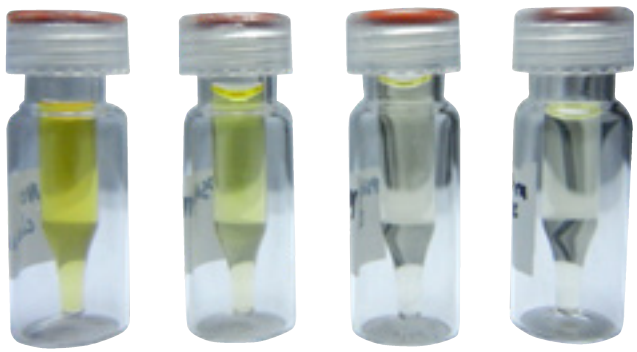
Compound	Avg. % Recovery (*n*=3)	RSD	Analysis
Acephate	86%	3%	LC
Acetamiprid	90%	2%	LC
Alachlor	110%	4%	GC
Aldrin	73%	2%	GC
Azinphos-methyl	79%	7%	GC
BHC- γ	84%	1%	GC
Boscalid	66%	7%	LC
Carbendazim	65%	2%	LC
Chlorpyrifos	100%	2%	GC
Chlorpyrifos-methyl	87%	3%	GC
Cycluron	85%	2%	LC
Cypermethrins (avg. isomers I-IV)	82%	8%	GC
DDT-p,p'	84%	6%	GC
Diazinon	86%	4%	GC
Dichlorvos	31%	72%	GC
Diflubenzuron	66%	2%	LC
Dimethoate	99%	2%	GC
Disulfoton	85%	5%	GC
Endosulfan I	92%	7%	GC
Endosulfan II	97%	23%	GC
Ethion	84%	14%	GC
Fenitrothion	102%	5%	GC
Fenoxanil	99%	60%	LC
Fosthiazate	85%	5%	LC
Heptachlor	73%	7%	GC
Hexachlorobenzene	45%	21%	GC
Iprodione	87%	4%	GC
Malathion	109%	4%	GC
Metalaxyl	79%	14%	GC
Methabenzthiazuron	58%	3%	LC
Methamidophos	72%	0%	LC
Methomyl	89%	5%	LC
Methoxychlor	90%	2%	GC
Mevinphos	57%	26%	GC
Monocrotophos	89%	4%	LC
Nitralin	67%	27%	LC
Oxamyl	89%	4%	LC
Parathion-methyl	97%	2%	GC
Permethrin I, II	not quantitated	–	GC
Phenthoate	110%	5%	GC
Phorate	70%	12%	GC
Phosalone	97%	5%	GC
Pirimicarb	90%	2%	LC
Pirimiphos-methyl	96%	3%	GC
Procymidon	59%	1%	LC
Profenofos	93%	3%	GC
Propaquizafop	70%	11%	LC
Quintozene	68%	8%	GC
Tetraconazole	46%	8%	LC
Uniconazole-P	179%	83%	LC
Vinclozolin	97%	4%	GC
Average	**82%**	**9%**	**–**

Results & Discussion

Background

After cleanup, the extract prepared for GC analysis showed substantially less color (**Figure 3**). For comparison, an aliquot of extract was also cleaned by dSPE using PSA/C18/GCB and PSA/C18. The PSA/C18/GCB and Ultra 2400 cleaned extracts were similar in color, while the extract cleaned using PSA/C18 was darker yellow, indicating the need for carbon to reduce pigmentation. GC-MS scan comparisons of the Ultra 2400, PSA/C18/GCB cleaned and uncleaned extracts are shown in **Figure 4**. Peak patterns are similar between the three extracts, however the overall amplitude of the peaks was reduced after cleanup. Most of the background peaks eluting prior to 15 minutes were removed by the Ultra 2400 cleanup. The reduction in background, measured as total peak areas, was 11% using PSA/C18/GCB and 21% using Ultra 2400.

Figure 3. QuEChERS extracts of ginger powder after cleanup by dSPE and Supelclean™ Ultra 2400 SPE



Pesticide Recovery

The pesticide recoveries and reproducibilities obtained after cleanup with Ultra 2400 are presented in **Table 5** and **Figure 5**. The average recovery obtained was 82%, with an average RSD of 9%. Of the 51 pesticides, 38 were within the 70-120% recovery range considered acceptable. Recovery of permethrin and uniconazole-P were affected by matrix interference. Procymidon was a very poor responding analyte by LC-MS/MS, and was difficult to detect at 10 ng/g. It is suspected that

recoveries of boscalid, carbendazim, diflubenzuron, nitralin and tetraconazole were reduced by the amount of sorbent to which the extract was exposed during cleanup. In past work done by the authors with cleanup of turmeric extract using the 3 mL Ultra 2400 SPE cartridge, these same pesticides had recoveries of >70%. Turmeric is much more oily and pigmented than ginger, and thus there was more matrix available to bind with active sites on the sorbents in the SPE cartridge. In the case of ginger, the relatively lower amounts of oil and pigment could have resulted in increased binding of these pesticides with the sorbents. A smaller 1 mL Ultra cartridge containing less sorbent weight could be used to increase recoveries.

Dichlorvos exhibited very low and variable recovery, and this same behavior has been observed by others using zirconia-based sorbents for cleanup.[6] This is most likely due to Lewis acid/base interaction between the Z-Sep and the phosphate group present in the structure of dichlorvos. Other pesticides containing phosphate groups: fosthiazate, methamidophos, mevinphos and monocrotophos, had better recoveries. All were recovered at >70%, except mevinphos, which was recovered at 57%. Both mevinphos and dichlorvos were analyzed by GC-MS/MS while the other phosphate containing pesticides were analyzed by LC-MS/MS. The difference in recoveries could be due to the variation in elution protocols used during the cleanups. Elution from the Ultra 2400 cartridge for GC analysis used acetonitrile containing formic acid, while LC used methanol:acetonitrile containing ammonium formate. Formic acid is a Lewis base, and is added to prevent interaction between the Z-Sep and weaker Lewis bases such as some acidic compounds. However, this was not entirely effective in the case of the phosphate groups in dichlorvos and mevinphos. The ammonium formate in the LC elution solvent acts as a Lewis base, and also acts to disrupt any weak cation exchange interactions that may be occurring between basic compounds and silanol groups present in the silica of the Z-Sep.[7] The addition of methanol to the elution solvent may also be contributing to increased recovery of the phosphate containing pesticides. It has been shown that methanol addition increases pesticide recovery when using Z-Sep sorbent; possibly by disrupting Lewis acid-base and/or electrostatic interactions.[8]

Figure 4. GC-MS scan analyses of ginger powder extracts

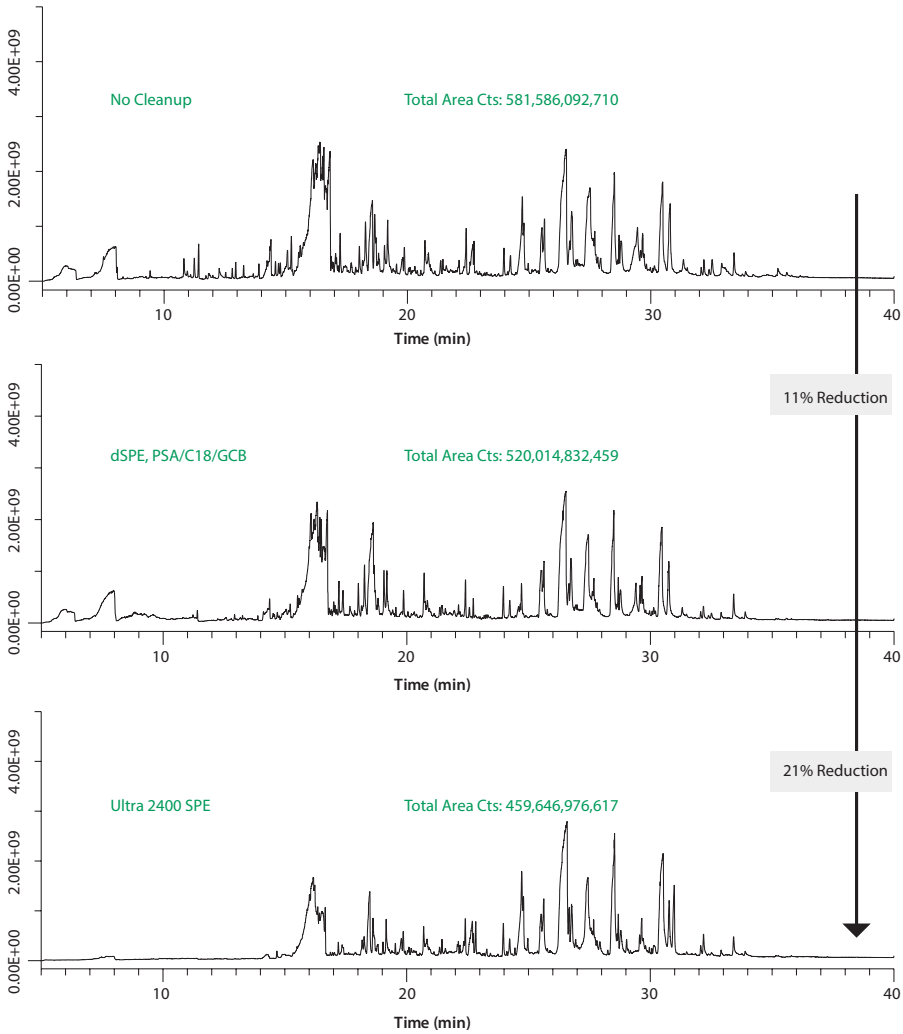


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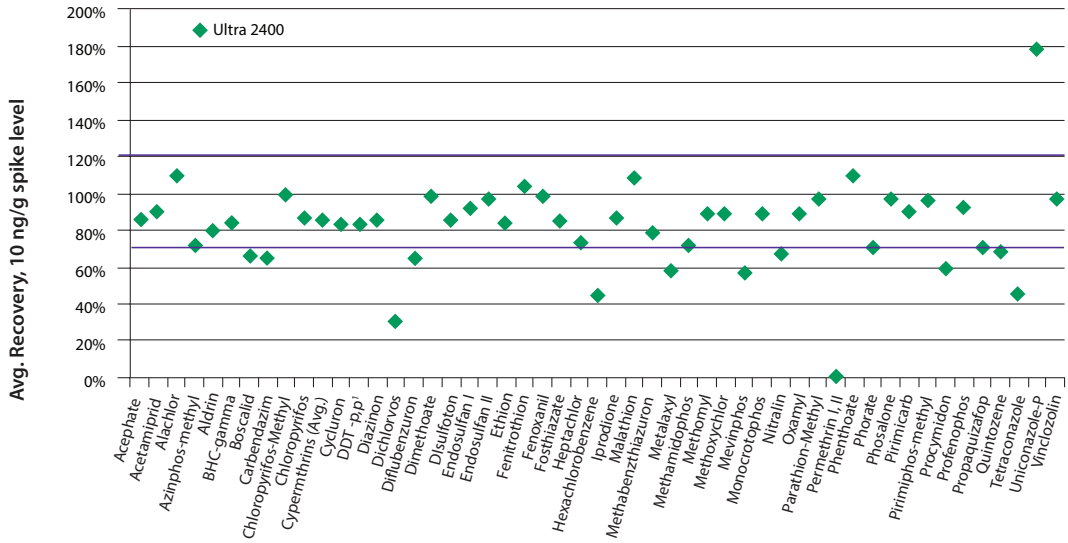
Figure 5. Average recoveries of pesticides from ginger powder spiked at 10 ng/g after cleanup with 3 mL Supelclean™ Ultra 2400 SPE



Recovery of the planar pesticide hexachlorobenzene (HCB) is traditionally problematic after cleanup using GCB. This compound is retained by conventional GCBs during cleanup. If using SPE cleanup, toluene in the elution solvent can increase recovery of this compound by displacing it from the carbon. However, toluene is then in the final extract, making it incompatible with HPLC analysis. Recovery of HCB after cleanup with the 3 mL Ultra 2400 cartridge was 45%. This is in the same range as recoveries reported by others for cleanup of acetonitrile extracts of botanicals using 6 mL dual layer SPE cartridges in combination with toluene-containing elution solvent.[9,10] To determine if recovery of HCB could be improved, the ginger extract was also cleaned using a smaller 1 mL Ultra 2400 cartridge. The elution protocol used was similar to that used for the 3 mL, with smaller solvent volumes. Recovery increased to 63%, indicating that, similar to other pesticides with recoveries <70%, the smaller 1 mL cartridge may provide a better balance between matrix and sorbent active sites for the cleanup of powdered ginger extract.

Conclusions

QuEChERS extraction of powdered ginger produces an extract containing enough background to require cleanup prior to chromatographic analysis. The use of Ultra 2400 SPE reduced this background, as evidenced by visual appearance of the extract and GC-MS-scan data. The greater capacity of the SPE cleanup reduced background more than dSPE using PSA/C18/GCB. Recoveries for 51 target pesticides, spiked at 10 ng/g, were in the range of 70-120% for 75% of the analytes. Recoveries of some of pesticides outside of this range could be improved through adjustments to the cleanup method such as use of a smaller, 1 mL Ultra SPE cartridge, and/or modifications to the elution solvent used. Compared to cleanup using larger 6 mL dual layer SPE cartridges containing GCB, the method using the 3 mL Ultra 2400 requires significantly less solvent: 11 mL vs. 20-30 mL. Recovery of the planar pesticide hexachlorobenzene was achieved without toluene in the elution solvent. Using the 3 mL cartridge, recovery from ginger was 45%; and this could be increased with use of the smaller 1 mL cartridge.

In summary, Supelclean™ Ultra SPE offers a cost effective alternative to the use of conventional 6 mL dual layer SPE containing GCB. The cartridge is available in 1 and 3 mL sizes, offering a choice to accommodate differing matrices and analyte lists.

References

1. Medical News Today, https://www.medicalnewstoday.com/articles/265990.php (assessed 07/08/2019).
2. http://faostat.fao.org/beta/en/#data/QC (accessed 07/08/2019)
3. Bzhelyansky, A. ; Presented at the 2016 AOAC Annual Meeting, SPDS Stakeholder Panel, Dallas, TX, Sept. 16, 2016.
4. Health Canada MRL database. www.hc-sc.gc.ca (accessed 07/08/2019).
5. Ali, B.H.; Blunden, G.; Tanira, M.O.; Nemmar, A. Some phytochemical, pharmacological, and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. *Food and Chem. Tox*. 2008, *46*, 409-420.
6. Hildmann, F.; Gottert, C.; Frenzel, T.; Kempe, G.; Speer, K., Pesticide residues in chicken eggs – a sample preparation methodology for analysis by gas and liquid chromatography/tandem mass spectrometry. *J. Chrom. A*. 2015, *1403*, 1-20.
7. Aurand, C.; Mi, C.; Lu, X.; Trinh, A.; Ye, M., *Troubleshooting analyte recovery when usng hybridSPE-precipitation technology*; Supelco Reporter Volume 27.2: 17-19.
8. Morris, B.D.; Schriner, R. B., Development of an automated column solid-phase extraction cleanup of QuEChERS extracts, using a zirconia-based sorbent, for pesticide residue analyses by LC-MS/MS. *J. Agric. Food Chem*. 2015, *63*, 5107-5119.
9. Chen, Y.; Lopez, S.; Hayward, D.G.; Park, H.Y.; Wong. J. W.; Kim, S.S.; Wan, J.; Reddy, R.M.; Quinn, D.J.; Steiniger, D. Determinatinon of multiresidue pesticides in botanical dietary supplements using gas-chromatography-triple-quadrupole mass spectrometry (GC-MS/MS). *J. Agric. Food Chem*. 2016, *64*, 6125-6132.
10. Hayward, D.G.; Wong, J.W.; Park, H.Y., Determinations for

pesticides on black, green, oolong, and white teas by gas chromatography triple-quadrupole mass spectrometry. *J. Agric. Food Chem*. 2015, *63*, 8116-8124.

Featured Products

Description	Cat. No.
Supelclean™ Ultra 2400 SPE Cartridges	
1 mL, pk of 108	**52779-U**
3 mL, pk of 54	**54281-U**
Supel™ QuE QuEChERS Products	
Citrate Extraction Tube, 12 mL, pk of 50	**55227-U**
Empty Centrifuge Tube, 50 mL, pk of 50	**55248-U**
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Ascentis® Express C18 HPLC Column, 10 cm × 2.1 mm I.D., 2 µm	**50813-U**
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QuEChERS Shaker and Rack Starter Kit, Schuko plug, AC input 230 V	**55438-U**
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Accessories	
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FocusLiner™, 4 mm I.D. with Taper (for Agilent), PK.1	**2879901-U**
Thermogreen® LB-2 Molded Septa, 11 mm, pk of 50	**28676-U**
Inlet Liner O-Rings for Agilent GC, pk of 10	**21003-U**
Gold-plated Inlet Seal for Agilent GC, pk of 2	**23318-U**
Capillary Column Nut for Agilent MS, pk of 5	**28034-U**

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FOOD & BEVERAGE

What Makes Wasabi so Hot?

New reference materials for Glucosinolates from PhytoLab now available through SigmaAldrich.com

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

PhytoLab is one of the leading manufacturers of phytochemicals internationally. We are proud to be able to offer their comprehensive portfolio of more than 1,300 extensively characterized and documented herbal reference substances of all classes of natural compounds. In Issue 5 of the Analytix Reporter we presented the compound class of pyrrolizidine alkaloids as an example of the extensive range covered by the PhytoLab portfolio. This time we will focus on a class of natural compounds that are responsible for the burning sensation we feel when eating mustard and horseradish: glucosinolates.

Glucosinolates are secondary plant metabolites that occur in a wide variety of plants mainly from the families of the Brassicaceae (e.g., horseradish (*Armoracia rusticana*), black mustard (*Brassica nigra*), wasabi (*Eutrema japonicum*), broccoli (*Brassica oleracea var. italica*), maca (*Lepidium meyenii*)), the Capparaceae (e.g., capers (*Capparis spinosa*)) and the Caricaceae (e.g., papaya (*Carica papaya*), but also from the Euphorbiaceae and Tropaeolaceae (e.g., garden nasturtium (*Tropaeolum majus*)). Besides being responsible for the pungent and bitter taste of these plants, the glucosinolates and their hydrolysis products also protect plants against herbivores and have been shown to have antimicrobial, antiviral, antifungal and anticarcinogenic properties. Due to their antimicrobial properties, herbal medicinal products containing nasturtium herb and horseradish root are used in the treatment of sinusitis, bronchitis and urinary tract infections.

All glucosinolates have a central carbon atom in common, which is bound via a sulfur atom to a glucose, and via a nitrogen atom to a sulfate group.

Furthermore, a substance-specific side chain (its structure depending on the amino acid applied in the biosynthesis) is bound to the central carbon atom. As the sulfate group is negatively charged, glucosinolates are most often isolated as their potassium salts.

Upon contact with the enzyme myrosinase and water (myrosinase is kept in a separate compartment in the cell, but can be released, for example, during cutting or chewing), the glucose moiety is cleaved. The remaining molecule can then undergo various spontaneous reactions, usually resulting in the corresponding isothiocyanate. Depending on the reaction conditions, thiocyanates, nitriles or oxyzolidine-2-thiones can also be formed.

For a reliable quantitative analysis of glucosinolates, well characterized reference substances are essential. Currently we offer seventeen glucosinolates, all of which come with a comprehensive certificate of analysis. Due to the negative charge of the molecule, the counter ion has to be taken into account. For all glucosinolates characterized as primary reference substances, potassium was determined quantitatively and considered as an impurity in the calculation of the absolute content, which therefore refers to the pure glucosinolate only.

Available Glucosinolate Reference Materials

Description	Package Size	Cat. No.
Epiprogoitrin	10 mg	**PHL89657**
Glucobarbarin	10 mg	**PHL89684**
Glucoberteroin	5 mg	**PHL83241**
Glucobrassicanapin	10 mg	**PHL83242**
Glucobrassicin	10 mg	**PHL80593**
Glucocheirolin	10 mg	**PHL89685**
Glucoerucin	10 mg	**PHL89686**
Glucoiberin	10 mg	**PHL89687**
Gluconapin	10 mg	**PHL89688**
Gluconasturtiin	10 mg	**PHL89689**
Glucoraphanin	10 mg	**PHL89215**
Glucoraphenin	10 mg	**PHL89690**
Glucosibarin	10 mg	**PHL89691**
Glucotropaeolin	10 mg	**PHL89216**
Progoitrin	10 mg	**PHL89765**
Sinalbin	10 mg	**PHL89793**
Sinigrin	25 mg	**PHL89279**

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FOOD & BEVERAGE

Separation of Steviol Glycosides by HPTLC

Introducing a new Stevia Extract Reference Material

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HPTLC (High-Performance Thin-Layer Chromatography) is a fast and efficient tool to create molecular fingerprints of complex chemical mixtures. Therefore it is particularly well suited for the analysis of plants and plant derived products. In a series of articles in Analytix and Analytix Reporter journals,[1-4] we showed several examples of HPTLC of plants used as dietary supplements or as herbal medicinal products such as Ginkgo, Ginseng or St. John's Wort.[5] That series is continued here with a fingerprint method for *Stevia rebaudiana* leaves, including the analysis of our new stevia extract reference material.

Extracts from the leaves of the *Stevia rebaudiana* plant have a long tradition of being used as a sweetener. Native tribes of Latin America have known and consumed it for centuries because of the sweet taste. Stevia extracts have approximately 300 times more intense sweetness than sucrose while only having a negligible effect on blood glucose. Therefore, in recent years, the plant has increasingly been used in other parts of the world as an alternative to artificial sweeteners.

Stevia is FDA approved as a dietary supplement and rebaudioside A is considered to be "Generally Recognized As Safe (GRAS)". The European Community has allowed the use of steviol glycosides as food additives since December 2011.

The WHO defined the acceptable daily intake of steviol glycosides at 4 mg per kg body weight.[6]

We recently launched a new extract reference material, developed and manufactured by HWI pharma services GmbH in Rülzheim, Germany:

Description	Quantified Components	Qualitatively Confirmed Components	Package Size	Cat.No.
Stevia extract	Stevioside	Rebaudiosides A, B, C and D, Dulcoside A, Rubusoside, Steviolbioside, Stevioside	500 mg	**06295001**

This new product complements our range of plant extract reference materials designed for use as a rapid identification and quantification method for typical constituents of plants used as food additives or as herbal medicinal products (see the complete offer at **SigmaAldrich.com/plantextracts**).

The Stevia extract reference material is provided with comprehensive documentation including a quantitative value for the major component Stevioside as well as qualitative conformation of various other constituents (Rebaudiosides A, B, C and D, Dulcoside A, Rubusoside, Steviolbioside, Stevioside). In addition to an HPLC method including a chromatogram with assigned peak identities, the documentation also contains an HPTLC method according to [5].

For the analysis, Supelco® HPTLC plates and reagents have been used. The analytical standards of the pure steviol glycosides are listed below. Please find a comprehensive listing of our entire phytochemical standards range at **SigmaAldrich.com/medicinalplants**.

HPTLC method

The scope of the method is the identification of a *Stevia rebaudiana* leaf dry extract reference material based on HPTLC fingerprints of steviol glycosides obtained with the HPTLC method by Wald and Morlock 2017[5] by comparison with the fingerprint of Stevia leaf. Additionally, chemical reference substances were used for identification of the zones of the chromatogram.

Instrumentation

Automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), Chromatogram Immersion Device 3, TLC Plate Heater 3, TLC Visualizer, *visionCATS* (the software offers a Method Library that includes an SOP for each method, an instrument method, and a comparison file with reference images).

(continued on next page)

Samples

Extract: 50 mg were suspended in 50 mL of methanol and sonicated for 10 min. The suspension was centrifuged, and the supernatant used.

Leaf: 0.5 g of powdered leaf was suspended in 30 mL of water and boiled for 10 min. The solution was filtered into a 50 mL volumetric flask and the volume was made up with water.

Standards

Standard solutions were prepared in a concentration of 0.3 mg/mL in methanol. (Note: This is 9.09–fold more concentrated than in [5])

Chromatography according to USP <203>

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (**1.05642**)
Sample application	Application with ATS 4, 10 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL for test solutions and standards
Developing solvent	Ethyl acetate, methanol and formic acid 93:40:1 (*v/v/v*)
Development	In the ADC 2 without chamber saturation and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride.
Developing distance	70 mm (from the lower edge)
Plate drying	5 min in the ADC 2
Documentation	With the TLC Visualizer under UV 366 nm and white light after derivatization.
Derivatization	Reagent name: 2-Naphthol
Reagent preparation (dipping):	2 g of 2-naphthol in 180 mL of ethanol and 12 mL of 50% sulfuric acid.
Reagent use:	The plate was immersed into 200 mL of 2-naphthol reagent using the Chromatogram Immersion Device (immersion time 0 s and immersion speed 3 cm/s) and then heated at 120°C for 5 minutes.

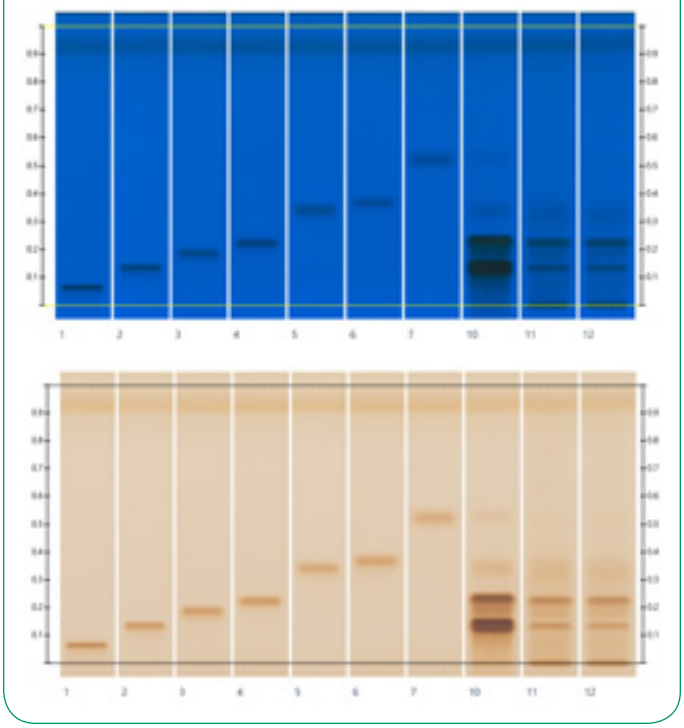
Results

The derivatized plates were viewed under UV light at 366 nm and white light (**Figure 1**.) In the fingerprint of the HWI extract (track 8), zones corresponding in color and position to those of the standards Rebaudioside A, C, Stevioside, Rebaudioside B and Dulcoside A (which are co-eluting), and Steviolbioside are seen. The fingerprint is similar to those of *S. rebaudiana* leaf (tracks 9 and 10). Rebaudioside D is only seen in the fingerprint of the leaf, particularly under UV 366 nm (very faint zone).

References:

1. Analytix 5 (2016): HPTLC Fingerprint Applications for Ginkgo Biloba
2. Analytix 1 (2017): Fingerprint Applications for *Hypericum perforatum*
3. Analytix Reporter 2 (2018): Fingerprinting of Medicinal Plants with TLC
4. Analytix Reporter 3 (2018): HPTLC Application for *Passiflora incarnata*
5. Wald JP, Morlock G. Quantification of steviol glycosides in food products, Stevia leaves and formulations by planar chromatography, including proof of absence for steviol and isosteviol. Journal of Chromatography A, 2017.
6. WHO Food Additives Series 54; 2006; page 117

Figure 1. HPTLC chromatograms after derivatization under UV 366 nm (top) and white light (bottom). **Track 1**: Rebaudioside D; **2:** Rebaudioside A; **3:** Rebaudioside C; **4:** Stevioside; **5:** Rebaudioside B; **6:** Dulcoside A; **7:** Steviolbioside; **8:** *Stevia rebaudiana* leaf dry extract reference material (HWI); **9:** *Stevia rebaudiana* leaf 1; **10:** *Stevia rebaudiana* leaf 2



Featured Products

Description	Package Size	Cat. No.
HPTLC Silica gel 60 F_{254}, 20 x 10 cm	50 ea	**1.05642**
Stevia Extract	500 mg	**06295001**
Analytical Standards for Stevia rebaudiana constituents		
Dulcoside A	10 mg	**90378**
Isosteviol	10 mg	**92273**
Rebaudioside A	10 mg	**38462**
Rebaudioside B	10 mg	**49747**
Rebaudioside C	10 mg	**30987**
Rebaudioside D	10 mg	**19189**
Rubusoside	10 mg	**62933**
Steviol	10 mg	**19345**
Steviolbioside	10 mg	**59754**
Stevioside	10 mg	**50956**

For a complete listing of your Stevia reference materials visit us at SigmaAldrich.com/stevia

Related Products

Description	Cat. No.
Solvents & Reagents	
Methanol gradient grade for liquid chromatography LiChrosolv®	**1.06007**
Ethyl acetate for liquid chromatography LiChrosolv®.	**1.00868**
Formic acid 98% - 100% for LC-MS LiChropur®	**5.33002**

For more information on our complete TLC offer, please see SigmaAldrich.com/TLC

FOOD & BEVERAGE

Are You Made of Sugar?

Examples of Ion Chromatography applications for sugar testing in food and environmental analysis

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Matthias Nold, Product Manager Reference Materials
Daniel Weibel, Product Manager Trace Organic Analysis & Sensorics, Analytix@merckgroup.com

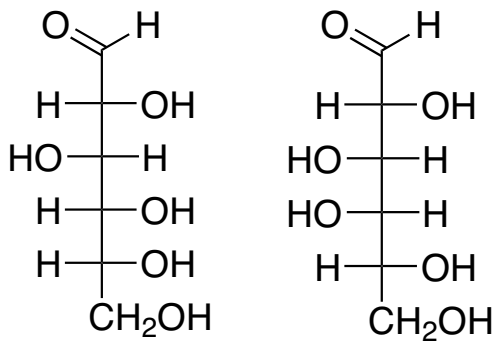
Carbohydrates constitute the biggest part of the biomass on Earth. They are produced by photosynthesis and are present in all plants and plant-based materials. The amount and composition of carbohydrates in a sample can reveal a wide range of information, depending on the context. As a result, they are subject to analysis in various industries.

In this article we demonstrate how ion chromatography is well-suited as an analytical technique for sugar analysis. All the eluents and standard solutions used for these applications are available from **SigmaAldrich.com/ic**

Carbohydrates are everywhere

In the food industry, carbohydrate and sugar content are notable for being key factors in determining the nutritional value of food and drink. In environmental analysis—to mention but one example—the anhydrosugar levoglucosan, which is produced by the pyrolysis of cellulose and acts as a tracer for biomass combustion, is determined in aerosols. These are just two of the many applications of carbohydrate analysis. Carbohydrates are composed of one or more monosaccharide units, each of which has a carbonyl group (aldehyde or ketone group) and several hydroxyl groups.[1] Because mono-, di-, and oligosaccharides are water-soluble, ion chromatography, which is performed in the aqueous phase, is particularly suitable for their analysis. It does not require extraction to the organic phase; thus, determination can be performed directly. However, a high-capacity column is necessary because sugars are relatively large molecules which are in many cases similar in structure (e.g., glucose and galactose; see **Figure 1**).

Figure 1. Structural formulae of glucose and galactose. The molecules differ only in the position of the OH group at the C4 atom (highlighted with an asterisk).



Sugars in Foods

Since December 2016, the European Union (EU) requires that nutritional values are indicated on all foodstuffs, with the exception of unprocessed products and products sold loose (regulation no. 1924/2006). What is already established practice, i.e., indicating the calorific value and certain nutrients, including sugar and carbohydrates, is set to become mandatory.

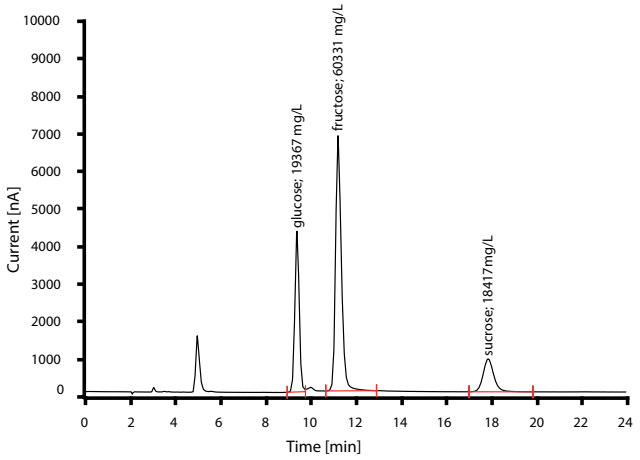
Along with starch, which is a polymer of glucose, the usable carbohydrates found in foodstuffs are largely in the form of sugars. According to the EU definition, this includes all mono- and disaccharides with the exception of polyvalent alcohols. The majority of sugars in foodstuffs are made up of the monosaccharides glucose, fructose, galactose, and the disaccharides sucrose, lactose, and maltose.

(continued on next page)

Apple Juice Analysis

The chromatogram in **Figure 2** was taken after the injection of apple juice, which was diluted (1:1000) with ultra-pure water. Apart from that, no sample preparation is necessary. The alkaline eluent (100 mM sodium hydroxide/10 mM sodium acetate, Cat. No. **78348**) ensures that the sugars are present in dissociated form (as anions) and can therefore be separated in the column using the ion exchanger.

Figure 2. Determination of glucose, fructose, and sucrose in apple juice. Except for simple dilution, no sample preparation is required.[2]

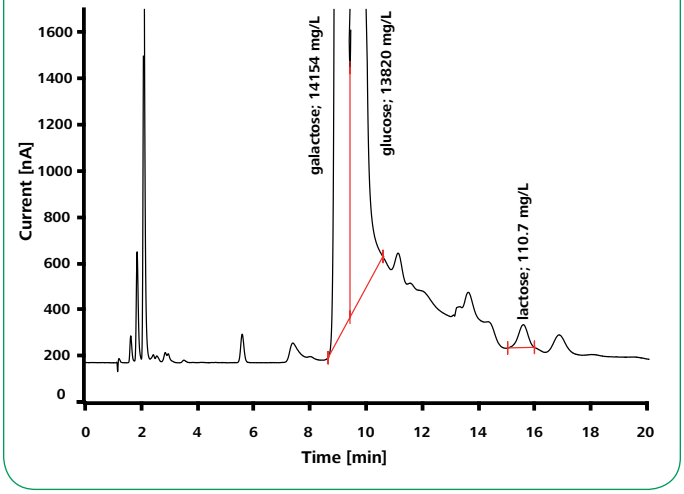


Because carbohydrates are electrochemically active, they can be detected amperometrically. During amperometric detection, the analytes are oxidized to a working electrode by applying a potential to the latter. This results in an electrical current that reveals the concentration. Over time, however, carbohydrates form deposits on the working electrode when a continuous potential is applied. The amperometric detector is therefore operated in PAD mode (pulsed amperometric detection). Here, a three-stage cyclic potential ensures that after measuring the current, i.e., after the determination stage, the electrode is cleaned from the adsorbed molecules and eventually conditioned.

Residual Lactose in 'lactose-free' Products

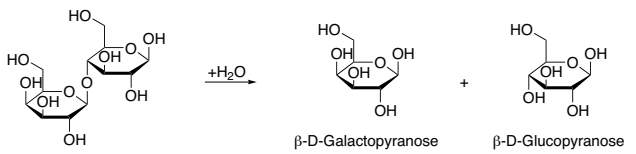
A key part of the quality control of products declared lactose-free is the determination of residual lactose. The ion chromatogram in **Figure 3** illustrates the determination of lactose in 'lactose-free' milk to which 100 mg/L lactose was added. Again, the separation takes place under strongly alkaline conditions (eluent of 5 mM sodium hydroxide/2 mM sodium acetate) and the analyte is detected by pulsed amperometry.

Figure 3. Determination of lactose traces in milk declared lactose-free, spiked with 100 mg/L lactose.[3]



The high concentrations of galactose and glucose illustrated in the chromatogram are a result of the enzymatic breakdown of lactose into these very monosaccharide constituents (**Figure 4**). Because of its protein-rich matrix milk must undergo dialysis before being analyzed, with the Metrohm Inline Sample Preparation. This is a fully automated process, and therefore does not involve any additional effort.

Figure 4. Lactose is composed of the monosaccharides galactose and glucose. The hydrolysis of lactose illustrated here is catalyzed by the enzyme lactase.



Carbohydrates as Tracers in Environmental Analysis

Fine dust limit values, which are used as health protection measures, are regularly being violated in many places. When looking for the culprit, the usual suspects are traffic and industry, but residential wood burning used for heating has also been linked to high fine dust values.[4] The tracer levoglucosan (**Figure 5**) is often determined in order to detect wood combustion.

Figure 5. Levoglucosan (1,6-Anhydro-β-D-glucopyranose) is produced in the pyrolysis of cellulose and is therefore commonly used as an indicator for biomass combustion.

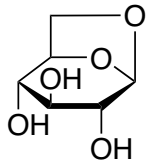
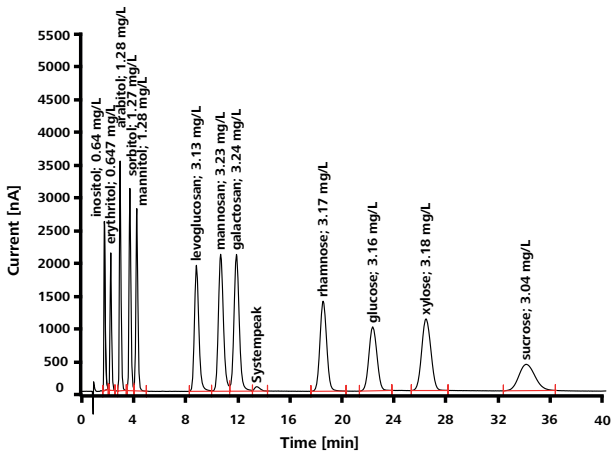


Figure 6 shows the determination of a standard solution in which levoglucosan, mannosan, and galactosan—all products of wood combustion—were analyzed, as were several biological sugars, alcohols, etc., which are typically found on aerosol particles. The high-capacity column achieves good separation of all substances, which then can be determined in a single analysis.

Figure 6. Determination of indicators for wood combustion (levoglucosan, mannosan, and galactosan) and biological sugars and alcohols, which are found in aerosols such as pollen.[5]



The new 'Carbohydrate Column'

The Metrosep Carb 2 chromatography column excels with its high ion exchange capacity, i.e., with the high number of ion exchange groups contained in its carrier material. This allows clean separation of the various sugars. Applications are found in a wide range of industries: water and environmental analysis, the pharmaceutical and food industry, forensics, the cosmetic industry, and the quality control of biofuels. In addition to carbohydrate analysis, the Metrosep Carb 2 is also suitable for determinations in samples with high salt content where lower-capacity columns fail, e.g., seawater.

For best performance, we have developed the alkaline IC eluent (Cat. No.**78348**) for the Metrosep Carb 2 column. We also offer a representative range of carbohydrate certified reference materials (CRM) solutions for IC.

References

1. Ebermann, R., Elmadfa, I. (2008): Lehrbuch Lebensmittelchemie und Ernährung. Vienna: Springer Verlag.
2. Metrohm Homepage Application Finder: «IC-amperometric sugar analysis in apple juice pursuant to EU regulation 1169/2011». Retrieved on January 17, 2019 from: https://www.metrohm.com/en/applications/AN-P-070
3. Metrohm Homepage Application Finder: «Lactose residues in "lactose-free" milk». Retrieved on January 17, 2019 from: https://www.metrohm.com/en/applications/AN-P-055
4. German Federal Institute for Risk Assessment (2015): «Analyse des quantitativen Einflusses der Holzverbrennung auf die Feinstaubkonzentration in Berlin und Brandenburg anhand des Tracers Levoglucosan (Levoglucosan)». Retrieved on May 18, 2015 from: http://www.bfr.bund.de/de/analyse_des_quantitativen_einflusses_der_holzverbrennung_auf_die_feinstaubkonzentration_in_berlin_und_brandenburg_anhand_des_tracers_levoglucosan_levoglucosan_-193056.html.
5. Metrohm Homepage Application Finder: «Anhydrosugars in addition to sugar alcohols and sugars from air filter sampling». Retrieved on January 17, 2019 from: https://www.metrohm.com/en/applications/AN-P-054

Featured Products

Description	Package Size	Cat. No.
Sodium acetate/Sodium hydroxide eluent for Metrosep Carb 2	1 L, 2.5 L	**78348**
Glucose Standard for IC	50 mL	**69222**
Fructose Standard for IC	50 mL	**72669**
Lactose Standard for IC	50 mL	**72622**
Sucrose Standard for IC	50 mL	**69631**
Galactose Standard for IC	50 mL	**72637**
Glycerol Standard for IC	50 mL	**72619**
Levoglucosan (1,6-Anhydro-β-D-glucose)	25 mg	**06724**

For a comprehensive overview of our product range for ion chromatography, please visit

SigmaAldrich.com/ic

FOOD & BEVERAGE

New Reference Materials for Elemental Analysis and Isotope Ratio Testing in Food

Expanded food matrix material offering

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

Everyone is concerned that the food we eat and the beverages we drink are safe and correctly labeled. Food scandals, which have included the detection of instances of food fraud and adulteration, frequently remind us that the quality of food is not to be taken for granted and should be monitored constantly by the food industry and regulators.

Enforcement of food regulations requires reliable and efficient testing methods. To ensure that these methods lead to traceable and comparable test results, well characterized reference materials need to be available.

As part of our comprehensive offering of reference materials for food and beverage testing, we provide a range of more than 150 food matrix materials intended for method development, method validation, or routine analysis. On our website **SigmaAldrich.com/foodmatrix** these products can be browsed by analyte type or matrix, making it easy to locate products for a certain area of interest.

This portfolio is now complemented with a new range of twelve products certified for element content of Carbon, Hydrogen, Nitrogen and/or Sulfur. Some of the products are certified for an isotope ratio of ^{13}C, ^{15}N and/or ^{34}S (see table for details).

Stable isotope analysis is often applied to detect adulteration of food, since the isotope ratio can be different depending on the source of a material.

The products are Certified Reference Materials (CRMs) manufactured under ISO/IEC 17025 and ISO 17034 double accreditation. The certified values for Carbon, Hydrogen, Nitrogen and Sulfur were determined by an elemental analyser calibrated to suitable materials from the National Institute of Standards and Technology (NIST), Maryland, USA. The isotope values are traceable to primary isotopic Certified Reference Materials (CRM) issued by IAEA Vienna.

Matrix	Parameters	Package Size	Cat. No.
Alfalfa	Carbon, Hydrogen, Nitrogen and Sulphur	30 g	**EMB2273**
Coconut Shell	Carbon, Hydrogen and Nitrogen	30 g	**EMB2168**
Olive Oil	$\delta\ ^{13}C$	5 g	**EMB2172**
Olive Stone	Carbon, Hydrogen and Nitrogen	30 g	**EMB2170**
Pasta	Nitrogen	100 g	**EMB2149**
Pasta	Nitrogen	10 g	**EMB2140**
Protein	Nitrogen	30 g	**EMB2154**
Rice Flour	Carbon, Hydrogen, Nitrogen and Sulphur	30 g	**EMB2278**
Sorghum Flour	Carbon, Nitrogen and Sulphur	30 g	**EMB2158**
Sorghum Flour	Carbon, Nitrogen and Sulphur, $\delta\ ^{13}C$, $\delta\ ^{15}N$, $\delta\ ^{34}S$	5 g	**EMB2159**
Wheat Flour	Carbon, Hydrogen and Nitrogen	30 g	**EMB2156**
Wheat Flour	Carbon, Nitrogen and Sulphur, $\delta\ ^{13}C$, $\delta\ ^{15}N$, $\delta\ ^{34}S$	5 g	**EMB2157**

For our complete range of reference materials for F&B testing, please see our new webpage below.

CHEMICAL INDUSTRY

New Sulfur Certified Reference Materials for Petrochemical Testing

Emily Withers, Marketing, Paragon, ew@paragon-sci.com
Matthias Nold, Product Manager Reference Materials, Analytix@merckgroup.com

Paragon Scientific is a UKAS accredited company specializing in the manufacture of Certified Reference Materials (CRMs) for petrochemical analysis. These products complement our broad portfolio of high-quality certified reference materials.

A comprehensive range of sulphur standards suitable for the most common internationally recognized test methods has now been added to this portfolio.

Background

Petroleum, or crude oil, is a valuable commodity because it is the feedstock for a wide range of products from fuels to plastics. Sulfur and its organic compounds are impurities found in crude oil, petroleum products and feedstock. The sulfur levels in these products vary from the highest amounts in the crude oils to trace levels in fuels.

Sulfur oxides formed during the combustion of gasolines or diesel fuels in internal combustion engines are damaging to the engine and to the environment. In the atmosphere, sulfur oxides convert to sulfuric acid by reaction with moisture, and harm vegetation, aquatic, animal and human life. Consequently, ultralow sulfur diesel (ULSD) fuel sold in North America must contain less than 15 ppm sulfur, as dictated by the US Environmental Protection Agency (EPA). In Europe, the sulfur content limit is even lower, at 10 ppm, as per the European Union's 'Euro V' Fuel Standard. Similar sulfur content limits are being adopted by other parts of the world to reduce air pollution and other environmental impacts.

Quantitative analysis of sulfur in crude oil, petroleum products and feedstock is extremely important to ensure compliance with regulatory bodies and legislation; failure to do so can result in costly fines. Analysis techniques for sulfur content measurement include wet chemistry, X-ray fluorescence, atomic spectroscopy and various thermal combustion methods with different detection limits. Regardless of the technique employed for measurement, robust analytical data is paramount for calibration and/or verification. The use of high-quality CRMs certified to ISO/IEC 17025 and ISO 17034 is critical to ensuring confidence in the measurement results achieved.

Paragon Scientific's Sulfur Certified Reference Materials

Paragon Scientific's Sulfur CRMs are suitable for calibration, verification, or for use in quality control procedures used for analyzing sulfur content in petroleum products. Our initial range includes sulfur in

- light mineral oil
- synthetic diesel
- heavy mineral oil
- kerosene (odorless)

with sulfur content ranging from 0 (Blank) through 5.0000% (50,000 ppm) for each material. More matrices are to be launched in the near future, including sulfur in isooctane and more.

Each Sulfur CRM provides traceability of measurement to recognized national standards, and to units of measurement realized at the National Physics Laboratory (NPL) or other recognized national standards laboratories. All sulfur content values are traceable to National Institute Standards and Technology (NIST), and each CRM provides low levels of uncertainty, ensuring maximum accuracy of data at hand.

Paragon also offers set sulfur kits for each matrix and the appropriate associated test methods, providing easy and efficient procurement solutions for end-users.

Key Benefits:

- Certified in strict accordance to ISO/IEC 17025 and ISO 17034 under UKAS accreditation
- Highest level of accreditation guarantee, providing the most credible certified data available worldwide
- Suitable for but not limited to internationally recognized test methods and protocols:
  ASTM: D2622, D3120, D4294, D5453, and D7039
  IP: 336, 496, and 497
- Fully traceable to international standards and low levels of uncertainty achieve maximum accuracy of data
- Supplied in 100 mL volume, tamper-evident glass packaging, providing assurance of sample integrity
- 12-month shelf life

The new portfolio comprises more than 100 products including kits. The entire product list can be found online at **SigmaAldrich.com/sulfurcrm**

Paragon Scientific also offers a large variety of certified reference materials for physical properties such as viscosity, density, cloud point, pour point, flash point, freezing point and many more. See more under **SigmaAldrich.com/paragon**

Our complete reference material portfolio is accessible via **SigmaAldrich.com/standards**

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