Food For Thought

Exploring the latest trends in food and beverage analysis.

Please click the circles to navigate

BETTER BREWING

BEER AHOY!

THE SWEET SPOT

WINNING WINES UNDER SCRUTINY

HPLC PAIN POINTS - PART 1

THE NEXT BIG (OR SMALL) THING

HPLC PAIN POINTS - PART 2

Better Brewing

Methods for filtering out yeast and CO^2 from beer

Many factors influence the beer brewing process and the quality of the final product. Wort filtration aids in assessing malt quality and reducing beer turbidity for downstream quality control (QC) analysis. This step enables manufacturers to manage product consistency and quality in line with their requirements.

Which factors determine quality during the brewing process?

Color, alcoholic strength, flavor, and clarity are the key factors that determine beer quality. These are the characteristics customers consider when choosing beer. For a manufacturer, the combination of raw ingredients and the specifics of the brewing process determine these characteristics.

QC checks at various stages ensure that the beer production process is on track to deliver a product consistent with internal standards. Such checks also allow the manufacturer to adjust for natural variations in ingredients as required.

These QC checks rely on standards recommended by bodies such as European Brewing Convention (EBC), Mitteleuropäische Brautechnische Analysenkommission (MEBAK™), and the American Society of Brewing Chemists (ASBC). The published methods describe how to prepare samples for QC, for example, by filtration, and how to conduct the analysis.

Table 1 lists MEBAK analytical methods that require filtration steps. Among the most critical of these methods is preparation of wort –and intermediate product in the brewing process - for quality analysis.

How does wort filtration help to improve beer quality?

Most beers are manufactured using barley malt as a primary starch source. Wort is a mixture resulting from the breakdown of this starch to sugars still contains various proteins and debris that require removal by filtration.

A coarse filter removes larger debris, while a finer filter removes coagulated proteins. These proteins (or other similarly sized particles), if left unfiltered, can lead to an unwanted change in flavor. They might also precipitate at a later stage in the manufacturing process, affecting the clarity of the final product.

The filtration speed and turbidity of resulting filtrate are indicators of quality for the brewer. Slower filtration reflects lower wort solubility, enabling the brewer to judge the quality of the malt. The wort filtrate turbidity indicates the efficacy of filtration.

Photometric QC testing of wort, as described by the ASBC Malt-4 method, requires a sample with sufficiently low turbidity. This method determines the extract of the malt, which assists in predicting fermentable extract, total acidity, pH, color, viscosity, total nitrogen, and free amino nitrogen.

What grades of filter paper are suitable for wort filtration?

Gravity filtration using cellulose paper filters is well suited for both regular wort filtration and sample preparation for QC. Various grades of filter paper meet specifications, but the speed and effectiveness of filtration can vary.

In a study to evaluate wort filtration, researchers at the Biotechnology School at Jiangnan University, Jiangsu, China, used three grades of Whatman brand filter paper—Grade 2V, Grade 597½, and Grade 2555½.

The researchers prepared two batches of coarse-filtered wort, adjusted to known turbidity levels of 10 and 30 respectively, and filtered using the three grades of filter paper. The study recorded turbidity before and after filtration, as shown in Table 2.

The results indicate that all tested filter papers are suitable for wort filtration. Grade 2V showed the greatest reduction in turbidity, potentially improving the accuracy of photometric analysis. However, it required a longer filtration time (up to 413 s vs 83 s and 52 s for Graes 597½ and 2555½, respectively).

These data demonstrate the use of three suitable grades of cellulose filter paper for wort filtration. The specific choice of filter paper depends on the individual brewer's requirements in terms of time and quality.

Table 1. MEBAK analytical methods that use filtration.

1.4.3.1	Soluble extract in wet spent grains obtained by pressing (rapid method)
1.4.3.2	Soluble extract in wet and dry spent grains obtained by rinsing (EBC)
1.4.4.2	Available residual extract
1.4.5	Iodine value of brewery spent grains
1.6.1	Solids in wort (Labor veritas method)
1.6.2	Solids or trub material (Field method)
1.6.3	Cold trub
2.6.2	Coaguble nitrogen (thermal coagulation of protein)
2.6.3.1	Nitrogen fractionation (precipitation with magnesium sulfate)
2.6.3.2	Nitrogen fractionation (precipitation with phosphomolybdic acid)
2.8.1	Limit of attenuation in wort (fermentation tube method)
2.8.2	Limit of attenuation in wort (reference method - EBC)
2.8.3	Limit of attenuation in wort (rapid method - EBC)
2.9.1	Degassing a sample (EBC)
2.10.3.2.1	Total glucose - hydrolysis method
2.12.2	Spectrophotometric (EBC)
2.14.2.2	Alcohol chill haze test, CHAPON (cold sensitivity)
2.16.3	Tannoids
2.17.3	Determination of hop bitter substances in wort and beer (EBC)
2.20.1	Membrane filterability test of beer
2.21.3.3	4-vinyl guaiacol and 4-vinyl phenol detection
2.21.8.3	Detection of SO2 with continuous flow rate
2.22.1	Chloride, sulfate, nitrate and phosphate in beer (EBC)
2.22.5	Sulfate ions

Whatman filter grade	Initial turbidity		Turbidity after filtration		Turbidity reduction (%)	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
2V	10.6	30.1	6.54	6.39	38.3	78.8
597½			8.02	7.25	24.3	75.9
2555½			7.24	8.96	31.7	70.2

Table 2. Turbidity reduction for wort batches by three grades of filter paper. Results are average of triplicate measurements.

Beer Ahoy!

When bottles of beer from the 1840s were found on a shipwreck, there was only one thought on the minds of analytical scientists: what chemicals do they contain?

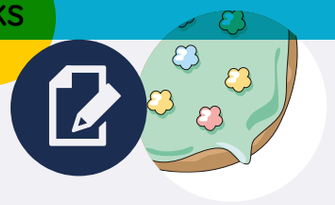
Scientists from the Technical Research Centre of Finland (VTT) and the Technical University of Munich have analyzed two bottles of beer found in the cargo of a shipwrecked schooner, which is estimated to have sunk in the Baltic Sea in the 1840s.

"In many ways, the beers were similar to modern beers," says Brian Gibson, a senior scientist at VTT and one of the study leaders. "They clearly contained malted grain and hops and were fermented with Saccharomyces yeast. They appeared quite clear suggesting some form of filtration, and the presence of iso-α-acids indicated that they had been boiled prior to fermentation, as is the case with all modern beers."

Gibson and his colleagues performed a number of analyses on the beers using a variety of chromatography and spectrometry techniques (1). No live yeast cells were found in the bottles, but they did find live, mainly lactic acid bacteria, which are typical beer contaminants. "These bacteria had apparently survived for 170 years without any additional source of nutrition. These long-lived strains are currently being studied at VTT and we hope to learn more about their biology and in particular to understand how they survived for such a long time. This information will hopefully help us to more effectively control brewery contamination levels in the future," says Gibson.

Find out more in our infographic.

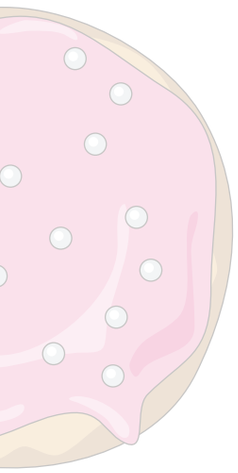
LINKS



Streamlining Sugar Polarimetry Analysis

Sponsored by GE

Produced by the Analytical Scientist



The Sweet Spot

Streamlining sugar polarimetry analysis

The sugar manufacturing process consists of a series of steps in which raw sugar is purified to produce granulated sugar and other refined products. The value of these products depends on meeting specific requirements for sucrose content, color, and other measures. Maximizing efficiency and accuracy in quality control (QC) of a sugar refinery is key to meeting these requirements.

What determines the quality of raw sugar?

Sucrose content and purity strongly influence the price paid by sugar mills or refineries. To measure these properties, organizations such as the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) publish methods for quality assessment in the manufacturing process. For labs performing these tests, speed and accuracy of measurements influence efficiency.

Sucrose content is measured with a polarimeter. For accurate analysis, particulates and other optically active substances need to be removed. This step is typically accomplished by clarification using lead acetate or a nonhazardous agent, followed by gravity filtration.

How does the choice of filter paper impact sugar QC testing?

Different filter papers might meet the required specifications for sugar filtration and produce similar polarimetry results. But they could also vary in filtration speed. To ensure that QC goes as quickly and smoothly as possible, filter choice matters.

An independent study, carried out by Salamon and Seaber Ltd., London, UK, investigated three different filter papers for their suitability for QC sample preparation of raw sugar in ICUMSA method GS 1/2/3/9-1. The tests compared consistency of the polarimetry measurements as well as filtration time.

The results, as shown in Figure 1, indicated no significant differences ($p > 0.05$) between the three tested papers in the consistency of polarimeter measurements. Filtration speed, however, did vary significantly ($p < 0.001$).

In these tests, the filtration time ranged from almost 70 minutes to under 20 minutes, with the shortest time achieved by the Whatman brand Grade 91 filter paper (Fig 2). This is approximately a three-fold increase in filtration speed, providing the opportunity for throughput improvements in QC analyses.

Sugar QC analysts can use the data from this independent study to improve their own workflows. The results demonstrate that achieving significant reductions in filtration time is possible without compromising filtration efficiency or consistency.

The data used to support this study was performed at Salamon and Seaber in 2014 and 2015. It can be made available upon request to TechSupportUK@ge.com

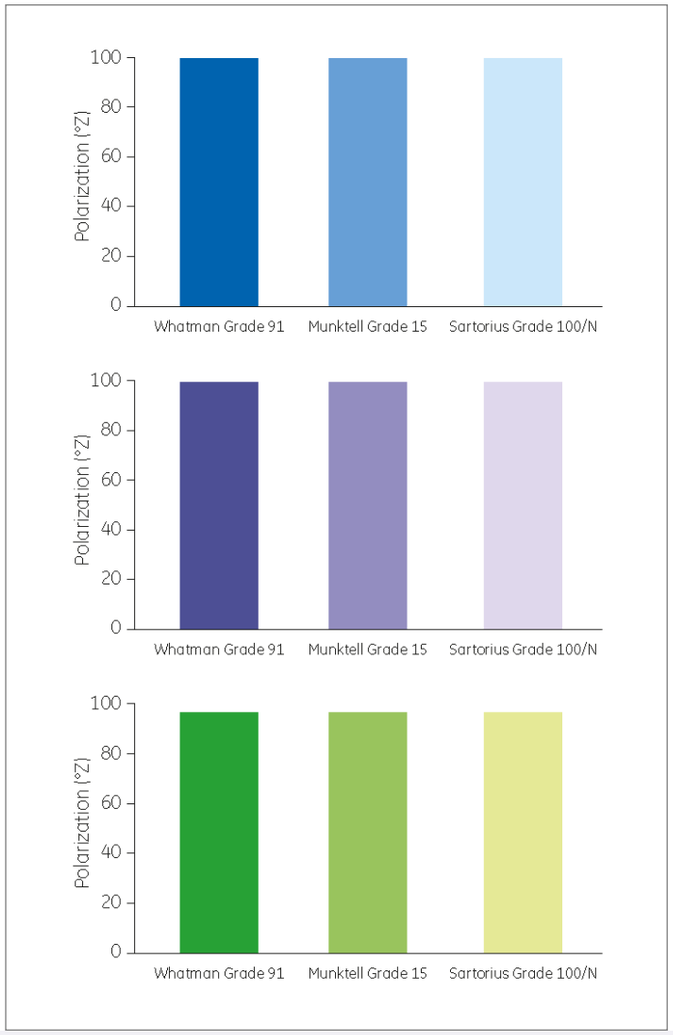
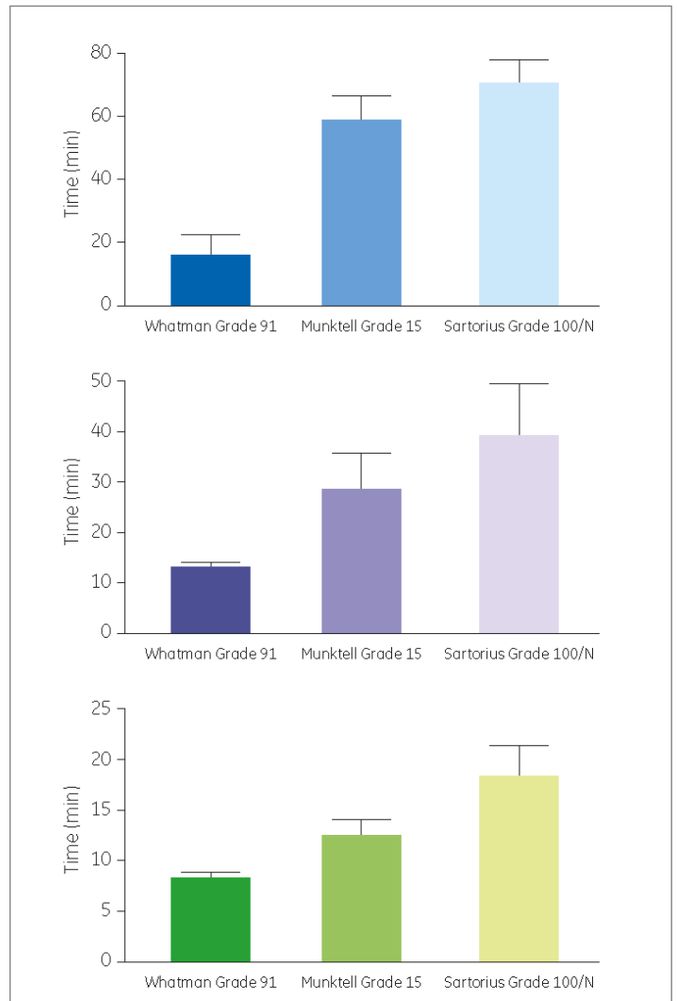


Figure 1. (Left) Optical rotation of 30 mL raw sugar solution samples after filtration. Samples are from A) Brazil B) Zimbabwe C) Guyana. Each bar represents the average of 10 replicates. Results were highly repeatable for all three filter types. Analysis using one-way analysis of variables.
Figure 2. (Below) Filtration time of 30 mL raw sugar solution samples using three filter papers (n=10). Filter selection significantly influences filtration speed ($p < 0.001$). Analysis using one-way analysis of variables. Sugar samples from Brazil (blue) Zimbabwe (purple) Guyana (green).



LINKS

Beer Quality Control: Total Acidity

Sponsored by GE

Produced by the Analytical Scientist

Winning Wines Under Scrutiny

Chemical profiles link production methods with characteristic flavors

Pinot noir wines all start off with Pinot noir grapes, and yet there is great variation in color and taste. To find out why, a team of analytical scientists from the University of Helsinki embarked upon research (that was in no way enjoyable) to analyze the chemical profiles of eight Pinot noir wines from across the world. They were able to determine some of the finer processing points, such as which sugars had been added and whether sulfur dioxide was added to prevent the wine from oxidizing.

Heli Sirén, a researcher from the university's department of chemistry, believes that if more information was included on the label, such as sugar content and organic acid content, it might also provide a clue as to how the wine would taste. For instance, the team found that biodynamically produced grapes fermented without sulfur dioxide and micro-oxygenation treated grapes gave the lowest organics contents – and it is the content of organic acids that can give wine a characteristic taste.

"I'm very interested in winemaking processes and in this study I wanted to look at what's happening at a molecular level," says Sirén. "It is commonly known that the flavors and colors of wine are influenced by aging and sunlight, but we also wanted to look at winemaking processes and whether producers use artificial improvements."

Analysis

8 wines were analysed to detect differences in their organic and inorganic compounds.
Methods used to conduct the analysis included, among others:

- liquid chromatography, ion chromatography
- capillary electrophoresis with direct and indirect UV detection
- UV/VIS spectrophotometry
- gas chromatography

19 organic acids and sugar acids were studied in the wines

Results

- The content of organic acids affects taste; for example, sweetness and acidity of wines tends to be based on sugar and organic acid concentrations.
- Wines made with new process technologies (biodynamic, and micro-oxygenation fermentation) contained the lowest concentrations of sugar and acids.
- The most acidic and alcoholic of the wines studied came from New Zealand; it contained high amounts of acetic, malic, lactic acid and acetaldehyde.

Benefits

Greater knowledge of compounds could improve the quality of wine… and influence wine storage and choice of wine to drink, particularly if information on sugar, acid and mineral content were to be included on a label alongside alcohol content

LINKS

Streamlining Sugar Polarimetry Analysis

Sponsored by GE

Produced by the Analytical Scientist

HPLC Pain Points - Part 1

Avoiding pitfalls in HPLC sample prep

High-performance liquid chromatography (HPLC) systems are widely used in analytical laboratories. Ensuring that systems are in good working order minimizes equipment downtime and increases lab efficiency.

Problems in an HPLC system can arise from several sources. The source of a given problem can often be identified by looking at the system itself or by checking downstream, by looking at the chromatogram.

Recognizing visible signs of in-system issues can identify potential problems and determine solutions that extend the usable lifetime of a column. Maximizing column lifetime has benefits in data quality, cost, and in reducing downtime.

What problems can occur in an HPLC system?

An HPLC system consists of multiple components, including an injector, flow path, pump, column, and detector unit.

A mobile phase (e.g., buffer) enters the system and passes through a pump that pressurizes the system. This mobile phase routes through to the injector to pick up the sample and carry it to the column. Both the sample and mobile phase are applied to the column for analyte separation before passing through the detector.

Problems occurring at any point in this path might influence the success of a run or affect the column quality and lifetime. Visible signs of issues from the HPLC system include:

- Increased back pressure
- Leakages and loss of pressure
- Inability to maintain a consistent flow rate

What causes these problems?

Particle contamination

Particles can make their way into an HPLC system via the sample or the mobile phase. These physical contaminants might be dust from the environment or other solids, such as precipitated protein and undissolved buffer components.

The result of physical contamination over time is two-fold:

- Blockages in the system, most likely at the HPLC column frit or the column itself.
- Wear and tear to the system components, including scratches, adding further particle contamination.

Increased back pressure is a clear indicator that part of the system is obstructed. The pump will try to maintain a fixed, accurate flow rate despite the blockage. But the instrument might eventually shut down to prevent damage.

Blockage of the column or frit also affects the uniformity of sample loading onto the column. These effects are visible from the chromatogram and explained in more detail in part 2 of the HPLC troubleshooting guide.

System components likely to suffer damage from particle contamination include injector valves, pump components, connectors, seals, and reciprocating parts. Depending on the location, damage might be visible as increased back pressure, leakages, or an inconsistent flow rate.

In addition to affecting quality of results, particle damage might require additional servicing/maintenance and column replacement. These delays and system downtime reduce lab efficiency.

Chemical contamination

Physical contamination of the HPLC system can build up over time to cause damage, resulting in the eventual need to replace columns and parts. However, chemical contamination has more immediate effects.

Some chemicals might bind irreversibly to the column, causing an increase in back pressure. Chemicals might also result in solubilization of the column resin, affecting separation and data quality. In both situations, the column will need replacing, which brings additional costs and equipment downtime.

Depending on the source of contamination, physical damage to the HPLC system and its components is another concern for users. This damage is also associated with equipment downtime and increased costs for replacement and servicing.

Dissolved gas

Although dissolved gas can be present in both the sample and the mobile phase, gas in the mobile phase generally causes many issues in an HPLC system.

If dissolved gas reaches the detector, bubbles can form as it comes out of solution, presenting as a drop in pressure. The interference from gas bubbles will be visible from the chromatogram. Part 2 of the HPLC troubleshooting guide covers these effects in more detail.

What solutions are available to minimize in-system problems?

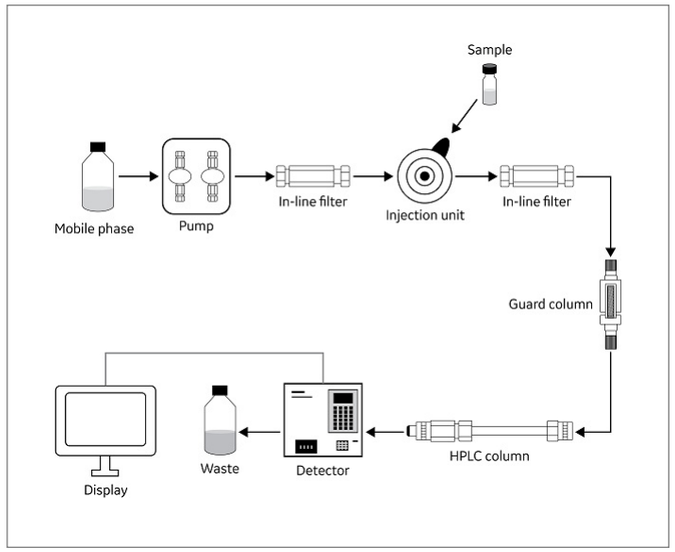
Particle contamination is a common issue affecting HPLC system components.

Filtration of the sample and mobile phase can help to combat particle contamination by reducing blockages and the need to replace system components, including the column.

Filtering the mobile phase through a 0.45 µm filter can reduce particles entering the system.

There are many considerations for sample preparation and filtration, but it is generally straightforward to remove particulates using a syringe or syringeless filter device. Prefiltration or a multilayer syringe filter can be an effective option for thick or particulate-laden samples. Using a precolumn filter and guard column can also aid the removal of particles to minimize damage and increase lab efficiency.

Inherently, columns and other components of HPLC systems have limited lifetimes. Adjusting protocols to extend this usable lifetime can minimize the frequency of replacement while maintaining data quality throughout.



LINKS

Beer Quality Control: Total Acidity

Sponsored by

Produced by the Analytical Scientist

The Next Big (or Small) Thing

What's the next big priority for LC development? We asked leading chromatographers what advances they would most like to see and why. Here's what they told us...

"I'd like to see the development of columns covering all LC modes with internal diameters (ID) of 1 mm, packed with particles (porous or superficially porous) and offering, with high reproducibility, the same efficiency as columns of 3 to 4.6 mm ID. To make this wish possible, we need instrumentation that provides dead volumes able to cope with such small IDs. Not only do we need optimal mobile phase flows in the order of 50 µL/min (20 times lower compared with the 1 mL/min for 4.6 mm ID columns) for R&D purposes, but there is also no fundamental reason not to implement such columns in QA/QC (green chemistry!)."
Pat Sandra, Emeritus Professor, Organic Chemistry, Ghent University; Founder and President, Research Institute for Chromatography, Kortrijk, Belgium.

"A great deal of research is focused on improving efficiency of separation. The other important practical aspect of SPME application would be to improve background and carry-over issues, which would require understanding the sources of column contamination, as well as improvements in the design of LC components to minimize carry-over. Longer term, I'd like to see improved fundamentals and instrumentation to facilitate on-line multi-dimensional separations, including heart cutting. The miniaturization of LC systems and use of alternative pumping systems, such as electro-osmotic pumping, are also important future directions."
Janusz Pawliszyn, Professor, Department of Chemistry, University of Waterloo, Ontario, Canada.

"During my 30 years in chromatography, I have been amazed by the technical improvements in (U)HPLC, but I get sticker shock at the costs and miss the ability to use modular LC components with any detector from any vendor. So my wish is for better modularity and interchangeability between vendor LC and detection systems."
Steven Lehotay, Lead Scientist, USDA Agricultural Research Service, Eastern Regional Research Center, Pennsylvania, USA.

"I would like:
1. An expert system that suggests the right column and mobile phase once you enter the structures you want to separate,
2. Routine LC in less than 10s,
3. Lipid isomer columns."

Bob Kennedy, Hobart H Willard Distinguished University Professor of Chemistry; Professor of Chemistry, Chair-Chemistry, College of LS&A; Professor of Pharmacology, Medical School, University of Michigan, Ann Arbor, USA.

"A transfer interface/strategy that makes fully uncoupled operation between the two separation processes in LC×LC possible, while still allowing complete and focused transfer of the eluent from the first dimension to the second, providing a flexible, universal and easy to optimize analytical platform."
Lourdes Ramos, Research Scientist, Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, Scientific Research Council (CSIC), Madrid, Spain.

"This Christmas, I wish Santa would bring me a really sensitive on-column UV absorbance detector with a physical diametric path length of 25 microns or smaller."
Sandy Dasgupta, Hamish Small Chair in Ion Analysis, Department of Chemistry and Biochemistry, University of Texas at Arlington, Texas, USA.

"Separations providing increased peak capacities and peak generation rates (essentially more resolution, and faster!), so as to enable analyses that provide increased dynamic range and speed for applications involving highly complex samples in conjunction with mass spectrometry, such as those in proteomics and metabolomics."
Dick Smith, Battelle Fellow and Chief Scientist, Biological Sciences Division, Pacific Northwest National Laboratory (PNNL), Washington, USA.

"I would most like to see highly efficient 3D printed columns. These computer-designed columns need to be identical, so we need suitable materials to create both the column and the filling at the same time, and high-speed high-resolution printers. By default, the filling must be a monolith."
Frantisek Svec, Facility Director, Organic and Macromolecular Synthesis, Lawrence Berkeley National Laboratory, Berkeley, USA.

"A universal LC-MS interface that allows the ionization of all compounds irrespective of their polarity, size, volatility and so on; plus, gives a more or less constant response for all species – so that universal calibration factors can be employed and compounds for which no standards are available can be quantified."
Hans-Gerd Janssen, Science Leader Analytical Chemistry, Unilever Research Vlaardingen, and Professor of Biomacromolecular Separations, van't Hoff Institute for Molecular Sciences, University of Amsterdam, the Netherlands.

"The desire for intact protein analysis has grown tremendously. We need more and new liquid chromatography stationary phase/support combinations and concepts to provide a wider range of selectivity for intact protein separations. Ideal products would be able to work over a wider pH range (especially above pH 8), have potential to recognize variable and changing protein conformations, and be extremely robust."
Kevin Schug, Shimadzu Distinguished Professor of Analytical Chemistry, University of Texas at Arlington, Texas, USA.

"My wish list would include:
1. 2D and 3D HPLC separation methods with a total peak capacity that can reproducibly separate thousands of compounds.
2. Preconcentration methods that can concentrate compounds based on compound class.
3. Column technology that is even more efficient than existing sub 2-micron particle technology.
4. Columns and preconcentration devices that can improve the dynamic range of analyses."

Susan Olesik, Dow Professor and Chair, Department of Chemistry and Biochemistry, The Ohio State University, USA.

"My big LC wish is for hardware that allows us to achieve the full potential of fast separations and miniaturization. For example, can we re-engineer how we introduce the sample (the injector) and the detector to take advantage of these performance gains?"
Emily Hilder, Director: Future Industries Institute, University of South Australia, Australia.

LINKS

Streamlining Sugar Polarimetry Analysis

Sponsored by GE

Produced by the Analytical Scientist

HPLC Pain Points - Part 2

Using UV analysis to avoid pitfalls in HPLC sample prep

Maintaining a high-performance liquid chromatography (HPLC) system in good condition provides users with consistent data quality and accuracy. A well-functioning system also minimizes downtime, helping to maintain lab efficiency.

As discussed in Part I, problems in HPLC systems might arise from several sources. When using a UV detector, the chromatogram can indicate the presence of a problem and provides clues about where to find the cause.

What problems can show up on a chromatogram?

A UV chromatogram plots absorbance at the selected wavelength over time. As the mobile phase carries the sample through the HPLC column, peaks indicate the relative abundance of compounds eluting from the column.

In the preferred scenario, sharp uniform peaks with clear separation and high signal to noise ratio will allow the identification of each analyte. Sometimes, however, the chromatogram might show distortions, including:

- Shouldered peaks
- Twin peaks or split peaks
- Tailed peaks
- Low signal to noise ratio

These distortions can add complexity to analysis, indicate upstream problems, and affect data accuracy and reliability.

What causes chromatogram distortions?

Particle contamination

Physical contamination of the HPLC system and column can present as peak tailing, splitting, and shouldering.

On an uncontaminated column, sample application is immediate. Uniform application in a short timeframe allows efficient separation, supporting consistent and accurate results.

Contamination of the column or frit can increase the time taken to apply a sample in comparison with a clean column. This increased time can lead to poor resolution of the analytes.

Chemical contamination

Any chemicals or compounds that absorb at the same wavelength as the analyte can distort chromatograms and confuse analysis. If contaminants elute at a similar time as the analyte, the chromatogram might report inaccurate absorbance values or unexpected peaks. Any contaminants in the mobile phase can also cause background noise.

Possible sources of contamination include extractables from filter devices or other system components. Filter materials that release extractables or housings with low solvent resistance have the potential to interfere with the chromatogram.

However, extractables only become a problem if they are detectable and co-elute with the analyte of interest. Other sources of contamination might include residue on glassware from previous experiments.

Dissolved gas

High pressure in the HPLC system keeps gas dissolved. Generally, dissolved gas is only likely to cause problems if it comes out of solution to form bubbles. These bubbles are most likely to arise at the detector where the pressure drops.

Detectors vary in their sensitivity to gas bubbles, but the effect is often evident as baseline noise on the chromatogram, leading to a low signal-to-noise ratio.

A low signal to noise ratio is a common indicator of a high proportion of dissolved gas. This noise can affect the reliability of peak identification, making HPLC analysis particularly difficult when the analyte is limited and has a low absorbance level.

How can problems in HPLC analysis be minimized?

Reducing sources of contamination

Filtering the sample can reduce particle contamination by preventing undissolved particulates from entering the system.

Solvent compatibility and level of extractables are considerations in selecting an appropriate filter device. Running a comparative test with and without a standard in place of the sample can assess the effect of extractables on the chromatograph.

Reversing the solvent flow is also a common technique to clear particulates from the column and frit. However, note that this technique can disrupt packing and affect separation efficiency.

Sufficient HPLC column packing and column equilibration procedures can help make sure sample application is uniform. These actions can also reduce the likelihood of bubbles forming in the column.

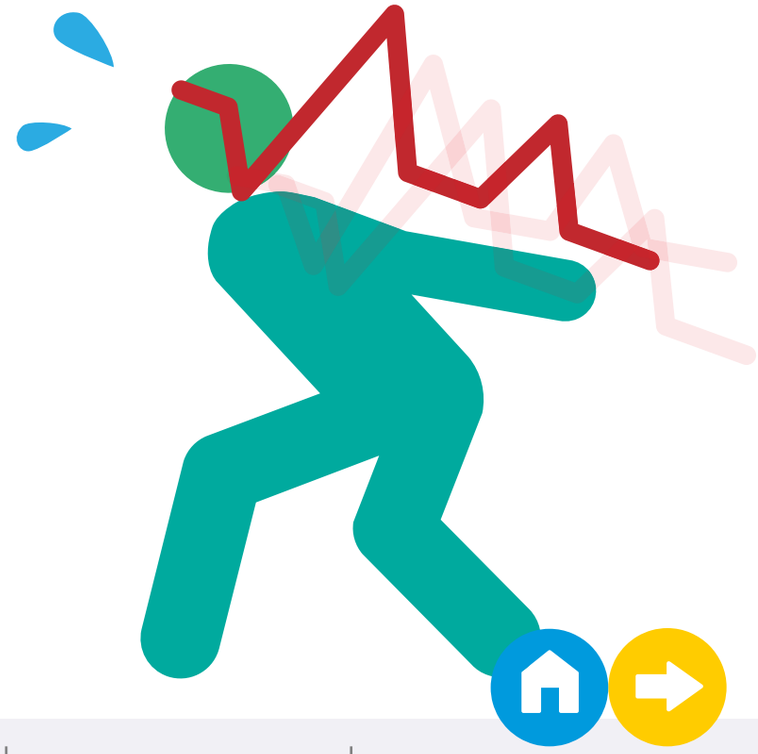
Degassing

It is common practice to degas the mobile phase before mixing it with the sample. Degassing with a cellulose filter minimizes bubble formation at the detector.

Some HPLC systems incorporate a degasser, but an alternative option is vacuum filtration of the mobile phase before use. Degassing before each HPLC run also reduces the likelihood that gases dissolved in the mobile phase reservoir between runs will affect results.

The quality and resolution of UV chromatogram data can provide users with feedback to help them achieve and maintain high-quality HPLC

Some HPLC problems can show up in the chromatogram, but others might be evident from the HPLC system itself. Examples of in-system problems are increasing back pressure, leakages and loss of pressure, and inconsistent flow rate.



LINKS

Beer Quality Control: Total Acidity

Sponsored by GE

Produced by the Analytical Scientist