Application Notes

Controlled supersaturation experiments to check the time that additives keep drugs in solution

Controlled Supersaturation experiments provide a way to create supersaturated solutions which can then be monitored to determine the precipitation rate and the time when precipitation starts. This is illustrated using bifonazole, a basic drug with pKa of 6.0.

Experiments with bifonazole

To run the method, a stock solution of sample is prepared in a solvent, e.g. 10 mM in DMSO. An aliquot of stock solution is then injected into an aqueous buffer to create a solution that is supersaturated. This technique is called "solvent-quench". The transmission of UV at multiple wavelengths is then monitored. While the solution remains supersaturated the UV signal shows constant absorbance values at all wavelengths. As soon as precipitation starts, the absorbance decreases at all wavelengths. **Figure 1** shows the behaviour of a supersaturated solution of bifonazole in aqueous buffer at pH 5. After absorbance has been measured, concentration is determined by applying previously-measured molar extinction coefficients.

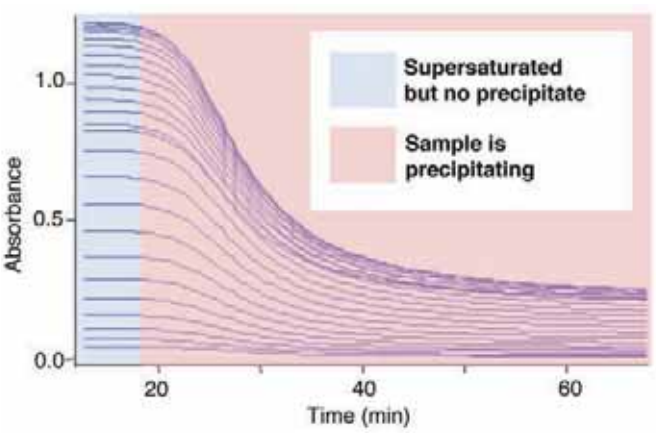

Figure 1: Controlled supersaturation of bifonazole by solvent quench, with UV monitoring of solution

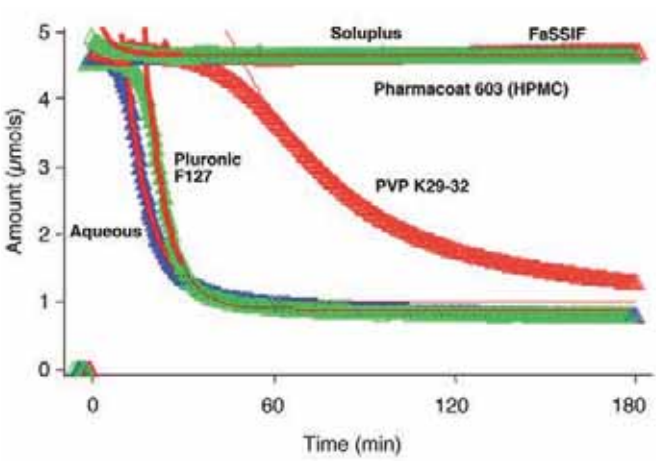

Figure 2: Experiments to investigate the effect of polymers and additives on the supersaturation of bifonazole

The solvent-quench method was used to investigate the ability of various polymers and additives to prolong the supersaturation of bifonazole at pH 5. **Figure 2** shows data from six experiments. To aid comparison the software automatically superimposed the curves such that "time zero" values coincided. The shortest duration of supersaturation occurred in aqueous solution. Pluronic 127 (BASF) slightly prolonged supersaturation. PVP K29-32 (Ashland) prolonged supersaturation for a longer time. However bifonazole remained supersaturated for three hours in the presence of Soluplus (BASF) and Pharmacoat 603 (Shin-Etsu). It also remained supersaturated in the presence of FaSSIF.

The benefits of automation

The data quality of solvent quench experiments is greatly enhanced by the automation of the Sirius inForm. In the experiments described here, 1 mL of acetate/phosphate buffer was automatically dispensed into a vial and 40 mL of aqueous 0.15 M NaCl solution was added, after which the pH was adjusted to 5.0 by the addition of 0.5 M HCl. The solution was stirred under inert gas while the temperature was brought to 37°C using the built-in Peltier controller. After equilibration, the dark spectrum and reference spectrum were measured at "time zero" using the in-situ UV probe. In the next step, 500 µL were aspirated from a vial of bifonazole stock solution and automatically dispensed under the level of solution. UV spectra were then monitored every 30 seconds until three hours from time zero had elapsed. Polymers and additives were weighed in solid form into the empty vials.

For more information:
Sirius Analytical
e sirius@sirius-analytical.com
w www.sirius-analytical.com
t +44 (0)1342 820720

Anthocyanin purification from blackcurrant with SCPC-100

Introduction

The blackcurrant is the edible berry of a shrub, which usually grows to 1-2 meters and can be found in Northern/Central Europe and Asia. Anthocyanins are found in high concentrations in the fruit and give its characteristic dark purple color. Delphinidin 3-O-glucoside, delphinidin 3-O-rutoside, cyanidin 3-O-glucoside and cyanidin 3-O-rutoside make up more than 97% of anthocyanin in the plant[1].

The crude extract is a complex matrix and the use of Centrifugal Partition Chromatography (CPC) enables purification of the targeted compound.

500mg of powder extract of blackcurrant was injected by CPC to purify anthocyanin.

Apparatus

An **Armen SCPC-100** with 100mL column capacity was connected and controlled via an **Armen Spot prep II** system equipped with 50ml/mn quaternary gradient pump, UV/Vis detector and fraction collector. AGCPC software was used.

Sample

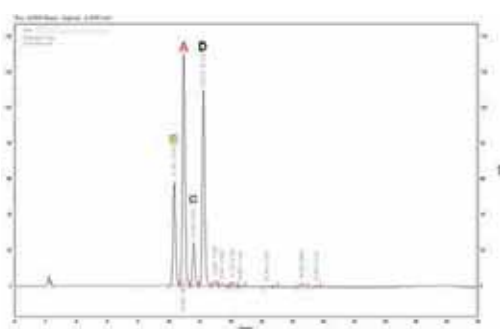

Figure 1: HPLC analysis 520nm of crude blackcurrant extract.

Table 1: Analytical HPLC conditions

HPLC column	:Purosphere RP18, 250X4.6mm, 5µm
Mobile phase A	:10% formic acid
Mobile phase B	:MeOH
Time program	:5%B (0.00 min)–60%B(20 min)- 100%B(25 min) -100%B(30 min)-5%B(35 min)-5%B(40 min)
Flow rate	:1 mL/mn
Injection volume	:10 µL
Temperature	:30°C

Crude extract was first analysed by analytical HPLC (**Figure 1**) to identify target anthocyanin to be purified. 4 major peaks A, B, C and D were detected at 520m with the following peak ratio: 39% A, 17% B, 7% C and 32% D. **A is the target compound to purify by CPC.**

Results

Table 2: CPC conditions

CPC column volume	:100 mL
Elution flow rate	:8 mL/mn
Extrusion flow rate	:30 mL/mn
Rotation speed	:2000 rpm
Solvent system	:BuOH/AcOEt/TFA 0.1%
Mode	:Ascending
Injection volume	:4 mL
Sample	:0.5 g in 2 mL upper + 2 mL lower
Detection	:520 nm

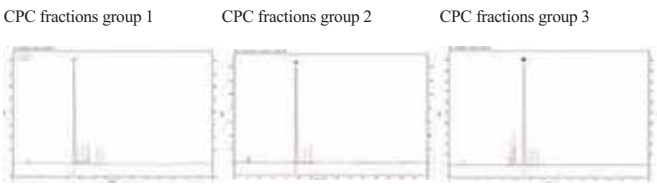

Figure 2: HPLC analysis 520nm of CPC fraction groups 1, 2 and 3

CPC solvent system was determined with the shake flask method to get a Kd=[HPLC peak area of A]stat/[HPLC peak area of A]mobile closed to one.

0.5g of sample was dissolved in 2ml of upper phase and 2ml of lower phase, filtered through a 0.45µm membrane filter and injected in CPC according to conditions describe in **Table 2**.

CPC Fractions obtained were analysed by HPLC and grouped according to HPLC purity of A, B, C and D. The grouped fractions were analysed by HPLC (**Figure 2**). Results are resumed in **Table 3**.

Table 3: Results

Duration	:30 mn
Solvent consumption	:500 mL
Purity HPLC 520 nm	:Group 2, 97% anthocyanin A
	:Group 1, 84% anthocyanin B
	:Group 3, 78% anthocyanin D

Conclusion

100 mL CPC column allows injection of 0.5g of crude blackcurrant mixture to get a few mg of pure anthocyanin A. In addition, the same run also enabled purification of two further anthocyanins. Therefore, multi gram injections could be performed on 250ml or 1L CPC column for scale up and small production of pure anthocyanin.

Reference

1. Slimestad, R.; Solheim, H. J.Agric.Food Chem. 2002, 50(11), 3228-3231."Anthocyanins from Black Currants (Ribes nigrum L.)"

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For further information, please contact: **gaudo@gilson.com**

Raman Analysis of Pharmaceutical Ingredients

A range of options is available for Raman analysis, including systems suitable for handheld, laboratory and educational applications. Systems typically include a spectrometer, laser, operating software and sampling accessories, while modular options are available for users to configure their own Raman systems. Setups are available for various wavelength ranges and resolution requirements.

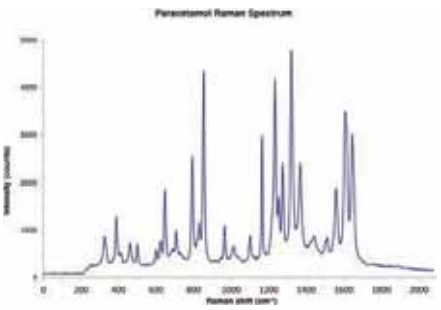

Figure 1: A modular Raman setup can be configured to measure paracetamol, an analgesic more commonly known as acetaminophen

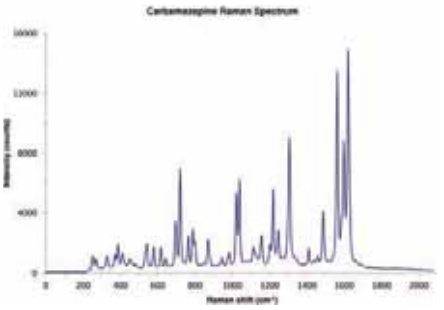

Figure 2: Spectral features of pharmaceutical ingredients such as carbamazepine are clearly discernible using Raman spectroscopy

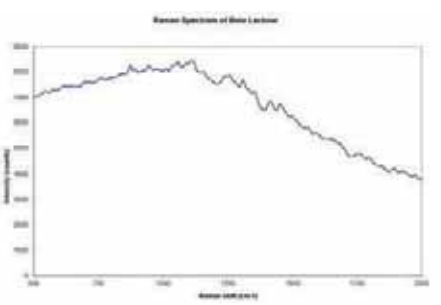

Figure 3: Raman analysis of excipients is a useful application in biomedical and pharmaceutical quality control

Introduction

Raman spectroscopy offers a number of benefits for testing and characterisation. It is rapid and non-destructive, requires only limited sample preparation and allows for sample volumes in the microliter range. In addition, Raman can be used to measure aqueous samples or samples with high moisture content, and allows researchers to capture data from a sample contained in plastic or other materials that are optically transparent to the wavelengths of interest.

Raman is particularly useful for pharmaceutical applications. For example, Raman techniques are used to discern characteristics of pharmaceutical raw materials, including active ingredients, binders, fillers, lubricants and other excipients. Raman is also useful for through-container measurements of pharmaceutical blister packs, pill bottles and vials.

Experimental Conditions

To illustrate the capabilities of our Raman systems we analysed paracetamol (acetaminophen) and carbamazepine, which are pharmaceutical active ingredients, and the excipients alpha and beta lactose. The samples studied consisted of simple organic compounds contained in standard, clear borosilicate scintillation vials. No additional preparation was necessary.

Samples were analysed using a modular Raman setup comprising a QE series spectrometer, a 785 nm laser with 500 mW output and a fiber optic probe. The spectrometer was set from ~780-940 nm and configured with a 50 µm slit for good optical resolution. High reflectivity optical bench mirrors were added to increase spectrometer sensitivity.

To collect signal, we placed the tip of the probe at the bottom of three glass vials containing the samples. We measured the samples at an integration time of 8 seconds and averaged three spectra.

Results

Our measurements confirmed that this Raman configuration can differentiate pharmaceutical active materials such as paracetamol (**Figure 1**) and carbamazepine (**Figure 2**) based on their spectral fingerprints. Also, the experiment helped demonstrate that, with proper method development and application of chemometric analysis, Ocean Optics Raman setups can be used to obtain semi-quantitative data of active ingredients in a pharmaceutical mixture.

In addition, our experiment showed that Raman response and fluorescence occurs in the lactose samples (**Figure 3**). Fluorescence is a common phenomenon in Raman measurements of some organic compounds and depends on the wavelength of the laser utilised.

Conclusions

The availability of both turnkey and modular Raman systems, complemented by sophisticated chemometric analysis packages and spectral libraries, makes Raman spectroscopy a versatile choice for a host of applications.

For more information:
Ocean Optics
info@oceanoptics.com
www.oceanoptics.com
US +1 727-733-2447 EUROPE +31 26-3190500 ASIA +86 21-6295-6600

Analysis of Compound Combination Experiments

With recent improvements in automation and liquid handling, compound combination experiments can be executed at higher throughput than ever before. However, software tools have not kept up with this increase and often still require either a manual, per-combination workflow or cover just the synergy calculation without the necessary pre-processing steps. In this application note we describe how compound combination experiments should ideally be analysed.

The earliest work formulating a systematic methodology for assessing combination effects was done by Loewe and Fraser (1928, 1872). The principal assumption is that two compounds combined in one organism may have a different effect than the two compounds individually. The whole purpose of compound combination studies is to identify and quantify effects such as synergism and antagonism in the organisms under study.

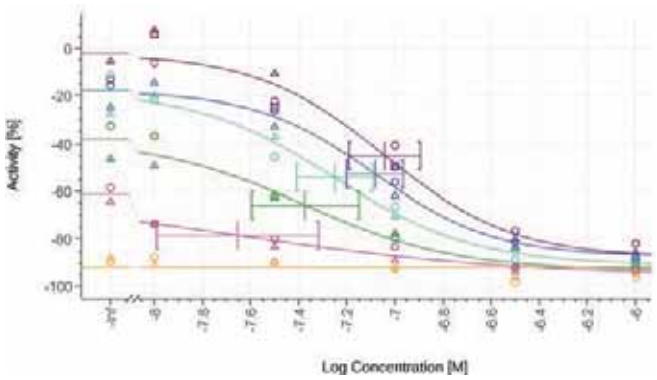

Figure 1: Overlay in Genedata Screener for combination screening: Different dose response curves of compound A at increasing concentrations of compound B, showing the shift of IC50 as dependent of compound

Plate layout

In compound combination experiments, each well carries either one compound (mono-therapeutic curves) or two (combination curves). The pipetting scheme can be classic with concrete concentration matrices on each plate, or optimised for higher throughput experiments where the layout is not obvious by looking at the plates. In either case, it is important to have a software system that stores the layout of the well content and provides this information to a suitable data analysis system.

Data normalisation

The raw data coming from the instrument has to be normalised with respect to control wells, for instance by applying a 2-point normalisation for inhibition experiments. Depending on the application, a more complex normalisation might be required, where for example cell growth is taken into account by incorporating the initial cell count measured shortly after cell seeding.

Curve fitting

With two compounds in every well, two sets of dose response curves have to be processed: one compound is fitted while the concentration of the other compound is kept constant. In cell growth assays, the response can exceed the stimulation control, and the fitting process has to account for this. It also has to take into account that if one of the compounds is considered an outlier and masked, the same well cannot be valid in the other fit. The fitted mono-therapeutic or combination curves can be overlaid for a first visual inspection of the results.

Model generation

Literature describes different models that can be used for synergy calculations; here we will just mention Loewe additivity, which differentiates and quantifies synergistic or antagonistic effects of compounds acting on the same target with the same mechanism.

Figure 2: Results of a single combination from a large combination screen as visualised in Genedata Screener

To calculate the additive effect for each combination well, the two mono-therapeutic curves are combined by applying the additivity model. Similarly, synergistic or antagonistic effects are predicted by applying the respective models. A plot showing the resulting activity matrix for the model can act as an important visual reference.

Effect assessment and quality control

The last step to determine synergism is to subtract the measured activities for each combination well from the result of the model calculation. The differences are summed up, and if the sum is zero, there is no synergism. For a large combination experiment with hundreds or thousands of combinations, the numerical results (often expressed as Synergy Scores) are sorted to identify promising combinations. The visualisation of measured and modelled data, as well as their differences, is a crucial QC step to make sure the individual combinations were measured successfully. An additional, common way to express synergy while assessing its proper measurement is through isobolograms.

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www.genedata.com/screener

How does the analysis of biomolecules benefit from UHPLC?

Evert-Jan Sneekes, Remco Swart and Mauro De Pra
Thermo Fisher Scientific, Germering, Germany

Ultra high performance liquid chromatography (UHPLC) provides improved separation speed, throughput, and sensitivity by employing stationary phase particles of around 2µm or smaller. UHPLC has found widespread use in the analysis of small molecules in pharmaceutical, food, and environmental areas. Are these analytical benefits also applicable to the separation of larger molecules, such as proteins and peptides? Biomolecules have, apart from their size, other differences to small molecules (e.g. charges, complex structures) that make the application of UHPLC less straightforward. This article discusses the rationale of using UHPLC in life sciences and demonstrate where the potential is. It also discusses the requirements needed for a true bio UHPLC system and shows examples of relevant biomolecule separations.

Introduction

An important physical parameter in the discussion of chromatographic performance of large molecules is the diffusion coefficient. As a consequence, chromatography, being a diffusion-controlled process, is highly influenced by the molecular size of the analyte. The increase of flow rates typically applied to increase throughput in UHPLC methods works for small molecules, but appears counter intuitive for the analysis of slower-diffusing molecules, such as proteins.

The most obvious difference between small and large molecules is their size, which is not only defined by the molecular weight (MW), but in the case of complex biomolecules also by their structure and hydrodynamic radius. The diffusion constants will decrease with molecular size.

Theoretically the effect of the difference in diffusion constants on the chromatographic efficiency can be visualised by the Van Deemter curves in **Figure 1A** (page 00). Here, a theoretical comparison on the optimal chromatographic conditions (i.e. optimal mobile phase velocity in the Van Deemter curve) for two analytes with a 100 times difference in molecular weight is given. For the same type of stationary phase the flow optimum is much lower for the protein and the range in which this performance is achieved is much narrower (see **Figure 1A**, page 00).

The Van Deemter curve has three components and the main underlying parameter for the differences between small and large molecules is the component that defines the interaction of the analyte with the stationary phase (also called C-term). This is why UHPLC and smaller particles are typically mentioned together; the shorter diffusion paths for smaller particles have a positive effect by lowering the C-term contribution. In practice this gives sharper peaks or allows higher flow rates with the same performance.[1]

Fortunately, this trend with smaller particle sizes is the same for proteins; the range for optimal performance increases (**Figure 1B**, page 00) as a result of the shorter diffusion pathway. The degree of improvement is much smaller for larger MW species. Therefore, UHPLC brings analysis speed for proteins into the ballpark of HPLC analysis speed of small molecules. In practice this means a difference in analysis time between e.g., 60 minutes and 15 minutes — but not bringing analysis time below 1 minute. (See the SCX separation example in **Figure 4**, page 00.)

In addition, there are other means to shorten the diffusion paths. The use of non-porous particle technology has a long tradition in protein separations. The tradeoff is that the sample–loading capacity is lower as a result of the smaller surface area. A compromise combining the best of both worlds are the solid core particles, where a small

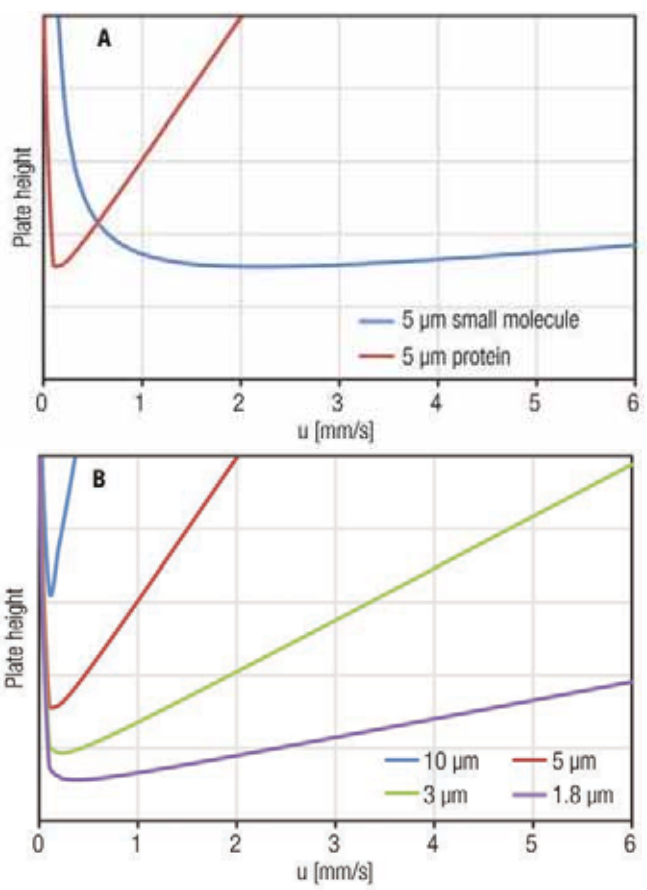

Figure 1: (A) Theoretical comparison between Van Deemter curve of two analytes with 100× MW difference ("small molecule" vs "protein") for the same column. (B) Comparison of optimal flow change with particle sizes for a protein.

Figure 2: UltiMate 3000 BioRS system, detail of the autosampler inert injection valve, and the Viper connection.

porous layer on the particles provides minimised diffusion paths and still provides acceptable sample loading capacity.

In all cases the use of smaller particles will result in increased pressure requirements from the system and that is where bio inert UHPLC equipment is required.

Bio UHPLC instrumentation

Biomolecules typically have multiple charges as well as a complex three dimensional structure. These properties allow and often necessitate the application of separation principles other than reversed phase. Therefore a bio UHPLC system does not only need to support UHPLC pressures but also the application of a wide range of chromatographic methods. A majority of analyses applied to proteins use solvents with extremes in pH and/or high salt concentrations. Any residual iron under these conditions will interfere with the analysis, foul the column, and in the end cause equipment malfunction.

The Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system is a dedicated bio UHPLC system, with an iron-free flow path to ensure stable operation under these harsh solvent conditions up to 1000 bar. This extends also to the columns, which are available in PEEK™ (Victrex PLC)-lined stainless steel, where the solvents and analytes are only in contact with inert PEEK and the stainless steel reinforcement allows the use of pressures that cannot be achieved by PEEK alone. All connections are made with a new addition to the Thermo Scientific™ Dionex™ Viper™ fingertight fittings family, offering the same performance and ease of use, but now with full biocompatibility.

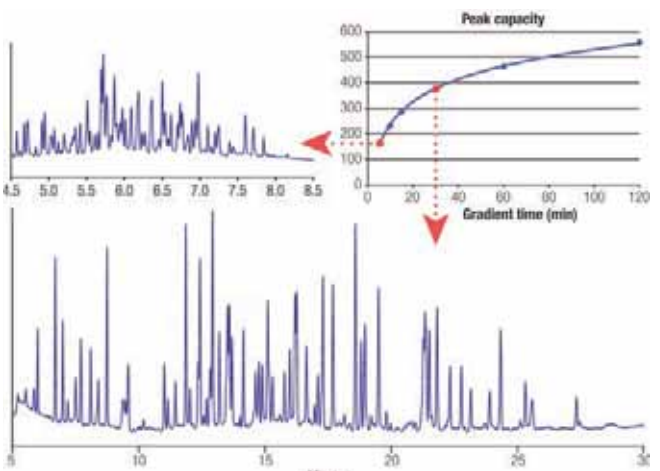

Figure 3: Peak capacity vs gradient time variation for a BSA digest separation on 2.1 × 250 mm Thermo Scientific™ Acclaim™ RSLC 2.2 µm C18 column.

UHPLC Separations of Biomolecules

A technology requires a goal in order to be useful. Bio UHPLC is most relevant for biopharmaceutical analysis where in-depth product characterisation is required. Generally multiple complementary techniques are required to characterise an entity or just a part of the biomolecule. The last section of this document will show some application examples of the UltiMate 3000 BioRS system in analyses typical for biopharmaceutical characterisation.

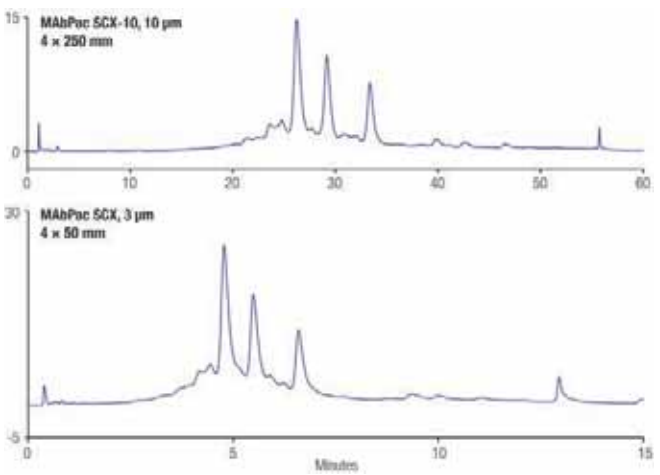

Figure 4: Comparison of SCX separation on 10 µm and 3 µm particle columns.

Peptide mapping – optimising analysis time and resolution

Peptide mapping is the analysis of enzymatically-generated protein fragments. In combination with mass spectrometric detection, peptide mapping is a powerful tool for elucidating the primary amino acid sequence. After enzymatic digestion, each protein is easily represented by 50 or more peptides, which have to be separated first to be identified. High resolution peptide mapping can be performed on the UltiMate 3000 BioRS system as shown in **Figure 2** (page 00). The use of a stationary phase with small particles will allow fine tuning for throughput or separation performance. The chromatograms in **Figure 3** (page 00) show the 5 minute and 30 minute separation with respective peak capacities.

Protein charge variant analysis – method speed-up

A common analysis in monoclonal antibody (MAb) characterisation is charge variant analysis. Here ion exchange chromatography is used to separate different charge states that can result from sequence truncations or differences in glycan structures. **Figure 4** (page 00) shows

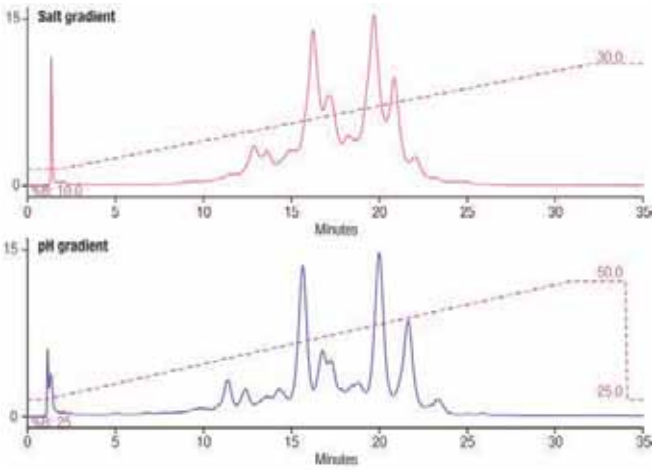

Figure 5: Comparison of salt-based vs pH gradient-based elution for the same MAb sample on identical MabPac SCX columns.

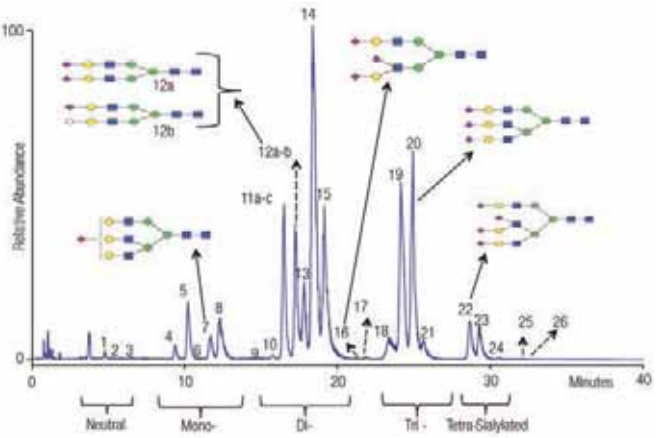

Figure 6: Separation of released glycans on GlycanPac AXH-1 columns and detected by LC-MS.

the separation of the same antibody sample on the Thermo Scientific™ MabPac™ SCX column with either 10 µm or 3 µm particles. The application of 3µm particles allowed the analysis to speed up without sacrificing resolution between the three major lysine truncation peaks or the detail between the major components. In this particular example the sample throughput has been increased by a factor of 4 using UHPLC.

Charge variant analysis with pH gradients

The example in Figure 4 is a salt-based elution with buffered eluents. Given the pI range of monoclonal antibiotics (MAbs) it is not uncommon that mobile phase systems have to be optimised for pH and salts when analysing different antibodies. This optimisation takes time and the method change reduces throughput. In addition, all new methods need to be thoroughly validated before use in the QC environment.

An alternative way to separate MAbs is to utilise pH gradient.[2] The MAb is loaded on the SCX column at a pH below the pI to ensure a positive charge on the protein. The proteins will bind to the negatively charged surface of the stationary phase. With a gradient the pH is increased over time, effectively reducing the overall positive charge until the pI of the MAb is reached, and at this point the MAb elutes from the column. The major benefit of this method is that it eliminates the need for pH optimisation.

Figure 5 (page 00) compares the salt with pH-based gradient separation of the same MAb on identical columns. Not only is the pH gradient method more generic and applicable to a wider range of samples but also, in this case, noticeably more detail is observed in the separated sample.

Since pH gradient methods use multiple buffer components that are not readily prepared, Thermo Fisher Scientific has developed a ready-to-use buffer kit. The buffer solutions have a specific make up that provide a linear pH variation when a linear AB gradient is programmed. The UltiMate 3000 BioRS system can also be equipped with a pH monitoring module (Thermo Scientific™ Dionex™ UltiMate™ 3000 PCM-3000 monitor) that can be used to validate the pH gradient formation.

Glycan separation

The last example is in the field of glycan analysis[3] and features the analysis of glycans by LC-MS. The Thermo Scientific™ GlycanPac™ AXH-1 columns provide a mixed mode chemistry for the separation of glycans. The charge form sialytion, as well as the general retention, are employed and these columns are available with sub 2 micron particles. Figure 6 (page 00) shows the separation of bovine fetuin glycan structures after enzymatic release from the protein. The distinct sialyted groups are visible as well as the separation within each group.

Conclusion

UHPLC is a technique with great acceptance for the analysis of small molecules. The title question "How does the analysis of biomolecules benefit from UHPLC?" was posed to explore and demonstrate that UHPLC can indeed offer advantages to the analysis of bio molecules. Apart from smaller particles, special solvents and separation principles beyond reversed phase also need to be supported. The UltiMate™ 3000 BioRS system represents a platform that is designed with multiple modules to allow configuration for in depth multidimensional analysis or high throughput quality control. The extensive column portfolio extends UHPLC capabilities beyond reversed phase by offering IEX or glycan separation materials in smaller particles and longer columns. Additionally, a ready-to-use buffer set is available to perform pH gradient charge variant analysis. The buffer system allows application of linear gradients to achieve a linear pH profile. With all these capabilities, the UltiMate 3000 BioRS is the system that will work in development, characterisation, and QC.

References

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2. Farnan , D.; Moreno , G. T. Anal. Chem. 2009, 81 (21), 8846–8857.
3. Fernandes, D.L. Eur. Biopharm. Review, Spring 2006, 100–104.

Evolving promising leads

Importance of outstanding SPR sensitivity for accurate binding results

Introduction

The precision of data from surface plasmon resonance (SPR)-based analyses, and the confidence with which results can be interpreted, is strongly coupled to the sensitivity of the SPR sensor. Biacore™ T200 system from GE Healthcare offers excellent sensitivity that enables detection of the lowest molecular-weight interactions and accurate measurements at very low concentrations. The system allows generation of high-quality data even when working with unstable and sensitive molecules such as membrane proteins.

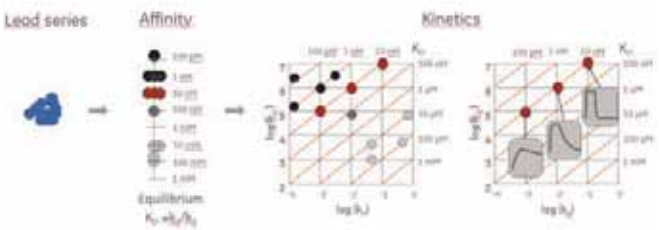


Figure 1: Binding affinity reflects the ratio of on- and off-rates (kinetics) and equal affinity interactions can have radically different kinetics

Advantages of kinetics in screening and characterisation

Interactions characterised by similar affinities can have very different kinetics. By resolving affinity into on- and off-rates, comprehensive information is obtained on how the dynamics of molecular interactions relate to protein function. On-rates reflect recognition between interacting partners, while off-rates indicate the stability of the complex. This information provides an extra dimension which can be crucial in supporting hit-to-lead development (Figure 1).

Precision through sensitivity: detect the smallest molecules

The need for technologies to reliably detect and profile interactions involving very small compounds is increasing in areas such as small molecule- or fragment-based drug discovery. When analysing interactions involving very small molecules in drug discovery, the sensitivity of Biacore T200 has no lower limit in terms of molecular weight, thereby reducing the risk of missing potentially interesting hits as well as confirming early failures (Figure 2).

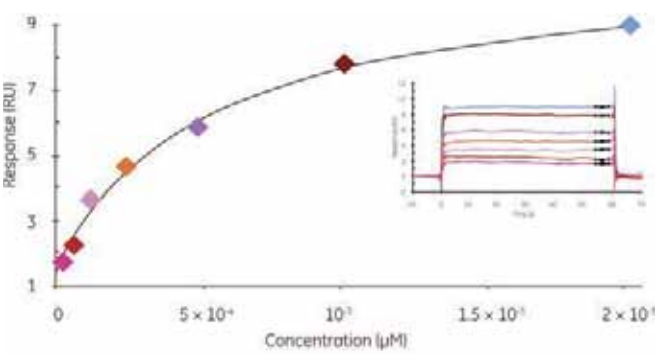


Figure 2: Binding of methanesulfonamide (molecular weight, (Mr) ~ 95) to carbonic anhydrase immobilized at a level of 7000 RU. Affinity (KD) was calculated to 0.49 mM

Flexibility in assay design: antibody characterisation without avidity

Full characterisation of antigen-antibody interactions is of great importance when assessing the suitability of antibodies as therapeutic, analytical, or diagnostic tools. Ranking of strong binders can be complicated by avidity effects, and the dissociation rate will appear slower than in reality (Figure 3A). To clearly differentiate strong-binding antibodies in terms of dissociation rates, it is necessary to use low levels of immobilised binding partner to obtain clean, avidity-free interaction studies (Figure 3B). Obtaining accurate data from the low immobilisation levels requires a highly sensitive SPR sensor for the analysis.

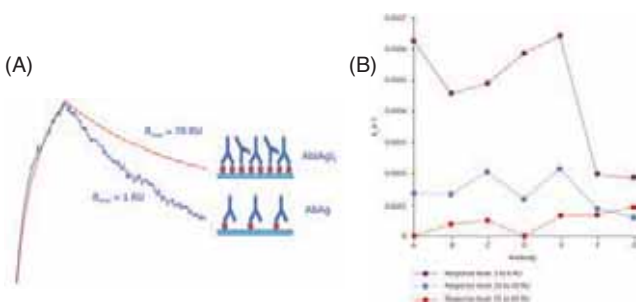


Figure 3: (A) Sensorgrams showing interactions between an antibody in solution and an immobilised antigen at high density (Rmax 70 RU) or low density (Rmax 1 RU). (B) Reliable ranking at low immobilisation levels to resolve antibodies with highly similar and slow dissociation profiles. At higher response levels, little or no difference is detectable.

The high sensitivity of Biacore T200 enables:

- Accurate results even from low levels of immobilisation when working with sensitive proteins
- Analysis of interactions characterised by on- and off-rates at the extremes of the kinetic scale
- Precise affinity determinations of interactions involving the smallest compounds
- Flexibility in antibody characterisation without complicating avidity effects

GE Healthcare

For further information, please visit:
www.gelifesciences.com/drugdiscoveryknowledgecenter

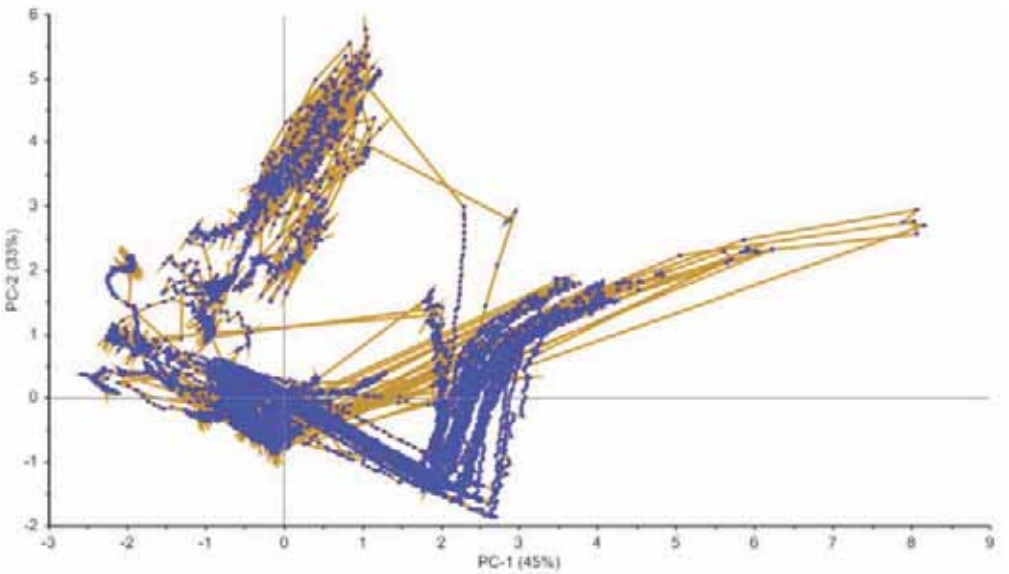


Figure 1: Combined Information of small-scale and large-scale batch trajectories in the PCA Score coordinate system

PAT success

PAT (Process Analytical Technology) is a tool within the QbD (Quality by Design) toolbox enabling real-time quality assurance. However the use of PAT is still not standard within the life sciences. The reasons may be many but typical explanations are that it is too time consuming, too costly, or there is simply no proof that it will benefit the operations. The premise for any PAT implementation is that it has to benefit the operations in an efficient and economically meaningful way.

The two fundamental requirements for successful PAT projects are relevant and cost effective tools in, addition to a justified business case. CAMO Software is working in this space to offer customers market leading software solutions for PAT applications.

The Unscrambler X is regarded as the best tool for analysis of historical PAT data with its wide selection of Chemometrics methods and intuitive user interface. CAMO also offers Process Pulse, which makes using Unscrambler models in online PAT applications easy. Process Pulse is scalable from the single user in a local laboratory to the fully connected enterprise version for the entire organization.

Recently CAMO went into an agreement with Lonza to provide them with an adapted enterprise version of Process Pulse. The solution connects directly to multiple data sources in the Lonza production lines and records and stores the data in secure databases. During data recording multivariate models can be run to track process performance and quality parameters, enabling real-time quality control. The recorded data is also available for future data analysis, troubleshooting and product traceability.

Data gathering and evaluation is becoming more important in the total life-cycle of a product and not just in the commercial scale production. Building processes start in the R&D labs and should be validated in the production plants. To close the gap between lab scale and production scale, the PAT data management software should be able to bring all data into one common platform.

The time consuming manual work will be supported and fully automated by the Process Pulse software. The main aspects are:

- Merging of multivariate and univariate data sets
- Bring data to a common time axis
- Identification of Batches
- Cleaning up of data sets
- Detection of interessting process steps
- Creation of Overlays

One example of combined data from lab scale and production scale shows the **Figure 1**. Due to the overlay of all batch trajectories the variance and the scaling effect can easily be demonstrated.

The integration of a PAT data management system into the development workflow is an important step to close the gap between R&D and production. And, process understanding becomes more integrated in daily business.

This Applican Note was co-authored by Dr. Tobias Merz, PAT Team Leader at Lonza AG

CAMO

For more information, please visit: **www.camo.com**

ActivityBase for results management in screening

A critical component of any scientific endeavour is the management of the results obtained. Inability to store and properly retrieve experimental results remains commonplace in research and development companies. This can lead to the needless repetition of experiments and, in some cases, incorrect conclusions being made due to storage of the data without context.

By screening a range of drug-like small molecules, you can build an understanding of agents and their effects on different cell types. Cells have a tendency to react in different ways, which necessitates the requirement for different analysis methods. All this compounds the need for effective data management.

Using both 96 and 364 well plate formats, cells are typically screened using single parameter and multi-parametric methods. An assay can even be performed on selected cells. Specifically, plates are subject to automated imaging and analysis, with multiple wavelengths being captured per field and typically multiple fields being captured per well. These associated component and composite images are subject to analysis, generating an output.

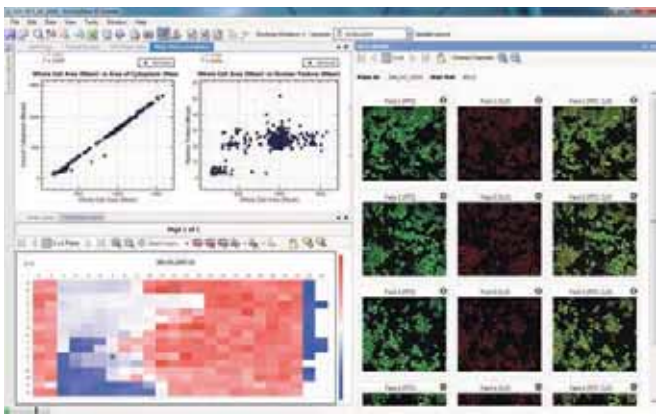
Need for results management and reporting

The key to success in any screening campaign is the ability to relate all appropriate information together. Raw data, quality assurance plate-based information, images (both composite and component), assay and protocol information must all be related to specific assays, plates and cells. Furthermore, derived results from the raw data must also be stored, with the ability to review and report on it.

Using ActivityBase in high content screening

Information generated by the image analysis tools is typically a file and is imported automatically into ActivityBase. Using import definitions, ActivityBase can be configured to work with a wide variety of HCS instrumentation (e.g. the In Cell 1000 Analyzer, GE). Upon import, the result information is associated with the appropriate screening campaign. Users have the option to either interactively or automatically exclude outlying values in the high and low control wells. The quality assurance Z is then generated, upon which a pass or fail decision can be made.

Reporting on single parameter datasets is relatively straightforward, but multiparametric reporting can be more difficult, especially when individual parameters have associated independent variances. This makes the quality assurance of plates challenging and highlights the need for robust data management. Once statistical criteria have been decided on for hit selection, a cherry picking list is generated and hits are retested. In potency analysis, a typical problem is hook effects which is due to cell toxicity at high compound concentration. These can be observed and rectified using the analysis tools within ActivityBase.



Benefits of ActivityBase

ActivityBase Suite is one platform for the test management of both high throughput (single parameter) and high content (single or multi-parametric) screening. The ability to work with different file types directly in the solution significantly reduces and almost eliminates transcription errors, which can have dramatic effects on result quality. However, the scientist remains in control of their results and can select the desired level of workflow automation. Quality assurance criteria can be applied to individual plates, or even whole batches, to select whether datasets should be included in analysis, based on control well values. Scientists also have the ability to observe specific erroneous data points and exclude from analysis if necessary.

Conclusion

The secure and robust management of intrinsically complex cell-based screening data is paramount to ensure confidence in results and subsequent decision-making. ActivityBase has long been regarded as the gold standard in biological high volume test data management, with a proven ability to adapt to numerous workflows in research laboratories over many years. These capabilities are proving equally applicable in many new types of high content assays, which are accelerated with the provision of the proven robust data management capabilities. Enabling scientists to analyse the characteristics of different cells-based assays, ActivityBase offers a compliant and accessible solution, allowing scientists to spend more time interpreting results and less time managing the data.

idbs

For more information, please visit: **www.idbs.com/epr**