

Implementation Of A High Throughput Chiral Pooling Strategy Generates Efficiency Gains For A Leading Purification Service Provider

How Reach Separations has increased productivity and profits in an increasingly competitive and regulated environment

Reach Separations is a Contract Research Organization (CRO) specializing in outsourced purification services to pharmaceutical, agrochemical, and fine chemical companies throughout the UK and Europe. Based at BioCity in Nottingham in the UK, Reach employs the most advanced liquid chromatography (UPLC), supercritical fluid chromatography (SFC), and mass spectrometry (MS) technologies for the separation, identification, and purification of achiral and chiral small molecules.

The specialty purification services offered by Reach are in increasing demand, particularly so in the pharmaceutical industry, as companies strive to improve the delivery time of compounds to market, while reducing research and development costs, including capital expenditure outlay. As the industry is looking for new sources of discovery and innovation with limited resources, there is a growing preference to outsource. Globally, the trend toward drug discovery outsourcing is growing rapidly, with the world market predicted to reach \$30 billion by 2020.¹

To keep pace with increasing market demand for purification services, Reach has rapidly expanded. Since startup in 2012, Reach has doubled the number of employees, increased lab space 5-fold and invested in new, state-of-the-art instrumentation. In the second year of operation, Reach supported 16 clients all based in the UK. The company now serves more than 90 clients across the UK, mainland Europe and the U.S. and, despite this hectic period of expansion, has succeeded in building an exceptional reputation for quality and timely completion of projects.



Reach relies on technologies like UPC² because of its enhanced resolving power and sensitivity, achieving impurity detection levels below 0.1%.



"In order to continue to grow our customer base and achieve our business goals, our labs must perform at peak efficiency day in and day out. High throughput and rapid turnaround of purified materials are vital to our success."

ALEX BRIEN

Senior Separations Scientist,
Reach Separations

Victoria Coulthard, Business Development Manager at Reach says, "Our basic philosophy is to focus on quick turnaround and to find innovative ways to prevent samples from stalling in the queue. Our goal is to always meet or preferably exceed customer expectations."

FIRST IN CLASS FOR CHIRAL PURIFICATION

A combination of purification expertise, fit-for-purpose lab equipment, highly motivated staff and a strong business model ensure that Reach can deliver exceptional service to their customers. For chiral compounds, Reach offers separation services from enantiomeric excess (EE) determinations through to the purification of hundreds of grams of enantiomer from racemic mixtures.

With pharmaceutical drug developers under pressure to adhere to regulatory guidelines on testing the single enantiomer rather than the racemate (for both generic and branded drugs), Reach has seen a significant increase in demand for their chiral purification services. Companies realize that it is cheaper, quicker, and easier to access the single enantiomer through chromatography rather than via asymmetric synthesis, particularly at early stage discovery.

THE NEED FOR INCREASED SPEED AND SENSITIVITY

To maintain the high efficiency with which Reach operates and ensure the continued quality of their customer service, the company regularly re-assesses their analytical strategy and implements fit-for-purpose laboratory equipment while applying sound scientific working practices. In late 2014, Reach upgraded their lab with state-of-the-art equipment that would help them optimize both their chiral and achiral separation workflows, improving the quality of data they provide to their customers, and speeding sample turnaround.

Reach adopted Waters ACQUITY UPLC® and ACQUITY UPC²® Systems and the ACQUITY® QDa® Mass Detector along with Empower® 3 Chromatography Data Software (CDS) because they were the best technology match for their lab providing the enhanced separation capabilities they needed. One year after the purchase, Alex Brien, Senior Separations Scientist, commented, "The ACQUITY UPLC has become the primary analytical instrumentation we use for the quick determination of purity of incoming and outgoing samples. With this system we can provide our customers with accurate purity determinations, confirmed by UV and mass spectral data."

He continued, "For chiral separations, the ACQUITY UPC² System provides the resolving power and sensitivity we need to detect and quantify enantiomeric mixtures and significantly

outperforms our legacy SFC technology. Before the advent of UPC²®, we couldn't achieve the sensitivity to confidently detect impurities below 0.1%. Now, we can provide our customers with 100% data accuracy, and in addition to the sensitivity gains, we have seen a greater than 99% increase in instrument uptime."

According to Alex, the sensitivity and reliability of the instrumentation were the overriding factors in choosing Waters technology. Their decision was also influenced by their confidence in Waters' applications and service teams. "Waters adds immeasurable technical and scientific expertise to both the implementation of the systems and our overall workflow," said Alex.

SUPERCRITICAL CO₂: THE MOBILE PHASE OF CHOICE FOR CHIRAL SEPARATIONS

Speeding the cycle time for chiral method development has become a key initiative in improving productivity, turnaround, and profitability at Reach. In the past, chiral separations were predominantly performed by normal-phase LC, using cellulose or amylose-based stationary phases. While normal-phase LC does have advantages for separating chiral compounds, the arguments against are persuasive. First, the ability to perform gradient separations is limited, and therefore isocratic separations are developed using different columns with different mobile phase combinations in series, which is time consuming. Second, equilibration times needed to achieve reproducible retention times can be lengthy, and the solvents typically used can be toxic and relatively costly to purchase and dispose of.

In recent years, SFC has become the separation technique of choice for chiral screening, enantiomeric excess determination, and chiral purification. There are several reasons for this: the supercritical CO₂ used as the mobile phase has a very low viscosity, allowing faster flow rates and therefore quicker run and equilibration times; the use of carbon dioxide and alcohol is safer for the environment; and furthermore, in purification, the volatility of CO₂ at room temperature and pressure mean that the CO₂ evaporates rapidly, leaving the purified compound in a low volume of co-solvent, significantly reducing the dry down time.



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EFFICIENCY GAINS THROUGH SAMPLE POOLING

Reach's chiral screening workflow had typically involved the sequential analysis of samples on HPLC using 3 different mobile phases across 6 chromatographic columns, with a total of 18 analytical runs carried out for every sample. However, with the demand for chiral work increasing, they realized there was a need to change their analytical approach. With the addition of the ACQUITY UPC² and the QDa Mass Detector, Reach saw the opportunity to implement a more efficient strategy involving the pooling of multiple samples.

Although sample pooling is not a new idea,² this approach is far from straightforward using LC separation with optical detection. This is primarily due to the time and standards needed to identify the pooled compounds, which elute with varying retention times, depending on the separation conditions. With access to mass detection, however, chiral pooling becomes feasible since every chromatographic peak is associated with mass information, allowing very quick identification.

Reach's high throughput screening strategy involves pooling a number of racemates for analysis at the same time using short, 6-minute, analytical runs. Three different mobile phases are evaluated across 9 analytical columns, therefore 27 separations per sample. This approach minimizes the time taken to determine the optimal separation method and provide the method conditions required to move forward to purification.

The aim is not to attain baseline separation of every compound in the sample, rather to assess the best conditions for separating isomers. The mass detector is essential in viewing the different isomers and helping to make quick decisions on the best separation conditions. Reach asks their customers to provide the molecular mass of the compounds they submit for analysis and it is straightforward to extract the mass chromatograms for specific samples from the pooled mixture and view the data.

"At first we had reservations about introducing a mass detector into our chiral screen," said Alex. "We had no experience with SFC-MS and real concerns with regard to sensitivity and robustness. However, we were up and running very quickly, and realized early on that it was the right decision. With the addition of the QDa Detector and the ability to detect mass data as well as UV spectral data, we are able to pool chiral samples and analyze as many as 6 compounds in a single screening run. We can also take advantage of the mass information to confidently determine the enantiomeric excess in the mixtures."



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Alex explained further, "Without the mass spectral data we obtain from the QDa Detector, we would have to screen one sample per vial on the UPC² System. Now, by pooling samples in a single vial, instead of carrying out multiple lots of 27 runs, we can do the same 27 runs with all samples at the same time. We can screen as many as 55 samples per day, providing a 10x increase in speed over conventional HPLC methods and 5x increase over conventional, sequential SFC runs."

In addition to increasing sample throughput, the approach taken by Reach has also helped to significantly reduce their costs associated with the purchase and disposal of solvents. The use of CO₂ and small amounts of modifier is less expensive than purchasing the solvents needed for normal phase chromatography and also reduces their overall solvent disposal costs. With UPC²/QDa-based chiral pooling, Reach estimates they are saving 50% in solvent costs compared to their previous approach.

A HIGHLY EFFICIENT WORKFLOW: FROM SAMPLE LOGIN TO DELIVERING RESULTS

In addition to introducing a high throughput screening approach, Reach has optimized their entire workflow from the time the samples enter the building to the time results are delivered to their customers.

Reach's Chiral Screening Workflow:

1. Client logs sample into the online LIMS system.
This is a simple web interface
2. Client samples are received into a safe and secure delivery area. Reach receives immediate notification that a parcel has arrived
3. Routinely pool 3-4 samples; this fits well with the current workload
4. Vials are placed then go into the UPC² sample manager

- Empower and the mass analysis tool with enhanced peak tracking are used in their standard acquisition and processing parameters
- Pooled vial is analysed on 9 different chiral selectors, also three different alcohols as modifier
- Data processed automatically but interpreted by a skilled chromatographer
- Using the XIC feature in the Empower mass analysis window enables straight forward viewing of the mass information
- The optimal separation is repeated on the single analyte (one 6-min isocratic run)
- The sample is then scaled up to prep
- A customised Empower report is then shared with the client

Their Empower® 3 Chromatography Data Software (CDS) is an important part of this workflow, working in harmony with their in-house LIMS to significantly improve efficiency, and enabling automation of laborious tasks associated with sample management, data processing, and reporting. Empower 3 also incorporates a dedicated mass analysis window with peak tracking for use with chromatographic systems coupled to an ACQUITY QDa Mass Detector, which displays chromatographic results and mass information.

This mass analysis interface allows the team at Reach to make quick decisions based on the data, a crucial aspect of reducing sample turnaround time. An example is shown in figure 1. The UV spectra and mass chromatograms for a sample of 6 pooled racemates are viewed using the Empower 3 mass analysis window. In this case, resolution of the isomers with a mass of 471 Da has been achieved. The compounds are identified using extracted mass chromatograms, whereby a known mass is entered into the software and only compounds containing that mass are displayed in a given channel. This allows the identification of compounds even if the relative concentrations vary significantly.

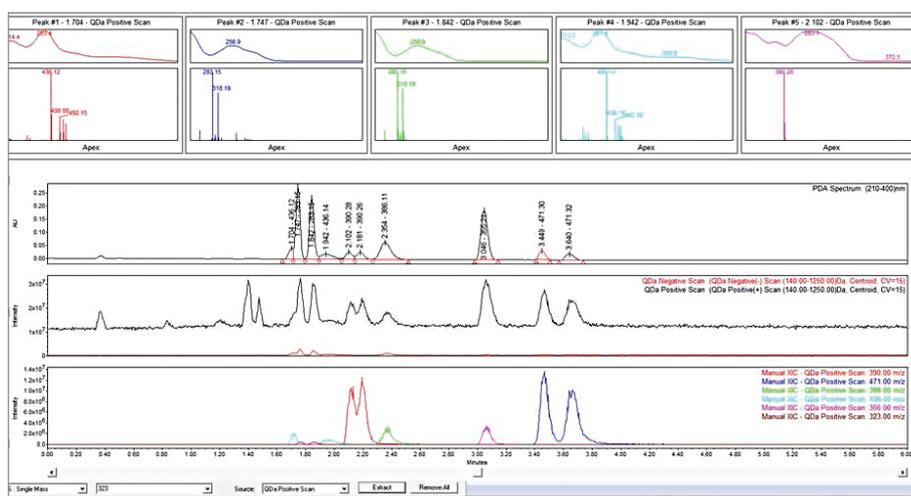


Figure 1. UV spectra and overlaid mass chromatograms for a sample of 6 pooled racemates, viewed using the Empower 3 mass analysis window. In this example, resolution of the isomers with a mass of 471 Da (dark blue trace in the lower window) is achieved. UV and MS spectral purity information is displayed at the top of the mass analysis window and can be used to confirm the purity of the peaks.

“The peak is detected and integrated based on the Empower software MS process method. A report is generated automatically and we can quickly look at the run and very easily identify by molecular weight where separation has been achieved and where it hasn't. The software is easy to use and fits very well within our workflow.”

Victoria Coulthard
Business Development Manager,
Reach Separations

"The peak is detected and integrated based on the Empower software MS process method," explains Victoria. "A report is generated automatically and we can quickly look at the run and very easily identify by molecular weight where separation has been achieved and where it hasn't. The software is easy to use and fits very well within our workflow."

In addition to the workflow benefits offered by Empower 3, the fast start up time and automated internal mass calibration functionality and resolution verification of the QDa Mass Detector are considered by Reach to be important features in a lab where speed is critical to delivery.

A BRIGHT FUTURE FOR REACH

Reach Separations has addressed the challenges of a dynamic specialized outsourced purification business and is flourishing in a highly competitive, fast-paced environment. The company's success is largely due to having a good understanding of their customers' needs, an extremely high level of expertise in purification, and a reputation for consistently delivering on their promise. The ability to react quickly to address the unique goals for each project sets them apart from the competition, ensuring that they are considered as the partner of choice for outsource purification.

Reach's partnership with Waters and their decision to invest in Waters instrumentation and informatics has facilitated a dramatic improvement in their analytical workflow for chiral screening. Their automated HPLC and SFC-based screening systems now allow them to screen over 200 chiral conditions in less than 24 hours giving a 98% success rate in terms of identifying the appropriate conditions for separation. The significant increase in sample throughput helps move compounds to the purification phase much faster and speeds the overall sample turnaround time.

Through the solid relationships they are building with their industry partners and customers across the pharmaceutical, fine chemical, agrochemical, and academic sectors, and with their clear insight and specialist capabilities, Reach Separations is well positioned for continued growth and success.

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