Fraction Trapping Liquid Chromatography (FT-LC):

Save Hours of Dry-Down Time! Desalt and Re-format your Prep HPLC Fractions in < 15 minutes.

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Preparative High Performance Liquid Chromatography (prep HPLC) is an established technique that has been used extensively by the pharmaceutical and chemical industry for many different applications and stages of the product development process. From early discovery through to late stage clinical development and even production, preparative chromatography plays a very important role in bringing a product to marke

The drug discovery and development process is a lengthy one. After target selection and assay development, it can take between 10 and 15 years to complete, at a cost of $500 million to $1Billion dollars. Of the 5000 compounds studied during lead discovery and optimisation/selection, perhaps only 5 will make it to clinical trials. Therefore, the recent trend has been to study more compounds earlier in order to have more candidates to trial. This approach however, has resulted in a more lengthy timescale with respect to chemistry i.e. synthesis and purification.

The speed of purification and level of purity of compounds, treated by prep HPLC, has increased dramatically over the last 20 years as techniques have advanced. Today, many thousands of compounds are purified by this method each day. The run times have decreased and throughput has improved now to a point where prep HPLC is no longer a bottleneck in the product development cycle.

The bottleneck today has shifted downstream, to the time taken to recover the purified product. A prep HPLC run can be completed in 15 minutes or less, however, it might take 8-24 hours to recover the purified compound and dry it, reconstitute it in the desired solvent and format it into a compound library. The reason for this additional time frame is due to the high water composition of the collected fraction, the need to change the product's structure, e.g. to remove compounds such as trifluoroacetic acid (TFA) and re-format the compound into another solvent or structural form.

However, Anachem has now developed a unique solution that will revolutionise prep HPLC by reducing this potentially overnight stage of the process, down to only 15 minutes. Following a tradition of taking existing Gilson instrumentation and further enhancing its capabilities, Anachem has designed its FT-LC system around the renowned Gilson prep HPLC platform. The FT-LC can dramatically and rapidly reduce the water content of fractions collected from the prep HPLC system from 88% water to less than 1% in under 15 minutes.

Figure 1: The Anachem/Gilson Fraction Trapping Liquid Chromatography System

FT-LC System & Software

The FT-LC system [Figure 1] is based on the industry standard Gilson prep HPLC system with application specific software. There are many hundreds of these prep systems in use in the UK and across the world. The FT-LC is composed of a standard prep HPLC gradient system with additional accessories to perform the trapping process. The system will work with columns from 4.6 mm to 50 mm ID and can process samples of a few milligrams up to several hundreds of milligrams of initially purified material in sample volumes from a few millilitres to litres.

The FT-LC system is easy to use. Samples are loaded into a sample loop by the liquid handler and pumped onto the trapping column where they are diluted with a specific mobile phase. The mobile phase selected to load the sample onto the trapping column is dependent on the compound's retention time from a previous HPLC run. This is a critical part of the process that eliminates the possible precipitation of the sample during the sample loading process, but ensures complete retention of the compound on the trapping column.

The protocol has been optimized to run automatically using two columns. Whilst column one is loading the first sample and processing this, the second column is being cleaned and regenerated by a series of

washing steps with acetonitrile and mobile phase solvents. These washing steps eliminate any possible carry over from the previous injection. On column one, the first sample is eluted using a strong volatile solvent of dichloromethane/methanol and collected on the liquid handler. The sample is eluted in <20 mls of volatile solvent and takes approximately 20 mins to dry down in a standard drier from GeneVac or a V10 instrument from Biotage.

Trapping Column Media

After extensive trials at Anachem and our partners in the project the media selected for use on the FT-LC system was a Polymer Labs 45 micron media. This material is extensively cross-linked providing a very high surface area and hence an excellent dynamic binding capacity for small molecule drug candidates. The base polymer is made of styrene-divinylbenzene which is very resistant to a wide selection of solvents, acids and bases, which is essential for this application. This column is now commercially available from Anachem and Varian Inc. under the trade name FlowTrap™.

Practical Examples Of Fraction Trapping

In order to test the performance of the system and the software protocol, we used a wide variety of test compounds with different chemical and physical properties.

Compound	Mol. Wt.	logP	ACN%
Caffeine	194.19	-0.13	10%
Propranolol.HCL	259.34	3.09	37%
Probenecid	285.35	3.30	67%
Benzophenone	182.21	3.18	85%
Warfarin (base prep)	308	3.40	14%
Dipyridamole	504	-1.22	30%

The dilution buffer is selected by the ACN%, i.e. the percentage of acetonitrile required to elute the compound during prep HPLC (or retention time). Compounds that elute before 40% acetonitrile can be diluted with water referred to as solvent one. Compounds that elute with >40% acetonitrile are diluted with an organic solvent/water mixture referred to as solvent two.

The compounds are eluted from the trapping column with a volatile solvent, using a second pumping system. The eluted compound is then collected into a user designated vessel in the collection rack.

Verification of Trapping Efficiency

In order to test the effectiveness of the trapping column, we performed a series of experiments on a wide variety of compounds to measure the recovery of the process and the water content of the collected fraction.

Study 1- Performing a loading optimisation study on the FT-LC trapping columns

The initial studies looked at the dilution ratio and loading flow rates on a 4.6 mm x 150 mm column. The optimal dilution ratio was found to be 1:5. This ratio gave the most reproducible and optimal results. Loading studies were performed using caffeine at 5 mg/ml. The total loading capacity of the column with caffeine was found to be approximately 50 mg, using a dilution ratio 1:5 and a total flow rate of 16 mls per minute (See figure 2).

The column loading capacity was also tested using propranolol.HCl and probenecid. Probenecid was diluted with solvent two. The loading capacity with these compounds was found to be a little less. The maximum capacity of the 4.6 mm x 150 mm column was found to 25-30mg.

The process was then scaled up to a 10 mm x 150 mm column in order to test loading of 100 mg of compound. The column worked very well and the process scaled up efficiently.

Study 2 - To Measure the Water Content of the Eluted Fraction from the Trapping Column.

The water content of our eluted fractions was measured using a Mitsubishi Karl Fisher CA-200 moisture meter from a1-envirosciences Ltd. The water content of all the organic solvents used in the study was measured to perform a blank test.

The same Karl Fisher analysis was performed on the eluted fractions within 10 minutes of their collection. This was done to minimise the effect that some solvents have for absorbing water.

Fractions were collected in 0.5 ml volumes across the entire chromatogram, with no fraction collector delay set. We measured the water content for each fraction for a blank injection of 20 ml water and for a sample of 20 ml of 100 mg benzophenone.

The water content of a typical caffeine or benzophenone fraction of 20 ml was found to be around 5%. An additional final polishing step can be automatically carried out on the FT-LC system, using a phase separation membrane. This removes the final 5% water content to further reduce sample dry down times, and add to the quality and the physical appearance of the compound. The phase

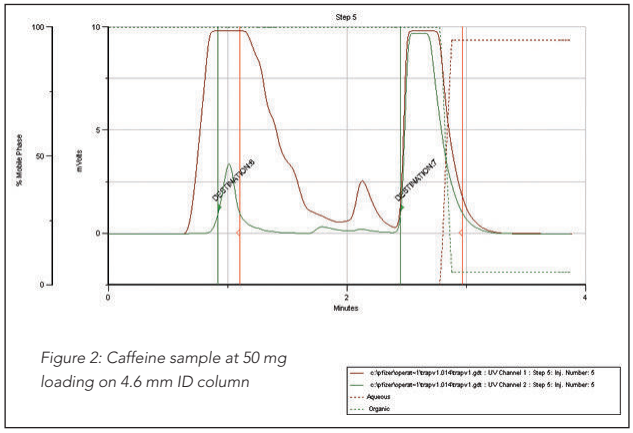

Figure 2: Caffeine sample at 50 mg loading on 4.6 mm ID column

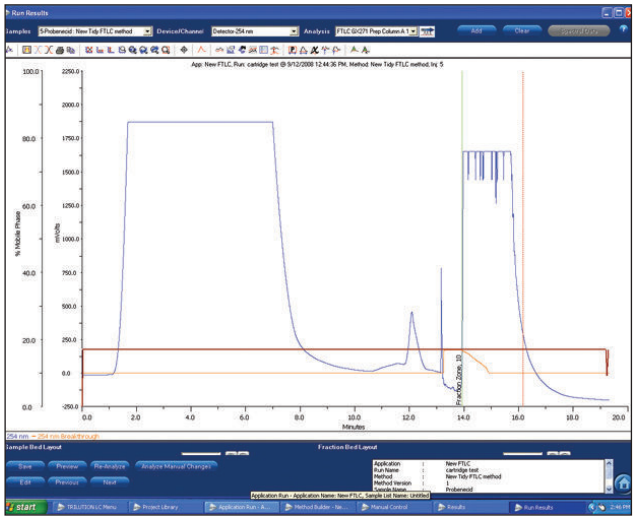

Figure 3: Probenecid sample at 100 mg loading on 10 mm ID column.

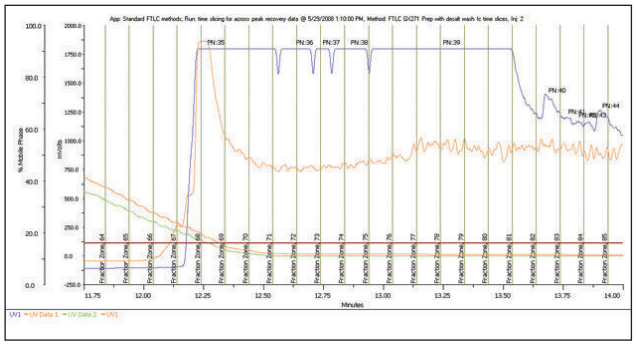

Figure 4: Benzopheone (blue trace) and a Blank run (orange trace). Fraction size 0.5ml. The water content of Fraction 70 was approximately 5%. At fraction 72 <0.4% of water ie. Solvent)

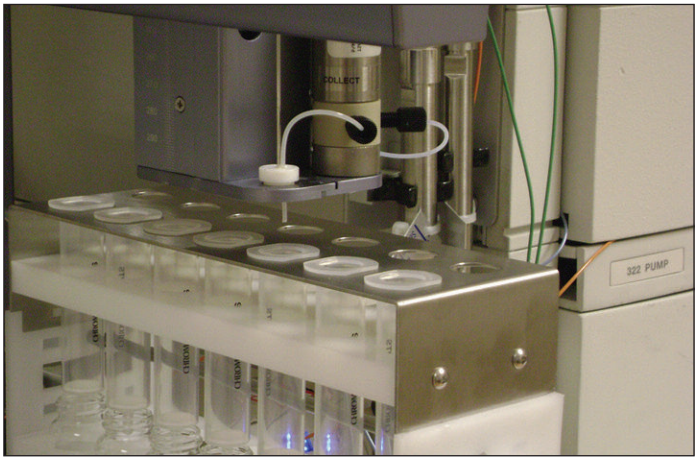

Figure 5: Phase Separation Membranes remove the remaining water

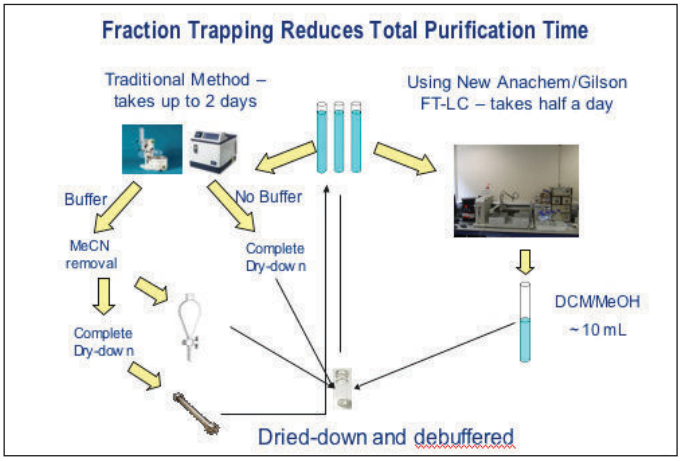

Figure 6: Anachem FT-LC Saves Time Compared to Traditional Prep HPLC methods

separation membrane allows the DCM/Methanol solvent through, containing the compound, but retains the water. Figure 5: Phase Separation Membranes remove the remaining water

Study 3 – To measure the recovery of compounds from the 10 mm ID FT-LC trapping column.

Recoveries from the FT-LC have been calculated for the total process from injection onto the FT-LC column, through to the final elution into the fraction collection tubes. Recoveries have been consistent with findings in prep HPLC of > 90% for the whole process.

Compound	% Recovery
Caffeine	99%
Propranolol.HCL	95%
Probenecid	95%
Benzophenone	95%
Warfarin (base form)	99%
Dipyridamole	95%

Impact of Fraction Trapping on Total Time of Purification Process

Pfizer, Sandwich has been using the Anachem FT-LC approach. In Figure 6 is a practical example of a Pfizer process, one using the traditional approach, the other using the Anachem FT-LC. The example shows how the FT-LC method took less than half a day compared to the traditional method which took up to two days.

Impact of Fraction Trapping on Quality of Purified Compound

In another example from Pfizer using the FT-LC, the resulting product quality has been improved over the existing methodology, as clearly shown in the Mass Spec traces in Figure 7.

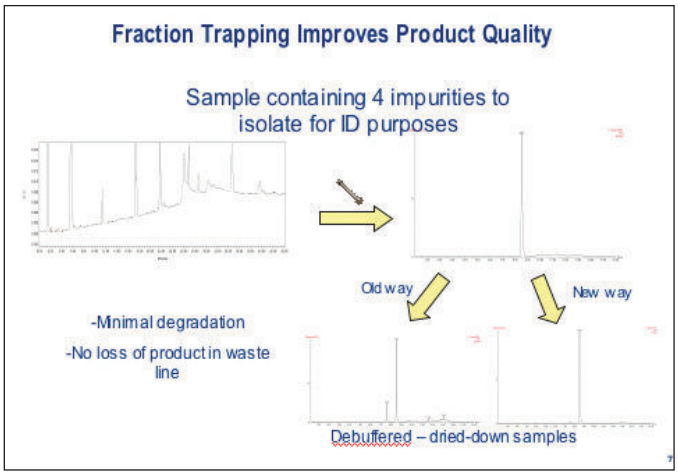

Figure 7: Anachem FT-LC Improves Product Quality

Conclusion

The FT-LC system has been running at Anachem now for over one year, and also at a large pharmaceutical company for several months. The system has proven to be very reliable. The FT-LC currently can be configured to work with 4.6 mm to 50 mm ID prep columns, working at flow rates between 5 - 200 mls/min and processing samples from 1-2 mgs to 1 gram in a single injection.

As discussed, the FT-LC will significantly reduce the water content of samples, while maintaining high recovery, and can even be used to de-salt the sample during the process. All this can be achieved in reduced time when compared to traditional methods.

The FT-LC trapping columns can capture a diverse range of compounds from very early polar eluting compounds to very hydrophobic compounds, making Anachem's Gilson-based FT-LC system a valuable new tool for all separation scientists and chemists.

We would like to thank several key individuals and acknowledge their work on this project and for the data they supplied to us over the last few years:

Pfizer Sandwich	Mark Taylor, Ben Matthews, Stephane Dubane, Mike Stace, Helen White, Susan Davidson
GSK	Bob Boughtflower, Tim Underwood, Keith Brinded
AstraZeneca	Steven Norris, Mike Giles
Polymer Labs	Graham Clever, Paul Boguszewski
Anachem Ltd	Jamie Swaile, Matthew Smith