Enabling facile, rapid and successful chromatographic Flash purification

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Flash liquid chromatography (Flash-LC) is an established technique that can have a very positive impact on productivity in the pharmaceutical research and development chemistry laboratory - provided it is used efficiently. Finding the optimal conditions for a normal phase or reverse phase Flash-LC separation can be a laborious process. The aim of this work was to develop a screening system to enable automated, rapid and reliable method development for preparative Flash chromatography. Implementation of these method screening systems into the chemists workflow has had beneficial productivity and quality impacts at different stages of drug development, and on scales ranging from laboratory to pilot plant.

Key words: Flash chromatography, automated method development, normal phase, reverse phase.

Introduction

Since first being introduced in 1978 by Still *et al.*[1], Flash chromatography, a medium performance liquid chromatographic (MPLC) purification technique, has evolved considerably, and has become a common tool for synthetic chemists in their everyday work. This is particularly true in modern industrial environments such as the pharmaceutical industry where tight timelines demand high productivity and quality. Flash-LC is a purification method of choice for large scale preparative separations and is used routinely on a laboratory scale (g-kg) and pilot plant scale (10's of kg). Advances made by instrument manufacturers have given access to reliable automated Flash-LC purification hardware for the laboratory and dependable pre-packed columns. Whilst most chemists are familiar with this technique, it is appropriate method development that remains the main hurdle in making the best use of it. Developing fit for purpose methods for routine laboratory work can be time consuming. Developing an optimised method to efficiently execute the more demanding applications of Flash-LC within the pharmaceutical industry, such as purification of materials to be used in non-clinical or clinical trials to tight purity requirements, can be particularly testing for those not skilled in the art.

Flash-LC employs particles with dimensions on order of 50 µm which give relatively low column efficiencies (N) compared with other LC techniques such as the ubiquitous high performance liquid chromatography (HPLC). Column efficiency cannot be relied upon in order to achieve a meaningful preparative Flash-LC separation. Instead a high selectivity (alpha) is required to achieve the resolution required to translate a Flash chromatographic method into pure product. The optimisation of selectivity is a key factor for successful method development for preparative applications. One very practical and effective means to affect selectivity for normal phase (NP) Flash-LC is by careful solvent choice - selecting solvent systems composed from a combination of solvents from the eight NP solvent selectivity families (based on Snyder's *et al.*[2] and Glajch *et al.*[3] solvent selectivity descriptors). For reversed phase (RP) Flash-LC the selectivity may be most affected by organic modifier choice or pH. Conventionally the method development process involves screening solvent combinations using TLC to determine the best solvent(s) and relative proportions in order to achieve the required separation. However, in order to achieve the required selectivity it is often necessary to use binary, ternary, or sometimes even quaternary solvent systems composed of a combination of solvents from the eight NP solvent selectivity families. Hence, even a routine method can involve a vast array of solvent combinations and proportions. Once the selectivity effects are known, other factors such as the chromatographic band shape, solubility, stability, solvent cost and environmental issues can be considered. The method development process can be arduous and the consistency and quality of the optimised method is dependant on the skill set of the individual.

The key to enabling facile, rapid and successful Flash-LC purifications is to be able to quickly identify the best conditions to maximise selectivity for a given separation problem using minimal effort and achieving consistent results independent of a chemist's experience with the technique. The aim was to deliver method development systems to rapidly and easily develop a method for normal or reverse phase Flash chromatography. The method screening systems would be implemented into intuitive and integrated purification workflows in order to encourage and enable the use of robust and successful purifications.

Experimental

All solvents used in this work are HPLC grade from Sigma-Aldrich (Gillingham, UK). The screening instruments were Agilent (Stockport, UK) 1100 HPLC systems (quaternary pumps) equipped with a solvent selection valve to enable screening of more solvents on the normal phase system. Detection was performed with a diode array UV/Vis detector. The evaporative light scattering detector used with the normal phase screening system was an ELS 1000 from PolymerLabs (Church Stretton, UK). The instruments were controlled using Chemstation Rev. B.03.01 and Easy-Access Rev. A.05.01 software. All Flash chromatography experiments were performed on a Biotage (Uppsala, Sweden) Isolera One equipped with a variable wavelength UV detector and two collections beds.

Solvent A "weak solvent"	Solvent B "strong solvent"	Snyder Selectivity group	Strength ε° (silica)
Heptane		0	
Toluene		VII	0.22
	Ethanol	II	0.65
	Ethyl Acetate	VIa	0.36
	tBME	I	0.32
	Acetone	VIa	0.53
	DCM	V	0.3
	Acetonitrile	VIb	0.52

Table 1: A list of the solvents used in the normal phase Flash screen. For use with basic analytes, additional methods can be selected that incorporate the base diethylamine with the other solvents.

Time (min)	% solvent A	% solvent B	% Ethanol
0	95	5	0
0.4	95	5	0
2.3	0	100	0
2.7	0	100	0

Time (min)	% solvent A	% solvent B
0	0	100
0.2	0	100
0.3	95	5

Table 2: Gradients used for the normal phase Flash screen: on the right, the column conditioning and equilibration gradient; on the left the gradient method used to generate the data.

Time (min) or ~column volume	% eluent A	% eluent B
0	95	5
2	95	5
17	5	95
20	5	5

Table 3: Reverse phase scouting gradient on a scaling Flash column. Since one minute approximately equals one column volume on the analytical scale, the same gradient method can easily be scaled to any preparative Flash column based on column volumes.

Sample A: This compound was a mixture of an intermediate from a drug substance synthetic route and contained one major desired product and three minor impurities that required purging. The sample was prepared by dissolving 5 mg in 1 mL of dichloromethane, and then analysed using the normal phase screening system.

Normal phase conditions:
Column: Luna Silica (2) 50 x 3.0 mm, 3 micron
Mobile Phase: See Table 1
Flow rate: 4 mL/min
(3.5 mL/min if Toluene is used)
Temperature: Ambient
Equilibration: see Table 2
Overall screening run time (6 methods): 45 min

Reverse phase conditions:
Column: Biotage C18 250 x 4.6 mm, 60 micron
Mobile Phase: See Table 3
Flow rate: 3 mL/min (~ 1 column volume/min, based on Biotage data)
Equilibration: 5 min equilibration at starting conditions
Temperature: Ambient
Eluents: A= Water/TFA (0.1 % vol) or Water/Ammonium Acetate (10 mM)
B= Acetonitrile or Methanol
Overall screening run time (4 methods): ~1.5 hours

Results and Discussion

Automated Normal Phase Flash method screening

An automated normal phase method screening system was built using standard HPLC instrumentation. Glajch et al.[3] demonstrated that solvents can be categorized using three descriptors (non-localized, base localized and dipole localized). These descriptors led to the classification of solvents for normal phase chromatography into eight selectivity families (Synder et al[2]). A comprehensive method development screen should ideally include a solvent from each of the eight selectivity families. However, some solvents are unfavourable in practice for reasons relating to safety, the environment or silica incompatibility. The method screening system includes solvents from several of the selectivity families (i.e. I, II, V, Via, VIb and VII) and performs a gradient with a choice of either heptane or toluene as the weak eluent (Tables 1 and 2). For basic analytes, the same methods can be run with basic additive conditions (using a dedicated column) by selecting the appropriate method in the instrument software. The diverse selectivity that can be obtained using different normal phase solvent systems is illustrated in Figure 1 using a typical reaction product (Sample A) from our laboratories. Sample A is an intermediate in an exploratory drug substance synthetic route and is comprised of four components, three of which are undesired impurities (designated 1, 2 and 3) that required purging. Significant selectivity differences can be observed between different solvents systems, leading to beneficial changes in resolution and even to differences in elution order (Figure 1). This approach enables rapid determination of the best solvent choice for optimal selectivity as well as facile

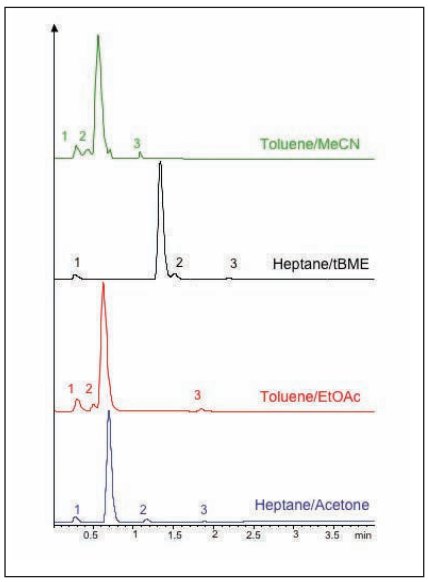

Figure 1: Example chromatograms showing the selectivity obtained using different normal phase solvent systems on silica for Sample A - a four component mixture containing impurities 1, 2 and 3.

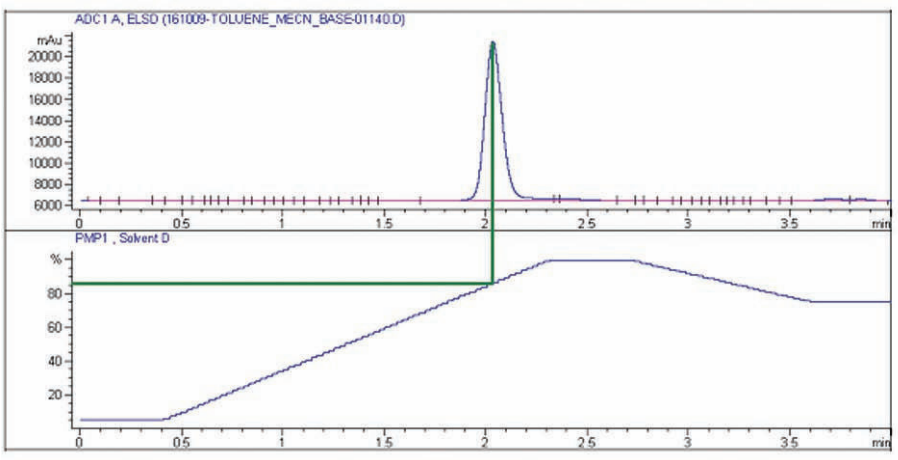

Figure 2: An example of how to estimate the isocratic Flash-LC solvent composition by relating the gradient retention time of any particular analyte from it chromatogram to the gradient profile. The report is engineered so that the estimated solvent composition that it predicts results in that analyte having a retention factor under those isocratic conditions that is suitable for a preparative purification process.

interpretation of gradient or isocratic conditions. Small scale purifications can be expedited by reproducing the gradient method of choice on automated laboratory Flash-LC equipment. Often however, isocratic conditions are favoured for use with simpler or larger scale flash purification equipment. Isocratic conditions for any particular analyte can be estimated from its retention time (see Figure 2). The reported gradient profile is engineered so that the estimated solvent composition that it predicts, results in the analyte having a retention factor under those isocratic conditions that is suitable for a preparative purification process. For absolute confidence in the selected method it is advisable to run one TLC to confirm the suitability of the solvent system and to estimate the sample loading prior to transferring the separation to a preparative process.

Speed and simplicity are key factors towards successful implementation of this screening approach with the end user. A standard screen of six methods takes approximately 45 minutes. The Chemstation software running the instrument is operated with Easy-Access software to facilitate open-access sample login and to build the method screen. It then automatically generates a report and emails it to the users.

It is important to note that UV detection can be an issue when working with normal phase solvents, especially those that have a very high UV cut-off (e.g. toluene or acetone). Alternative HPLC detectors such as evaporative light scattering detection (ELSD) provide complementary information to UV when solvent UV background is prohibitive or because the analyte lacks a significant chromophore.

Reverse Phase Flash method screening

Bonded Flash silica, including C18 bonded silica for Reverse Phase Flash-LC, is readily available in pre-packed commercial columns (Crane *et al.*[4]). It offers a useful complementary method to the normal phase separation mode as analyte selectivity can be very different, as can the solvent compatibility and solubility (e.g. for alkanes, ethyl acetates, ketones or ethers). Moreover, since reverse phase HPLC is often used as an analytical technique of choice to follow chemical reactions, this analytical method can often provide the foundation for perfectly reasonable RP Flash purification and an integrated purification workflow.

A method screening system was designed and built using standard HPLC equipment that enabled an unskilled user to quickly assess the feasibility of a reverse phase Flash purification. The screen comprises four sets of conditions performed on a scaling Flash column (4.6mm x 250mm) using a generic gradient (see Table 3). The same generic gradient is pre-installed on the automated laboratory Flash instrument to allow for simple transfer.

The screening system is designed to scout for, or verify, RP phase conditions as well as provide an estimate of the preparative loading for a successful Flash purification. This negates the need for a loading study and facilitates a rapid purification workflow. The preparative loading is estimated from the resolution between critical components of choice on the chromatogram. For absolute ease of use by the end users, the resolution is expressed in time units (e.g. T in minutes) and the preparative loading expressed as percent weight on (RP) silica (see Figure 3).

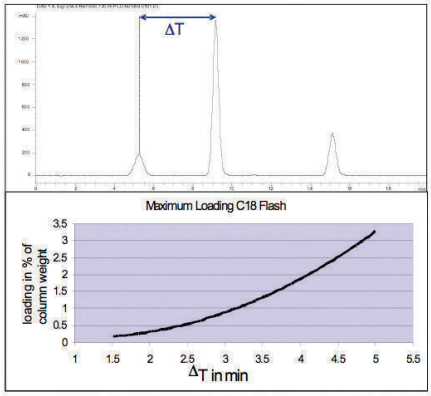

Figure 3: A chart used to estimate preparative loading expressed as percent weight on (RP) silica from a chromatogram produced by the RP Flash method screening system.

This approach has been implemented and is particularly well suited to enabling rapid purification workflows on the laboratory scale (see Figure 4).

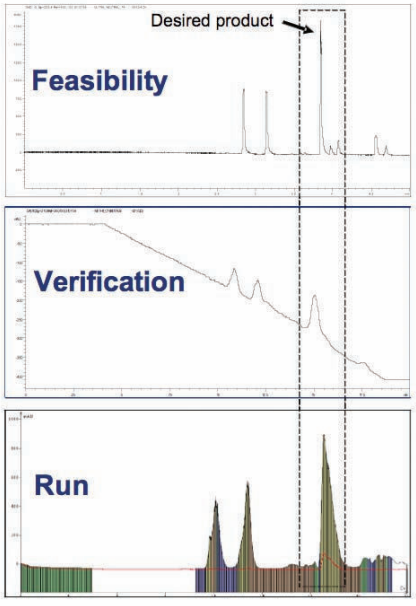

Figure 4: A schematic of the workflow for a laboratory scale RP-Flash purification: 1. Feasibility step, if a reversed HPLC method is used to monitor a chemical reaction it can be used to assess feasibility by demonstrating selectivity for critical components; 2. Verification and optimisation step, the same sample is run on the Reverse Phase Flash screen, suitability of the RP Flash silica is confirmed and optimal loading can be estimated; 3. Run purification, the sample is loaded on the Flash reverse phase cartridge and exhibits the expected scale-up, enabling predictable peak tracking and collection of the desired product(s).

Again, speed and simplicity are key factors towards successful implementation of this technique with the end user. A standard screen of four methods takes approximately 90 minutes. Automation of the method screening, method verification and loading study combined with automated laboratory Flash-LC equipment makes for a RP Flash purification workflow that is light, reliable and rapid.

Conclusion

Method screening systems have been put in place to facilitate the use of preparative Flash-LC in order to realise efficiency gains and achieve quality specifications in the chemistry laboratory compared to traditional approaches. Comprehensive and automated method screening gives facile and rapid access to optimal separation conditions. Any chemist is capable of accessing consistent and optimal purification conditions independent of their depth of knowledge in Flash-LC method development.

The implementation of an automated and integrated Flash-LC method screen has had a significant impact on working practices in our research and development laboratories. The approach has removed technical barriers for the chemists which has resulted in greater uptake of the technique. It has enabled the development of higher quality methods in shorter timeframes so increasing the speed to and quality of purifications run by Flash-LC in our laboratories. At laboratory scale, an integrated approach combines automated screening and purification equipment to facilitate rapid access to pure materials. For large scale Flash chromatography (e.g. pilot scale) the method screening approach consistently generates methods with a strong foundation for further optimisation, reducing the overall method development time.

In this way, appropriate application of Flash-LC method development has realised increases in the individual chemist's and the overall project's productivity.

References

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