

Method Development for Reproducible Flash Purification of Pharmaceuticals by UHPLC and HPLC

by J. Reilly, Q. Liu, C. Howson, K. Boss, B. Shin, M. Palermo, Jay Larrow, Ming Qian, Thierry Mann
Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, USA.

This work demonstrates that efficient and reproducible low pressure flash purifications can be enabled by scaling from generic analytical UHPLC and HPLC conditions. Examples of challenging achiral, reverse phase small molecule, cyclic peptides, and chiral separation examples demonstrate the success of this approach on a routine basis. Flash chromatography is a preferred approach for many chemists in the pharmaceutical industry, although the reverse phase mode is not often adopted due to limited expertise in method development. Improvements in manufacturing technology of flash column stationary phases have enabled an efficient 'scout to flash' open access workflow to permit chemists to choose this path in place of a HPLC semi-prep solution.

Introduction

The use of low pressure flash chromatography was first described in 1978 [1]. The particle size of the stationary phase (40-63 μm) was much finer than in gravity fed packed open column chromatography (63 – 200 μm) which was an experiment originally invented by Tswett in 1906 for separation of plant pigments through a glass column packed with calcium carbonate [2]. Later in 1983 it was demonstrated that flash columns using 15 – 40 μm stationary phases gave much better resolution and efficient chromatography. It was also demonstrated that the use of spherical over irregular shaped particles improved reproducibility and efficiency [3].

During recent years, this flash chromatography methodology has become an open access semi-automated purification method for many medicinal chemistry crude achiral purifications either in normal phase or reversed phase mode. Technology advancements have led to flash purification being a relatively cost effective solution where inexpensive plastic cartridge columns packed with irregular silica are in some laboratories considered disposable after only one separation [4, 5]. Often flash chromatography is utilised to purify large batches 50-100g of material quickly in one purification run. Organic chemists often use silica TLC to scout conditions for normal phase separations, but have less experience or are reluctant in scaling for reverse phase conditions. We demonstrate that scouting to flash purification from

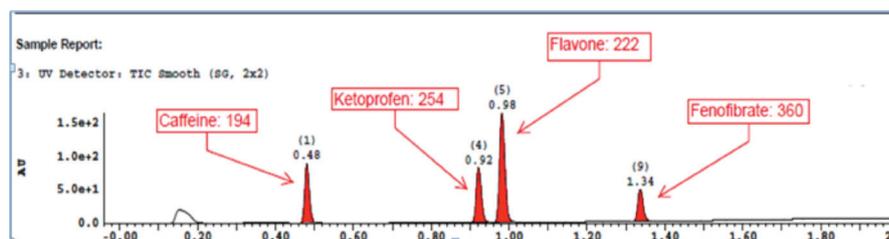
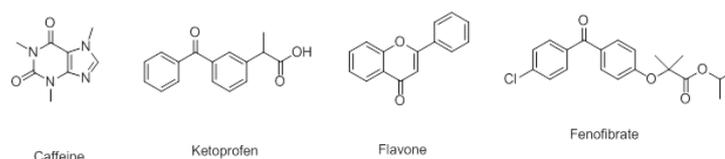


Figure 1. Analytical QC Testmix on 2 min 2-98% ACN in Water (0.1%TFA) gradient

generic open access reverse phase UHPLC separations is achievable and reproducible on commercially available instruments. It is important, as demonstrated in our laboratory previously [6], that analytical reverse phase instrument performance is routinely monitored and tracked for reproducible retention times. Retention time reproducibility is vital in analytical methods to allow a choice for the most suitable focused gradients for flash preparative separations. The use of flash chromatography for chiral separations has been initially reported over 20 years ago where basic analytes were crudely separated on an in-house synthesised carbamate-coated flash silica (40 - 63 μm) [4-5]. We have demonstrated that we can easily pack glass column cartridges with 20 μm immobilised Chiralpak-AD silica and scout purifications from a 10 μm immobilised Chiralpak-AD analytical HPLC method on an puriFlash 450 system.

Instrumentation

Reverse Phase UHPLC-MS Analytical

Waters Acquity UHPLC Binary system, PDA detector, SQ Mass Spec
Column: Waters Acquity BEH C18 1.7 μm 30 x 2.1 mm

Chiral Analytical HPLC

Agilent 1100 with PDA detection, Chiralpak-AD 10 μm 250 x 4.6 mm column
Flow: 1.0 mlmin⁻¹
Column Temperature: 50°C

Reverse Phase Flash Purification columns:

Teledyne ISCO 150g GOLD C18 Aq (30 μm)
Redisep column 85 mlmin⁻¹
Teledyne ISCO 150g GOLD C18 (30 μm)
Redisep column 85 mlmin⁻¹
Teledyne ISCO 100g GOLD C18 (30 μm)
Redisep column 60 mlmin⁻¹
Interchim 300g C18 (15 μm) Interchim – Flow rate 160 mlmin⁻¹

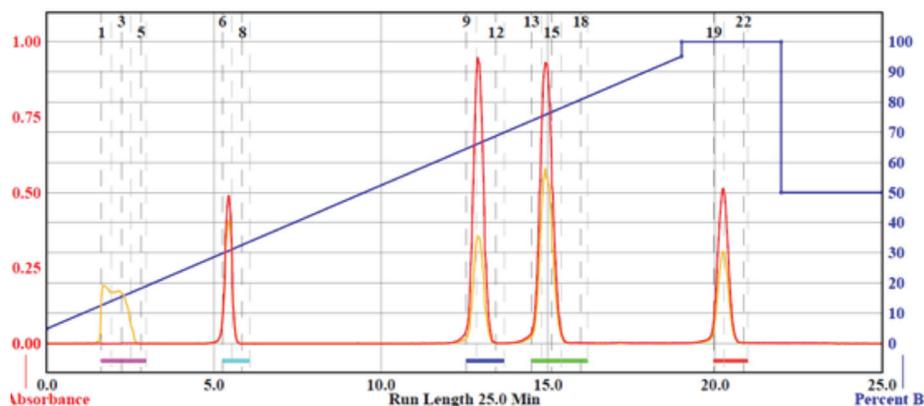


Figure 2. Flash Chromatography QC Testmix on 20 min 5-95% ACN in Water (0.1%TFA) gradient - Teledyne ISCO 150g GOLD C18 Aq (30µm) Redisep column

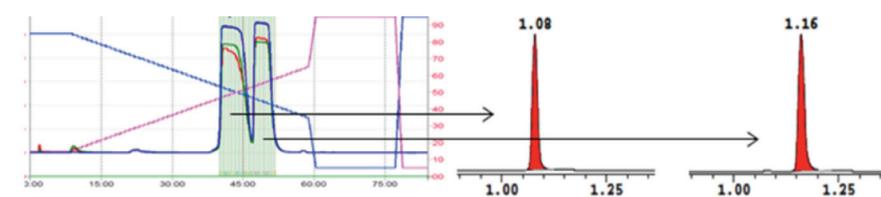


Figure 3. Flash Chromatography 6 g injection on 60 min 15-65% ACN in Water (0.1%TFA) shallow gradient - 300g C18 (15µm) Interchim - Flow rate 160 ml min⁻¹

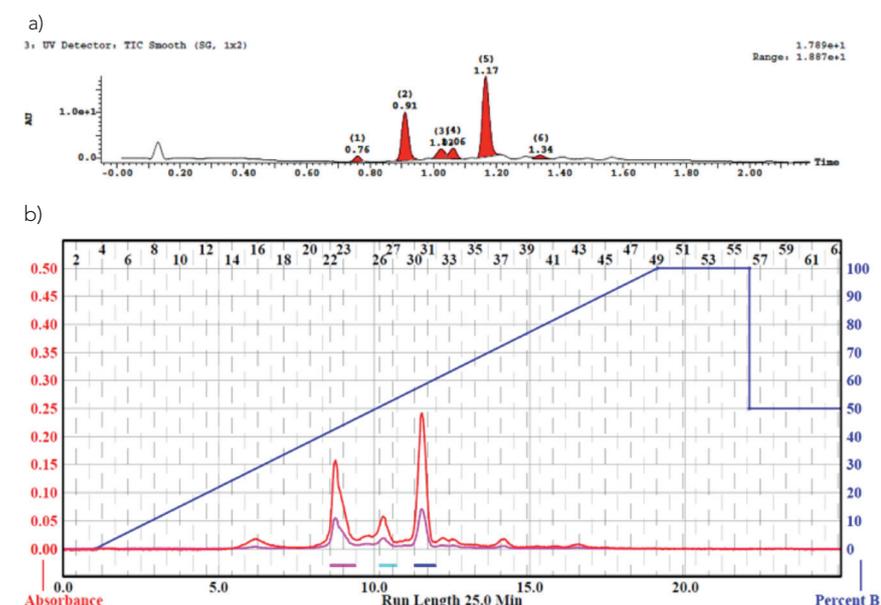


Figure 4. a) Analytical 'scout' run on 2 min 2-98% ACN in Water (0.1%TFA) gradient.
b) Flash on 20 min 0-100% ACN in Water (0.1%TFA) gradient

Chiral flash purification column:

SureFlash Glass Column, packed with Daicel Chiralpak AD 20µm 250 x 15 mm

Teledyne ISCO Flash Purification System:

CombiFlash Rf UV detection and collection

Interchim Flash Purification System:

puriFlash 450 system with UV detection and collection

UHPLC-MS as a 'Scout' for Flash Purification

As we have previously published we track chromatographic performance for UHPLC-

MS instruments by running QC samples across multiple standardised instruments with generic 2 min analysis [6]. We decided to use this as a basis to determine whether we can scale up a UHPLC separation to flash chromatography. Particularly challenging was the separation between ketoprofen (peak 2) and flavone (peak 3), of our QC Testmix which are just baseline resolved on UHPLC in Figure 1 when run on 1.7 µm Waters BEH C18 column.

We demonstrate that by loading a 240mg total injection (60mg of each standard dissolved in 3ml of DMSO top loaded

injection) on a 150g C18 flash column (30µm) we can easily separate all four components in a 20-minute run as shown in Figure 2. This was an extremely encouraging result as it proved we could scale from 1.7µm analytical 'scout' to flash 30µm stationary phase.

The chromatography has been shown to be reproducible using the same ISCO column for a series of different separations over several weeks with maximum of 30 injections. It is important to flush and store the column with 50% acetonitrile in water post-purification, this has shown to significantly enhance column longevity. A large benefit of using reverse phase flash chromatography is the significant increase observed in loading capacity and resulting increase in purification productivity because of an increase in surface area of C18 phase. The ability to purify large amounts in one single injection whilst maintaining resolution is a definite advantage, over having to pool multiple fractions from multiple injections using HPLC due to purification and lyophilisation time reduction. We have also noted that sample recovery is also consistently higher, presumably due to reduced losses in fraction transfer with test mix recovery of 97% recorded. A further advantage is that the operational expenses for a standard flash chromatography system make this an attractive alternative for many laboratories to scale up separations. An example is demonstrated in Figure 3 where we significantly scaled up the challenging separation between ketoprofen and flavone by running 6g purification in one single injection. This was achieved on a 300 g C18 column by running a shallower focused gradient which results in pure material in one single run. A further advantage of a single injection reverse phase flash purification is the reduced fraction volume in comparison to HPLC due to sample loading increase.

In general, we are using the reverse phase flash methodology on more small scale reactions (<100 mg) using a single injection on the 100g C18 AQ (30 µm) column scouting from our standard Open Access 2-minute generic. One small molecule chemistry example is shown below in Figure 4.

Recently we have been also investigating this technology as an alternative for cyclic peptide and macrocycle separations. We have found this to be extremely helpful for complex reaction purifications. The purification of a cyclic peptide is shown in Figure 5 where a 'scout' from a high pH analytical method was used to successfully obtain 62 mg of pure product (>99 %) from a 1.3 g crude sample.

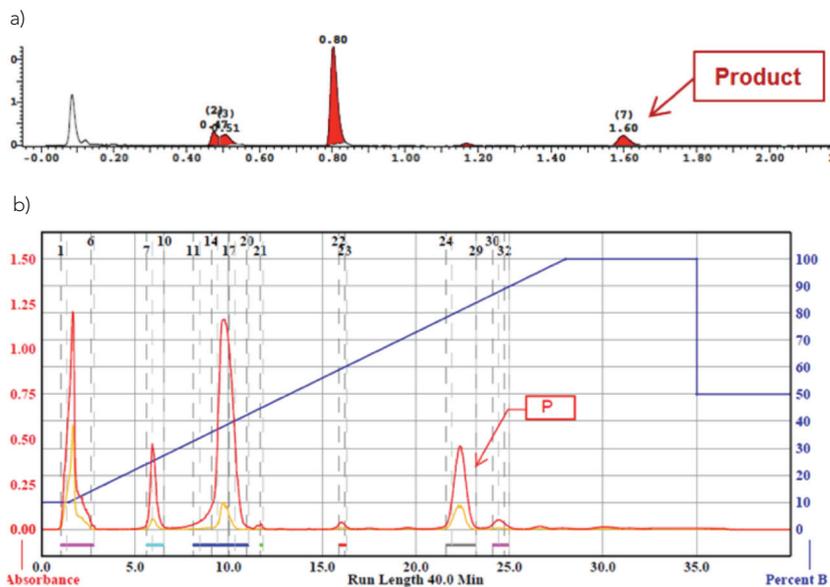


Figure 5. a) Analytical 'scout' run on 2 min 2-98% ACN in Water (0.1% NH₄OH) gradient.

b) Flash on 28 min 10-100% ACN in Water (0.1% NH₄OH) gradient.

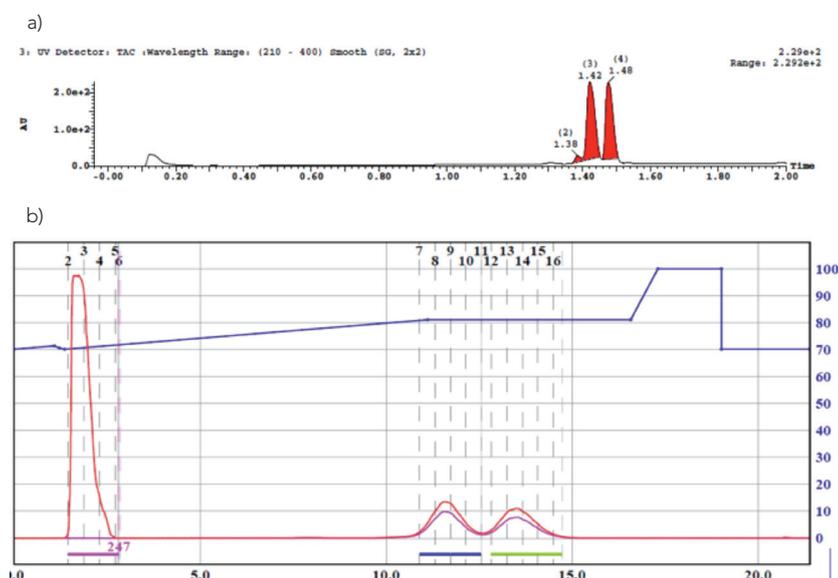


Figure 6. a) Analytical 'scout' run on 2 min 2-98% ACN in Water (0.1% NH₄OH) gradient.

b) Flash on 17 min 70-80% ACN in Water (0.1% NH₄OH) specialised gradient

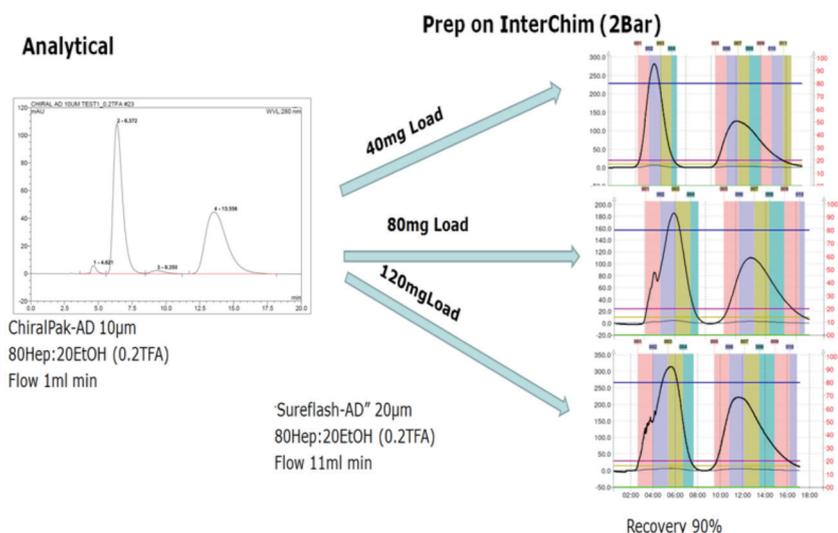


Figure 7. Chiral Analysis and Flash loading study of warfarin using SureFlash flash cartridge

This was achieved by a single injection on a 100g GOLD C18 (30 μ m) Redisep column.

Often HPLC purifications can be particularly challenging for non-polar lipophilic poorly water soluble compounds (in-house data) due to the insolubility observed within sample loading. The benefit of flash chromatography for these types of samples is that the sample is manually pre-loaded onto the cartridge prior to separation. One example is shown in Figure 6 of a 130 mg diastereoisomer, just separable on UHPLC with 0.06-minute separation, that failed on reverse phase HPLC due to poor sample solubility. This sample required a specialised 70-80% acetonitrile gradient, but worked successfully on flash.

HPLC as a 'Scout' for Chiral Flash Purification

We wanted to take this analytical 'scout to flash' technology one step further and test whether we could achieve automated reproducible chiral separations using SureFlash glass columns packed with chiral stationary phase. This has been previously reported using a HPLC instrument running 4 ml min⁻¹ on non-commercial internally synthesised coated columns at very small scale for a selection of beta blockers [7]. Our remit was to separate racemates in a single injection on commercially available stationary phase using flash at <100 mg scale. A SureFlash axial compression glass cartridge with dimensions 15 x 250 mm was custom packed with Daicel ChiralPak AD 20 μ m chiral stationary phase. This column was used throughout this study and set up on the puriFlash 450 system. SureFlash columns are stable up to 80 bar/1160 PSI and use axial compression to compensate for voids at the inlet. We have demonstrated that we could scale from HPLC 10 μ m to SureFlash 20 μ m and have separated many racemates using this technology. The benefit of using 20 μ m stationary phase is ability to increase loading for single injections. The analytical method was scaled up from a 250 x 4.6 mm analytical to a 250 x 15 mm flash column and 40 mg, 80 mg and 120 mg loading was achieved for warfarin in a single injection as shown in Figure 7. Based upon scaling factors if we were to scale to a 250 x 50 mm column sample loads in excess of 1 gram per injection could be achieved for this separation.

Conclusion

We have demonstrated that scaling to flash chromatography from generic reverse phase UHPLC-MS analytical methods is routinely achievable. The ability to 'scout' using generic analytical conditions for small and large scale reverse phase flash chromatography purifications

has had significant project impact recently at Novartis and is being adopted by many medicinal chemists. Larger macromolecules and cyclic peptide separations perform equally as well as small molecule separations. Flash chromatography units can deliver highly efficient and reproducible pharmaceutical separations when scaled from analytical methods. The ability to also use these systems for chiral separations has also been demonstrated.

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