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Scalable Technology for the Extraction of Pharmaceutics: Outcomes from a 3 year collaborative industry/academia research programme☆

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ABSTRACT

This paper reports on some of the key outcomes of a 3 year £1.5 m Technology Strategy Board (TSB) funded research programme to develop a small footprint, versatile, counter-current chromatography purification technology and methodology which can be operated at a range of scales in both batch and continuous modes and that can be inserted into existing process plant and systems. Our consortium, integrates technology providers (Dynamic Extractions) and the scientific development team (Brunel) with end user needs (GSK & Pfizer), addressing major production challenges aimed at providing flexible, low capital platform technology driving substantial cost efficiency in both drug development and drug manufacturing processes. The aims of the Technology Strategy Board's high value manufacturing programme are described and how the academic/industry community were challenged to instigate step changes in the manufacturing of high value pharmaceuticals. This paper focusses on one of the themes of the TSB research programme, "Generate a Comprehensive Applications Portfolio". It outlines 15 applications from this portfolio that can be published in the public domain and gives four detailed case studies illustrating the range of application of the technology on the separation of (1) isomers, (2) polar compounds, (3) crude mixtures and (4) on the removal of impurities. Two of these case studies that were scaled up demonstrate between 10 and 20% lower solvent usage and were projected to have significant cost savings compared to conventional solid phase silica gel chromatography at procss scale demonstrating that the latest high performance countercurrent chromatography technology is a competitive platform technolgy for the pharmaceutical industry.

1. Introduction

This paper highlights the major outputs from a 3 year Technology Strategy Board high value manufacturing research programme managed by end users from the pharmaceutical industry (GSK and Pfizer), technologically driven by a supply company (Dynamic Extractions Ltd.) with research support from academia (Brunel University). The research programme aims to drive substantial cost efficiency in both drug development and drug manufacturing processes by developing commercial counter-current separation technology to the point where it will allow selective purifications to be achieved at loadings of approximately 1 kg/day at the laboratory scale.

In contrast to high-performance liquid chromatography, counter current separations [1–5] do not require expensive packing materials; are more tolerant of particulate matter and have the benefit of excellent reproducibility. The project will demonstrate the broad applicability of the technology to pharmaceutical separation problems and specific examples will more closely examine the capability of the technology for increased sample loading and scale-up.

This paper will not go into detail on how the technology works as this has been described elsewhere [1–5], but this is a maturing technology with developing supply companies that are continuously improving the technology robustness so that it becomes the platform technology the industry needs.

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2. The aims of the TSB high value manufacturing programme

The Technology Strategy Board's high value manufacturing research programme highlights that "manufacturing globally is a key provider of wealth and employment" and that "manufacturing is also one of the primary mechanisms for realising wealth from new technologies and is therefore critical to the UK". It also maintains that manufacturing is highly competitive and gravitates to countries of lowest overall cost. Therefore, manufacturing in comparatively high wage economies, such as the UK, has had to change radically to remain globally competitive.

The TSB set up a "High Value Manufacturing" competition to fund "step changes in competitiveness" in January 2009 focussing on innovation in four broad areas which they called the "four pillars of high value manufacturing": (1) products; (2) production processes; (3) service systems and (4) value systems planning to invest in projects that will, over the duration of the project, bring about major changes – STEP CHANGES – in the competitiveness of the participating companies.

This competition inspired us to set up our consortium of end-users, supply companies and academics to address the issues facing the pharmaceutical industry. From a chemical development point of view, the pharmaceutical industry was facing the following major challenges: (1) to make significant quantities of materials for early phase testing very rapidly, with good purity and cost effectively and (2) to develop production processes that were both robust and highly cost effective. We proposed the introduction of new technology to the pharmaceutical industry that would create a much more flexible, small footprint, integrated purification technology which would be scalable, transferable, containable and more environmentally friendly. Being a TSB proposal, they were interested in the business opportunities addressed – i.e. not just in development but in manufacture as well. These were as follows: (a) rapid and cost-effective access (compared to existing technologies) to significant amounts of drug substances for development purposes resulting in large reductions in early development timelines and associated costs; (b) ease of technology transfer from small scale R&D stages (pre-clinical) to commercialisation; (c) portability of processes that can be validated in the UK and transferred anywhere globally without changing the process itself hence maximising commercial benefit and minimising the associated regulatory issues; (d) an environmentally friendly process which contains the product, that can be easily cleaned or disposed of, and uses orders of magnitude less solvent than conventional processes; (e) large scale, robust and cost-effective manufacturing technology that will permit batch or continuous purification, broadening processing options and lowering overall production costs.

We proposed a step-change in the manufacture of high value pharmaceuticals, which would be critical if UK companies wanted to maintain global competitiveness in the face of the move of manufacturing bases to low cost countries. We proposed our liquid flow technology as having applications in a variety of key areas: (1) with reductions in process development timelines and costs by up to 50% by allowing rapid access to purified materials; (2) allowing potentially significant savings in manufacturing process costs by offering simplified manufacturing processes with fewer steps; increased yields as a result of the processing of liquors that would otherwise have been discarded; the removal of impurities allowing potential access to less expensive starting materials/less expensive synthetic routes and the capability to input very high quality materials into crystallisation processes potentially lowering risks associated with solid state issues; (3) involvement of equipment designers/manufacturers at every stage in development would facilitate equipment design enhancements and (4) preparative chromatographic separations were growing at an annual rate of greater than 20% (Strategic Directions International Inc.), which was of major interest to the Pharma industry. A successful outcome to the project would globally stimulate the market at all scales, leading to significant increases and growth in sales of the technology. Critically, the team felt that the project would allow a substantial body of experience to be gained with counter-current chromatography and associated equipment allowing major advances to be made in equipment capability and reliability. The end-users would become familiar with the scope and flexibility of using liquid processing streams and further new ideas and innovative uses of the technology would develop.

The TSB HVM competition was launched in January 2009. It was a two stage process. Our expression of interest entitled "Scalable Technology for the Extraction of Pharmaceuticals (STEP)" was ranked in the top ten of more than 300 entries and our final proposal was eventually funded in June 2009 with the project starting on September 1st, 2009.

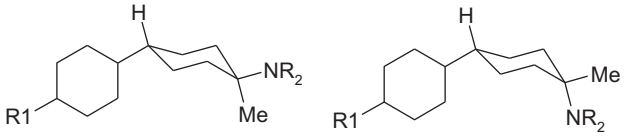
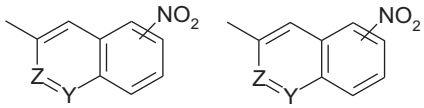
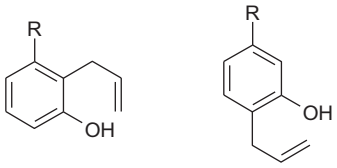
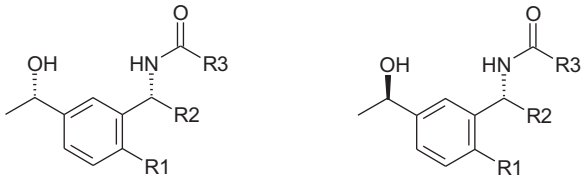
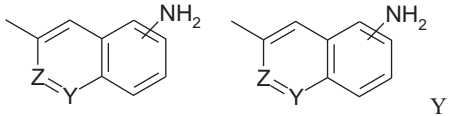
3. The TSB-STEP research programme

The initial proposal advocated 5 end user applications and proposed a phased programme of development and validation at the analytical scale in the first year (Phase 1); laboratory scale in the second year (Phase 2) and pilot/process scale in the third year (Phase 3). As the project evolved it became clear that the end-user companies required a much larger applications portfolio to persuade senior management to invest more resource into the project. The research programme, being end-user driven, evolved to become theme driven where the themes were dictated by the needs of the end-user industries in the consortium. These themes were (1) instrument integration and automation; (2) generate a comprehensive applications portfolio; (3) new solvent system development; (4) improve instrument reliability; (5) continuous processing and scale-up; (6) demonstrating value and (7) management – including a dissemination and exploitation plan. Themes (1) and (4) involving instrument development and Theme (6) demonstrating value will be presented at SPICA 2012 in Brussels on October 2nd, 2012; Themes (2), (3) and (5) were presented at the CCC2012 conference in Hangzhou, China. Theme (2) will be reported in this paper while Themes (3) and (5) will be in separate publications in either the SPICA or CCC2012 special editions respectively [6,7].

4. Generating a comprehensive applications portfolio

In total more than 30 applications were processed as part of the TSB research programme. Tables 1–4 list 15 of these that have been approved for general release divided into isomeric compounds (1–5 – Table 1); highly polar compounds (6 and 7, Table 2); crude mixtures (8–12, Table 3) and removal of impurities (13–15, Table 4) – the others are commercially sensitive. Of the 30 applications more than 85% achieved their target separation. Of those listed in Tables 1–4, applications 1, 10, 13 and 14 were the case studies 1, 2, 3 and 4 presented at CCC2010 in Lyon, France and described in detail in the first TSB-STEP publication in the CCC2010 special issue of the Journal of Chromatography A [8]. One application from each of the groupings here has been selected for a detailed description. Case Study 1: applications 2 from isomeric compounds in Table 1; Case Study 2: application 7 from highly polar compounds in Table 2; Case Study 3: application 9 from crude mixtures in Table 3 and Case Study 4: application 15 from removal of impurities in Table 4.

Table 1
Details of example applications involving isomeric compounds used for HPCCC assessment.

Example	Mixture	Loading	Solvent system	Result
1		10 mg	Hept:DCM:MeCN [5:0.5:4.5]	Baseline separation. Not optimised. Non aqueous system required for solubility
2	 Y and Z = C or N	50 mg	HEMWat SS11 + 0.1% HCl – RP	Scaled to Spectrum semi-prep column. 260 mg injection to give 77% recovery of product containing <0.5% of unwanted isomer
3		300 mg	HEMWat SS19 [3:2:3:2] – NP	Product contains <1% of unwanted isomer
4		No separation	No systems identified	Range of solvent systems investigated
5	 Y and Z = C or N	15 mg	DCM:MeOH:0.2% aq. Formic acid + Et3N – pH 3.5: Propan-2-ol [6:2:4:3] – NP	Sample had poor solubility in most organic solvents. Product contains <0.5% unwanted isomer

5. Application case studies

5.1. Case Study 1: separation of isomers

5.1.1. Separation

The crude sample (application 2, Table 1) contains a mixture of two isomers, about 60% of the required isomer (ratio of required to unwanted isomer is 1.8:1). The aim was to purify the required isomer with less than 0.5% of the unwanted isomer. Any other impurities were to be kept for further analysis.

5.1.2. Solvent system selection

Initial scouting runs on a DE Mini (18 ml column, 0.8 mm bore) using an automated solvent mixing on demand system homed in

Table 2
Details of example applications involving polar compounds used for HPCCC assessment.

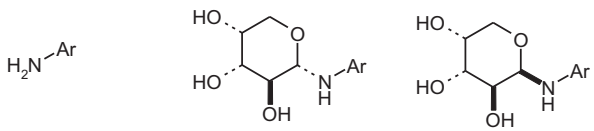
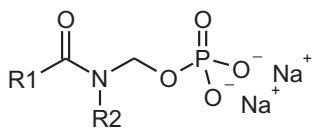
Example	Mixture	Loading	Solvent system	Result
6	 Parent Impurity A 12% Impurity B 30%	20 mg	MeCN:n-PrOH:21% aq. $(NH_4)_2SO_4$ [2.5:5:11] – NP	Impurity A isolated in 90% purity impurity B isolated in 60% purity
7	 + Unknown impurities at 0.8% and 1.5%	27 mg	n-BuOH:MeCN:1M aq NaOAc [4:1:5] – NP	Unknown impurities reduced to <0.1%

Table 3
Details of example applications involving crude mixtures used for HPCCC assessment.

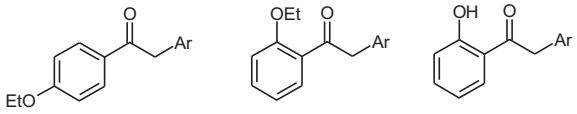
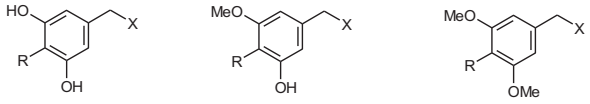
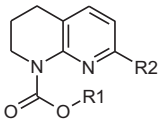
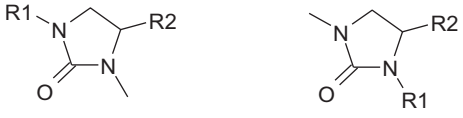
Example	Mixture	Loading	Solvent system	Result
8	 + two unknowns	56 mg	Isocratic HEMWat SS18 [6:5:6:5] then gradient from SS18 to SS8 [1:9:1:9] then Isocratic HEMWat SS8 – NP	Four components obtained at >95% purity, one component 60% purity
9		55 mg	HEMWat SS18 [6:5:6:5] – NP	Baseline separation
10	 Three chemistry steps performed without purification to give intermediate of purity 64%	200 mg	HEMWat SS19 [3:2:3:2] – NP	Product purity 98% separation scaled-up to Spectrum semi-prep column. 1.8 g injection gave 50%, w/w recovery, equivalent to approximately 80% recovery based on purity
11	API + excess reagent + by-product	Partial separation	Hexane:EtOAc:MeOH: water:THF containing 1% NH_4OH [2:2:2:2:1]	Excess reagent separated. By-product not separated
12	Mother liquors containing 7%, w/w API	200 mg	HEMWat SS17.5 [12:11:12:11] – NP	Product purity 96.4%

Table 4
Details of example applications involving removal of impurities used for HPCCC assessment.

Example	Mixture	Loading (mg)	Solvent system	Result
13	Reaction intermediate containing 20% of an unexpected process related impurity	290	HEMWat 23 [4:1:4:1] – NP	Crude purity of target 73% by peak area. Final product purity >98% in 30 min with 95% recovery. Predicted throughput on mid system 60 g/h
14	Synthetic Metabolite (87%) containing multiple impurities including critical bromo impurity	200	HEMWat14 [1:2:1:2] – NP	Product isolated in 96.5% purity and containing <0.5% of bromo impurity
15		450	HEMWat SS17 [1:1:1:1] – NP	Product contains <1% unwanted isomer and impurities removed

on a HEMWat #11 (1:4:1:4) phase system [9] operating in reversed phase mode with an additive of 0.1% HCl.

5.1.3. Loading studies and scale up

Loading studies were first performed on the DE Mini (18 ml column, 0.8 mm bore) with a flow rate of 1 ml/min at 2100 rpm with an initial sample loading of 4 mg in 0.1 ml (Fig. 1a). Loading was then increased to 20 mg in 0.5 ml (not shown) to 50 mg in 0.5 ml loaded in a 1:1 mix of mobile and stationary phases (Fig. 1b). Fractions after 28 min in Fig. 1b contained <0.5% of the unwanted isomer. The process was then scaled up to the DE Spectrum (134 ml column, 1.6 mm bore). As the length of the Spectrum column is almost twice that of the Mini, volumetric scale up (ratio of the column volumes – 7.4×) could not be applied and linear scale up (4×) would be conservative.[1] In this case flow rate was scaled up linearly (4 ml/min) and sample loading somewhere between the two at 5.2× to 260 mg in 3 ml. The result is shown in Fig. 1c where it can be seen that the separation is longer, due to the longer column but the resolution better. The HPLC analysis is shown in Fig. 2 for (a) the starting material; (b) fractions from 8 to 42 min; (c) fractions from 43 to 62 min and finally (d) fractions from 63 to 78 min. The fraction in Fig. 2c gave a purity of 96.8% with no detectable amount of unwanted isomer (<0.1%). The mass balance gives a 120 mg recovery of the target compound from a total of 260 mg crude injected. The peaks eluting before 1.5 min were not taken into consideration

[1] Volumetric scale up uses the ratio of the column capacities; linear scale up uses the ratio of the cross-sectional areas [9].

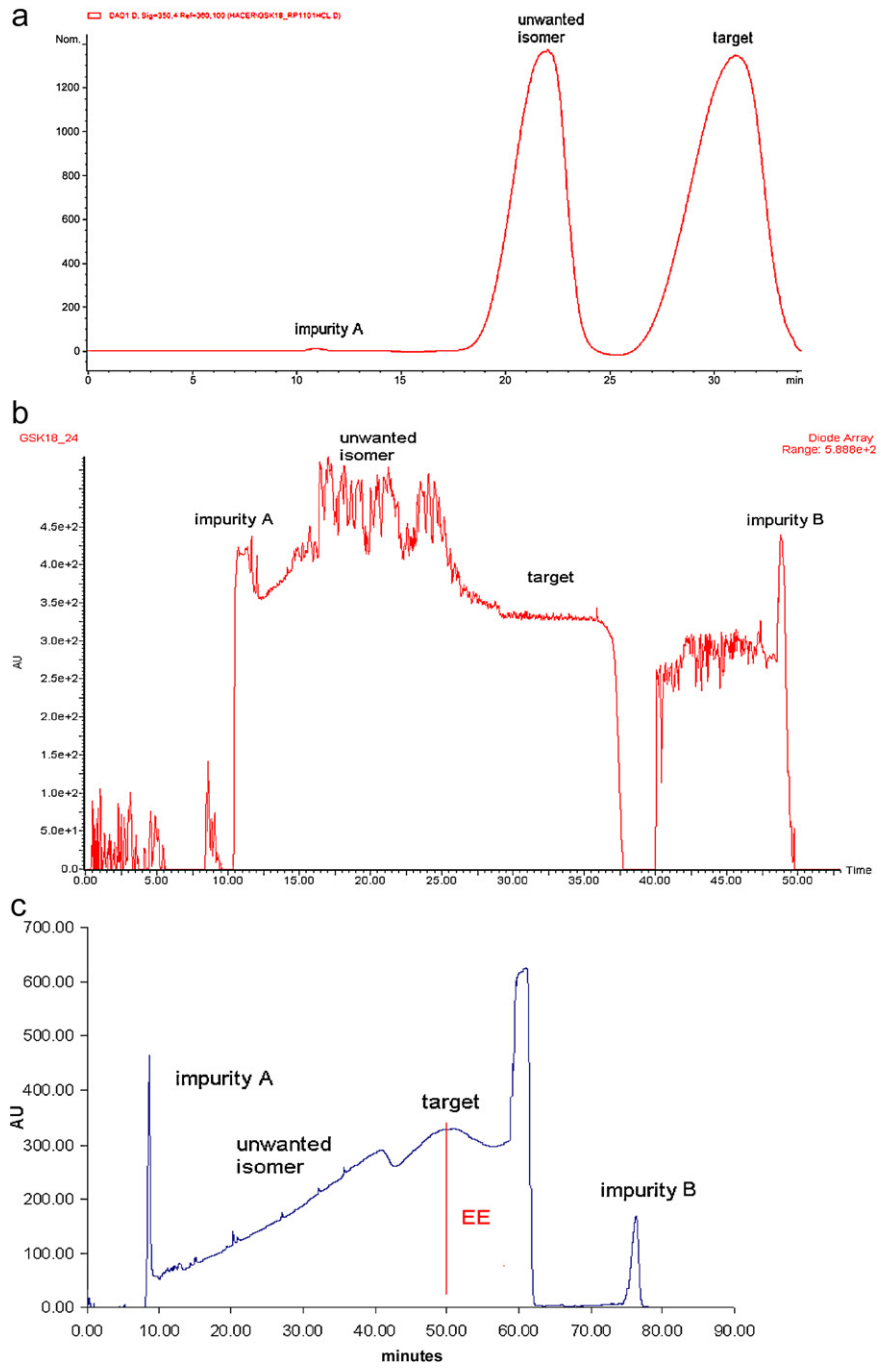


Fig. 1. HPCCC loading and scale-up study using the analytical column of the DE Mini instrument for sample from application 2 in Table 1 for loadings of (a) 4 mg and (b) 50 mg. Analytical HPCCC run conditions: column: 18 ml, 0.8 mm bore; solvent system: hexane:ethyl acetate:methanol:water with 0.1% HCl (HEMWat – SS11) [1:4:1:4]. Operational mode: reversed phase (RP); flow, 1 ml/min; rotation, 2100 rpm; temperature, 30 °C, isocratic, elution extrusion; sample concentration 40 mg/ml. Semi-preparative scale up to the DE Spectrum instrument using the same sample, phase system and operating mode for a loading of (c) 260 mg. Semi-prep run conditions: column: 134 ml, 1.6 mm bore; flow, 4 ml/min; rotation, 1600 rpm; temperature, 30 °C, isocratic, elution extrusion; sample concentration 100 mg/ml.

since they are solvent systems peaks, which is why the height of these peaks remains the same on all HPLC chromatograms.

5.2. Case Study 2: separation of polar compounds

5.2.1. Separation

Application 7 from Table 2 is a sample containing a bis-sodium salt of an API (target) and two unknown impurities at 0.8% and 1.5%. The aim was to isolate the API and reduce these impurities to less than 0.1%. Fig. 3a shows the starting material (R_t = 1.96 min) with impurities at 1.84 min (1.5%) and 2.07 min (0.76%). The 2.22 peak is a degradant which forms during the HPLC process.

5.2.2. Solvent system selection

Several solvent systems were tried to get the partition coefficient in the appropriate range ($0.5 < K_D < 2.0$). The sample partitioned into the lower phase (LP) for HEMWat phase systems 17, 14 and 10, therefore the target was highly polar. As samples dissolved well in mixtures containing DCM and MeCN, polar phase systems using these solvents were tried. The DCM phase systems tended

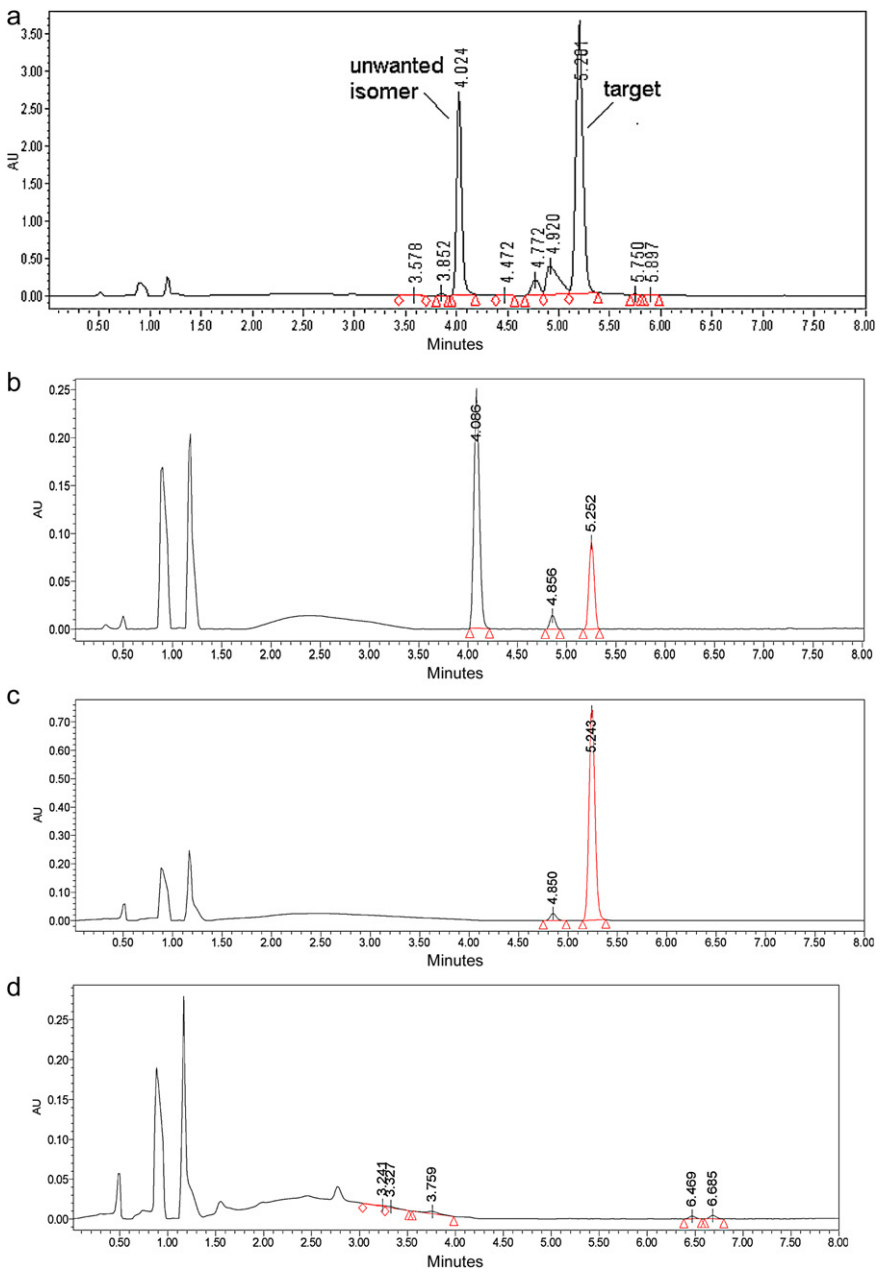


Fig. 2. HPLC analysis for Case Study 1, application 2 in Table 1 for (a) crude starting material, (b) fractions from 2 to 42 min, (c) fractions from 43 to 62 min (target) and (d) fractions from 63 to 78 min. A Gemini NX, 3 μ, C18 column was used 110 Å (50 × 4.6 mm). Mobile phase A – 0.1% aq. TFA, B – MeCN; Method: 5–25%B over 5 min, 90%B for 1 min then 2 min equilibration at 5%B. Flow rate: 1 ml/min; detection 220 nm.

to emulsify, but the MeCN systems with the addition of salt [10] were successful with the final optimised solvent system chosen as n-BuOH:MeCN:1 M aq. NaOAc (4:1:5) giving a target partition coefficient of K_D = 1.6 (R_t = 1.96 – Fig. 3a) with impurities partitioning at K_D = 1.23 (R_t = 2.22) and K_D = 2.42 (R_t = 1.84).

5.2.3. The HPCCC separation

The chromatogram for a 27 mg injection of crude (55%, w/w purity (15 mg) of target compound) in 1 ml of stationary (lower) phase is shown in Fig. 4 with the following run conditions: Column: Dynamic Extractions Midi HPCCC analytical column/coil (22.5 ml, 0.8 mm bore); flow rate: 0.5 ml/min; rotational speed: 1400 rpm (240 g); temperature: 30 °C; stationary phase volume retention (S_f): 82%.

In Fig. 4, the 2.07 min impurity elutes in fractions 1–5 between 26 and 45 min. The desired product elutes next between 70 and 90 min which approximately matches the predicted retention time calculated from the partition study results. The 1.84 min impurity is retained in the stationary phase. Fig. 3b is the HPLC chromatogram of fraction 13 (between 74 and 76 min). Fractions 10–20 present a similar purity profile. The impurity at 2.22 is a degradation product of the main peak under HPLC conditions.

5.3. Case Study 3: separation of crude mixtures

5.3.1. Separation

The aim was to separate a monohydroxy compound from an approximately 2:5:1 mixture (see HPLC chromatogram of the crude – Fig. 5) of dihydroxy, monohydroxy and dimethoxy compounds illustrated in Table 3, application 9.

Test tube partitioning studies for 5 different HEMWat phase systems (Fig. 6) showed that HEMWat #18 (heptane:ethyl

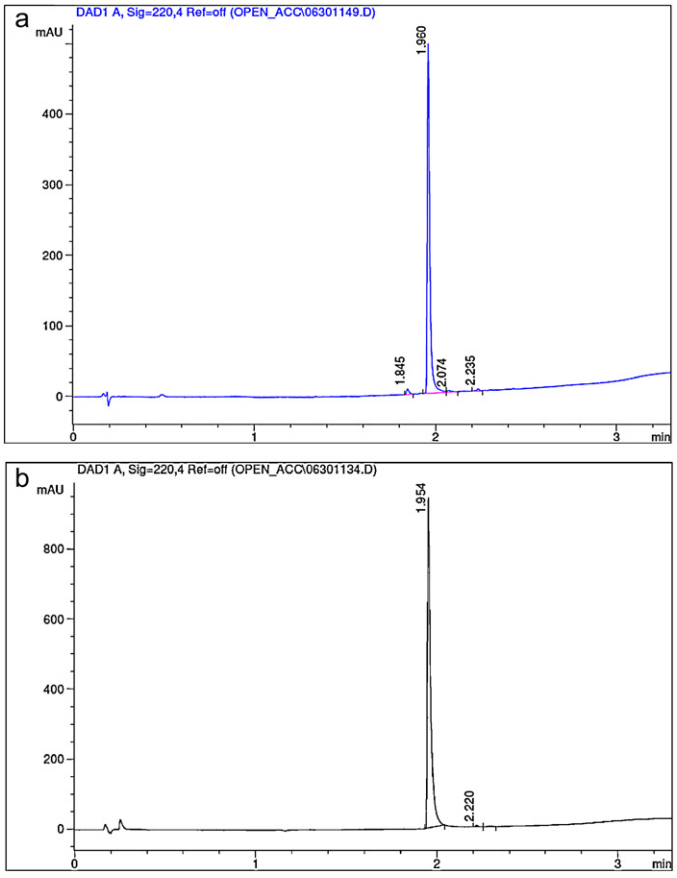
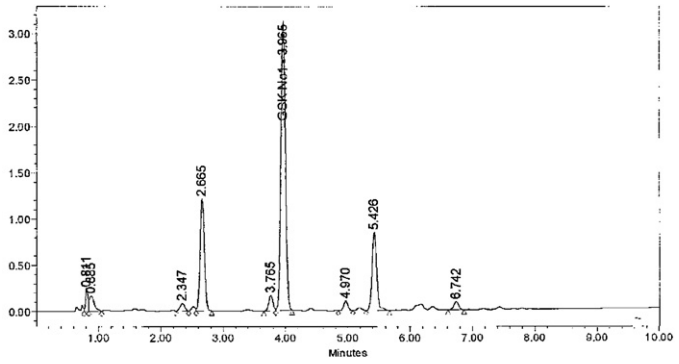


Fig. 3. HPLC chromatogram of (a) starting material for Case Study 2 (application 7, Table 2) with target at 97.07% purity (1.96 min) and impurities at 1.53% (1.84 min), 0.76% (2.07 min) and 0.65% (2.24) and (b) HPLC chromatogram of fraction 13 showing 99.48% pure target material. An Agilent Zorbax SB-C18, 1.8 μ column was used (50 × 3.0 mm). Mobile phase A – 0.5%, v/v trifluoroacetic acid in water. Mobile phase B – 0.05%, v/v trifluoroacetic acid in acetonitrile; method: 0–95%B over 2.5 min, 95%B for 0.2 min then 1.3 min equilibration at 0%B. Flow rate: 1.5 ml/min.



	Peak Name	RT	Area	% Area	Height
1		0.811	639933	2.13	243094
2		0.885	966718	3.21	167420
3		2.347	441723	1.47	85146
4		2.665	5691275	18.91	1213083
5		3.765	780949	2.59	168705
6	GSK No1	3.965	16243847	53.96	3115165
7		4.970	495712	1.65	105343
8		5.426	4378805	14.55	843423
9		6.742	462591	1.54	87949

Fig. 5. HPLC chromatogram of starting material for Case Study 3 showing an approximate 2:5:1 ratio between the dihydroxy, monohydroxy and dimethoxy compounds illustrated in Table 3, application 9. A Waters Symmetry C18, 3.5 μ column was used (75 × 4.6 mm) at 30 °C. Mobile phase A – 0.05%, v/v trifluoroacetic acid in water. Mobile phase B – acetonitrile. Method: 50–90%B over 10 min, then 4 min equilibration at 50%B. Flow rate: 1.0 ml/min.

acetate:methanol:water – 6:5:6:5) gave partition coefficients in the appropriate range for CCC (K_D = 0.5–2.0, Log_{10} (K_D) = −0.3 to +0.3).

A DE Midi analytical column (25 ml, 0.8 mm bore) was used for the sample loading studies. Fig. 7a shows that the main components are well separated with contaminant 2 eluting first, the target compound second and contaminant 1 eluting third.

5.3.2. Sample loading

The effect of increasing sample loading is shown in Fig. 7a–c for 10.9 mg (a), 55 mg (b) and 220 mg (c). The first chromatogram

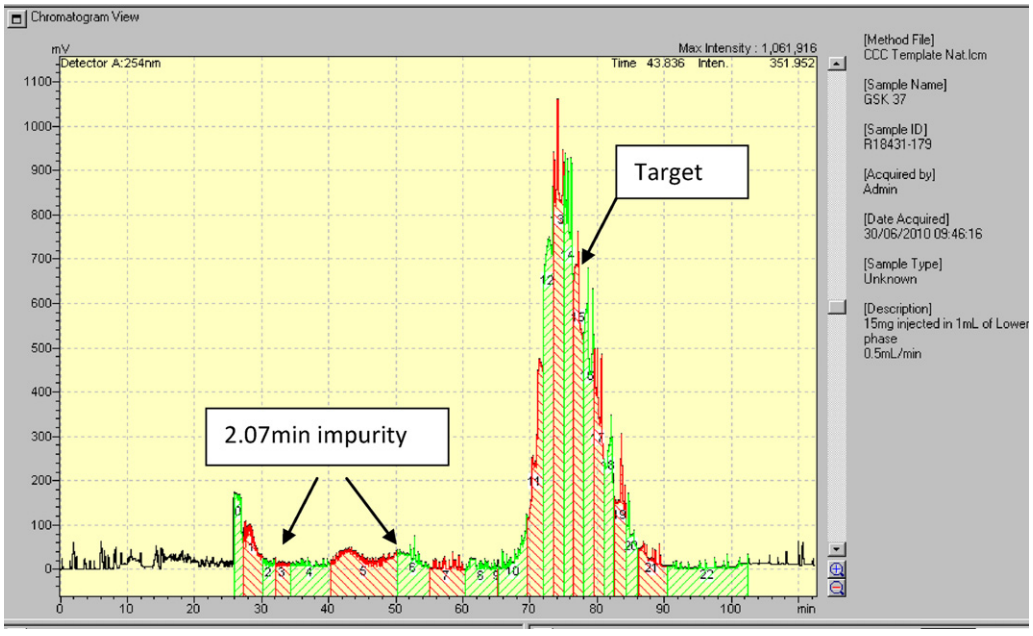


Fig. 4. HPCCC chromatogram for Case Study 2 using the analytical column of the DE Midi instrument for sample from application 7 in Table 2. HPCCC run conditions: column: 22.5 ml, 0.8 mm bore; solvent system: n-BuOH:MeCN:1 M aq. NaOAc [4:1:5]; stationary phase retention (S_f): 82%; operational mode: normal phase (NP); method: flow (0.5 ml/min), rotation (1400 rpm); temperature (30 °C), isocratic, elution extrusion; sample loading 27 mg of crude in 1 ml of stationary (lower) phase.

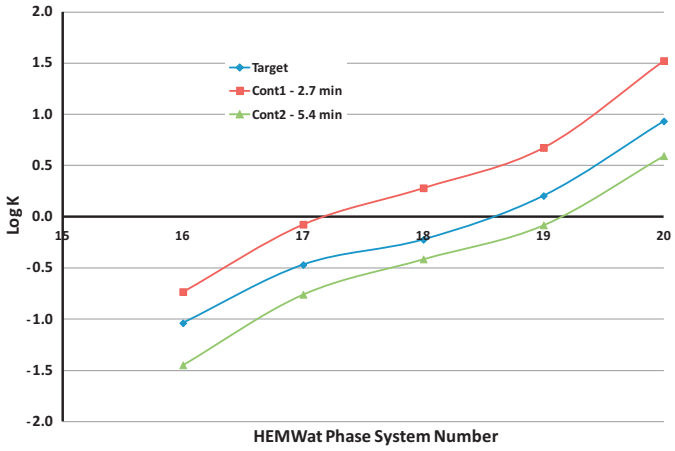


Fig. 6. Variation of Log_{10} *K* with HEMWat phase system number for the target compound and 2 impurities for application 9 in Table 3. HEMWat phase system 18 (6:5:6:5) was chosen for the HPCCC runs of Fig. 7.

(Fig. 7a) shows that all three compounds can be eluted, but as impurity 1 is so well retained in the lower phase the flow was doubled to 4 ml/min to elute it earlier. Increasing the loading to 55 mg broadened the target peak considerably showing that the run could be stopped after 30 min with impurity 1 still in the column (Rs_{12} = 2.3 compared to 3.1 at 10.9 mg loading). This could then be quickly flushed out as fresh stationary phase was used to fill the column for a total cycle time of 45 min. Purities in these first two loadings was ~100%, but when loading was increased to 220 mg in 5 ml with some MeOH added to help solubility – the purity of the target peak reduced to 85% (Rs_{12} = 1.3 compared to 2.3 for 55 mg loading).

5.3.3. *Projected further scale up and cost comparisons*

With a total cycle time of 45 min including refilling with fresh stationary phase and a sample loading of 110 mg (i.e. between those illustrated in Fig. 7b and c where resolution Rs was interpolated to be approximated 1.9) it would be possible to get a throughput of starting material of 24.4 g/h leading to a recovery of 15.3 g/h target compound. This can be volumetrically scaled up [11] as shown in Table 5. In a pilot plant environment the Maxi would enable purification of 2.6 kg/day. Note that this is much less than Case Study 4 (Section 5.4) where solubility was much better and assumes the development of a pilot scale Maxi operating at 240 g for 24 h/day.

A material cost comparison for the purification of 16 kg of the target compound is given in Table 6. The solid phase chromatography used 16 runs loading 1 kg on a 20 kg silica gel cartridge column. The dihydroxy impurity was very strongly retained on the silica gel and therefore a new cartridge was used for each purification resulting in a high cost for stationary phase which would be avoided using HPCCC. In this case HPCCC uses 20% less solvent and is only 15% of the cost of silica gel chromatography. Note that only solvent and stationary phase costs have been taken into account.

Table 5
The estimated throughput for analytical, preparative and pilot scale HPCCC instruments for application 9, Table 3.

Instrument	Column volume	Throughput	Throughput (g/week)
Midi	25 ml	0.15 g/h	4.6[a]
Midi	980 ml	6 g/h	180[a]
Maxi	4.6 L	28 g/h	0.7[b]
Maxi	18 L	110/h	2.6[b]

[a] Assumes lab instrument runs for 30 h/week.
[b] Assumes 240 g instrument in pilot plant and 24 h operation.

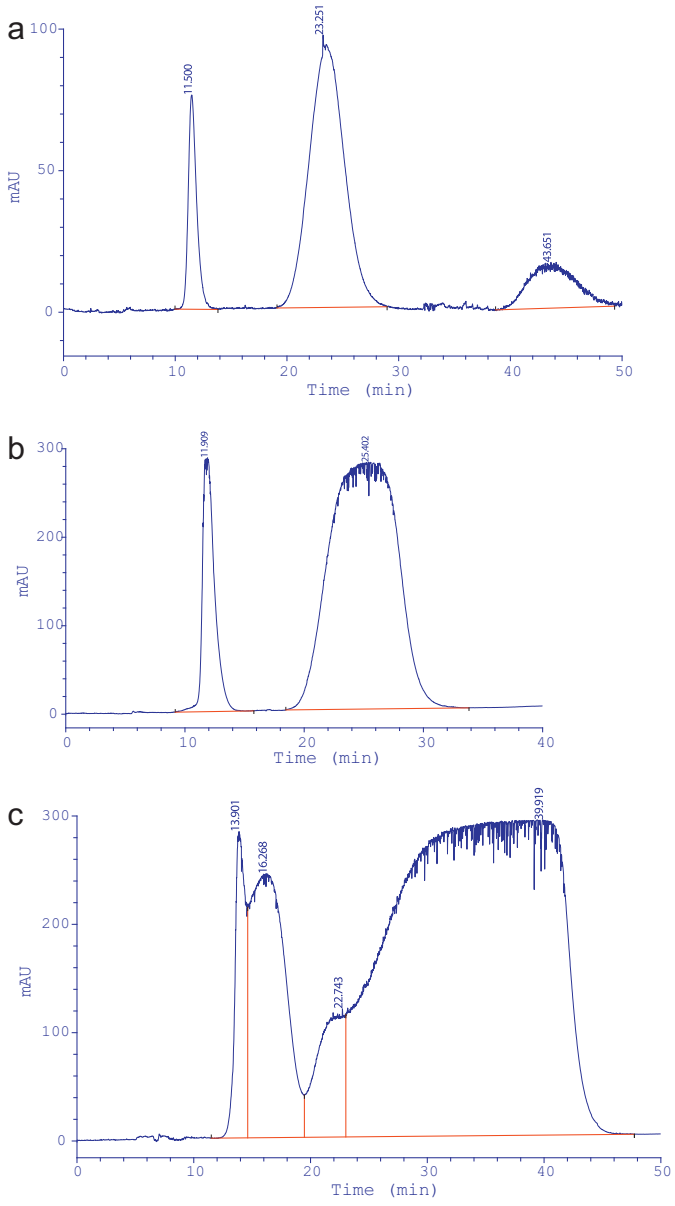


Fig. 7. HPCCC loading study for Case Study 3 using the analytical column of the DE Midi instrument for sample from application 9 in Table 3 (a) 10.9 mg, (b) 55 mg and (c) 220 mg. HPCCC run conditions: column: 25 ml, 0.8 mm bore; solvent system: hexane:ethyl acetate:methanol:water (HEMWat – SS18) [6:5:6:5]. Operational mode: normal phase (NP); method: flow (2 ml/min to 32 min then 4 ml/min), rotation (1400 rpm); temperature (30 °C), isocratic, elution extrusion; sample concentration 43 mg/ml. All sample loaded in stationary phase with a small amount of MeOH to aid solubility.

Equipment costs, disposal costs, workforce costs, overheads, energy and environmental costs have not been included.

5.4. *Case Study 4: removal of impurities*

5.4.1. *Separation*

Application 15 from Table 4 is an intermediate purification of a crude sample containing a pair of regio-isomers in the approximate ratio 5:1 of the required to the unwanted isomer. The material has previously been separated by silica gel chromatography with 40 kg processed in 8 runs on 20 kg columns with the unusually high loading of 25%, w/w. Could CCC match this performance? There was 58% of the target isomer in the crude (Fig. 8a) and the aim was to

Table 6
The material costs comparison of MPLC vs. HPCCC for application 9, Table 3.

Input material	Cost/L or kg (unspecified units)	Silica chromatography		CCC	
		Volume (weight)	Cost (units)	Volume (L)	Cost (units)
Heptane	4	12,000 L	48,000	3300	13,000
Ethyl acetate	1	3000 L	3000	2700	2700
Methanol	0.4	0 L	–	3300	1300
Water	0	0 L	–	2700	0
Total organic solvent	–	15,000 L	51,000	12,000	17,000
Solid stationary phase	190	320 kg	61,000	0	0
Materials cost (units)	–		112,000		17,000

achieve a purity for the target compound of >98% purity or <2% of the unwanted isomer and impurities.

5.4.2. Loading studies and scale-up to semi-prep

After initial partition studies it was found that hexane:ethyl acetate:methnol:water (HEMWat #18) [1:1:1:1] gave distribution ratios (K_D) of 0.71 and 1.41 in normal phase for the target and unwanted isomer with an α of 2. Loading studies were performed using the DE Spectrum (Dynamic Extractions Ltd., 890 Plymouth Road, Slough, UK) instrument with its analytical coil (23 ml) operating at 1 ml/min, 1600 rpm and 30 °C. The results are shown in Fig. 9 for (a) 100 mg, (b) 200 mg and (c) 450 mg sample loading in the mobile phase operating in normal phase mode. It can be seen that loading can be increased to 450 mg and that 98.8% purity (Fig. 8b and inset in Fig. 9c) is achieved with a yield of 95% from fractions 20 to 27 in Fig. 9c.

This was then scaled up to the DE Spectrum semi-prep column (134 ml) on the same instrument with the same phase system operating at 6 ml/min, 1600 rpm and 30 °C in normal phase in isocratic elution–extrusion mode. Initially volumetric scale up was used with a loading of 2.6 g in 18 ml, but it was found that even higher loading was possible (4 g in 26 ml – Fig. 10) achieving a purity of 98.6% with 87% recovery therefore giving greater overall throughput at acceptable yield.

5.4.3. Projected further scale up and cost comparisons

With a run time of about 28 min and allowing a further 20 min for extrusion and refilling – a cycle time of 48 min is feasible leading to a throughput of 5 g/h. This can be volumetrically scaled up [11] as shown in Table 7. In a pilot plant environment the Maxi would enable purification of 40 kg of material in <1 week once the 240 g version of Maxi has been constructed – a follow-on development resulting from the TSB-STEP research programme. This will then be a realistic alternative to conventional chromatography.

A material cost comparison for the purification of 1 kg of intermediate sample is given in Table 8. In this case CCC is competitive with silica gel chromatography, with CCC using 10% less solvent and only being 16% of the cost of silica gel chromatography. Note that only solvent and stationary phase costs have been taken into

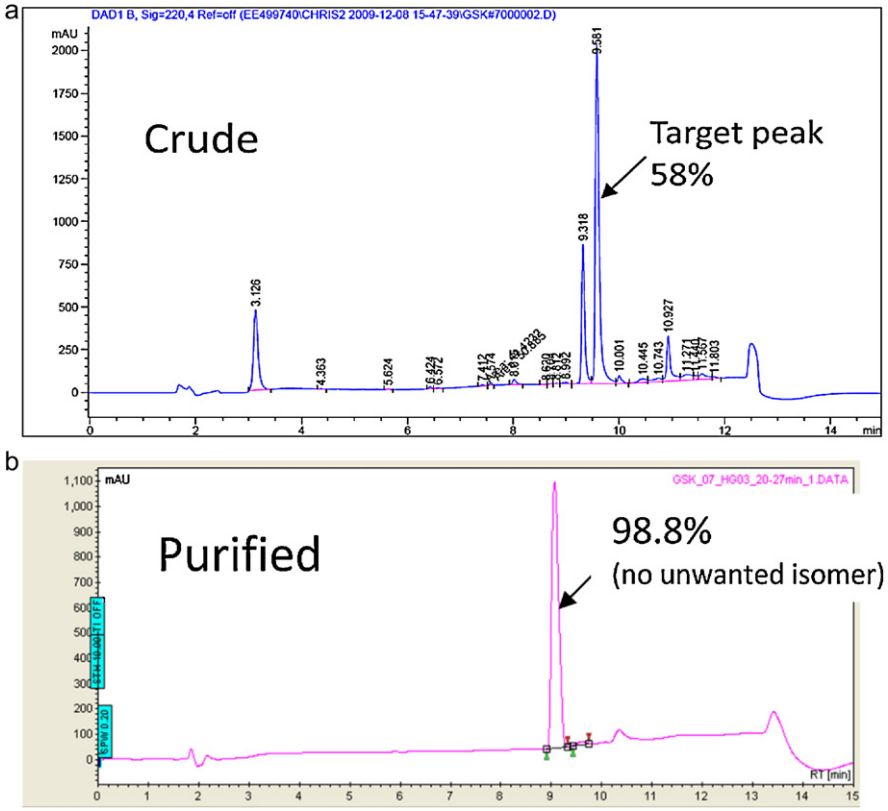


Fig. 8. HPLC traces of sample from Case Study 4, application 15 in Table 4 (a) crude with 58% target present and (b) fractions 20–27 purified to 98.8% from Spectrum semi-prep run (Fig. 9c). HPLC conditions: Gemini NX, 5 μm, C18, 110 Å (150 × 4.6 mm); mobile phase A = water + 0.1% TFA, B = MeCN + 0.1% TFA; method: 5–95%B over 10 min, 5 min equilibration at 5%B; flow rate: 1 ml/min; detection: 220 nm.

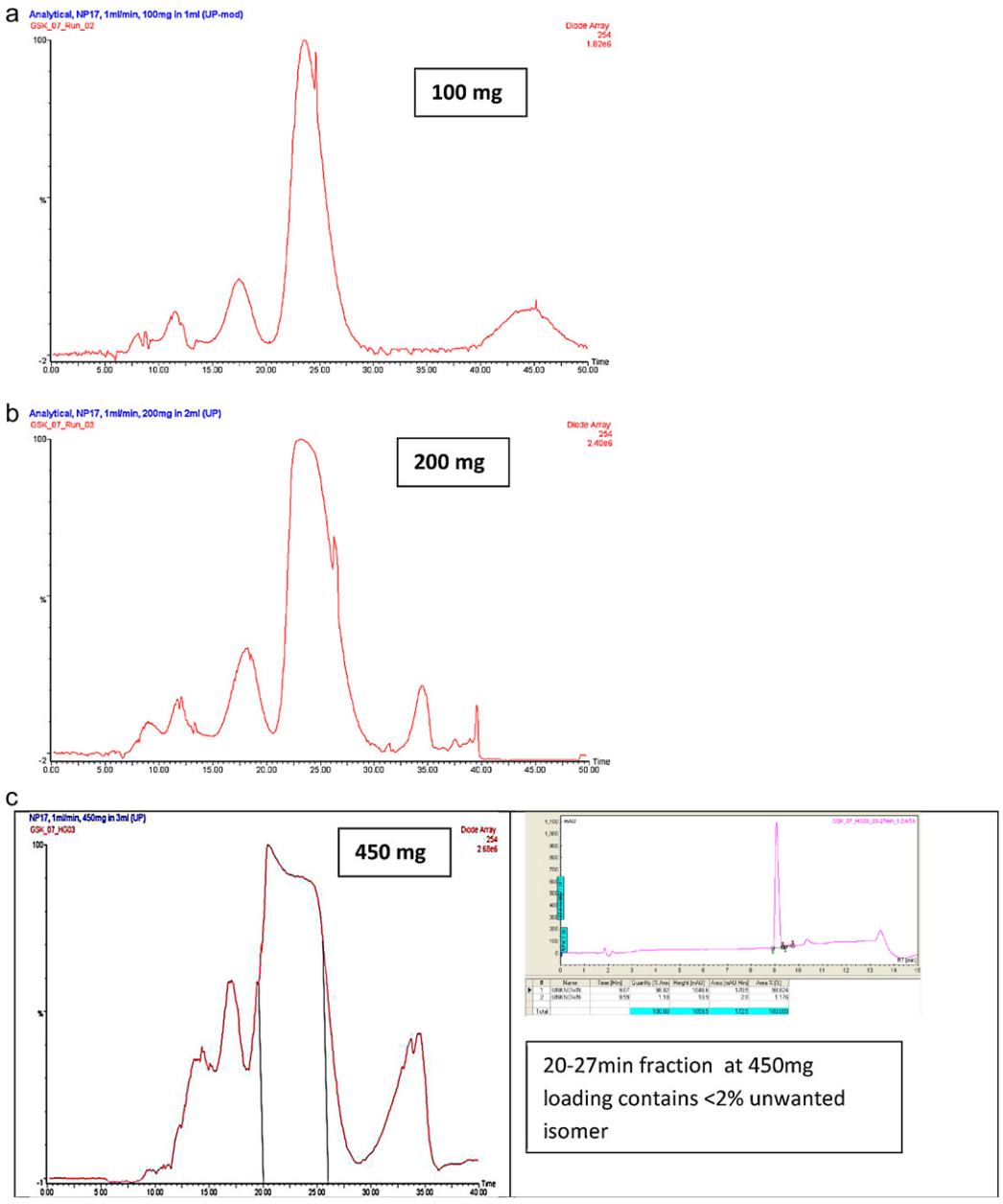


Fig. 9. HPCCC loading study using the analytical column of the DE Spectrum instrument for sample from application 15 in Table 4 (a) 100 mg, (b) 200 mg and (c) 450 mg with HPLC trace from Fig. 8b inset. HPCCC run conditions: column: 23 ml, 0.8 mm bore; solvent system: hexane:ethyl acetate:methanol:water (HEMWat – SS17) [1:1:1:1]; stationary phase retention (S_f): 79%; operational mode: normal phase (NP); method: flow (1 ml/min), rotation (1600 rpm); temperature (30 °C), isocratic, elution extrusion; sample loading (a) 100 mg in 1 ml; (b) 200 mg in 2 ml and (c) 450 ml in 4 ml. All sample loaded in mobile phase dissolving the sample in the following order: EtOAc (34%) and then MeOH (2.5%) before slow addition of hexane.

Table 7
The estimated throughput for semi-preparative, preparative and pilot scale HPCCC instruments for application 15, Table 4.

Instrument	Column volume	Throughput	Throughput
Spectrum	134 ml	5 g/h	150 g/week[a]
Midi	980 ml	37 g/h	1.1 kg/week[a]
Maxi	4.6 L	175 g/h	4.2 kg/week[b]
Maxi	18 L	685/h	16.4 kg/week[b]

[a] Assumes lab instrument runs for 30 h/week.
[b] Assumes 240 g instrument in pilot plant and 24 h operation.

Table 8
The material costs comparison of MPLC vs. HPCCC for application 15, Table 4.

Input Material	Cost/L or kg (unspecified units)	Silica chromatography		CCC	
		Volume (weight)	Cost (units)	Volume (L)	Cost (units)
Heptane	4	76 L	304	34	136
Ethyl acetate	1	19 L	19	28	28
Methanol	0.4	0 L	–	23	9
Water	0	0 L	–	22	0
Total organic solvent	–	95 L	323	85	173
Solid stationary phase	185	4 kg	740	0	0
Materials cost (units)	–		1063		173

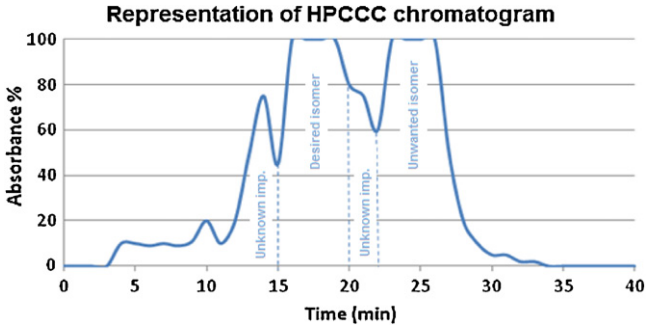


Fig. 10. Representation of HPCCC chromatogram for Case Study 4 indicating target compound and impurities for the scale-up to the DE Spectrum semi-prep column. Run conditions: column: 134 ml, 1.6 mm bore; solvent system: hexane:ethyl acetate:methanol:water (HEMWat – SS17) [1:1:1:1]. Operational mode: normal phase (NP); method: flow (6 ml/min), rotation (1600 rpm); temperature (30 °C), isocratic, elution extrusion; sample loading, 4 g in 26 ml. Recovery, 87%. Same sample preparation strategy as for analytical runs.

account. Equipment costs, overheads, energy and environmental costs have not been included.

6. Conclusions

The TSB-STEP research programme described in this paper has proved to be a very successful teaming involving end users, supply companies and academics. What started as a phased study involving analytical, preparative and process scale objectives soon changed to become theme driven user led research programme focussing on product integration and automation, generating a comprehensive applications portfolio, new solvent system development, improving instrument reliability, continuous processing and scale up and demonstrating value. This paper has focussed on the "generating a comprehensive applications portfolio" theme. It lists 15 of the 30+ applications studied in the research programme that can be put in the public domain. These have been categorised into isomer separations, polar separations, separations from crude extracts and removal of impurities. Case studies have been taken from each of these categories and, where scale up was projected, it was demonstrated that in specific cases HPCCC would use between 10% and 20% less solvent. In particular, due to the elimination of the cost of silica based stationary phase required for conventional chromatography, the use of HPCCC was projected to lead to significant cost savings at scale. 85% of the applications undertaken by the consortium were successful and, of the ones that were not, only one failed completely and the rest were partially successful. This research has shown that the high performance countercurrent chromatography instruments now being developed are capable of becoming a valuable platform technological resource for the pharmaceutical industry offering for some applications a step-change reduction in costs.

Acknowledgements

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