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Scalable Technology for the Extraction of Pharmaceutics (STEP): The transition from academic knowhow to industrial reality

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ABSTRACT

This paper addresses the technological readiness of counter-current chromatography (CCC) instruments to become platform technology for the pharmaceutical industry. It charts the development of the prototype technology since its inception in 1966, through conceptual improvements in the 1980s that led to higher speed separations in hours as opposed to days. It then describes the engineering improvements that have led to the development of high performance counter-current chromatography with the potential for scale-up to process scale for manufacturing products in industry with separation times in minutes rather than hours. A new UK Technology Strategy Board high value manufacturing £1.5m research programme to take CCC through to technology readiness level 8 (i.e. as platform technology for continuous 24×7 operation by industry) is introduced. Four case studies are given as examples of successes from its expanding applications portfolio, which is mainly confidential. Finally, the hurdles for the uptake of new technology by industry are highlighted and the following potential solutions given: rapid method development, automation, continuous processing and instrument reliability and robustness. The future challenge for the CCC community will be to address these development needs urgently if CCC is to become the platform technology it deserves to be.

1. Introduction

The manufacture of high value pharmaceuticals, whether from natural products, fermentation processes or chemical/biochemical synthesis, involves multiple, down stream processing steps to ensure final product quality. This paper gives an overview of progress made to date with an R&D collaborative programme to develop small footprint, continuous extraction technology based on counter current chromatography (CCC) which can be used to lower downstream processing costs. Extracted material is always maintained in an easily managed liquid stream which is able to cope with crude extracts and particles without any pre-conditioning. The potential benefit of this technology is to reduce operating costs by minimising the number of processing steps, lowering solvent usage and also dispensing with expensive solid support materials.

The basic science, developed with industry and supported by the Research Councils, has already been successfully demonstrated; scale up is feasible in batch and continuous process mode at the laboratory and pilot scale and the technology is now at readiness level 4 [1]. This collaborative programme [2] is driven by the needs of the pharmaceutical industry and integrates technology providers and the scientific development team. It is funded by industry and part funded by the UK government.

Since project inception in September 2009 excellent progress has been made in developing a portfolio of industry applications which illustrate the utility of CCC and allow a direct comparision to both preparative HPLC and medium pressure chromatography. A number of hurdles that could potentially delay the uptake of the technology have been identified and plans are in place to address these.

While there will be limitations from a commercial confidentiality point of view on what can be reported, this paper charts the development of the technology from basic research at Brunel University, through to its commercial development at Dynamic Extractions Ltd and the current collaborative government funded development programme that may see the first commercial use of

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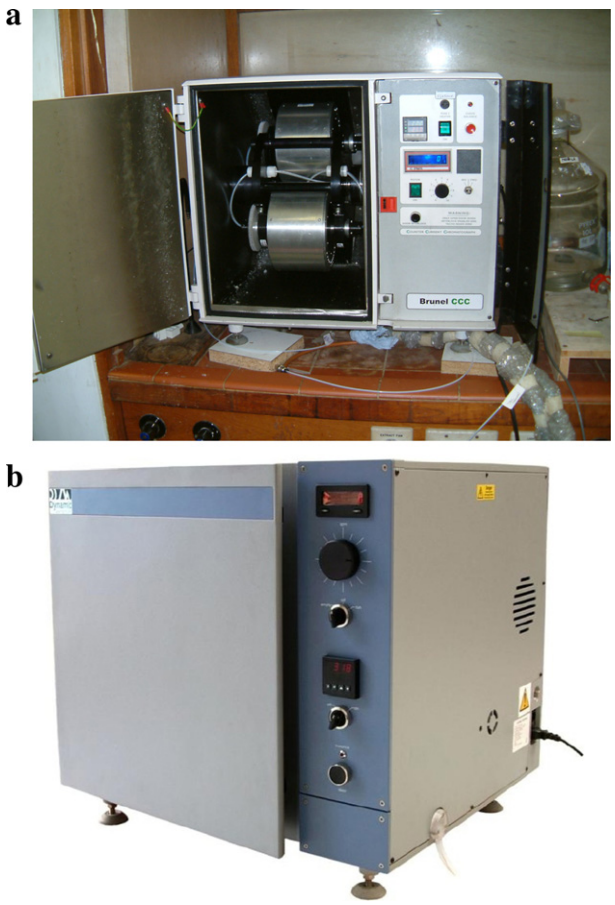

Fig. 1. (a) Brunel CCC prototype 1 L instrument and (b) the commercially available Dynamic Extraction's Midi – planet radius 110 mm, preparative column volume 940 mL, tubing bore 4 mm, max speed 1400 rpm (240 *g*).

the technology in production in the pharmaceutical industry within the next five years.

2. Background

Counter-current chromatography was first introduced by Ito in Nature in 1966 [3]. He demonstrated how two immiscible liquids placed at either end of a coiled tube would move in opposite directions with respect to one another (counter-current) when the tube was slowly rotated in a force field. He placed a sample between them and demonstrated a separation of proteins using this counter-current process. Since then the term counter-current chromatography (CCC) has been widely used for all processes that have developed from this fundamental principle. First Ito's "I" type centrifuges and later in the 1980s his "J" type centrifuge which led to much faster and more competitive separations in hours as opposed to days with Craig's CCD instruments and other continuous flow methods like locular or droplet CCC which could take days to produce the desired separation. Ito's "J" type centrifuge in the 1980s produced a step change in usage of the technology [4] and led to a number of small companies producing commercial instruments: (1) the PC Inc single column instrument developed by Peter Carmeci who was fortunate to have the same initials as Partition Coefficient – hence PC Inc and (2) the 3-column PharmaTech instrument developed by Edward Chou, who was a great pioneer of CCC technology and was largely responsible for its global uptake. Sadly both Peter Cameci and Edward Chou died prematurely, but their contribution to the commercialization of the technology lives on.

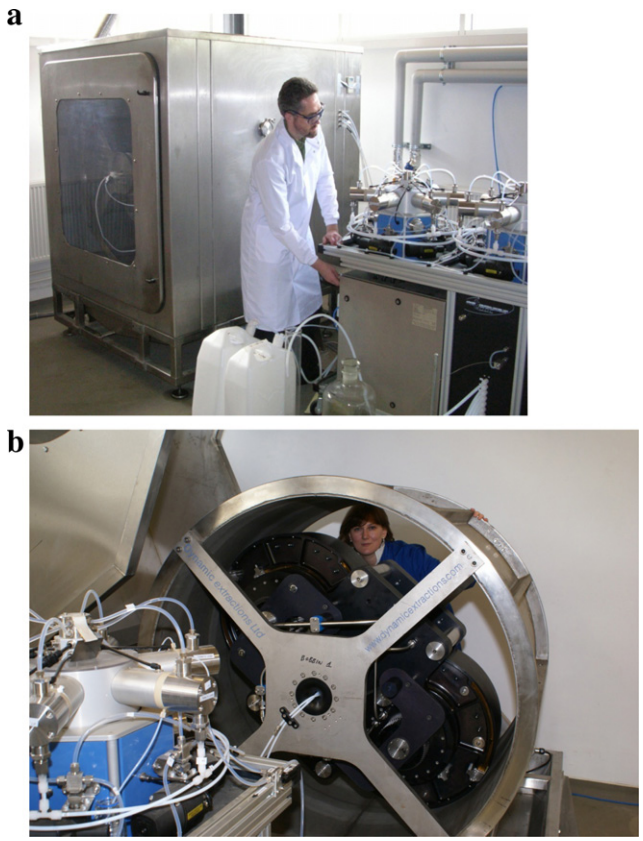

Fig. 2. (a) 4.6 L Maxi CCC prototype and (b) 18 L commercial Dynamic Extraction Maxi – both have a planet radius of 300 mm; tubing bore 10 mm and max speed 850 rpm (240 g).

The Brunel Institute for Bioengineering (BIB) team was the first group to focus on the engineering developments of the CCC centrifuges. Its initial research was funded by a major BBSRC[1] LINK grant (Grant No. 100/BCE08803) with industrial end-users AstraZeneca, Zeneca Agrochemicals (later Sygenta), Glaxo Wellcome (later GSK) and Shell. This research examined the feasibility of scale up by constructing special multilayer bobbins of varying tubing bore for the Brunel CCC instrument (Fig. 1a) the forerunner of the Dynamic Extractions Midi (Fig. 1b). This was shortly followed by an industry led EPSRC-IMI[2] programme (Grant No. GR/R013143/01) which led to the construction of the first, intrinsically safe, 4.6 litre CCC centrifuge (Fig. 2a). The engineering challenge was to design bobbins that could withstand cyclic "g" fields of between 290 and 530 *g* with cyclic loads of between 13 and 25 metric tonnes at 600 rpm which would double if speed were increased to 850 rpm.

Both grants supported studies on the engineering fundamentals of these devices allowing the relationships between key operating variables such as mobile phase flow rate, column rotational speed and column bore to be established [5–9]. These and follow-on studies led to kg scale scale-up studies [10] and volumetric scale-up [11] which was followed by a small business research initiative with Dynamic Extractions Ltd to look at the further scale-up of CCC by building a set of bobbins for the 4.6 litre Maxi with various bore sizes up to 18 mm. Further scale-up to process scale with throughputs as high as 25 kg/day appear feasible [12] and later

[1] BBSRC – Biotechnology and Biological Sciences Research Council.
[2] EPSRC-IMI – Engineering and Physical Sciences Research Council Innovative Manufacturing Initiative.

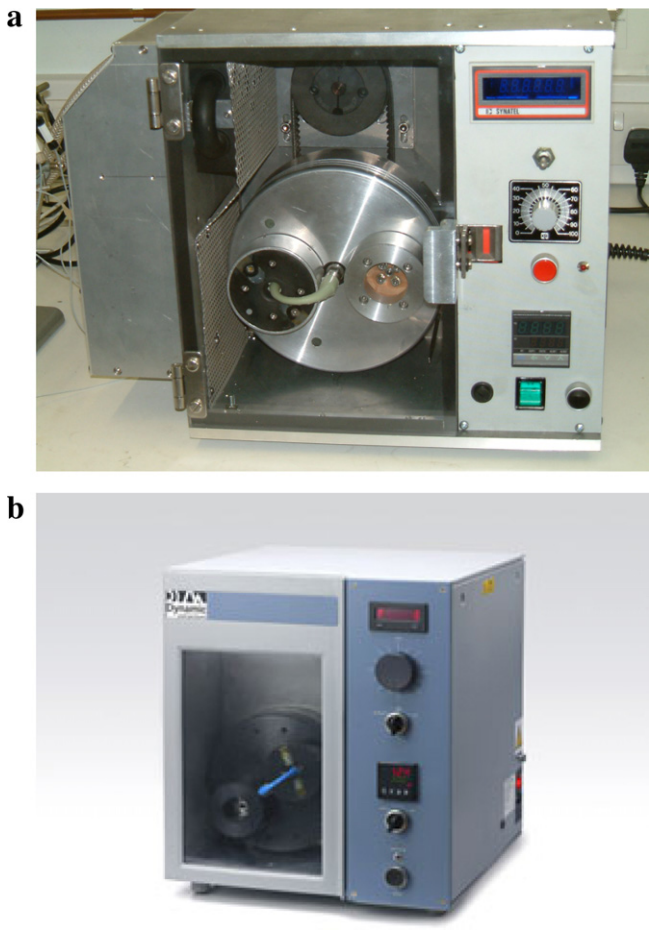

Fig. 3. (a) Mini CCC prototype – column volume 5.4 or 17.8 mL, tubing bore 0.8 mm, max speed 2100 rpm (240 *g*) and (b) commercial Dynamic Extraction Mini – column volume 18.8 mL, tubing bore 0.8 mm, max speed 2100 rpm (240 *g*).

the development of the 18 litre Maxi was achieved and demonstrated [13,14] with a cyclic load only twice that of the 4.6 litre Maxi. Meanwhile, with funding from the EPSRC Instrument Development Programme (GR/M48345/01) the Institute demonstrated that it is also possible to scale down counter-current chromatography (CCC) from a process that could fractionate large quantities of sample in a few hours to one that could fractionate small quantities in minutes. Prototype Milli-CCC systems were designed, developed and tested (Fig. 3a) both with model sample phase systems [15,16] and on real CCC-MS applications [17,18]. The process and its design improvements, such as an encased lubricated gearbox for quiet running and moulded flying leads to increase life, were patented and a company, Dynamic Extractions Ltd, formed in 2002 to commercialise the whole range of CCC instruments (Figs. 1–3b). In 2006 the company expanded, moving to its own premises in Slough.

3. Technology readiness

The NASA technology readiness levels first introduced by Sadin et al. [1] are shown in Fig. 4. While they relate to flight hardware and NASA mission readiness, there are useful parallels with the readiness for hardware for the manufacture of drugs in the pharmaceutical industry. Both are interested in robust hardware that does not fail. Failure would result in the loss of valuable product (or in the case of NASA, its crew). A rough translation for the pharmaceutical industry would be (1) basic research; (2) technology concept and basic design; (3) proof of concept and laboratory studies; (4) breadboard (prototype) validation in the laboratory;

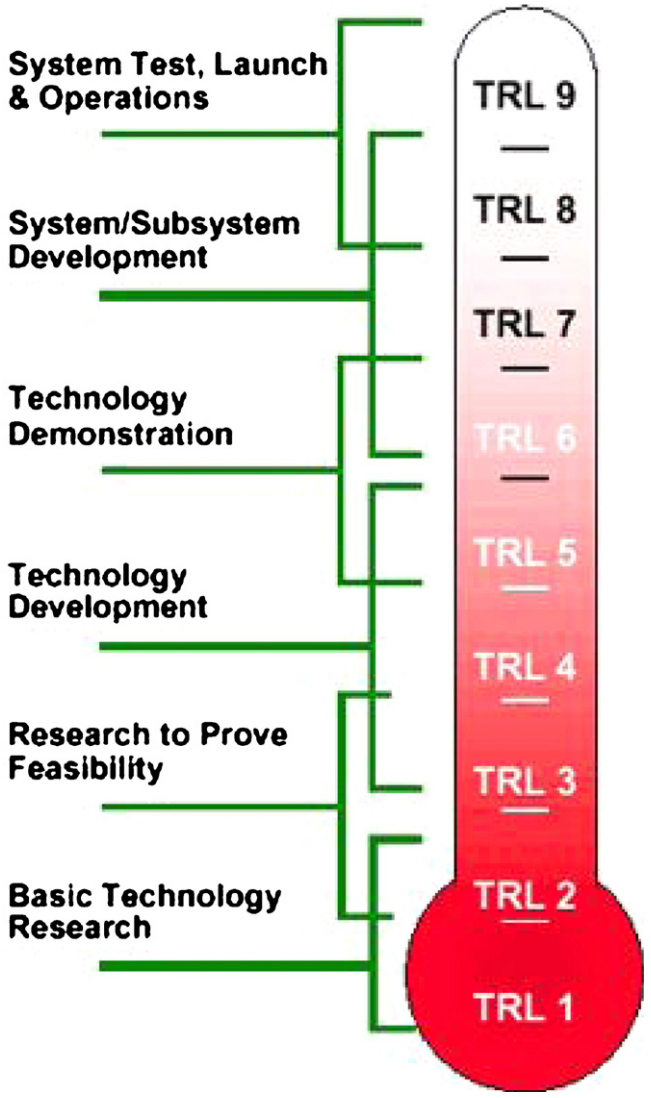

Fig. 4. NASA technology readiness scale [1]. Brunel prototypes took the technology to TR4, Dynamic Extractions Ltd through levels 5–7 and the current TSB-STEP research programme is taking the technology through to technology readiness level 8.

(5) breadboard or prototype validation in pharmaceutical environment; (6) system/prototype demonstration in a pharmaceutical environment; (7) system/prototype/product demonstration in an operational manufacturing environment; (8) final system/product completed and tested in a manufacturing environment; and (9) system/product operational in a manufacturing environment.

4. The TSB-STEP research programme

The research performed at Brunel covers technology readiness levels 1–4, while Dynamic Extractions covers technology readiness levels 5–7. Recently Brunel with Dynamic Extractions, GlaxoSmithKline and Pfizer has won a £1.5m TSB-HVM award for "Scalable Technology for the Extraction of Pharmaceuticals (STEP)" to take dynamic extraction technology through to technology readiness level 8. The intention is to develop high performance counter-current extraction (HPCCE) processing systems and new continuous processing and production techniques for material isolation and purification and provide a new platform technology for the future. The objective is to achieve a step change in speed and scale-up of isolations, generating a portfolio of practical applica-

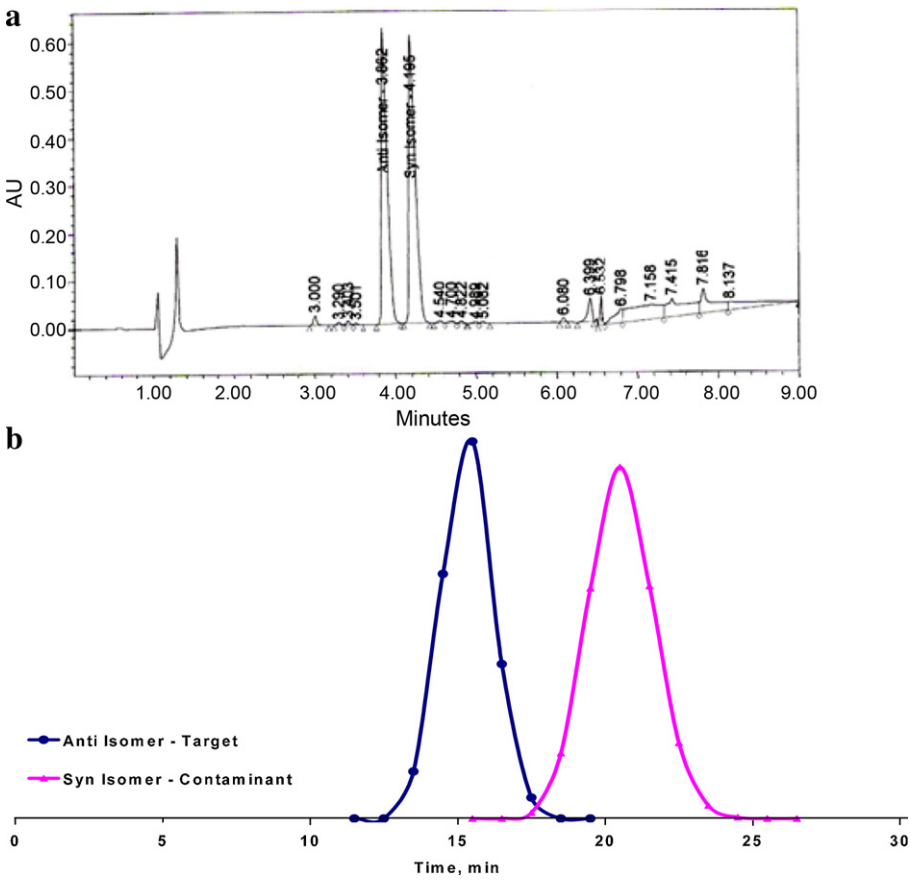

Fig. 5. Case study 1 – Separation of syn/anti-isomers (a) HPLC analysis of crude and (b) CCC chromatogram showing isolation of anti-isomer to >98% purity. Run conditions: DE Mini HPCCC instrument; column volume 18.8 mL, bore 0.8 mm; rotational speed 2100 rpm (241 *g*); solvent system: heptane–dichloromethane–acetonitrile (5:0.5:4.5); flow rate 1 mL/min; normal phase (NP); sample loading 50 μL of 200 mg/mL solution in DCM:ACN (1:1); detection wavelength 220 nm.

tions at various scales; demonstrating a reduction of processing complexity and cost (i.e. fewer processing steps) and develop and integrate robust, easy to use systems.

The approach was to align the technology to the pharmaceutical industry by having an end-user driven programme (Dr. Keith Freebairn of GSK is the lead and manager of the programme) which would encourage a broader industrial uptake. The project is now in its second year and will run for three years in total. As can be seen from the authors on this paper, this research programme is a team effort with collaborative activity from all the participant organizations. Pfizer, in addition to UK representation from Sandwich also has staff involved from the Groton site in the USA.

5. Application case studies

At the initial stage of the project it was important to establish the boundaries of the investigation for HPCCC as a separation and purification technology within pharmaceutical research, discovery and manufacture. Consequently, a wide range of applications has been tested to push the technology to its limits. These applications represent a range of different polarity and solubility issues with different types of impurities. So far approximately 15 applications have been examined of which 11 have produced promising separations and reached their targets, 3 have been partially successful and only one has failed. A few of these are now given as case studies.

5.1. Separation of syn/anti isomers (Fig. 5)

5.1.1. Separation

This is an example of a non-polar application. There was 32% of the target anti isomer in the crude (Fig. 5a). A baseline separation was achieved in the DE Mini instrument isolating the anti-isomer to greater than 98% purity (Fig. 5b).

5.1.2. Run conditions

DE Mini HPCCC (Dynamic Extractions Ltd, Slough); column volume 18.8 mL, bore 0.8 mm; rotational speed 2100 rpm (241 *g*); solvent system: heptane–dichloromethane–acetonitrile (5:0.5:4.5); flow rate 1 mL/min; normal phase (NP) with upper less polar phase mobile; sample loading 10 mg in 50 μL of 200 mg/mL solution in DCM:ACN 1:1; detection wavelength 220 nm; run time 25 min.

5.2. Isolation of intermediate from a telescoped synthesis (Fig. 6)

5.2.1. Separation

The aim was to find a high throughput method as an economical alternative to NP chromatography. 1.8 g of a mixture containing 64% of the starting material (Fig. 6a) was injected into a DE Spectrum instrument with the organic-rich mobile phase of a heptane:ethyl acetate:methanol:water (HEMWat 3:2:3:2) phase system and was fractionated in a run time of 50 min. 75% of the target compound was recovered (Fig. 6b) at 97.9% purity with an estimated throughput of 15.1 g/h. The HPCCC chromatogram is given in Fig. 6c. The first eluting peak is the target isomer.

5.2.2. Run conditions

A DE Spectrum HPCCC instrument (Dynamic Extractions Ltd, Slough) was used: column volume 134 mL; flow 6 mL/min; rotational speed 1600 rpm; phase system:heptane:ethyl acetate:methanol:water – HEMWat 19 (3:2:3:2); sample loading 1.8 g in 12 mL UP/LP 1:1; normal phase (NP); run time 50 min.

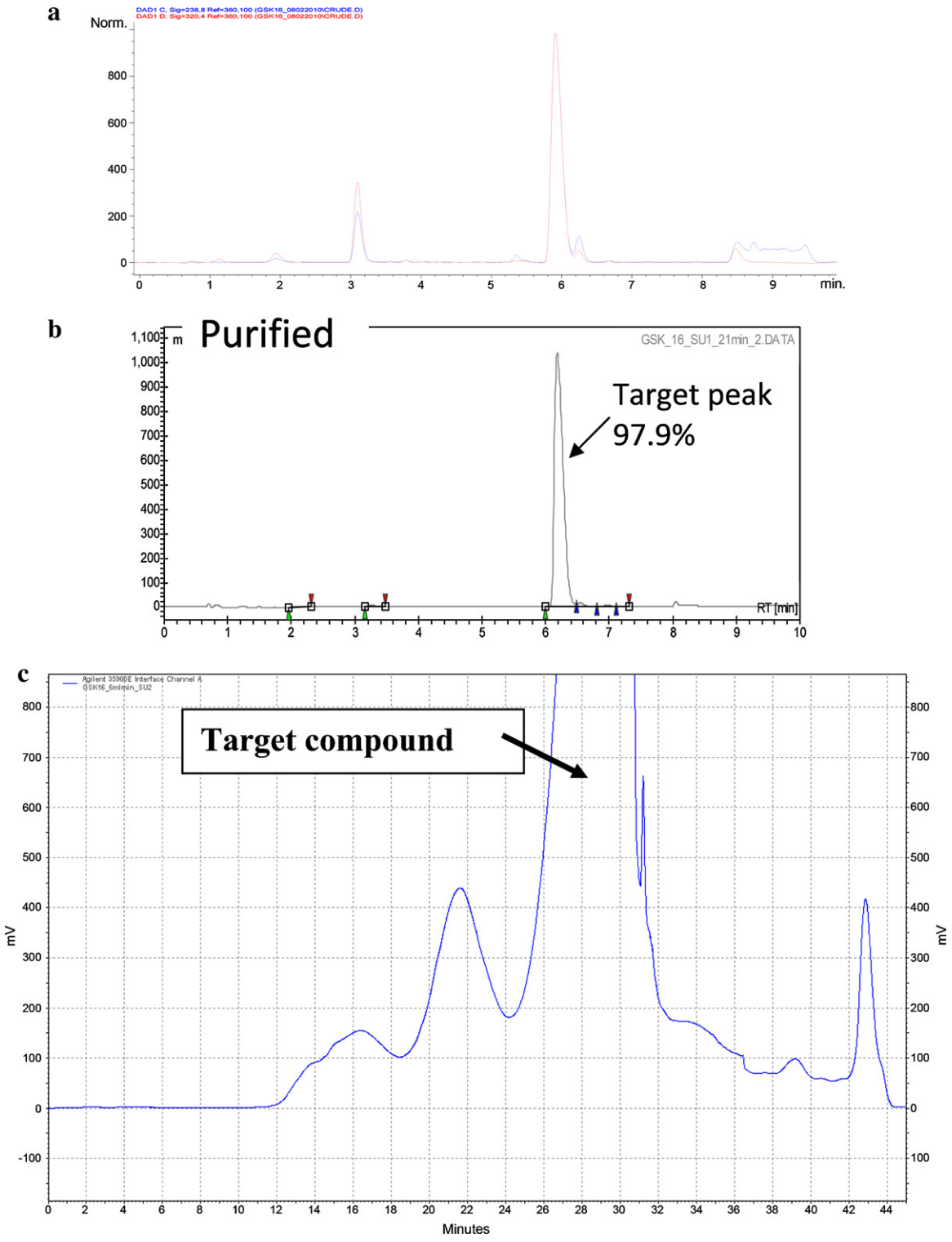

Fig. 6. Case study 2 – Intermediate from a telescoped synthesis (a) HPLC analysis of starting material, (b) HPLC analysis of recovered target compound showing 97.9% purity and (c) HPCCC chromatogram showing the target compound. Run conditions: DE Spectrum HPCCC instrument; column volume 134 mL; flow 6 mL/min; rotational speed 1600 rpm; phase system: heptane:ethyl acetate:methanol:water – HEMWat 19 (3:2:3:2); sample loading 1.8 g in 12 mL UP/LP 1:1; normal phase (NP).

5.3. Separation of isomers (Fig. 7)

5.3.1. Separation

Fractionation was required to remove an impurity (20%) which was related to the target which was originally isolated by low selectivity liquid–liquid extraction and NP flash chromatography. The re-work was unplanned and cost two weeks late delivery. The crude contained 74% of the target (Fig. 7a) and was purified to >98% with 95% recovery (Fig. 7b) in a DE Mini HPCCC instrument with a run time of 30 min (Fig. 7c) and an estimated throughput when scaled from the DE Mini to DE Midi of 60 g/h (1.4 kg/day) for serial injection with optimised cycle times.

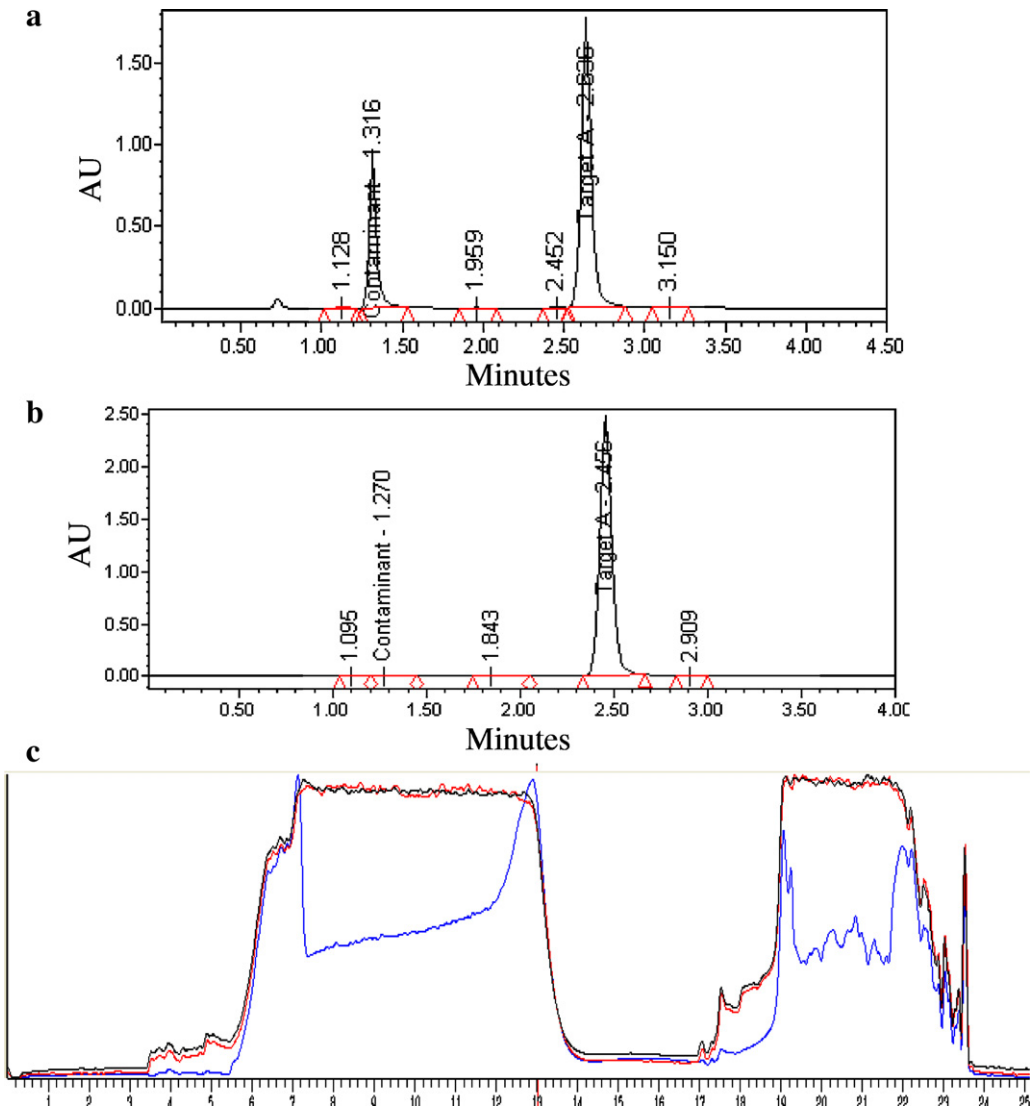

Fig. 7. Case study 3 – Separation of isomers (a) HPLC of crude containing 74% of target; HPLC of purified fraction, (c) HPCCC trace indicating target. Run conditions: DE Mini HPCCC instrument; column: 18.8 mL; solvent system: HEMWat 23 (4:1:4:1); normal phase (NP); flow: 2 mL/min; rotational speed: 2100 rpm; bore: 0.8 mm; sample loading 290 mg in UP:LP (2:1); recovery 95%. Spectrophotometer analysis 250 nm (blue); 254 nm (black and red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

5.3.2. Run conditions

Instrument: DE Mini HPCCC unit (Dynamic Extractions Ltd, Slough); column: 18.8 mL; solvent system: HEMWat 23 (4:1:4:1); normal phase; flow: 2 mL/min; rotational speed: 2100 rpm; bore: 0.8 mm; sample loading 290 mg in UP:LP (2:1); recovery 95%; run time 25 min. Spectrophotometer analysis 250 nm (blue); 254 nm (black and red).

5.4. Isolation of a peroxy-substituted compound (Fig. 8)

5.4.1. Separation

Fractionation was required to remove a bromo-impurity from the crude, which had 87% of the target compound present (Fig. 8a). The aim was to isolate the required target to >95% purity with less than 0.5% of any unspecified impurity and none of the bromo-analogue eluting. This target was achieved (Fig. 8b). The target isomer eluted between 26 and 50 min with the bromo-impurity eluting previously between 15 and 25 min (Fig. 8c). This run demonstrated that the target was extremely well separated from the bromo-impurity by the selectivity of the chosen solvent system.

5.4.2. Run conditions

Instrument: DE Mini HPCCC unit (Dynamic Extractions Ltd, Slough); Column: 18.8 mL; solvent system: HEMWat 14 (1:2:1:2); normal phase; flow: 1 mL/min; rotational speed: 2100 rpm; bore: 0.8 mm; sample loading 200 mg in 2 mL LP; recovery 59%; run time 45 min.

6. Hurdles to the uptake of new technology

There is considerable expertise in conventional solid phase preparative chromatography in the pharmaceutical industry. At the laboratory scale in particular solid phase preparative chromatography is the technique of choice for preparative isolations. There is a high level of confidence in this technique as a result of a strong skills base and a clear history of application success. In contrast to this well established capability, there is very little experience of counter current chromatography in the pharmaceutical industry. Given this situation, what can be done to drive change?

The consortium's approach has been to build as large a portfolio of CCC applications as possible allowing a direct contrast to the existing capability. These contrasts will also allow the key

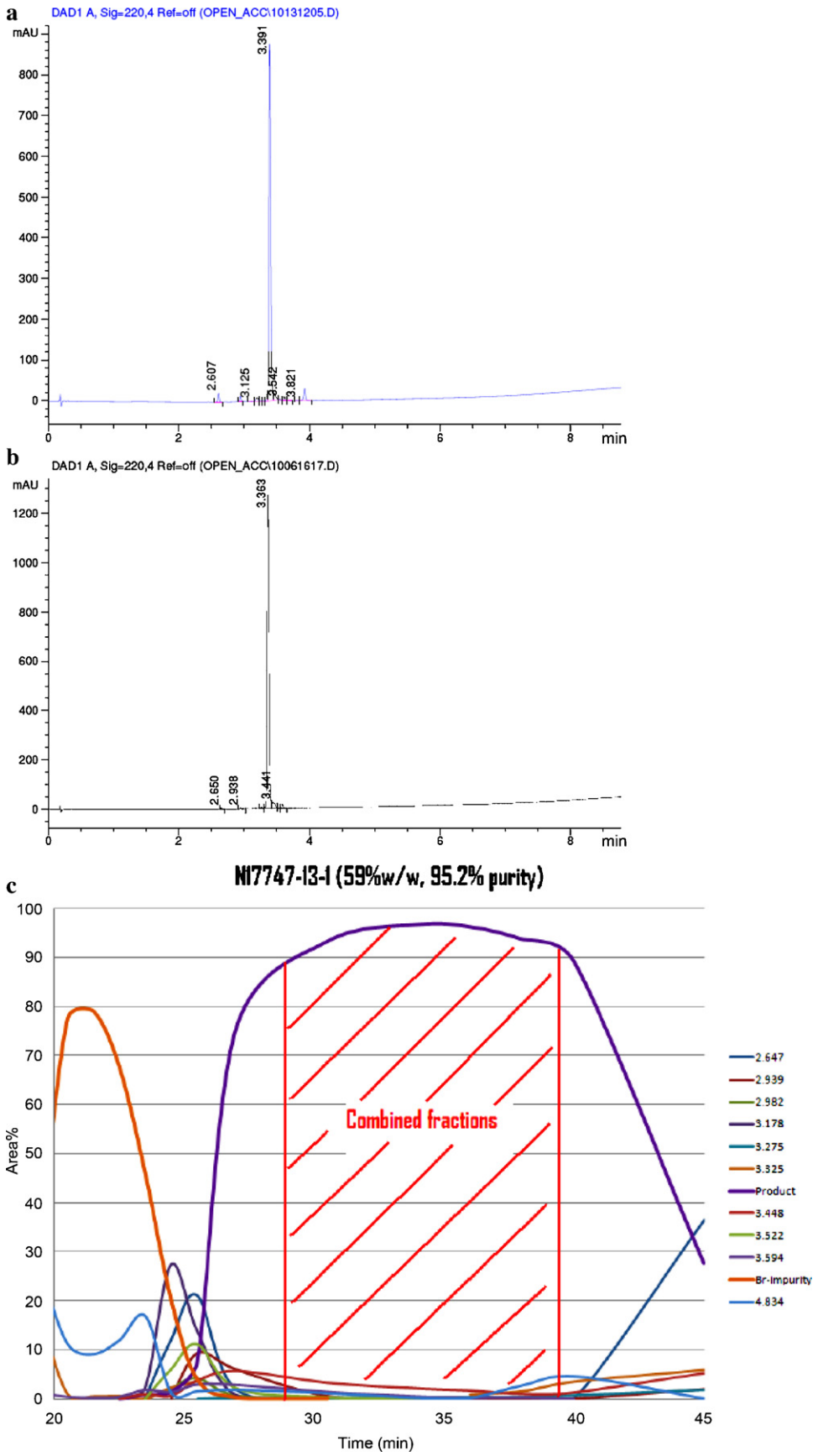

Fig. 8. Case study 4 – Isolation of peroxy-substituted compound (a) HPLC trace of starting material; (b) HPLC trace of the target fractions from HPCCC and (c) the HPCCC chromatogram showing the target isomer eluting between 26 and 50 min. Run conditions: DE Mini HPCCC instrument; column: 18.8 mL; solvent system: HEMWat 14 (1:2:1:2); normal phase (NP); flow: 1 mL/min; rotational speed: 2100 rpm; bore: 0.8 mm; sample loading 200 mg in 2 mL LP; recovery 59%.

differentiators of CCC to be understood. There are a multitude of different considerations here that include topics such as reliability of retention behaviour/scalability; solvents costs; consumable costs (cheap liquid phases rather than expensive solid phases); potential to process very dirty materials including solids; potential to handle compounds unstable on silica/insoluble compounds and waste handling (solvent disposal issues); equipment reliability, ease of use, separation speed, separation capability and cost. A clear view of these differentiators only emerges with experience and clearly this experience will only be gained over an expanded time scale.

At the manufacturing scale, it is unusual to see chromatographic processes being used to isolate materials. There are again a multitude of factors at play here. Separation processes in manufacturing will inevitably be more complex than conventional processing and process reliability must be high. By their nature chromatographic processes are complex and will need rigorous control which in turn will add expense and increase skill requirements. In addition to these hurdles there is a strong reluctance to make changes to existing processes because of the requirement for re-registration which again adds both expense and uncertainty. Very rigorous regulatory requirements in manufacturing generate a strong reluctance to venture 'into the unknown'.

The barriers to adopting new separation technology then, particularly in a manufacturing environment, are high. Given this situation, the substantial energy barrier will only be surmounted where there is a very clear business benefit and where the goal cannot be achieved by other means.

7. Conclusions

The TSB-STEP research programme has just completed its first year and has two more years to go. A pharmaceutically relevant applications portfolio continues to build and data obtained to date indicates some interesting potential for CCC technology. Effort is currently focussed on rapid method development, automation, continuous processing and instrument reliability and robustness.

Now is the time (in a recession) to promote the new robust high performance CCC technology, emphasising its green potential and its potential cost savings. It is also time for the CCC community to move out of their comfort zones and present their work at non-CCC conferences and work more closely with industry and the end-users of the technology. There will be a lot of focussed hard work required in the next two years if CCC is to become the platform technology it deserves to be.

Acknowledgements

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