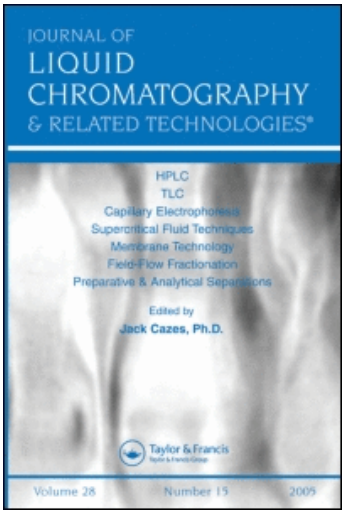


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Minimising Solvent Usage in High Speed, High Loading, and High Resolution Isocratic Dynamic Extraction

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**To cite this Article** Garrard, Ian J. , Janaway, Lee and Fisher, Derek(2007) 'Minimising Solvent Usage in High Speed, High Loading, and High Resolution Isocratic Dynamic Extraction', Journal of Liquid Chromatography & Related Technologies, 30: 2, 151 — 163
**To link to this Article: DOI:** 10.1080/10826070601077252
**URL:** http://dx.doi.org/10.1080/10826070601077252

*Journal of Liquid Chromatography & Related Technologies*®, 30: 151–163, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070601077252

# Minimising Solvent Usage in High Speed, High Loading, and High Resolution Isocratic Dynamic Extraction

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**Abstract:** The development of the planetary-motion countercurrent chromatography machine into a high speed, high loading and high resolution dynamic extraction centrifuge has allowed the scale-up of the technique from analytical to pilot (kg/day). At this scale, solvent usage is a major consideration for both economical and environmental ("green") reasons. The analysis by gas chromatography of the composition of the two phases in solvent systems allows each layer to be made separately as required, minimising solvent wastage. Furthermore, as operation is generally isocratic, analysis of the recovered condensate from evaporated fractions allows the mobile phase to be easily reconstituted ready for re-use in the centrifuge. Such recycling further minimises solvent usage during preparative and pilot-scale separations.

**Keywords:** Countercurrent chromatography, CCC, Dynamic extraction, Recycling

## INTRODUCTION

It is becoming increasingly important for industry to improve efficiency, reduce costs and shorten the time taken to develop purification processes. The Brunel Institute for Bioengineering (BIB) has developed a range of dynamic extraction instruments running from analytical to pilot scale capable of processing up to 10 kg/day, and is currently exploring the prospect of process-scale dynamic extraction (100 kg/day).

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Dynamic extraction technology (DE) is a recent development of countercurrent chromatography (CCC). Unlike CCC, with dynamic extraction the separations are provided in minutes rather than hours, the equipment is more robust than previous CCC instruments, and the scale-up to pilot level has been shown to be quick and easy.[1,2] It is being commercialised by Dynamic Extractions Ltd (www.dynamicextractions.com) and three scales of DE centrifuges are currently available, known as MINI, MIDI and MAXI (Table 1).

In DE centrifuges, tubing is wound on a drum which is centrifugally rotated in planetary motion. The tubing is initially filled with the solvent phase intended to be the stationary phase and the mobile phase is pumped over it. Up to 85% of the stationary phase can be retained in the coil. The planetary motion sets up alternating zones of mixing and settling along the length of the tube, that travel in waves synchronously with the rotation. Samples injected with the mobile phase undergo many partitioning steps per minute before they elute. This provides for high resolution separations based on differential partitioning behaviour as characterised by the distribution ratio (D = concentration of solute in stationary phase/concentration of solute in mobile phase). Dynamic extraction can be used in normal and reverse phase mode. Material that elutes very slowly can be recovered by pumping out the stationary phase, without any compound losses, whilst maintaining resolution.

The key advantages of the technology are:

1. High loading capacity with short processing times.
2. No losses arising from irreversible adsorption onto solid matrices. All sample components are recoverable.
3. One step purification of compounds from a crude extract is possible, without the need for prior sample clean-up procedures. Even particulates are tolerated so filtering a crude sample is not necessary.
4. A wide range of polarities can be processed due to the range of solvents that may be used. The literature reports examples with a logP scope of at least −7 to 17.[3]
5. Can be extended to the separation of proteins and other biomolecules, using aqueous-aqueous two-phase systems but further development is required.
6. No changes to the chromatography over time, as a fresh "column" is created each run, making it easier to consistently satisfy the regulatory authorities.
7. The coils are tough and the machinery robust. A set of coils would be expected to last the lifetime of the centrifuge and so maintenance and running costs are low.
8. Far lower solvent usage compared with solid phase chromatography systems operating at the same scale. The solvent usage can be reduced still further with the recycling technique described in this paper.

***Table 1.*** The range of dynamic extraction centrifuges with their specifications

| Instrument | MINI | MIDI | MAXI |
|---|---|---|---|
| Scale of application | Analytical | Preparative | Pilot |
| Processing rate | mg/hr | g/hr | kg/day |
| Main rotor radius (mm) | 50 | 110 | 300 |
| Volume of coils (mL) | 17 | 840 | 4600 |
| Coil bore (mm) | 0.8 | 4.0 | 10 |
| Max rotor speed (rpm) | 2100 | 1400 | 850 |
| Flow range (mL/min) | 0.25–2 | 5–80 | 100–1000 |
| Typical elution time (min) D = 1 peak | 20 | 20 | 20 |



Appropriate phase systems are selected by screening a graded range of solvent systems to identify one with a suitable distribution ratio for the compound of interest (generally in the range D = 0.2 to 5).[4]

For use in the centrifuge, the solvent system is traditionally made up as a two-phase system at the temperature at which the instrument is to be operated. After mixing, the two phases are allowed to equilibrate before they are separated and put into different vessels for use as the mobile or stationary phase, depending on the mode of operation – normal or reverse phase. This is a time-consuming process that is particularly cumbersome at a large scale. Furthermore, since more mobile phase is used than stationary phase (typically greater than 10:1), this method creates a large amount of redundant stationary phase. Therefore the scaling up of dynamic extraction to pilot and process scale, with the larger volumes of solvent used, make it important to develop strategies to minimise the usage of solvents, both for economical and environmental ("green") reasons.

In this paper, we describe a method to overcome these various problems. The solvent composition of both the upper and lower phases of the selected system was determined by gas-liquid chromatography. Then the amount of each phase required for DE was made up separately, without the need to make a complete system and separate it into the two phases. It was also possible to make the phases up at room temperature based on the analysis data obtained at the operating temperature. This avoided the necessity for temperature equilibration during phase preparation, although of course the individually-made phases were temperature-equilibrated to the operating temperature before use. On the DE centrifuges, no difference was detected between phases made individually as described and those made in the classical manner as a two-phase system which is then separated.

In addition to the above efficient, single phase preparation, we have been able to minimise solvent usage further with an efficient recycling of the solvents. The solvent recovered from the evaporation of eluted fractions in

the product recovery steps was analysed and then reconstituted into the required phase system for reuse in the DE purification process.

## EXPERIMENTAL

### Materials

Heptane, ethyl acetate, butanol and chlorobenzene were AR grade; water, methanol and THF were HPLC grade, all from Fisher Chemicals.

### GC Analysis

An Agilent 6890 gas chromatograph (GC) fitted with an HP Innowax column was operated under the following conditions which were developed specifically for the analysis of solvent systems containing heptane, methanol, ethyl acetate, water and butanol (Table 2).



***Table 2.*** The conditions used for GC analysis of the solvent systems

| Condition | Value |
|---|---|
| Oven initial temp: | 45°C |
| Oven initial time: | 2 minutes |
| Oven ramp rate: | 20°C/min |
| Oven final temp: | 125°C |
| Total run time: | 7 minutes |
| Equilibration time: | 3 minutes |
| Inlet mode: | Split |
| Inlet temp: | 200°C |
| Inlet pressure: | 8.50 psi |
| Split ratio: | 82.2:1 |
| Split flow: | 120 mL/min |
| Carrier gas: | Hydrogen |
| Column description: | HP Innowax polyethylene glycol, Agilent No 19091 N-133 |
| Column dimensions: | 30 m length, 250 um nominal diameter |
| Film thickness: | 0.25 um |
| Average velocity: | 41 cm/sec |
| Front detector: | Flame ionisation detection (FID) |
| FID temperature: | 250°C |
| FID gas flow: | Hydrogen 30 mL/min, air 450 mL/min |
| FID makeup gas: | Nitrogen |
| Makeup gas flow: | 30 mL/min |
| Back detector: | Thermal conductivity detection (TCD) |
| TCD temperature: | 200°C |
| TCD gas flow: | Reference flow 30mL/min, makeup flow 2 mL/min |
| Injection volume: | 1 μL |

To calibrate the GC, each of the solvents heptane, ethyl acetate, methanol and butanol was made up using a Perkin Elmer liquid handling robot at the following percent volume, using chlorobenzene as the makeup solvent: 1, 2, 5, 8, 10, 15, 25, 30, 35, 40, 50, 60, 70, 80, 100%. This was done twice to have two replicates, and each was injected twice onto the GC in order to give 4 calibration points per level, which were averaged. Although 15 levels for the calibration curve might seem somewhat excessive, it was felt necessary to ensure that the calibration curve was linear throughout the complete range from 1 to 100% solvent (or at least to establish the region of linearity). A reasonably high split ratio at the GC injection port was selected to ensure that the detectors did not top out at the higher percentage volumes.

For calibration with water, the same % volumes were made up in duplicate but using tetrahydrofuran (THF) as the makeup solvent. A blank THF sample was also analysed on the GC in order to obtain a background value for residual water in the THF solvent. Although barely detectable since a fresh bottle of dry THF was used, this value was subtracted from each water calibration result for accuracy.

All solvents were calibrated on both detectors (FID and TCD) separately, apart from water which does not detect on the FID. A linear calibration curve with a forced fit through the origin was used, with the $R^2$ correlation value above 0.9994 in every case apart from the water calibration, where $R^2$ was 0.9945.

All solvents contained other minor peaks that were not calibrated. These could be up to, or over, 1% of the total peak area in some cases and also varied from batch to batch. Heptane and butanol contained the most and the largest contaminant peaks, methanol the fewest.



### Preparation of Solvent Systems

Using a Perkin Elmer liquid-handling robot in a thermostat controlled room set at 22°C, 4 mL of each of the 28 solvent systems in the solvent selection table (Table 3, obtained from reference)[4] was made up in duplicate. 1 mL of each layer was put into separate GC vials and each injected onto the GC using the conditions described above. Thus each solvent system layer had two replicates with two injections of each, four values in total, with each value apart from water determined on both the FID and the TCD detectors. These values were all averaged in order to obtain overall values for the percentage composition of the upper and lower layers of each solvent system at that temperature.

## RESULTS AND DISCUSSION

### Upper and Lower Phase Composition of Solvent Selection Table

The solvent compositions of the upper and lower phases are shown in Tables 4 and 5. For each phase system, these values have been added to find the total

***Table 3.*** Table for selecting a DE solvent system graded from polar (no 1) to nonpolar (no 28). Quantities (in mL) required to make 4 mL of system

| No | Heptane | EtOAc | MeOH | Butanol | Water |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 2 | 2 |
| 2 | 0 | 0.4 | 0 | 1.6 | 2 |
| 3 | 0 | 0.8 | 0 | 1.2 | 2 |
| 4 | 0 | 1.2 | 0 | 0.8 | 2 |
| 5 | 0 | 1.6 | 0 | 0.4 | 2 |
| 6 | 0 | 2 | 0 | 0 | 2 |
| 7 | 0.1 | 1.9 | 0.1 | 0 | 1.9 |
| 8 | 0.2 | 1.8 | 0.2 | 0 | 1.8 |
| 9 | 0.29 | 1.71 | 0.29 | 0 | 1.71 |
| 10 | 0.33 | 1.67 | 0.33 | 0 | 1.67 |
| 11 | 0.4 | 1.6 | 0.4 | 0 | 1.6 |
| 12 | 0.5 | 1.5 | 0.5 | 0 | 1.5 |
| 13 | 0.57 | 1.43 | 0.57 | 0 | 1.43 |
| 14 | 0.67 | 1.33 | 0.67 | 0 | 1.33 |
| 15 | 0.8 | 1.2 | 0.8 | 0 | 1.2 |
| 16 | 0.91 | 1.09 | 0.91 | 0 | 1.09 |
| 17 | 1 | 1 | 1 | 0 | 1 |
| 18 | 1.09 | 0.91 | 1.09 | 0 | 0.91 |
| 19 | 1.2 | 0.8 | 1.2 | 0 | 0.8 |
| 20 | 1.33 | 0.67 | 1.33 | 0 | 0.67 |
| 21 | 1.43 | 0.57 | 1.43 | 0 | 0.57 |
| 22 | 1.5 | 0.5 | 1.5 | 0 | 0.5 |
| 23 | 1.6 | 0.4 | 1.6 | 0 | 0.4 |
| 24 | 1.67 | 0.33 | 1.67 | 0 | 0.33 |
| 25 | 1.71 | 0.29 | 1.71 | 0 | 0.29 |
| 26 | 1.8 | 0.2 | 1.8 | 0 | 0.2 |
| 27 | 1.9 | 0.1 | 1.9 | 0 | 0.1 |
| 28 | 2 | 0 | 2 | 0 | 0 |



percentage, this giving an indication of the experimental error. Two main factors were known to contribute to this value not equalling 100%. First there is the ever-present experimental error, both systematic e.g. the calibration curve being slightly out, and random e.g. slight variations in injection volume. Second is the presence of the uncalibrated contaminant peaks present in all the solvent components. This latter factor will, of course, make the total percentages for the calibrated compounds equal less than 100%.

For the upper layer values, it can be seen that the lowest total percentage was solvent system No. 20 at 96.15% while the highest by far was solvent system No1 at 103.82%. The next highest after that was solvent system No26 at 101.53%. Overall, the mean total percentage value is 99.07% (probably indicating the presence of the solvent contaminant peaks by being under 100%) with a %RSD (% relative standard deviation) of 1.99.

***Table 4.*** The average values for the % volume composition of the upper layers of solvent systems no 1 to 28

| Solvent system no | % Methanol | % Ethyl acetate | % Heptane | % Water | % Butanol | Total |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 | 16.77 | 87.04 | 103.82 |
| 2 | 0.00 | 15.74 | 0.00 | 17.49 | 68.11 | 101.34 |
| 3 | 0.00 | 32.35 | 0.00 | 16.44 | 49.47 | 98.26 |
| 4 | 0.00 | 51.18 | 0.00 | 13.71 | 33.10 | 97.98 |
| 5 | 0.00 | 73.51 | 0.00 | 9.09 | 16.30 | 98.90 |
| 6 | 0.00 | 97.40 | 0.00 | 3.16 | 0.00 | 100.55 |
| 7 | 0.74 | 90.22 | 5.00 | 2.04 | 0.00 | 98.00 |
| 8 | 1.32 | 84.95 | 10.10 | 1.66 | 0.00 | 98.03 |
| 9 | 1.78 | 80.53 | 14.83 | 1.16 | 0.00 | 98.29 |
| 10 | 2.01 | 78.53 | 16.62 | 1.20 | 0.00 | 98.35 |
| 11 | 2.37 | 74.61 | 20.44 | 1.06 | 0.00 | 98.48 |
| 12 | 2.75 | 68.38 | 26.23 | 0.86 | 0.00 | 98.23 |
| 13 | 3.04 | 63.20 | 29.66 | 0.74 | 0.00 | 96.64 |
| 14 | 3.18 | 57.15 | 36.30 | 0.52 | 0.00 | 97.15 |
| 15 | 2.84 | 48.44 | 45.29 | 0.41 | 0.00 | 96.97 |
| 16 | 2.48 | 40.02 | 53.85 | 0.31 | 0.00 | 96.65 |
| 17 | 2.33 | 32.83 | 61.51 | 0.28 | 0.00 | 96.96 |
| 18 | 1.84 | 25.94 | 69.12 | 0.17 | 0.00 | 97.07 |
| 19 | 1.40 | 19.62 | 76.84 | 0.10 | 0.00 | 97.96 |
| 20 | 1.00 | 13.59 | 81.51 | 0.06 | 0.00 | 96.15 |
| 21 | 0.97 | 10.05 | 89.55 | 0.04 | 0.00 | 100.61 |
| 22 | 0.89 | 8.02 | 91.35 | 0.03 | 0.00 | 100.29 |
| 23 | 0.87 | 5.91 | 94.11 | 0.02 | 0.00 | 100.92 |
| 24 | 0.70 | 4.54 | 96.22 | 0.02 | 0.00 | 101.47 |
| 25 | 0.87 | 4.02 | 96.66 | 0.02 | 0.00 | 101.57 |
| 26 | 0.90 | 2.47 | 98.14 | 0.01 | 0.00 | 101.53 |
| 27 | 1.47 | 1.33 | 97.83 | 0.01 | 0.00 | 100.65 |
| 28 | 2.51 | 0.00 | 98.48 | 0.00 | 0.00 | 101.00 |



For the lower layer values, the lowest total percentage was solvent system No. 1 at 95.44% with the highest being solvent system No. 21 at 99.85%. None of the lower layer systems had total percentages above 100%. The mean total percentage was 97.57% and the %RSD 1.09.

For routine use in making up the solvent systems, the average values detailed in Tables 4 and 5 were adjusted to make the totals 100%. This action was justified in that each solvent system was created by mixing just the solvents heptane, ethyl acetate, methanol, water and butanol so the totals should add up to 100% as nothing else was added. This adjustment compensates for the error introduced by the presence of contaminant peaks in each solvent, although it assumes that each solvent component contains the same proportional error. That is not necessarily the case, as it has already been noted that the

***Table 5.*** The average values for the % volume composition of the lower layers of solvent systems no 1 to 28

| Solvent system no. | % Methanol | % Ethyl acetate | % Heptane | % Water | % Butanol | Total |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 | 86.97 | 8.47 | 95.44 |
| 2 | 0.00 | 2.19 | 0.00 | 86.48 | 7.29 | 95.96 |
| 3 | 0.00 | 4.10 | 0.00 | 85.88 | 5.97 | 95.95 |
| 4 | 0.00 | 5.56 | 0.00 | 85.98 | 4.41 | 95.96 |
| 5 | 0.00 | 6.66 | 0.00 | 87.03 | 2.64 | 96.32 |
| 6 | 0.00 | 7.57 | 0.00 | 89.15 | 0.00 | 96.72 |
| 7 | 4.46 | 8.02 | 0.00 | 84.52 | 0.00 | 96.99 |
| 8 | 8.85 | 8.19 | 0.00 | 80.21 | 0.00 | 97.25 |
| 9 | 12.71 | 8.18 | 0.00 | 76.80 | 0.00 | 97.69 |
| 10 | 14.41 | 8.56 | 0.00 | 74.43 | 0.00 | 97.40 |
| 11 | 17.47 | 9.00 | 0.00 | 71.20 | 0.00 | 97.67 |
| 12 | 21.56 | 10.09 | 0.00 | 66.02 | 0.00 | 97.66 |
| 13 | 24.60 | 10.28 | 0.00 | 62.63 | 0.00 | 97.51 |
| 14 | 27.64 | 12.51 | 0.01 | 58.23 | 0.00 | 98.39 |
| 15 | 32.62 | 14.97 | 0.03 | 50.87 | 0.00 | 98.48 |
| 16 | 36.52 | 17.51 | 0.13 | 44.81 | 0.00 | 98.97 |
| 17 | 40.56 | 18.83 | 0.25 | 39.51 | 0.00 | 99.16 |
| 18 | 42.24 | 19.21 | 0.35 | 35.72 | 0.00 | 97.52 |
| 19 | 47.32 | 19.25 | 0.49 | 31.32 | 0.00 | 98.38 |
| 20 | 52.98 | 17.72 | 0.72 | 26.80 | 0.00 | 98.20 |
| 21 | 59.23 | 16.19 | 1.07 | 23.36 | 0.00 | 99.85 |
| 22 | 61.68 | 14.73 | 1.20 | 20.47 | 0.00 | 98.09 |
| 23 | 67.45 | 12.20 | 1.77 | 17.12 | 0.00 | 98.55 |
| 24 | 71.33 | 10.65 | 2.59 | 13.87 | 0.00 | 98.44 |
| 25 | 73.40 | 9.36 | 3.11 | 12.27 | 0.00 | 98.13 |
| 26 | 77.34 | 6.52 | 4.98 | 8.36 | 0.00 | 97.21 |
| 27 | 79.48 | 3.34 | 9.62 | 4.19 | 0.00 | 96.62 |
| 28 | 72.49 | 0.00 | 24.92 | 0.00 | 0.00 | 97.41 |

heptane contained more extra peaks than say, the methanol. However, such adjustment to 100% does not compensate for any systematic error that may be present in the experiment for it keeps the relative proportions of the components at the same values. Overall though, it was deemed beneficial to adjust the values to 100% in order to make the table more practically useful.

The results are represented graphically in Figures 1 and 2 below. For solvent systems No. 6-28, which consist purely of heptane-ethyl acetate-methanol-water, it can be seen that it is the heptane-ethyl acetate ratio that drives the upper layer polarity and predominantly the methanol-water ratio that drives the lower layer polarity.

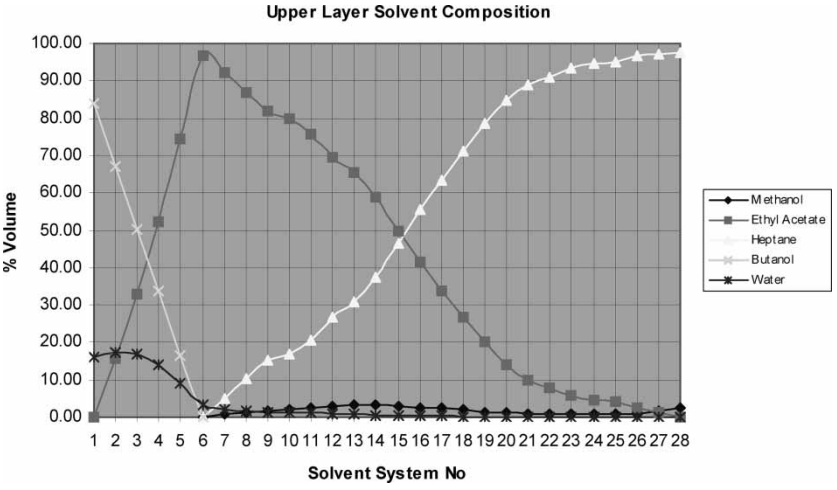


*Figure 1.* Graphical representation of the % volume results of the composition of the upper layers of solvent systems 1 to 28.

## Preparation of Phases for Large Scale Dynamic Extractions

Using these analysis for phase systems for a range of separations, separate mobile and stationary phases have been prepared and used to obtain similar separations to those obtained with systems made by the traditional phase separation method. An example of this is provided by our recent large scale

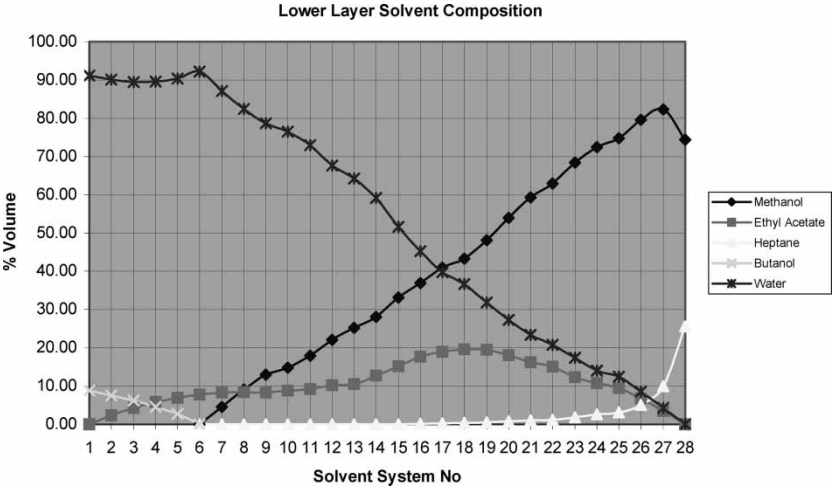


*Figure 2.* Graphical representation of the % volume results of the composition of the lower layers of solvent systems 1 to 28.

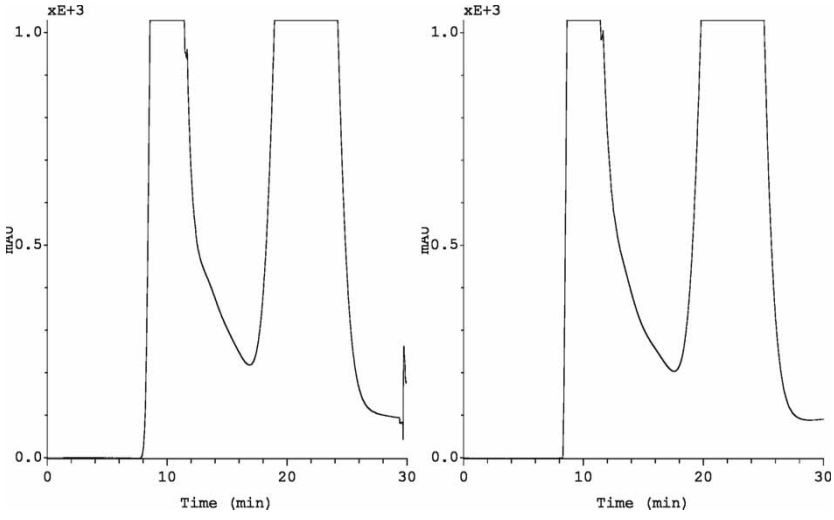


***Figure 3.*** A crude glucosinolate mixture separated on DE using solvent phases made in the classical manner (left) compared to solvent phases made individually according to their composition (right). DE MINI centrifuge, 17.4 mL PTFE coil, 0.8 mm bore, 2100 rpm, 30°C, 1.5 mL/min flow rate, upper phase mobile, 50μL injection of crude syrup in stationary phase at c . 200 mg/mL concentration.



preparation of glucoraphanin from broccoli seed extracts.[5] A phase system of 1-propanol/acetonitrile/31% (w/v) aqueous ammonium sulphate (1:0.5:2.2) was used in normal phase operation. The solvent composition of each phase was determined by GC and the ammonium sulphate by gravimetric analysis using, in this case, the contract analysis laboratories ITS Testing Services (UK), Sunbury, West London.

Similar chromatograms were obtained with solvent phases prepared by the classical phase separation method and individual creation of the phases based on the analysis (Fig. 3).

## Solvent Recycling

In a particular application, solvent system No23 (heptane-ethyl acetate-methanol-water, 4:1:4:1) was used with a number of repeat runs on the DE MIDI centrifuge, with the upper organic phase as the mobile one. The first batch of fractions were collected and evaporated on a large-scale Buchi-type rotary evaporator to produce the dried target compound and 10.2 litres of solvent condensate, normally regarded as waste. This was mixed to ensure a uniform consistency and 1 μL was injected (in duplicate) onto the gas chromatograph using the method described above. The averaged results (adjusted to make 100%) were found to be; heptane 95.92, ethyl acetate 3.92, methanol 0.12, water 0.04 (all % vol).

Referring to the table of the upper layers of the solvent systems (Table 4), the composition of solvent system No. 23 is quoted as: heptane 94.11, ethyl acetate 5.91, methanol 0.87, water 0.02 (all % vol). Adjusted to 100%, the figures become heptane 93.26, ethyl acetate 5.86, methanol 0.86, water 0.02 (all % vol).

It was then a relatively simple mathematical process to determine the volumes of pure solvent to be added to the condensate in order to turn it back into the composition of the upper phase for re-use in the next preparative runs. It was assumed, for the purposes of the calculation, that the water concentration could be ignored (0.04%). In this example, the calculation was based around the heptane content so that only ethyl acetate and methanol were added. Any of the components could be used as the base value, however it makes sense to select one in which the percentage volume in the condensate is greater than the desired percentage volume of the mobile phase. In this case, there was 95.92% heptane in the condensate and the desired percentage was 93.26%.

To perform the calculation, the % volume of each component in the recovered condensate was multiplied by the total volume (10,200 mL) to obtain the volume of each component in the total (i.e. 4 mL water, 12 mL methanol, 400 mL ethyl acetate and 9784 mL heptane). Since the calculation was based on the heptane content, and the desired heptane % volume was 93.26%, the volume of heptane present in the condensate (9784 mL) was taken to equal 93.26% of the total. This allows a total volume of the new phase to be calculated (i.e. 10,491 mL). Since the desired % volume of ethyl acetate was 5.86%, and the total volume was going to be 10,491 mL, then 614 mL of ethyl acetate must be present in the total. The recovered condensate contained 400 mL, and thus a further 214 mL of ethyl acetate was added in order to make the new upper phase layer. The same calculation was then done for the methanol. That is, since the desired % volume of methanol was 0.86%, and the total volume was going to be 10,491 mL, then 90 mL of methanol must be present in the total. The recovered condensate contained 12 mL, and thus a further 78 mL of methanol was added in order to make the new upper phase layer.

So in order to transform the 10,200 mL of recovered condensate from the rotary evaporator into fresh upper phase for solvent system No. 23, 214 mL of ethyl acetate and 78 mL of methanol were added. With the temperature kept constant at the operating temperature, this then formed a single layer ready to be used as the mobile phase for subsequent DE runs.

To perform the required calculations automatically, a simple Excel spreadsheet was written. The total volume and % volume composition of the recovered condensate are entered and the program instantly calculated the volume of methanol and ethyl acetate to be added in order to take the composition back to the original mobile phase ready for re-use.

No difference was detected between the runs initially performed with fresh solvent and subsequent runs preformed with the recycled solvent reconstituted into the mobile phase (Figure 4).

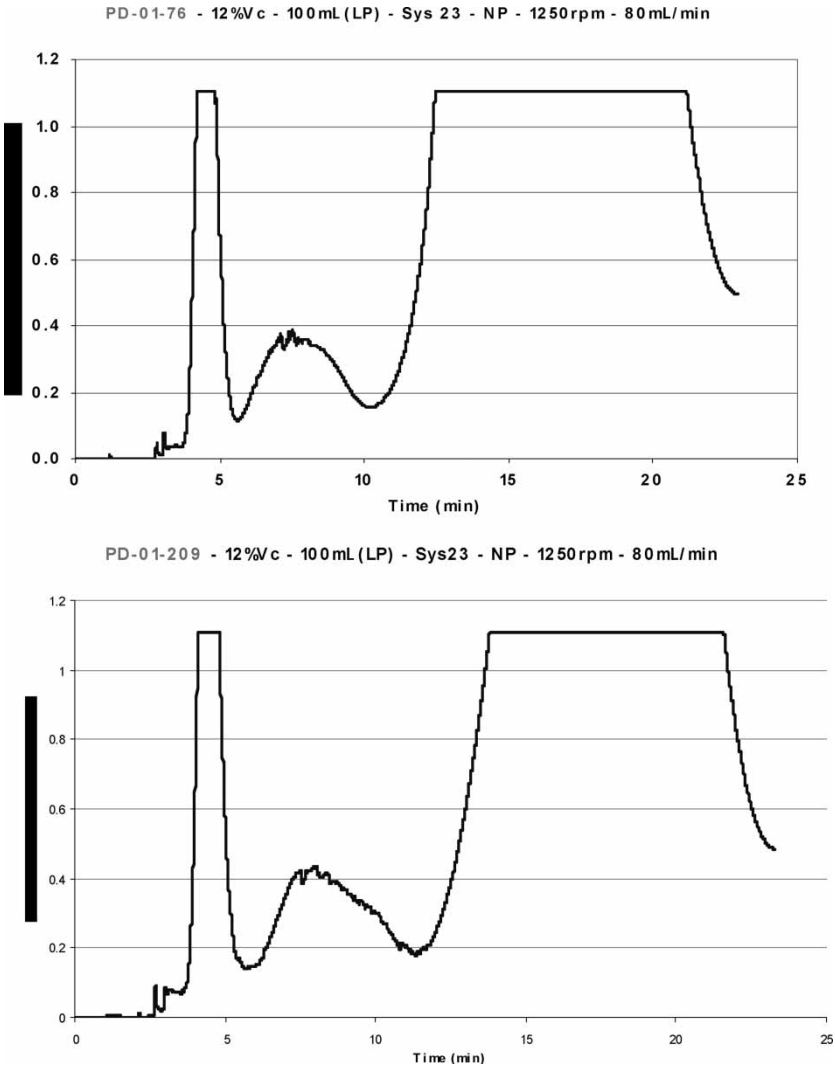


***Figure 4.*** A synthetic mixture separated on DE using fresh solvent (top) compared to recycled solvent that was reconstituted into the mobile phase (bottom). DE MIDI centrifuge, 840 mL s/s coil, 4 mm bore, 1250 rpm, 30°C, 80 mL/min flow rate, upper phase mobile, 100 mL injection (12% of coil volume).

In this particular campaign, a total of 1.7 kg of crude material was purified. Without recycling, more than 750 litres of purchased solvent were required. In actual fact, with the recycling described in this paper, no more than 120 litres of solvent were used. Different separations performed on different scales would be able to recycle different percentages of solvent. Not only does this technique

save on the costs of running the purification, it also minimises the environmental impact. It should be noted that this recycling process could be applied to any technology that runs isocratic solvent conditions.

## CONCLUSION

This analysis of the precise composition of the upper and lower layers of all the solvent systems in the selection table has provided:

1. An insight into the mechanisms that cause the changes in polarity as the table is traversed from No. 1 to No. 28.
2. The ability to make any solvent system one layer at a time and thus minimise solvent wastage.
3. A method that allows the analysis of condensate from evaporated fractions and thus the recycling of solvent systems for minimal wastage during preparative runs.



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Manuscript 0607