References

	TITLE	AUTHOR	YEAR	ABSTRACT	JOURNAL
1	Countercurrent chromatographic isolation of lolitrem B from endophyte-infected ryegrass (Lolium perenne L.)	Grancher, D	2004	This paper describes a new method of purification of the Lolitrem B, a tremorgenic mycotoxin produced in planta by the endophytic fungus Neotyphodium lolii. The method is based on the large-scale isolation of the toxin by countercurrent chromatography (CCC). The lolitrem B content in endophyted ryegrass seed, 11 microg/g or 11 ppm, is extracted by stirring finely ground seeds with ethanol for 3 h at room temperature. The concentrated crude extract contains about 0.6 mg/g or 600 ppm of lolitrem B. It is then submitted to CCC purification with a biphasic four-solvent liquid system. A 160-fold enrichment was obtained in one step producing a raffinate containing 10% or 100 mg/g of the toxin. Further purifications were then performed by thin layer and low pressure liquid chromatography. Twenty-eight micrograms of lolitrem B with a 96% purity grade were obtained from 8 kg of seeds (yield 32%).	Journal of Chromatography A
2	Technology transfer and scale up of a potential cancer preventive plant dynamic extraction of glucoraphanin. D.	Fisher, D	2005	Glucosinolates are anionic, hydrophilic β-thioglucoside N-hydroxysulfates, which are abundant plant secondary metabolites found in cruciferous plants and are of particular interest for their chemoprotective, antioxidant and antibiotic activities. The purification of gluoraphanin (GR), the predominant glucosinolate in broccoli, was achieved using high-speed countercurrent chromatography (HSCCC) with a high salt, highly polar phase system of 1-propanol-acetonitrile-saturated ammonium sulphate-water (1:0.5:1.2:1) on a preparative scale (850 mL capacity) Pharma-Tech HSCCC instrument at ca 700 mg glucosinolates per run in about 3 hours. To scale up the production of glucosinolates the technology was transferred from Johns Hopkins to Brunel Institute for Bioengineering (BIB). Within three days a 50×scale up was achieved with comparable target compound recovery and purity using a MIDI-dynamic extraction centrifuge (928 mL capacity) with run times of 30 min and processing loads of 30 mL of ca 50% w/w viscous solutions (15 g injected). The 34 runs processed 589 g of extract producing a total of 52.6 g of 98% pure GR.	Journal of Liquid Chromatography & Related Technologies
3	Hydrodynamics of PEG-Phosphate Aqueous Two-Phase Systems in a J-Type Multilayer Countercurrent	Al-Marzouqi, I	2005	Recently, we have shown that countercurrent chromatography (CCC) is an effective method for the purification of plasmid DNA vaccines and gene therapy vectors.1Kendall, D., Booth, A. J.,Levy, M. S. and Lye, G. J.2001. Separation of supercoiled and open-circular plasmid DNA by liquid-liquid countercurrent chromatography. Biotech. Lett., 23: 613–619. As a basis for further work, this paper studies the hydrodynamics of various PEG-Salt aqueous-aqueous two-phase systems in a Brunel J-type countercurrent chromatograph. The degree of stationary phase retention, S f , once a hydrodynamic equilibrium is achieved has been studied as a function of mobile phase flow rate (0.5–2.0 mL·min−1), coil rotational speed (500–850 rpm), column volume (92.3 and 167.3 mL), choice of mobile phase (PEG or phosphate), and mobile phase pumping direction (Head-Tail or Tail-Head). Three different aqueous two-phase systems (ATPS) were studied, which consisted of PEG 300-K_2HPO_4, PEG 600- K_2HPO_4, and PEG 1000-K_2HPO_4, having density and viscosity ratios between 1.15–1.13 and 0.27–0.12, respectively. HighS f values in comparison to previous aqueous-aqueous studies with CCC were obtained of up to 73.7%. These high S f values were obtained when the lower aqueous phase was pumped from Tail(periphery)⊠Head(centre), opposite to the direction normally recommended for most organic-aqueous systems in J-type CCC machines.2Sutherland, I. A.,Muytjens, M., Prins, M.and Wood, P. 2000. A new hypothesis on phase distribution in countercurrent chromatography. J. Liq. Chromatogr. & Rel. Technol., 23(15):2259–2276.[Taylor & Francis Online],[Web of Science ®] This is believed to be due to the high settling times and low density/high viscosity difference of aqueous-aqueous systems compared to organic-aqueous systems. Du3Du, Q., Wu, C., Qian, G.,Wu, P. and Ito, Y. 1999.Relationship between flow rate of the mobile phase and retention of the stationary phase in countercurrent chromatography. J Chromatogr A, 835:231–235.[CrossRef], [Web of Science ®] plots of the data showed the essential linear relationship between S f and √F, provided that S f >20%. Data obtained from Du plots for each phase system could also be used to satisfactorily predict S f as a function of column rotational speed. This work gives an insight into the behaviour of aqueous phase systems in J-type CCC machines and is useful as a basis for process design and scale-up.	Journal of Liquid Chromatography & Related Technologies
4	How to achieve rapid separations in counter-current chromatography	Chen ,L	2006	A new generation of high performance coil planet centrifuges is now making it possible to realize the enormous potential of liquid–liquid partition chromatography for the rapid and predictable scale up of separation processes. This paper uses a separation of flavonoids to demonstrate how rapid fractionations can be obtained at high flow rates with limited loss of resolution and with significant increase in throughput. Furthermore, it is shown that chromatograms at various flows can be modeled so that optimum conditions can be rapidly assessed.	Journal of Chromatography A
5	Developments in the application of counter-current chromatography to plant analysis	Marston, A	2006	Counter-current chromatography is a very versatile separation technique which does not require a solid stationary phase. It relies simply on the partition of a sample between the two phases of an immiscible solvent system. Some of the more recent applications of the method to the separation of plant-derived natural products are described here. Crude plant extracts and semi-pure fractions can be chromatographed, with sample loads ranging from milligrams to grams. Aqueous and non-aqueous solvent systems are used and the separation of compounds with a wide range of polarities is possible. The technique is complementary to other chromatographic methods and is compatible with gradient systems. The possibilities for solvent selection are almost limitless but some guidelines for the choice of successful systems are presented.	Journal of Chromatography A
6	Feasibility of scaling from pilot to process scale	Ignatova, S	2007	The pharmaceutical industry is looking for new technology that is easy to scale up from analytical to process scale and is cheap and reliable to operate. Large scale counter-current chromatography is an emerging technology that could provide this advance, but little was known about the key variables affecting scale-up. This paper investigates two such variables: the rotor radius and the tubing bore. The effect of rotor radius was studied using identical: length, β-value, helix angle and tubing bore coils for rotors of different radii (50 mm, 110 mm and 300 mm). The effect of bore was researched using identical: length, helix angle and mean β-value coils on the Maxi-DE centrifuge (R = 300 mm). The rotor radius results show that there is very little difference in retention and resolution as rotor radius increases at constant bore. The tubing bore results show that good retention is maintained as bore increases and resolution only decrease slightly, but at the highest bore (17.5 mm) resolution can be maintained at very high flow rates making it possible for process scale centrifuges to be designed with throughputs exceeding 25 kg/day.	Journal of Chromatography A
7	Counter-current chromatography separation scaled up from an analytical column to a production column	Wood, P	2007	An analytical separation was performed on an analytical J-type counter-current chromatography (CCC) instrument using a 5.4 ml column, with a 1 ml/min mobile phase flow rate. This separation had a resolution of 0.69 and was achieved in 10 min. The same separation was performed using two 2300 ml columns connected in series at a flow rate of 850 ml/min using a production scale J-type centrifuge. This production scale separation was also obtained in 10 min with a resolution of 0.71. This represents an 850 times increase in productivity. This paper presents these separations and the underlying scale up theory.	Journal of Chromatography A
8	Rapid purification and scale-up of honokiol and magnolol using high-capacity high-speed counter-current chromatography	Chen ,L	2007	In this paper, a rapid separation approach has been developed using high-capacity high-speed counter-current chromatography (high-capacity HSCCC) to isolate and purify honokiol and magnolol, which are the main bioactive constituents from Houpu. The optimization of the solvent selection process, sample loading volume and flow rate is systematically studied using analytical high-capacity HSCCC. The optimized parameters obtained rapidly at analytical scale were used for a 1000× scale-up preparative run using pilot scale high-capacity HSCCC in a MAXI-DE centrifuge. A crude sample of 43 g was successfully separated and the fractions were analysed by high-performance liquid chromatography (HPLC). This large scale preparative single step run yielded 16.9 and 19.4 g of honokiol and magnolol with purities of 98.6 and 99.9%, in only 20 min. This is the first time that high-performance counter-current chromatography has been used to purify multiple gram grade bioactive compounds in less than 1 h and at such high concentrations of final products (10.8 g/l for magnolol and 7.0 g/l for honokiol).	Journal of Chromatography A
9	Recent progress on the industrial scale-up of counter-current chromatography	Sutherland, I	2007	The pharmaceutical industries are looking for rapid methods of purification and predictable scale-up for their drug development process that will cut their costs and enable them to reduce the time to market. In this paper, recent progress is reviewed in the development and demonstration of two types of industrial scale centrifugal liquid–liquid chromatography: hydrostatic and hydrodynamic. Industrial scale hydrostatic processes by Partus Technologies and Armen Instrument are just emerging. Results demonstrating scalability are presented for hydrodynamic processes by Dynamic Extractions. The review concludes that the time is now right, with this appropriate commercial support, for high performance counter-current chromatography to emerge as a major enabling technology for industry.	Journal of Chromatography A
10	Preparative purification of anti-tumor derivatives of honokiol by high-speed counter-current chromatography	Luo, Y	2008	In our program to synthesize a series of novel derivatives as potential analogs of honokiol for anti-tumor treatment, we have found that at least three of the derivatives of honokiol showed more potency to inhibit the proliferation of K562 leukemia cells and SPC-A1 adenocarcinoma cells. As a critical step to our further series synthesis of derivatives of honokiol, three derivatives of honokiol composed of two isomers and one compound with two formyl groups, which were hardly separated by common purification methods, needed to be rapidly separated and purified. The present work describes analytical and preparative high-speed counter-current chromatography (HSCCC) for the isolation and purification of these three C-formylation derivatives of honokiol, named 3'-formylhonokiol, 5-formylhonokiol and 3',5-diformylhonokiol, respectively. The solvent system for HSCCC separation was composed of hexane-ethyl acetate-methanol-water with the ratio of 1:0.4:1:0.4 (v/v). The one-step purification produced 157.8 mg, 121.6 mg and 21.2 mg of 3'-formylhonokiol, 5-formylhonokiol, 3',5-diformylhonokiol from crude sample of 400mg with purities of 98.6%, 99.2% and 99.6%, respectively, in an elution time of 2.5 h. The purities and structural identification were determined by HPLC, (1)H NMR, (13)C NMR and mass spectroscopy. Their anti-proliferation effects on K562, A549 and SPC-A1 cell lines were evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.	Journal of Chromatography A
11	How to realise the linear scale-up process for rapid purification using HPCCC	Yuan, Y	2008	This study used the isolation of six constituents from Selaginella tamariscina as an example to demonstrate how to achieve rapid and predictable linear scale-up processes in both normal- and reversed-phase high-performance counter-current chromatography. After systematic optimization of solvent systems, sample concentration, sample loading volume, rotation speed and flow rate on the analytical Mini-DE centrifuge, the optimized parameters obtained were directly transferred to the preparative Midi-DE centrifuge, with nearly the same purities, resolutions and elution times but with 50 times the throughput. Amentoflavone (446.7 mg, 97.8%), robustaflavone (21.6 mg, 89.4%), bilobetin (80.7 mg, 92.7%), hinokiflavone (15.1 mg, 85.5%), isocryptomerin (34.8 mg, 89.6%) and an apigenin-diglucoside (46.3mg, 96.4%) were obtained with amounts and purities shown in parentheses as analysed by HPLC. The process, therefore, offers an efficient and rapid method of obtaining sufficient quantities of target compounds with significantly increased throughput after a linear scale-up.	Journal of Chromatography A
12	Linear scale-up of the separation of active components from Oroxylum indicum using high-speed counter-current chromatography	Yuan, Y	2008	High-speed counter-current chromatography was used to separate and purify flavonoids from the ethyl acetate extract of Oroxylum indicum. After the optimization of separation conditions on analytical instrument, including the two-phase solvent system, rotation speed, flow rate, sample volume and sample concentration, a linear scale-up procedure was performed at preparative grade. Chrysin (160.9 mg, 97.3% in purity), baicalein (130.4 mg, 97.6% in purity), baicalein-7-O-glucoside (314.0 mg, 98.3% in purity), baicalein-7-O-diglucoside (179.1 mg, 99.2% in purity), and a new chrysin-diglucoside (21.7 mg, 98.8% in purity) were obtained from 911.6 mg ethyl acetate extract of Oroxylum indicum by only one step. These five compounds were identified using high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance. With the improvement of the throughput for 53 times after such a scale-up, the resolution and the separation time were kept as the same as those of the analytical grade separation. Therefore, the linear scale-up provided an efficient method for the separation of natural products.	Journal of Chromatography A
13	Preparative separation of a terpenoid and alkaloids from Tripterygium wilfordii Hook. F. using hpccc. Comparison of various elution and operating strategies	Ye, H	2008	This paper describes how high-performance counter-current chromatography (HPCCC) was used strategically for the separation of Tripterygium wilfordii Hook. f. Due to the complexity of Chinese herbal medicines, the initial ethanol crude extract was fractionated into seven fractions using medium-pressure liquid chromatography (MPLC). One terpenoid (triptolide) and three alkaloids (peritassine A, wilforgine and wilforine) were further separated from one of the MPLC fractions. This fraction (1.25 g) yielded 8 mg of triptolide and 28 mg of peritassines A after one HPCCC column pass and 30 mg of wilforgine and 120 mg of wilforine after a second column pass with respective purities of 97%, 93.6%, 95.0% and 94.4%, which were determined by high-performance liquid chromatography (HPLC). This was a one-step HPCCC separation, using an n-hexane-ethyl acetate-methanol-water (4:5:4:5, v/v) solvent system, where increases in theoretical plates have been sacrificed in favour of increasing throughput. Structures were identified by electrospray ionization mass spectrometry (ESI-MS), (1)H nuclear magnetic resonance [(1)H NMR] and (13)C nuclear magnetic resonance [(13)C NMR]. Comparison of three different modes of eluting compounds retained in the liquid stationary phase: elution extrusion; dual mode and simple pump-out showed that simply pumping out the column contents at high flow gave better resolution and was eight times faster than the other two well-utilised methods. Triptolide and peritassines A were isolated for the first time from Tripterygium wilfordii Hook. f.	Journal of Chromatography A
14	Flow rate gradient high-speed counter-current chromatography separation of five diterpenoids from Triperygium wilfordii and scale-up	Peng, A	2008	In this paper, high-speed counter-current chromatography (HSCCC) instruments with different gravitational forces were applied for the separation of bioactive compounds from Triperygium wilfordii Hook.f. The critical parameters including sample concentration, sample volume and flow rate were first optimized on an analytical Mini-DE HSCCC system, and then scaled up to a preparative TBE 300A HSCCC system.	Journal of Chromatography A
15	Practical solvent system selection for counter-current separation of pharmaceutical compounds	Dubant, S	2008	Counter-current chromatography (CCC) is a technique that shows a lot of potential for large scale purification. Its usefulness in a "research and development" pharmaceutical environment has been investigated, and the conclusions are shown in this article. The use of CCC requires the development of an appropriate solvent system (a parameter of critical importance), a process which can be tedious. This article presents a novel strategy, combining a statistical approach and fast HPLC to generate a three-dimensional partition coefficient map and rapidly predict an optimal solvent system. This screen is performed in half a day and involves 9 experiments per solvent mixture. Test separations were performed using that screen to ensure the validity of the method.	Journal of Chromatography A
16	Preparative isolation and purification of three rotenoids and one isoflavone from the seeds of Millettia pachycarpa Benth by high-speed counter-current chromatography	Ye, H	2008	Both analytical and preparative high-speed counter-current chromatography (HSCCC) were used to isolate and separate chemical bioactive constituents from the seeds ofMillettia pachycarpa Benth, a famous traditional Chinese medicine. Three rotenoids and one isoflavone were successfully purified for the first time by HSCCC with a two-phase solvent system composed of n-hexane-ethyl acetate–methanol–water (HEMWat) (1:0.8:1:0.6, v/v/v/v). The separation parameters were first performed on the analytical HSCCC and optimized conditions were then scaled up to preparative HSCCC. The separation produced 160.2 mg tephrosin, 14.6 mg 4⊠,5⊠-dimethoxy-6,6-dimethylpyranoisoflavone, 109.4 mg deguelin, 6.7 mg 6a,12a-dehydrodeguelin with respective purities of 95, 93, 95, 95%, in one single run from 400 mg crude extract of the seeds of M. pachycarpa Benth. The purity of the isolated compounds was analyzed by high-performance liquid chromatography (HPLC) and their structures were identified by electrospray ionization mass spectrometry (ESI-MS); 1H nuclear magnetic resonance (1H NMR) and 13C nuclear magnetic resonance (13C NMR) analysis. This paper is an excellent example of the role that CCC is playing in isolating active compounds for pre-clinical trials of new chemical entities, even when scaling up between centrifuges from different manufacturers.	Journal of Chromatography A
17	Separation of Flavonoids from the leaves of Oroxylum indicum by HSCCC	Yuan, Y	2008	A high-speed counter-current chromatography system (HPCCC) capable of rapid processing has been employed to separate seven flavonoids from a methanolic extract of the leaves ofOroxylum indicum by a one-step isocratic elution using a chloroform–methanol–water (9.5:10:5) two-phase system. LC, MS and NMR have identified the components from the extract as chrysin, baicalein, baicalein-7-O-glucoside, baicalein-7-O-diglucoside, chrysin-7-O-glucuronide, baicalein-7-O-glucuronide, and a chrysin-diglucoside. Baicalein-7-O-glucuronide and chrysin-7-O-glucuronide have been separated from this plant by HSCCC for the first time. The present study also reports a new chrysin-diglucoside from the leaf extract. The results demonstrate that HSCCC is a powerful separation tool and can contribute to identifying and quantifying plant ingredients.	Chromatographia
18	Multiple dual-mode counter-current chromatography applied to chiral separations using a (S)-naproxen derivative as a chiral selector	Rubio, N	2009	Countercurrent chromatography (CCC) is a liquid–liquid chromatographic technique without a solid support. Several alternative elution modes can be applied to take advantage of the special nature of the liquid stationary phase. Among these dual-mode (DM) and multiple dual-mode (MDM) consist of switching alternatively between Reversed and Normal Phase operation during the experiment (once for DM and several times for MDM). In this paper, MDM has been applied to the chiral CCC separations of two racemic mixtures, (±)-N-(3,4-cis-3-decyl-1,2,3,4-tetrahydrophenanthren-4-yl)-3,5-dinitrobenzamide and N-(3,5-dinitrobenzoyl)-(±)-leucine, using (S)-naproxen N,N-diethylamide as chiral selector (CS). Although the behaviour of the two analytes differed, improved resolution factors were successfully obtained. Results are rationalized on the basis of the distinct partition behaviour of the CS/enantiomer complexes in the biphasic system.	Journal of Chromatography A
19	Stationary Phase Retention and Peak Elution in CCC	Wood, P	2009	Countercurrent chromatography (CCC) devices, both J-type high-speed countercurrent chromatography (HSCCC) centrifuges and centrifugal partition chromatography (CPC) machines, are now used to manufacture pharmaceutical products where the sample loading is increased to the point where peaks are not fully resolved. The higher the sample loading, the more asymmetric the peaks of a separation become, causing a loss of resolution and the merging of peaks. In case of the peaks that are not fully resolved peak shaving (precise fraction collection) is used to obtain the desired purity of a target solute. Peak shaving works efficiently if the asymmetric nature of peak elution is acknowledged.	Encyclopaedia of Chromatography
20	Performance comparison using the GUESS mixture to evaluate counter-current chromatography instruments	Guzlek, H	2009	Comparing the performance of different counter-current chromatography (CCC) J-type centrifuges has and will always be difficult. This is due to the number of variables such as speed of rotation, swung radius, β-value, column bore, column length that can be chosen during the design of an instrument. This situation is further complicated by the implication that some of these variables are intrinsically linked, such that if one is changed another or others can also change. The chromatographer has no influence on these hardware parameters once the instrument designer has chosen them. However, the chromatographer wants a CCC column that retains the most liquid stationary phase in order to achieve the best separation of the components in a mixture. What matters most is column performance in terms of: sample loading per injection, speed of separation, purity of target and yield of target. The instrument that has the best performance in all these areas is called a "high-performance" CCC system. This paper demonstrates to the modern chromatographer that a "high-performance" CCC instrument has shorter, lower volume columns that are rotated faster to provide quicker separations, even with the same sample loading.	Journal of Chromatography A
21	Role of counter-current chromatography in the modernization of Chinese herbal medicines	Sutherland, I	2009	This review focuses on the growing popularity of using counter-current chromatography (CCC), with its liquid stationary phase, as one of the prime methods for isolating compounds from Chinese herbal medicines (CHMs). 198 publications are reviewed covering 108 different plant species from 56 plant families. These describe the isolation of 354 different molecules across a wide range of polarities, chemical classes and molecular weights (in the range 100–1000 Da). The suitability of CCC for the separation of active compounds from CHM, the phase systems used, how CCC has developed in China, compounds isolated, CCC instrumentation, performance, operational issues and innovations, all supported by detailed cross-referencing, are described. It is concluded that CCC is making an increasingly important contribution to the modernisation of Chinese herbal medicines.	Journal of Chromatography A
22	Multiple dual-mode counter-current chromatography applied to chiral separations using a (S)-naproxen derivative as a chiral selector	Lu, Y	2009	In countercurrent chromatography (CCC) the choice of the liquid system is the heart of any separation. It corresponds to the selection of the mobile phase and the stationary phase at the same time. Any change in one phase composition induces a change in the other phase composition which renders the choice of the appropriate liquid system difficult and lengthy. A scale of compositions of the heptane-ethyl acetate-methanol-water quaternary liquid system was referred to by letters from A to Z and called the Arizona (AZ) liquid system. Each composition of the AZ system has the same heptane/ethyl acetate and methanol/water volume ratios. It is shown that there is a continuous polarity change from the hydrophilic A composition (ethyl acetate-water) to the hydrophobic Z (heptane-methanol) mixture by measuring the distribution constant K(D) of a known test mixture. For all compounds, the log K(D) is linearly increasing with the water content of the lower aqueous phase of the composition used. The slopes of the log K(D) versus percent H(2)O have very different values which means that the chromatographic selectivity changes with liquid system compositions. The AZ system was associated to the elution-extrusion method to design a procedure to identify rapidly the appropriate solvent composition able to fractionate correctly a complex natural extract. With the use of an integrated three-coil CCC column (40 mL each coil) able to test three AZ compositions in parallel, it is shown that the optimum AZ composition is found in half a day using less than a liter total volume of solvents. Two natural extracts are rapidly screened using the proposed protocol. An extract of Piper longum L. of intermediate polarity was fractionated in five usable portions using the 3/2/3/2 (Q) composition of the AZ system. A polar extract of Polygonum cuspidatum was also separated in five fractions using the 1/6/1/6 (D) composition. In both cases, a 140 mL CCC column was used for a direct scale-up transfer with the same liquid system. Purified fractions were subjected to an antioxidant activity assay and liquid chromatography with UV and mass spectrometry detection (LC-UV/MS) analysis to determine the molecular weight, number, and quantity of compounds in the active fractions. Four fractions of P. cuspidatum showed excellent antioxidant activity. They were rapidly produced at the milligram level by the 140 mL CCC column and fractionated by semipreparative high-performance liquid chromatography (HPLC) in individual compounds that were each identified by NMR and MS and reevaluated for confirmation of bioactivity. The rapid screening CCC protocol associated to the	Journal of Analytical Chemistry
23	New 18-l process-scale counter-current chromatography centrifuge	Sutherland, I	2009	A new Dynamic Extractions Maxi-counter-current chromatography (CCC) centrifuge with a column volume of 18-l has been installed in the Advanced Bioprocessing Centre at Brunel. This instrument has four times the capacity of the 4.6-l Maxi-CCC centrifuge which has been operating robustly for 3 years. Tests using the model sample system benzyl alcohol and p-cresol with a heptane:ethyl acetate:methanol:water (HEMWat) phase system (1.4:0.1:0.5:1.0) show that resolution is almost double with this new high capacity device. Commissioning tests with a mixture of caffeine, KD = 0.21; ferulic acid, KD = 0.82; umbelliferone, KD = 1.2 and vanillin, KD = 1.49 using a HEMWat phase system of 1:1.5:1:1.5 on the 9-l column show that resolutions equivalent to analytical instruments will be possible using the full 18-l capacity. They also show that predictable scale-up from simple test tube tests is feasible with knowledge of the stationary phase retention for the planned process scale run.	Journal of Chromatography A
24	Isolation of the new minor constituents dihydropyranochrom one and furanocoumarin from the fruits of Peucedanum alsaticum L. by high-speed counter-current chromatography	Skalicka-Wozniak, K	2009	A preparative high-speed counter-current chromatography (HSCCC) method was successfully used for isolation of two new minor compounds – alsaticol and alsaticocoumarin A. A two-phase solvent system composed of n-hexane-ethyl acetate–methanol–water (1:1:1:1) was developed. Compounds were obtained from the dichloromethane extract of Peucedanum alsaticum fruits and their identification was performed with NMR and MS methods. Optimized HSCCC offers a rapid method of obtaining new natural compounds.	Journal of Chromatography A
25	Rapid and high-throughput purification of salvianolic acid B from Salvia miltiorriza Bunge by HPCCC	Zhang, M	2009	A large-scale purification of salvianolic acid B from Salvia miltiorrhiza Bunge is presented. The method development began with selection of the solvent system, then optimization of the operating parameters and ended up with linear scale-up from an analytical to a preparative instrument. Three factors were used for method optimization and scale-up estimation: purity, process throughput and process efficiency. Preparation was achieved using a two-phase solvent system comprising hexane-ethyl acetate-methanol-acetic acid-water (1.5:1.5:1.5:0.0.0059.5, v/v). The preparation yielded 475 mg of salvianolic acid B with a purity of 96.1% from 1.5 g of crude extract. The process throughput of crude was 2.23 g/h while process efficiency per gram of target compound was 0.769 g/h. Two factors-process environmental risk factor and process evaluation factor were used for evaluation of the separation process.	Journal of Chromatography A
26	Preparative separation of capsaicin and dihydrocapsaicin from Capsicum frutescens by HSCCC	Peng, A	2009	Capsaicin and dihydrocapsaicin are two main bioactive components of Capsicum frutescens and are widely used as food additives and drugs in China and India. Due to their similarity in structures, isolation of capsaicin and dihydrocapsaicin with traditional methods such as silica gel column chromatography, normal-phase thin-layer chromatography (TLC) becomes difficult. This study involves separating capsaicin and dihydrocapsaicin with sufficient purity and recovery using high-speed counter-current chromatography (HSCCC) with a solvent system composed of n-hexane-ethyl acetate-methanol-water-acetic acid (20:20:20:20:2, v/v/v/v/v). Separation parameters such as sample volume, and sample concentration were first optimized on analytical HSCCC, and then scaled up to preparative HSCCC. 0.65 g capsaicin and 0.28 g dihydrocapsaicin were obtained from 1.2 g crude extract and their purities were 98.5 and 97.8%, respectively. The recoveries of the two compounds were 86.3 and 85.4%, respectively. The purity of the isolated compounds was analyzed by high-performance liquid chromatography (HPLC) and their structures were identified by (1)H nuclear magnetic resonance (NMR) and (13)C NMR analysis.	Journal of Separation Science
27	Intermittent counter-current extraction as an alternative approach to purification of Chinese herbal medicine	Hewitson, P	2009	This paper describes intermittent counter-current extraction, a novel method of using a conventional twin column counter-current chromatograph to either split a sample into two groups of compounds or extract and enrich a target compound from a crude extract. The first method is demonstrated by splitting a model mixture of four compounds into two groups. The second method is demonstrated by the extraction and enrichment of a high value target compound, triptolide, from a Chinese herbal medicine crude extract of Tripterygium wilfordii Hook. f., where it is found at low concentration (2%). This was achieved by retaining and enriching the target compound within the column while washing away all other components of the crude material. The success of the first method allowed the second method to be carried out without the need for costly preliminary experiments with the high value sample. 188 mg of triptolide at greater than 98% purity was separated from 9.2 g of crude extract, using 10 l of solvent in a 3-h separation.	Journal of Chromatography A
28	Scale-up of counter-current chromatography: Demonstration of predictable isocratic and quasi-continuous operating modes from the test tube to pilot/process scale	Sutherland, I	2009	Predictable scale-up from test tube derived distribution ratios and analytical-scale sample loading optimisation is demonstrated using a model sample system of benzyl alcohol and p-cresol in a heptane:ethyl acetate:methanol:water phase system with the new 18 L Maxi counter-current chromatography centrifuge. The versatility of having a liquid stationary phase with its high loading capacity and flexible operating modes is demonstrated at two different scales by separating and concentrating target compounds using a mixture of caffeine, vanillin, naringenin and carvone using a quasi-continuous technique called intermittent counter-current extraction.	Journal of Chromatography A
29	Advantages of a small-volume counter-current chromatography column	Berthod, A	2009	Counter-current chromatography (CCC) works with a support-free liquid stationary phase. This allows for preparative separations and purifications. However, there are serious technical constraints because of the need to keep a liquid stationary phase in a column. Centrifugal fields are used. A new commercial hydrodynamic 18mL column made with a narrow-bore 0.8mm Teflon tubing was evaluated by comparing it with older hydrodynamic CCC columns and a similar 19mL column but made with 1.6mm Teflon tubing. A small-volume CCC column allows for reliable and fast solute partition coefficient determination. When resolution is required, both high efficiency and liquid stationary phase retention are needed. Unfortunately, these two requirements bear technical contradictions. A column coiled with a narrow tubing bore will provide a high chromatographic efficiency while a column containing wider tubing bore will achieve higher stationary phase retention. In all cases, increasing the magnitude of the centrifugal field also increases the stationary phase retention. The solution is to build centrifuges able to produce high fields that will provide acceptable liquid phase retention with narrow-bore tubes. The new 18mL 0.8mm tubing bore column is able to rotate as fast as 2100rpm generating a 240xg field. The two older CCC columns cannot compete with the new one. However, the small 19mL column with 1.6mm bore tubing can be useful when fast results are desired without top resolution.	Journal of Chromatography A
30	Two-column volume elution-extrusion countercurrent chromatography and rational parallel method for rapid screening of complex natural extracts	Lu, Y	2009	In countercurrent chromatography (CCC) the choice of the liquid system is the heart of any separation. It corresponds to the selection of the mobile phase and the stationary phase at the same time. Any change in one phase composition induces a change in the other phase composition which renders the choice of the appropriate liquid system difficult and lengthy. A scale of compositions of the heptane-ethyl acetate-methanol-water quaternary liquid system was referred to by letters from A to Z and called the Arizona (AZ) liquid system. Each composition of the AZ system has the same heptane/ethyl acetate and methanol/water volume ratios. It is shown that there is a continuous polarity change from the hydrophilic A composition (ethyl acetate-water) to the hydrophobic Z (heptane-methanol) mixture by measuring the distribution constant KD of a known test mixture. For all compounds, the log KD is linearly increasing with the water content of the lower aqueous phase of the composition used. The slopes of the log KDversus percent H2O have very different values which means that the chromatographic selectivity changes with liquid system compositions. The AZ system was associated to the elution-extrusion method to design a procedure to identify rapidly the appropriate solvent composition able to fractionate correctly a complex natural extract. With the use of an integrated three-coil CCC column (40 mL each coil) able to test three AZ compositions in parallel, it is shown that the optimum AZ composition is found in half a day using less than a liter total volume of solvents. Two natural extracts are rapidly screened using the proposed protocol. An extract of Piper longum L. of intermediate polarity was fractionated in five usable portions using the 3/2/3/2 (Q) composition of the AZ system. A polar extract of Polygonum cuspidatum was also separated in five fractions using the 1/6/1/6 (D) composition. In both cases, a 140 mL CCC column was used for a direct scale-up transfer with the same liquid system. Purified fractions were subjected to an antioxidant activity assay and liquid chromatography with UV and mass spectrometry detection (LC-UV/MS) analysis to determine the molecular weight, number, and quantity of compounds in the active fractions. Four fractions of P. cuspidatum showed excellent antioxidant activity. They were rapidly produced at the milligram level by the 140 mL CCC column and fractionated by semipreparative high-performance liquid chromatography (HPLC) in individual compounds that were each identified by NMR and MS and reevaluated for confirmation of bioactivity. The rapid screening CCC protocol associated to the	Journal of Analytical Chemistry
31	A Novel Approach to Modelling Counter Current Chromatography	Guzlek, H	2010	Literature lists a number of counter-current chromatography (CCC) models that can predict the retention time and to a certain extent the peak width of a solute eluting from a CCC column. The approach described in this paper distinguishes itself from previous reports by relating all model parameters directly to column dimensions and experimental settings. Most importantly, this model can predict a chromatogram from scratch without resorting to traditional calibration using empirical values. The model validation with experimental results obtained across a range of CCC instruments demonstrated that the solute retention time, peak width, and peak resolution could be predicted within reasonable accuracy. Additionally, the effect of several process parameters, such as mobile phase flow rate, rotational speed of the column or β-value, showed that the model is robust and applicable to a wide range of CCC instruments. Overall, this model proved to be a useful tool for parameter estimation and, most significantly, separation optimisation.	Journal of Chromatography A
32	Critical b-values for all Coil Planet Centrifuges	Wood, P	2010	I-type, J-type and non-synchronous centrifuges are all coil planet centrifuges. Analysing the motion of I-type and J-type centrifuges has advanced the understanding of how to manufacture and use these centrifuges. This paper analyses the motion of non-synchronous centrifuges producing equations of motion that can be applied to all coil planet centrifuges. This has also produced simple equations to determine the critical β-values for any coil planet centrifuge. This paper also demonstrates that I-type centrifuges also have 2 critical β-values when it was thought that β-value did not influence the understanding of the processes within I-type centrifuges. For the I-type instrument both of these critical values are at bobbin radii approaching infinity. In practice this means all I-types function within one β-value range hence the unilateral distribution and type/effectiveness of the mixing is consistent. Finally the paper shows the influence that the tangential velocity has on the Archimedean screw effect and thus the unilateral distribution of the upper and lower phases in the columns of coil planet centrifuges. This explains why the maximum stationary phase retention in an I-type centrifuge is limited to 50%.	Journal of Chromatography A
33	Predictable and linear scale-up of four phenolic alkaloids separation from the roots of Menispermum dauricum using hpccc	Luo, H	2010	This paper describes how distribution ratios were used for prediction of peak elution in analytical high-performance counter-current chromatography (HPCCC) to explore the method for separation and purification of bioactive compounds from the roots of Menispermum dauricum. Then important parameters related to HPCCC separations including solvent systems, sample concentration, sample loading volume and flow rate were optimized on an analytical Mini-DE HPCCC and finally linearly scaled up to a preparative Midi-DE HPCCC with nearly the same resolutions and separation time. Four phenolic alkaloids were for the first time obtained by HPCCC separation with a two-phase solvent system composed of petroleum ether-ethyl acetate-ethanol-water (1:2:1:2, v/v). This process produced 131.3 mg daurisolin, 197.1 mg dauricine, 32.4 mg daurinoline and 14.7 mg dauricicoline with the purity of 97.6%, 96.4%, 97.2% and 98.3%, respectively from 500 mg crude extract of the roots of M. dauricum in a one-step separation. The purities of compounds were determined by high-performance liquid chromatography (HPLC). Their structures were identified by electrospray ionization mass spectrometer (ESI-MS) and nuclear magnetic resonance (NMR).	Journal of Chromatography B
34	A new non-synchronous preparative counter-current centrifuge–the next generation of dynamic extraction/chromatography devices with independent mixing and settling control.	Ignatova, S	2010	A new and significantly more robust design of non-synchronous coil planet centrifuge is introduced where the degree of mixing between two immiscible phases can be changed independently from the "g" field required to separate out the phases. A hypothesis that an optimum ratio between the speed of the bobbin and the speed of the rotor can be found to optimise the efficiency of the separation for a given force field is upheld for an intermediate polarity phase system. This paves the way for extensive further research to find the optimum non-synchronous conditions for a range of different phase systems that are desirable for the separation of large molecules, proteins and biologics but can tend to emulsify in the standard "J" type centrifuge systems currently available and routinely in use for aqueous organic phase systems. A step change of up to 30% in resolution and 90% in plate efficiency is demonstrated.	Journal of Chromatography A
35	Preparative isolation and purification of ginsenoside Rf, Re, Rd and Rb1 from the roots of Panax ginseng with a salt containing solvent system and flow step-gradient by hpccc coupled with elsd	Qi, X	2010	Ginseng is a popular herb worldwide and has had varied uses in traditional Asian medicine for thousands of years. There are several different species of the herb, but all share the same constituents. Ginsenosides, the most extensively studied chemical components of ginseng, are generally considered to be one of the most important active ingredients of the plant. In this study, we have developed fast and efficient methodology for isolation of four known ginsenosides Rf, Rd, Re and Rb1 from Ginseng by high performance counter-current chromatography (HPCCC) coupled with evaporative light scattering detection (ELSD). The crude sample for HPCCC was purified firstly from a ginseng extraction using macroporous resin. The enriched saponin fraction (480 mg) was separated by using methylene chloride-methanol-5 mM aqueous ammonium acetate-isopropanol (6:2:4:3, v/v) as the two-phase solvent system and yielded 10.7 mg of Rf, 11.0 mg of Rd, 13.4 mg of Re and 13.9 mg of Rb1. The purity of these ginsenosides was 99.2%, 88.3%, 93.7% and 91.8%, respectively analyzed by HPLC-DAD-ELSD, and their structures were characterized by electrospray ionization mass spectrometry (ESI-MS) and compared with standards. Ammonium acetate was used to shorten the separation time and eliminate emulsification together with a flow step-gradient. The salt can be removed by re-dissolving the sample using acetone.	Journal of Chromatography A
36	Isolation and Purification of bioactive materials using HPCCC	Jung, D	2010	Many successive liquid-liquid extractions occur enabling purification of the crude material to occur. In high performance counter-current chromatography (HPCCC), crude material is partitioned between two immiscible layers of solvent phases. The stationary phase (SP) is retained by hydrodynamic force field effect and the mobile phase (MP) is pumped through the column. Purification occurs because of the different solubility of the components in the liquid mobile and stationary phases. There are many key benefits of liquid stationary phases such as high mass and volume injection loadings, total sample recovery, and easy scale-up. Many researchers showed that predictable scale-up from simple test is feasible with knowledge of the stationary phase retention for the planned process scale run. In this review we review the recent advances in HPCCC research and also describe the key applications such as natural products and synthetics (small or large molecules).	Journal of Chromatography A
37	Separation of honokiol and magnolol by intermittent counter current extraction	Peng, A	2010	Recently, intermittent counter-current extraction (ICcE) has been developed and shown its advantage in improving resolution between targeted compounds. However, how to choose suitable parameters to increase the throughput has not been systematically studied yet. In present work, we first calculated theoretically the conditions to carry out ICcE elution mode. Then, honokiol and magnolol were separated as model compounds using ICcE elution mode to confirm our conclusion. After parameters like sample concentration and sample feed were optimized in analytical high-performance counter-current chromatography (HPCCC), the separation process was scaled up to preparative HPCCC successfully. 12.8 g honokiol and 16.1g magnolol were separated from 30 g mixture with purities of 98.6% and 93.7%. And the throughput of target isolation of ICcE elution mode was at least 3.75 x higher than isocratic elution mode with the same HPCCC instruments. Our results confirmed our theory calculation and demonstrated the enormous potential of ICcE on preparative separation of binary mixture.	Journal of Chromatography A
38	Comprehensive separation and identification of chemical constituents from Apocynum venetum leaves by hpccc and hplc-ms	Zhang, Y	2010	High-performance counter-current chromatography (HPCCC) and high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) was efficiently utilized for the separation and identification of the chemical components with a wide range of polarity from the mixed extract of Chinese medicinal herb Apocynum venetum. For HPCCC separation, four sets of solvent systems, n-hexane-ethyl acetate-acetonitrile-water (1.5:3:5:2:4:5, v:v:v:v), ethyl acetate-methanol-water (5:2:5, v:v:v) and n-butanol-methanol-water (5:1:5, v:v:v) were used for the one-step separation by four stages. The HPCCC separation was initiated by filling the column with the lower phase of n-hexane-ethyl acetate-acetonitrile-water (1.5:3:5:2:5, v:v:v:v) as a stationary phase followed by elution with the upper phase of n-hexane-ethyl acetate-acetonitrile-water (1.5:3:5:2:5, v:v:v:v) to separate the hydrophobic compounds (tail to head). Then the mobile phase was switched to the upper phase of ethyl acetate-acetonitrile-water (5:3:7, v:v:v) to eluted the moderate hydrophobic compounds, then the mobile phase was switched to the upper phase of ethyl acetate-methanol-water (5:2:5, v:v:v) to eluted the moderate hydrophilic compounds, and finally the hydrophilic compounds still retained in the column was eluted by the upper phase of n-butanol-methanol-water (5:1:5, v:v:v). A total of 16 named compounds including adhyperforin, hyperforin, amentoflavone, biapigenin, quercetin, avicularin, acetylated isoquercetin, acetylated hyperoside, astragalin, trifolin, isoquercetin, hyperoside, quercitrone, rutin, chlorogenic acid and quercetin-3-O-β-D-glucosyl-β-D-glucopyranoside were successfully separated via the four sets of solvent systems in one step operation for 130 min. The compounds separated by HPCCC were identified by comparing with mixed standards data of HPLC-MS as well as NMR data.	Journal of Chromatography B
39	Purification of honokiol derivatives from one-pot synthesis by HPCCC	Shi, J	2010	This paper describes the application of high-performance counter-current chromatography (HPCCC) as a fast, useful and economic alternative for the separation and purification of seven honokiol derivatives (two of them are isomers), which were synthesized by a one-pot procedure. Five honokiol derivatives were successfully separated by n-hexane-ethyl acetate-methanol-water solvent system at three different volume ratios in a step-gradient elution. Two derivatives were obtained through a cycle elution mode. The whole separation process produced 366.3 mg, 323.6 mg, 242.8 mg, 216.2 mg, 203.5 mg, 185.8 mg and 279.3 mg of 3'-formylhonokiol (1), 2'-methoxy-3'-formylhonokiol (2), 2'-methoxyhonokiol (3), 4-methoxyhonokiol (4), 3',5-diformylhonokiol (5), 2',4-dimethoxy-3'-formylhonokiol (6) and 2',4-dimethoxyhonokiol (7) from crude sample of 3 g with purities of 98.7%, 99.3%, 98.6%, 98.2%, 99.0%, 98.4% and 99.2%, respectively. The purities and structural identification were determined by HPLC, (1)H NMR, (13)C NMR and mass spectroscopy.	Journal of Chromatography A
40	Combination of normal phase liquid chromatography and HPCCC for preparation of ginsenoside RO from panax ginseng with high recovery and efficiency	Cheng, Y	2010	Ginsenoside-Ro which belongs to the oleanane-type saponins has anti-inflammatory, anti-platelet, anticomplementary and immunomodulatory activities. The present paper described the preparation of ginsenoside-Ro from panax ginseng with high recovery and efficiency by combination of normal-phase medium-pressure liquid chromatography (NP-MPLC) and high-performance counter-current chromatography (HPCCC). The crude sample was preliminarily chromatographed by NP-MPLC to enrich ginsenoside-Ro to the purity of 70.2% with 95.0% recovery. Then the enriched sample was further purified by HPCCC with a solvent system composed of ethyl acetate-isopropanol-0.1% formic acid (3:1:5, v/v), where 61mg ginsenoside-Ro was obtained from 100mg enriched sample with 96.0% purity and 83.4% recovery. The overall recovery of ginsenoside-Ro was 79.2%, whose structure was identified by liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) finally.	Journal of Chromatography A
41	Retention of fluorinated chiral selectors in biphasic fluorinated solvent systems and its application to the separation of enantiomer by countercurrent chromatography	Perez, A	2010	Ethoxynonafluorobutane (ENFB) has been used as a component of new biphasic solvent mixtures. The suitability of several mixtures as solvent systems in countercurrent chromatography was tested. The applicability of the ENFB/2-PrOH/H2O mixture to the separation of enantiomers, in combination with a fluorinated chiral selector (CS), was evaluated. N-Perfluorooctanoyl-l-proline-3,5-dimethylanilide (2), analogous to the previously used N-dodecyl-l-proline-3,5-dimethylanilide (1), was synthesized for this purpose. The capacity of the new solvent system to retain the fluorinated CS in the fluorinated phase used as stationary was examined. Chiral selector 1 was applied in analogous conditions for comparative purposes. Additionally, MTBE/phosphate buffer solvent system was also used with the two CSs. The ENFB/2-PrOH/H2O (25:35:40) mixture was found to be adequate in the enantioseparation of DNB-Leu and DNB-Leu-tBu. Enantioselectivity was maintained in the fluorinated solvent system without compromising eluting time. The complete separation of DNB-Leu-tBu was achieved and no leaks of CS to the mobile phase were detected.	Journal of Chromatography A
42	Scalable Technology for the Extraction of Pharmaceutics (STEP): The transition from academic knowhow to industrial reality	Sutherland, I	2011	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.	Journal of Chromatography A
43	Comparison of preparative reversed phase liquid chromatography and countercurrent chromatography for the kilogram scale purification of crude spinetoram insecticide	DeAmicis, C	2011	Reversed phase HPLC (RP-HPLC) and high performance countercurrent chromatography (HPCCC) were compared for the pilot scale purification of two semi-synthetic spinosyns, spinetoram-J and spinetoram-L, the major components of the commercial insecticide spinetoram. Two, independently performed, 1 kg, purification campaigns were compared. Each method resulted in the isolation of both components at a purity of >97% and yields for spinetoram-J and spinetoram-L of >93% and ≥63% of theoretical, respectively. The HPCCC process produced a 2-fold higher throughput and consumed approximately 70% less solvent than preparative scale RP-HPLC, the volume of product containing fractions from HPCCC amounted to 7% of that produced by HPLC and so required much less post-run processing.	Journal of Chromatography A
44	Gradient elution in counter-current chromatography: A new layout for an old path	Ignatova, S	2011	Gradient elution in CCC is a powerful tool, which needs further systematic development to become robust and easy to use. The first attempt to build a correlation between gradient elution profile and distribution ratio (K(D)) values for model mixtures containing typical representatives of pharmaceutical compounds is presented in this paper. The three step estimation of the solvent system composition of a heptane-ethyl acetate-methanol-water (HEMWat) series is described. The estimation is based on simple measurements of initial and final stationary phase retention for gradient elution run, calculating gradient distribution ratio and correlating it with static K(D) against HEMWat number.	Journal of Chromatography A
45	Evaluation of dual flow counter-current chromatography and intermittent counter-current extraction	Ignatova, S	2011	The aim of this research is to compare two continuous extraction technologies, intermittent counter-current extraction (ICcE) and dual flow counter-current chromatography (DFCCC), in terms of loading and throughput using the GUESSmix, and show the advantages and disadvantages of the two methods. A model sample containing caffeine, vanillin, naringenin and carvone, with a total load of 11.2 g, was employed with a hexane-ethyl acetate-methanol-water (2:3:2:3) phase system to evaluate an ICcE method on a preparative (912 ml coil volume) DE-Midi instrument. While DFCCC was carried out on a specially designed preparative (561 ml coil volume) bobbin installed in a similar Midi instrument case. While similar throughputs of 7.8 g/h and 6.9 g/h were achieved for the ICcE and DFCCC methods respectively, ICcE was demonstrated to have a number of advantages over DFCCC.	Journal of Chromatography A
46	Application of accelerated solvent extraction coupled with hpccc to extract and online isolation of chemical constituents from Hypericum perforatum L	Zhang, Y	2011	Accelerated solvent extraction (ASE) coupled with high-performance counter-current chromatography (HPCCC) was successfully used for the extraction and online isolation of five chemical constituents from the plant Hypericum perforatum L. The upper phase of the solvent system of ethyl acetate-methanol-water (5:2:5, v:v:v) was used as both the ASE solvent and the HPCCC stationary phase. Two hydrophobic compounds including 28.4 mg of hyperforin with a HPLC purity of 97.28% and 32.7 mg of adhyperforin with a HPLC purity of 97.81% were isolated. The lower phase of ethyl acetate-methanol-n-butanol-water (5:2:2:5:12, v:v:v:v) was used as both the ASE solvent and CCC stationary phase. Three hydrophilic compounds of 12.7 mg of 3,4,5-O-tricaffeoylquinic acid with a HPLC purity of 98.82%, 15.2 mg of 1,3,5-Otricaffeoylquinic acid with a HPLC purity of 99.46% and 42.5 mg of 3-O-caffeoylquinic acid with a HPLC purity of 96.90%, were obtained in a one-step extraction-separation process with less than 3 h from 10.02 g of raw material of H. perforatum. The targeted compounds isolated, collected and purified by HPCCC were analyzed by high performance liquid chromatography (HPLC), the chemical structures of all five compounds above mentioned were identified by UV, MS and NMR.	Journal of Chromatography A
47	Separation of patuletin-3-O-glucoside, astragalin, quercetin, kaempferol and isorhamnetin from Flaveria bidentis (L) Kuntze by elution-pump-out high performance counter-current	Wei, Y	2011	Flaveria bidentis (L) Kuntze is an annual alien weed of Flaveria Juss. (Asteraceae) in China. Bioactive compounds, mainly flavonol glycosides and flavones from F. bidentis (L) Kuntze, have been studied in order to utilize this invasive weed. Analytical high-performance counter-current chromatography (HPCCC) was successfully used to separate patuletin-3-O-glucoside, a mixture of hyperoside (quercetin-3-O-galactoside) and 6-methoxykaempferol-3-O-galactoside, astragalin, quercetin, kaempferol and isorhamnetin using two runs with different solvent system. Ethyl acetate-methanol-water (10:1:10, v/v) was selected by analytical HPCCC as the optimum phase system for the separation of patuletin-3-O-glucoside, a mixture of hyperoside and 6-methoxykaempferol-3-O-galactoside and astragalin. A Dichloromethane-methanol-water (5:3:2, v/v) was used for the separation of quercetin, kaempferol and isorhamnetin. The separation was then scaled up: the crude extract (ca 1.5 g) was separated by preparative HPCCC, yielding 12 mg of patuletin-3-O-glucoside at a purity of 98.3%, yielding 9 mg of a mixture of hyperoside and 6-methoxykaempferol-3-O-galactoside constituting over 98% of the fraction, and 16 mg of astragalin (kaempferol-3-O-glucoside) at a purity of over 99%. The pump-out peaks are isorhamnetin (98% purity), kaempferol (93% purity) and quercitin (99% purity). The chemical structure of patuletin-3-O-glucoside and astragalin were confirmed by MS and ¹H, ¹³C NMR.	Journal of Chromatography A
48	Development of a strategy and process parameters for a green process in counter-current chromatography: Purification of tanshinone IIA and cryptotanshinone from Salvia miltiorrhiza Bunge as	Zhang, M	2011	A strategy for the development of a green process using counter-current chromatography technology is presented in this paper. The strategy began with solvent system selection, followed by linear scale-up from an analytical to a preparative process with optimized operating parameters. A two-stage separation using a multi-injection method was performed with a solvent system of hexane-dichloromethane-methanol-water (4:0.75:4:1) for the 1st stage and a hexane-ethanol-water (4:2:2) for the 2nd stage. A 191.8 mg of tanshinone IIA was purified, with a 97% purity and 34.4% recovery and a 276.7 mg of cryptotanshinone was separated, with a 95% purity and 31.8% recovery from 2.1g of crude extract. Process parameters (throughput, efficiency, environmental risk factor and general process evaluation) and mass factors (mass intensity, separation mass efficiency and greenness) of a target were developed for monitoring of the counter-current chromatography process.	Journal of Chromatography A
49	Separation and Purification of Quinolone Alkaloids from the Chinese Herbal Medecine Evodia rutaecarpa (Juss.) Benth by HPCCC	Zhong, S	2011	Preparative separation of quinolone alkaloids in Evodia rutaecarpa (Juss.) Benth was conducted by high performance counter-current chromatography (HPCCC) with a pair of two solvent systems consisting of n-hexane-methanol-water-acetic acid (2:1:1:0.2, v/v) and (5:4:2:0.1, v/v). Consequently, 31.78 mg 1-methyl-2-nonyl-4-(1H)-quinolone (I), 59.25 mg 1-methyl-2-(6-undecenyl)-4-(1H)-quinolone (II), 333.27 mg evocarpine (III), 101.13 mg 1-methyl-2-(6,9-pentadecadienyl)-4(1H)-quinolone (IV), 132.17 mg dihydroevocarpine (V), and 86.99 mg 1-methyl-2-(10-pentadecenyl)-4(1H)-quinolone (VI) were obtained from 1.3 g of the crude extract. The structures of these compounds were identified by mass spectrometer (MS), nuclear magnetic resonance (1H NMR and 13C NMR).	Separation Science and Technology
50	Preparative separation of chromones in plant extract of Saposhnikova divaricata by HPCCC	Gui, Y	2011	Four chromones, prim-O-glucosylcimifugin, 4'-O-β-D-glucosyl-5-O-methylvisamminol, cimifugin and sec-O-glucosylhamaudol, were isolated and purified from Saposhnikovia divaricata for the first time by high-performance counter-current chromatography (HPCCC) using a system consisting of ethyl acetate/n-butanol/ethanol/water (1:1:0.1:2, v/v/v/v). The separation parameters were first performed on the analytical HPCCC and the optimized conditions were then scaled up to preparative HPCCC. A total of 72.1 mg of prim-O-glucosylcimifugin, 27 mg of 4'-O-β-D-glucosyl-5-O-methylvisamminol, 14.1 mg of cimifugin and 1.1 mg of sec-O-glucosylhamaudol were purified from 960 mg of the n-butanol extract of S. divaricata, each at over 90% purity as determined by high-performance liquid chromatography (HPLC). The structures of four compounds were identified by their retention time, the liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) in the positive ion mode, and confirmed by NMR. The characteristic LC-ESI-MS fragmentation patterns of the four compounds were discussed, and found to be a very specific and useful tool for the structural identification of chromones from S. divaricata	Journal of Separation Science
51	Intermittent counter-current extraction–effect of the key operating parameters on selectivity and throughput	Hewitson, P	2011	Intermittent counter-current extraction (ICcE) has proved itself as a method for splitting compounds into streams and/or concentrating compounds in the column. In this paper a model mixture sample based on a modified GUESSmix (containing salicin, caffeine, aspirin, coumarin, salicylic acid, carvone, ionone and biphenyl) was separated into two eluent streams across a range of HEMWat phase system polarities from the polar system 11 through to non-polar system 23. ICcE could provide throughput of over 1 kg/day with this model sample, at the preparative scale. Changing the time cycle to adjust where the sample mixture is split into two streams was demonstrated. It is established that for the continuous running of ICcE, on a conventional twin bobbin counter-current chromatograph instrument, it is necessary to adjust the dead volumes of the flying leads to maintain similar phase retention in each column so the instrument does not become hydrodynamically and mechanically unbalanced due to the difference in densities between the upper and lower phases.	Journal of Chromatography A
52	Toroidal coil chromatography: The effect of scale-up and 'g' field on stage efficiency	Sutherland, I	2011	Selected test results have been taken from various publications and resolution and stage efficiency measured using an established model. All experiments used the same sample and, where possible, the same sample loading. The results show that stage mixing efficiencies have increased from 1.1% in 1998 to greater than 25% in the latest scaled-up version of a Toroidal coil chromatography (TCC) instrument working at 240g.	Journal of Chromatography A
53	High salt solvent systems in separation of betanin and its derivatives from red beet (Beta vulgaris L.) by HPCCC	Spórna-Kucab, A	2012	A study on a separation of betanin and its decarboxy- and dehydro-derivatives obtained from red beet roots (Beta vulgaris L.) using analytical high-performance counter-current chromatography (HPCCC – Dynamic Extractions Ltd., UK) was performed. The HPCCC process was accomplished in the 'tail to head' mode with three highly polar solvent systems with high salt concentrations: 1-propanol-acetonitrile-saturated ammonium sulphate-water (v/v/v/v, 1:0.5:1.2:1); ethanol-acetonitrile-1- propanol-saturated ammonium sulphate-water (v/v/v/v/v, 0.5:0.5:0.5:1.2:1) and ethanol-1-butanol-acetonitrile-saturated ammonium sulphate-water (volume ratio, 0.5:0.5:0.5:1.2:1). HPLC analysis was performed in a conventional reversed phase mode with diode-array (DAD) detection to characterize the composition of obtained fractions. The applied solvent systems enabled the separation of the betalain pigments with high effi ciency for the fi rst time. In the mode of separation selected, the more hydrophobic compounds eluted fi rst as expected. Moreover, for the fi rst time, the applied HPCCC solvent systems generated a separation of 2-decarboxy-betanin from 17- and 2,17-bidecarboxy-betanin as well as from neobetanin and betanin.	Challenges of Modern Technology
54	Isolation and structural elucidation of indole alkaloids from Geissospermum vellosii by mass spectrometry	Mbeunkui, F	2012	Alkaloids from the stem bark of Geissospermum vellosii possess a variety of therapeutic properties including antimalarial activities, activity as a sexual stimulant and inhibition of the proliferation of HIV and herpes viruses. Methods currently used to isolate the active components from G. vellosii are time-consuming, labor intensive, and result in low recovery. In addition, there is a lack of sensitive and accurate analytical methods for the structural characterization and identification of alkaloid components in minor quantities. A combination of high performance counter-current chromatography and ESI tandem mass spectrometry (MSn) was established to isolate alkaloids from the stem bark of G. vellosii, and study their electrospray ionization mass spectrometry fragmentation behavior. Five indole alkaloids were successfully isolated and identified by nuclear magnetic resonance and mass spectrometry. The multi-stage tandem mass spectrometric data were used to study their fragmentation pattern and set a model for detailed structure characterization of related indole alkaloids. The presence of the even mass fragment ion suggestive of an odd number of nitrogen at m/z 144 corresponding to C10H9N was characteristic to indole alkaloids. The results of the experiments demonstrated that the combination of high performance counter current chromatography and ESI-MSn is a sensitive, selective and effective approach for rapid isolation and characterization of alkaloids from G. vellosii.	Journal of Chromatography B
55	Isolation of the minor and rare constituents from fruits of Peucedanum alsaticum L. using HPCCC	Skalicka-Wozniak, K	2012	A high-performance counter-current chromatography (HPCCC) method was applied for the first time for the preparative separation and purification of three rare compounds which occur as minor constituents in the fruits of Peucedanum alsaticum L.: 5-substituted coumarin notoptol and two dihydropyranochromones: divaricatol and ledebouriellol. A scale-up process from analytical to preparative in a very short time was developed. In order to purify a range of rare and minor compounds with different polarity two separate experiments were performed, one in reverse phase, the other in normal phase, using the same crude extract. A two-phase solvent system composed of n-hexane/ethyl acetate/methanol/water (1:1:1:1 v/v) was developed. The components purified and collected were analyzed by high-performance liquid chromatography. The method yielded 0.7 mg of notoptol, 1.46 mg of ledebouriellol at purity of 99.5%, and 10 mg of mixtures of divaricatol, alsaticol and alsaticocoumarin, where divaricatol present 22% by peak area. These amounts were obtained from 1 g of the crude extract in a single run. This is the first time when minor notoptol, ledebouriellol, and divaricatol were isolated in a single run using HPCCC method and first time when these were identified in plant from Peucedanum genus.	Journal of Separation Science
56	Application of step-wise gradient HPCC for rapid preparative separation and purification of diterpene components from Pseudolarix kaempferi Gordon	He, S	2012	In general, simultaneously separation and purification of components with a broad polarity range from traditional Chinese medicine (TCM) is a challenge by an ordinary high-speed counter-current chromatography (HSCCC) method. In this paper, we describes a rapid and efficient separation method of combining three-step gradient elution and two-step flow-rate gradient elution using high-performance counter-current chromatography (HPCCC) to separate 8 diterpene compounds simultaneously within 80 min in a single run from the alcohol extract of Pseudolarix kaempferi Gordon. This separation process produced 166 mg pseudolaric acid B O-β-D-glucopyranoside (PABGly), 152 mg pseudolaric acid C (PAC), 8 mg deacetylpseudolaric acid A (deacetylPAA), 5 mg pseudolaric acid A O-β-D-glucopyranoside (PAAGly), 484 mg pseudolaric acid B (PAB), 33 mg pseudolaric acid B methyl ester (PAB methyl ester), 10mg pseudolaric acid A (PAA) and 18 mg pseudolaric acid H (PAH) from 1.0 g crude sample with purities of 98.6%, 99.6%, 92.3%, 92.2%, 99.2%, 99.4%, 98.3%, 91.0%, respectively. Our study indicates that the suitable combination of step-wise gradient elution and flow-rate gradient elution using HPCCC is an effective strategy to separate complex components from natural products.	Journal of Chromatography A
57	Isolation of bitter acids from hops (Humulus lupulus L.) using counter-current chromatography	Dahlberg, C	2012	Commercially available hops (Humulus lupulus L.) bitter acid extracts contain a mixture of three major congeners (co-, n-, and ad-) in addition to cis/trans diastereomers for each congener. Individual isomerized α-acids were obtained by the consecutive application of two separate countercurrent chromatography methods. First, individual isomerized α-acid congeners as a mixture of cis/trans diastereomers were obtained using a solvent system consisting of hexane and aqueous buffer. The second purification, capable of separating cis/trans diastereomers, was accomplished using a quaternary solvent system; an alternative procedure using β-cyclodextrin followed by countercurrent chromatography was also investigated. The NaBH(4) reduction of the purified isomerized α-acid compounds followed by countercurrent chromatography purification resulted in individual ρ iso α-acids (>95%). Similarly, catalytic hydrogenation of the purified isomerized α-acid compounds followed by countercurrent chromatography purification produced individual tetrahydro isomerized α-acids (>95%). Reported herein is a widely applicable approach that focuses on three critical variables--solvent system composition, pH, and buffer-to-sample ratio--that enable the efficient purification of individual bitter acids (≥95%) from commercially available hops extracts.	Journal of Separation Science
58	Preparative separation of hyperoside of seed extract of Saposhnikovia divaricata by HPCCC	Li, Li	2012	Hyperoside was isolated and purified from the seeds of Saposhnikovia divaricata for the first time by high performance counter-current chromatography (HPCCC) using a solvent system of ethyl acetate-n-butanol-ethanol -water (1:1:0.1:2, v/v/v/v). By injecting ca. 500 mg of n-butanol extract of S. divaricata seeds for five times, a total of 232.7 mg of hyperoside was purified from 2.5 g of the n-butanol extract of S. divaricata seeds, at 96.3% purity as determined by high-performance liquid chromatography (HPLC). The identification of the purified compound was achieved by congruent retention time, ultraviolet (UV) spectra and the data of high-performance liquid chromatography-electrospray ion source mass spectroscopy (LC-ESI-MSn) in the positive mode with that of the authentic standard and literature reports.	Journal of Medicinal Plants
59	Using HPCCC combined with preparative hplc for the separation of bioactive compounds from the water extract of Gentiana macrophylla Pall	Wu, W	2012	In this paper, a combined high performance counter-current chromatography (HPCCC) and preparative high-performance liquid chromatography (HPLC) method was employed for rapid separation and enrichment of bioactive constituents from a water extract of Gentiana macrophylla Pall. With a two phase solvent system composed of ethyl acetate-n-butanol-water-acetic acid (2: 3: 5: 0.6, v/v), the water extract of G. macrophylla Pall was fractionated into six fractions with three targets isolated and four others highly concentrated, which were then further purified by preparative-HPLC. As a result, 37 mg deglucoserrulatoside, 22.4 mg loganic acid, 3.9 mg isoorientin, 22.4 mg swertiamarin, 52.3 mg gentiopicroside, 27.5 mg sweroside, and 7.9 mg macrophylloside D with the purity of 95.3%, 90.2%, 98%, 98%, 99.2%, 98.8%, and 98.4%, respectively, were isolated from the water extract of Gentiana macrophylla Pall. The structures were confirmed by UV spectra, MS, as well as NMR measurements.	Separation Science and Technology
60	Cost efficient and process-efficient separation of geniposide from Gardenia jasminoides Ellis by HPCCC	Zhang, M	2012	Gardenia jasminoides Ellis has been used for traditional Chinese medicine to treat all forms of febrile diseases. A cost-efficient and process-efficient separation of the bioactive molecule geniposide from G. jasminoides Ellis by high-performance counter-current chromatography (HPCCC) has been developed. Separation by preparative HPCCC was performed with a two-phase solvent system composed of ethyl acetate-butanol-water (0.4:1.6:2, v/v). The separation yielded 445 mg of geniposide with a purity of 95.2% from 4.0 g of crude extract. A factor of cost efficiency was developed and used for an economic evaluation of the separation process. The cost efficiency for geniposide was 0.070 g h−1 $−1. The separation process by HPCCC was demonstrated to be more economical and efficient than those methods by HSCCC and LC.	Separation and Purification Technology
61	Scalable Technology for the Extraction of Pharmaceutics: Outcomes from a 3 year collaborative industry/academia research programme	Sutherland, I	2013	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. © 2013 Elsevier B.V.	Journal of Chromatography A
62	Preparative separation of flavonoids in plant extract of Smilacis Glabrae Roxb. by HPCCC	Zhang, H	2013	Four flavonoids, isoastilbin, astilbin, isoengeletin, and engeletin were isolated and puri- fied simultaneously from Smilacis Glabrae Roxb. for the first time by high performance counter-current chromatography using a system consisting of n-hexane–n-butanol–water (1:2:3, v/v/v). A total of 392.6 mg of astilbin, 71.4 mg of isoastilbin, 47.4 mg of engeletin, and 10.3 mg of isoengeletin were purified from 1.89 g of the ethyl acetate extract of Smilacis Glabrae Roxb. in six runs, each at over 94.51% purity as determined by HPLC. The structures of the four compounds were identified by their retention time, the LC-ESI-MSn in the negative ion mode, and confirmed by 1H-NMR experiments. The characteristic LC-ESI-MS fragmentation patterns of the four compounds were discussed.	Journal of Separation Science
63	Scaled up countercurrent chromatography separation of secondary metabolites from Schinus therebinthifolius Raddi	Costa, F	2013	Schinus therebinthifolius Raddi (Anacardiaceae) is a tree of medium size native to South and Central America, whose fruits known as pink pepper, are widely used for cooking. In folk medicine it has been used to treat ulcers, arthritis, as well as in leprosy therapy. Previous phytochemical investigations have described the isolation of polyphenols, fatty and terpenoid acids1. Countercurrent chromatography (CCC) is a liquid-liquid partition chromatography in which the stationary liquid phase is retained in the apparatus without the use of a solid support, while the mobile phase is pumped through the coil. Scaled up CCC was performed to isolate metabolites from a CH2Cl2 extract of S. therebinthifolius berries. Evaluation of parameters was done on an analytical Mini-DE centrifuge (17.4 mL; 0.8 mm i.d.) using heptane-EtOAc-MeOH-H2O 6:1:6:1 (v/v/v/v) as the solvent system. Sample concentration (25, 50, 100, 125 mg/mL), volume (2.5% and 5% of coil volume) and flow-rate (0.5 and 1.0 mL/min) were studied. After systematic optimization, a linear scale-up was calculated to Spectrum-DE (143.5 mL; 1.6 mm i.d.) and Midi-DE (912.5 mL; 4.0 mm i.d.) centrifuges, keeping the same g force value by adjusting the rotational speed. Detection of all runs was performed by TLC and UV (λ 210nm). Results indicated that the scale-up settings gave good chromatographic resolution, with phase retention of about 75%. The optimal concentration value of 100 mg/mL was kept in all experiments. All CCC runs yielded directly the isolation of two pure major triterpene acids, identified as 3β-masticadienolic acid and masticadienoic acid (Figure) by NMR and MS data2. Other minor compounds were also enriched and further purified. Identification of these is in progress. In conclusion, a CCC purification method was developed, optimised and scaled-up to increase throughput 130 times, whilst maintaining the run duration and separation efficiency.	Planta Medica
64	Separation of five oligostilbenes from Vitis amurensis by flow gradient HPCCC	Ko, J	2013	A rapid and efficient high-performance counter-current chromatography (HPCCC) method was developed to separate five oligostilbenes from the roots of Vitis amurensis. An n-hexane/ethyl acetate/methanol/water (4:8:4:10, v/v/v/v) was selected as an optimal two-phase solvent system of which the upper phase was used as the stationary phase and the lower phase was used as the mobile one. Partition coefficient values for the target compounds under these optimized conditions were 0.28 (1, ampleopsin A), 7.12 (2, (+)-g-viniferin), 2.26 (3, vitisin A), 5.38 (4, wilsonol C), and 11.23 (5, vitisin B). Flow-rate gradient HPCCC (4 mL/min in 0–70 min, 8 mL/min in 70–250 min) was applied to isolate the target compounds in as high purity as possible within the shortest possible run time. Under these conditions, ampelopsin A (12.1 mg), (+)-g-viniferin (10.4 mg), vitisin A (2.8 mg), wilsonol C (3.2 mg), and vitisin B (37 mg) were isolated with >95% purity from 150 mg of enriched oligostilbene extract. Although the KD of the last eluted compound, vitisin B (KD = 11.23), was relatively large, it was eluted in 115–145 min using the two-phase solvent system. This study shows that HPCCC is an efficient tool for the isolation and purification of natural products.	Journal of Separation Science
65	Preparative separation of triterpene alcohol ferulates from rice bran using HPCCC	Liu, M	2013	A novel method for the separation of two major triterpene alcohol ferulates from rice bran oil (RBO) was developed using a high performance counter-current chromatography (HPCCC). A two-phase solvent system of n-hexane-acetonitrile (1:1, v/v) was applied to purify cycloartenyl ferulate (CAF) and 24-methylene cycloartanyl ferulate (24-mCAF) from RBO. The yields were 20.50±2.60 mg CAF and 12.62±1.15 mg 24-mCAF from 390 mg RBO through a two-step separation procedure. The purities of the two compounds were 97.97±0.90% and 95.50±0.75%, respectively, as determined by high performance liquid chromatography (HPLC). Their chemical structures were confirmed by ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS), and (1)H, (13)C and 2D nuclear magnetic resonance (NMR). This represents the first report on direct separation of CAF and 24-mCAF from RBO by HPCCC.	Journal of Food Chemistry
66	Versatile solvent systems for the separation of betalains from processed Beta vulgaris L. juice using CCC	Spórna-Kucab, A	2013	Two mixtures of decarboxylated and dehydrogenated betacyanins from processed red beet roots (Beta vulgaris L.) juice were fractionated by high performance counter-current chromatography (HPCCC) producing a range of isolated components. Mixture 1 contained mainly betacyanins, 14,15-dehydro-betanin (neobetanin) and their decarboxylated derivatives while mixture 2 consisted of decarboxy- and dehydro-betacyanins. The products of mixture 1 arose during thermal degradation of betanin/isobetanin in mild conditions while the dehydro-betacyanins of mixture 2 appeared after longer heating of the juice from B. vulgaris L. Two solvent systems were found to be effective for the HPCCC. A highly polar, high salt concentration system of 1-PrOH-ACN-(NH4)2SO4 (satd. soln)-water (v/v/v/v, 1:0.5:1.2:1) (tail-to-head) enabled the purification of 2-decarboxy-betanin/-isobetanin, 2,17-bidecarboxy-betanin/-isobetanin and neobetanin (all from mixture 1) plus 17-decarboxy-neobetanin, 2,15,17-tridecarboxy-2,3-dehydro-neobetanin, 2-decarboxy-neobetanin and 2,15,17-tridecarboxy-neobetanin (from mixture 2). The other solvent system included heptafluorobutyric acid (HFBA) as ion-pair reagent and consisted of tert-butyl methyl ether (TBME)-1-BuOH-ACN-water (acidified with 0.7% HFBA) (2:2:1:5, v/v/v/v) (head-to-tail mode). This system enabled the HPCCC purification of 2,17-bidecarboxy-betanin/-isobetanin and neobetanin (from mixture 1) plus 2,15,17-tridecarboxy-2,3-dehydro-neobetanin, 2,17-bidecarboxy-2,3-dehydro-neobetanin and 2,15,17-tridecarboxy-neobetanin (mixture 2). The results of this research are crucial in finding effective isolation methods of betacyanins and their derivatives which are meaningful compounds due their colorant properties and potential health benefits regarding antioxidant and cancer prevention. The pigments were detected by LC-DAD and LC-MS/MS techniques.	Journal of Chromatography B
67	Isolation of anti-tumor compounds from the stem bark of Zanthoxylum ailanthoides Sieb. & Zucc. By silica gel column and CCC	Cao, X	2013	Silica gel column chromatography combined with high performance counter-current chromatography (HPCCC) was employed for the separation of potential anti-tumor compounds from a petroleum ether fraction of a crude extract of Zanthoxylum ailanthoides Sieb. & Zucc. This traditional Chinese medicine was recently found to display high inhibitory activity against A-549 human cancer cells in vitro and Lewis lung cancer in vivo. A 75% aqueous ethanol extract of the stem bark of Z. ailanthoides was fractionated with petroleum ether, ethyl acetate and n-butanol. In this paper, the petroleum ether fraction was pre-separated by silica gel column chromatography with a petroleum ether-ethyl acetate gradient. Two fractions were further separated and purified by HPCCC using n-hexane-ethyl acetate-methanol-water (3:1:2:1, v/v) and petroleum-ethyl acetate-methanol-water (8:6:7:7, v/v). Finally, coumarins and lignans including luvangetin, xanthyletin, hinokinin and asarinin were isolated and identified by MS, (1)H and (13)C NMR. In total, 56mg of xanthyletin (1), 140mg of hinokinin (2), 850mg of luvangetin (3) and 74mg of asarinin (4) were obtained from approximately 50g of petroleum ether extract, in 96.0%, 94.0%, 99.0% and 94.0% purity, respectively, as determined by HPLC. The separation method proved to be efficient, especially for those minor components.	Journal of Separation Science
68	Isolation of chlorogenic acid from Mutellina purpurea L. herb using HPCCC	Sieniawska, E	2014	The aim of the study was to explore proper isolation conditions of chlorogenic acid from the herb of Mutellina purpurea L. – a new source of this bioactive molecule. The accelerated solvent extraction (ASE) with 40% aqueous solution of methanol combined with high-performance counter-current chromatography (HPCCC) was utilised for the efficient extraction and the separation of chlorogenic acid from the M. purpurea herb in less than 30 min. The structure of the obtained compound was confirmed by mass spectrometry and NMR analysis. The preparative HPCCC was performed using the mixture of ethyl acetate, butanol and water (4:1:5, v/v/v) in the reverse-phase mode. The chlorogenic acid was isolated from this herb for the first time, yielding 96% purity. The ASE with 40% methanol combined with HPCCC separation was proven to be a useful tool for quick and efficient isolation of chlorogenic acid from M. purpurea.	Natural Product Research
69	Iriflophenone-3-C-glucoside from Cyclopia genistoides: Isolation and quantitative comparison of antioxidant capacity with mangiferin and isomangiferin using on-line hplc antioxidant assays	Malherbe, C	2014	The benzophenone, iriflophenone-3-C-glucoside, was isolated from Cyclopia genistoides using a combination of fluid-fluid extraction, high performance counter-current chromatography (HPCCC) and semi-preparative high performance liquid chromatography (HPLC). The microplate oxygen radical absorbance capacity (ORAC) assay, with fluorescein as probe, was adapted for use in an on-line HPLC configuration. The method was validated using a mixture of authentic standards including iriflophenone-3-C-glucoside, and the xanthones, mangiferin and isomangiferin. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was included in the mixture for calculation of Trolox equivalent antioxidant capacity (TEAC) values. Using the on-line HPLC-ORAC assay, as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS(+)) on-line assays, the antioxidant activity of iriflophenone-3-C-glucoside and isomangiferin was demonstrated for the first time. Iriflophenone-3-C-glucoside presented no radical scavenging ability against DPPH, but scavenged ABTS(+) and peroxyl radicals (TEACABTS of 1.04 and TEACORAC of 3.61). Isomangiferin showed slightly lower antioxidant capacity than mangiferin against DPPH (TEACDPPH of 0.57 vs. 0.62), but higher capacity against ABTS(+) (TEACABTS of 1.82 vs. 1.67) and peroxyl radical (TEACORAC of 4.14 vs. 3.69) than mangiferin. The on-line HPLC-ORAC assay was shown to be more sensitive for radical scavengers, but at the same time less selective for rapid radical scavengers than the DPPH assay.	Journal of Chromatography B
70	Two step separation of Nostotrebin 6 from Cultivated soil Cyanobacterium (Nostoc sp.) by HPCCC	Cheel, J	2014	High performance countercurrent chromatography (HPCCC) was successfully applied for the separation of nostotrebin 6 from cultivated soil cyanobacteria in a two-step operation. A two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (4:5:4:5, v/v/v/v) was employed for the HPCCC separation. In the first-step operation, its neutral upper phase was used as stationary phase and its basic lower phase (1% NH3 in lower phase) was employed as mobile phase at a flow rate of 1 mL/min. In the second operation step, its neutral upper phase was used as stationary phase, whereas both its neutral lower phase and basic lower phase were employed as mobile phase with a linear gradient elution at a flow rate of 0.8 mL/min. The revolution speed and temperature of the separation column were 1,000 rpm and 30 °C, respectively. Using HPCCC followed by clean-up on Sephadex LH-20 gel, 4 mg of nostotrebin 6 with a purity of 99% as determined by HPLC/DAD-ESI-HRMS was obtained from 100 mg of crude extract. The chemical identity of the isolated compound was confirmed by comparing its spectroscopic data (UV, ESI-HRMS, ESI-HRMS2) with those of an authentic standard and data available in the literature.	Journal of Molecules
71	Normal phase HPCCC for the fractionation of dissolved organic matter from a freshwater source	Sandron, S	2014	Normal-phase high-performance counter-current chromatography (HPCCC) is used to obtain a preliminary fractionation of components in dissolved organic matter (DOM) from a freshwater source. The HPCCC solvent system involved a normal-phase approach with water/methanol (1:1) as the lower stationary phase and hexane/ethyl acetate (1:1) as the upper mobile phase. The critical experiment parameters were optimised: revolution speed 1800 rpm and flow rate 0.15 mL/min. Under these conditions 50 µL of a 0.50 mg/mL DOM solution was loaded. The detection wavelength was monitored at 330 nm in order to isolate the main portion of DOM, which includes substances such as carboxyl-rich alicyclic molecules. By optimising this system it was possible to isolate materials that, according to GC-MS, can be related to molecules with an analogous structural background. Where fraction analysis was not suitable for GC-MS, RP-HPLC with UV absorbance detection was used, showing unique chromatograms for each fraction at both 210 and 330 nm.	Journal of Separation Science
72	Rapid screening and detection of XOD inhibitors from S. tamariscina by ultrafiltration LC-PDA-ESI-MS combined with HPCCC	Wang, J	2014	Xanthine oxidase (XOD) catalyzes the metabolism of hypoxanthine and xanthine to uric acid, the overproduction of which could cause hyperuricemia, a risk factor for gout. Inhibition of XOD is a major treatment for gout, and biflavonoids have been found to act as XOD-inhibitory compounds. In this study, ultrafiltration liquid chromatography with photodiode-array detection coupled to electrospray-ionization tandem mass spectrometry (UF-LC-PDA-ESI-MS) was used to screen and identify XOD inhibitors from S. tamariscina. High-performance counter-current chromatography (HPCCC) was used to separate and isolate the active constituents of these XOD inhibitors. Furthermore, ultrahigh-performance liquid chromatography (UPLC) and triple-quadrupole mass spectrometry (TQ-MS) was used to determine the XOD-inhibitory activity of the obtained XOD inhibitors, and enzyme kinetics was performed with Lineweaver-Burk (LB) plots using xanthine as the substrate. As a result, two compounds in S. tamariscina were screened as XOD inhibitors: 65.31 mg amentoflavone and 0.76 mg robustaflavone were isolated from approximately 2.5 g S. tamariscina by use of HPCCC. The purities of the two compounds obtained were over 98 % and 95 %, respectively, as determined by high-performance liquid chromatography (HPLC). Lineweaver-Burk plot analysis indicated that amentoflavone and robustaflavone were non-competitive inhibitors of XOD, and the IC 50 values of amentoflavone and robustaflavone for XOD inhibition were 16.26 µg mL(-1) (30.22 µmol L(-1)) and 11.98 µg mL(-1) (22.27 µmol L(-1)), respectively. The IC 50 value of allopurinol, used as the standard, was 7.49 µg mL(-1) (46.23 µmol L(-1)). The results reveal that the method for systematic screening, identification, and isolation of bioactive components in S. tamariscina and for detecting their inhibitory activity using ultrafiltration LC-ESI-MS, HPCCC, and UPLC-TQ-MS is feasible and efficient, and could be expected to extend to screening and separation of other enzyme inhibitors.	Analytical and Bioanalytical Chemistry
73	Preparative separation of isoflavones in plant extract of Pueraria lobata by HPCCC	Li, B	2014	Five isoflavones, puerarin, daidzin, daidzein, 3⊠-hydroxy-puerarin and 3⊠-methoxy-puerarin were isolated and purified simultaneously from Pueraria lobata for the first time by high performance counter-current chromatography (HPCCC) using a system consisting of n-butanol-ethyl acetate-n-butanol-ethanol-water (0.5 : 2 : 1 : 0.5 : 3.5, v/v/v/v/v). In total, 155 mg of puerarin, 41 mg of daidzin, 12 mg of 3⊠-hydroxy-puerarin, 6 mg of 3⊠-methoxy-puerarin and 106 mg of daidzein were purified from 5.1 g of the ethyl acetate extract of P. lobata; the purities of the five isoflavones as determined by high performance liquid chromatography (HPLC) were 98.77%, 96.53%, 97.59%, 90.21% and 98.36%, respectively. The structures of the five compounds were identified by their retention time and the electrospray ionization multi-stage tandem mass spectrometry (ESI-MSn) in the positive ion mode, and confirmed by 1H-NMR experiments. The characteristic ESI-MS fragmentation patterns of the five compounds are discussed.	Journal of Analytical Methods
74	A general method for the separation of triphenylphosphine oxide and reaction products using high performance counter-current chromatography	Edwards, N	2014	A standardised separation methodology was developed for the purification of crude reaction mixtures containing triphenylphosphine oxide (TPPO) using high performance counter-current chromatography (HPCCC). A solvent system consisting of hexane/ethyl acetate/methanol/water (5:6:5:6) was used in 1 column volume elution-extrusion mode. The HPCCC methodology was compared with classical RP HPLC purification using a set of 12 representative Mitsunobu reaction mixtures. HPCCC was seen to yield a 65% increase in the average recovery of the target component whilst providing similar final target purities to those obtained by HPLC. By eliminating the need for method development for individual samples, the HPCCC methodology described within provides a simple and efficient means for the purification of the entire family of TPPO-containing reaction products.	Journal of Chromatography A
75	The CO2 microalgae biorefinery: high value products and biofuels using halophilic microalgae in the "D-Factory"	Harvey, P	2014	Representing the largest (100s ha) of current commercial cultivation technologies for any microalga, Dunaliella is cultivated commercially in highly saline non-potable waters for ?-carotene. After harvesting, the cells are spray-dried then sold as a high-value preparation of ?-carotene that meets FDA approval. However ?-carotene is not the only compound of commercial interest: Dunaliella cells also produce a range of other carotenoids, oxycarotenoids, lipids, proteins and other compounds of commercial value as well as glycerol: up to 80% of their mass, depending on biological and environmental conditions. Glycerol has emerged as a new biofuel for an entirely new environmentally-sustainable, biofuel industry as well as an intermediate to replace various fossil oil-based bulk chemicals. Driving down costs by recovering the entire biomass of Dunaliella cells from saline cultivation water poses one of the challenges for the D-Factory, because Dunaliella cells are motile, and do not possess an external cell wall, making them susceptible to cell rupture. We present results from experiments aimed at examining the effect of increasing g-force on the harvesting of the entire cell contents of Dunaliella cells using cytosolic glycerol as a marker for evidence of cell rupture.	New Biotechnology
76	Isolation of aspalathin and nothofagin from rooibos (Aspalathus linearis) using hpccc: Sample loading and compound stability considerations	De Beer, D	2015	Aspalathin and nothofagin, the major dihydrochalcones in rooibos (Aspalathus linearis), are valuable bioactive compounds, but their bioactivity has not been fully elucidated. Isolation of these compounds using high-performance counter-current chromatography (HPCCC), a gentle, support-free, up-scalable technique, offers an alternative to synthesis for obtaining sufficient amounts. An HPLC-DAD method was adapted to allow rapid (16 min from injection to injection) quantification of the four major compounds (aspalathin, nothofagin, isoorientin, orientin) during development of the isolation protocol. The traditional shake-flask method, used to determine distribution constants (KD) for target compounds, was also adapted to obtain higher repeatability. Green rooibos leaves with a high aspalathin and nothofagin content were selected as source material. Sample loading of the polyphenol-enriched extract was limited due to constituents with emulsifying properties, but could be increased by removing ethanol-insoluble matter. Furthermore, problems with degradation of aspalathin during HPCCC separation and further processing could be limited by acidifying the HPCCC solvent system. Aspalathin was shown to be fairly stable at pH 3 (91% remaining after 29 h) compared to pH 7 (45% remaining after 29 h). Aspalathin and nothofagin with high purities (99% and 100%, respectively) were obtained from HPCCC fractions after semi-preparative HPLC.	Journal of Chromatography A
77	New solvent systems for gradient counter-current chromatography in separation of betanin and its derivatives from processed Beta vulgaris L. Juice	Spórna-Kucab, A	2015	Betalains, natural plant pigments, are beneficial compounds due to their antioxidant and possible chemoprotective properties. A mixture of betalains: betanin/isobetanin, decarboxybetanins and neobetanin from processed red beet roots (Beta vulgaris L.) juice was separated in food-grade, gradient solvent systems using high-performance counter-current chromatography (HPCCC). The decarboxylated and dehydrogenated betanins were obtained by thermal degradation of betanin/isobetanin from processed B. vulgaris L. juice under mild conditions. Two solvent systems (differing in their composition by phosphoric acid and ethanol volume gradient) consisting of BuOH-EtOH-NaClsolution-H2O-H3PO4 (v/v/v/v/v, 1300:200-1000:1300:700:2.5-10) in the 'tail-to-head' mode were run. The flow rate of the mobile phase (organic phase) was 1.0 or 2.0 ml/min and the column rotation speed was 1600 rpm (20 °C). The retention of the solvent system stationary phase (aqueous phase) was ca. 80%. The system with the acid and ethanol volume gradient consisting of BuOH-EtOH-NaClsolution-H2O-H3PO4 (v/v/v/v/v, 1300:200-240:1300:700:2.5-4.5) pumped at 2.0 ml/min was the most effective for a separation of betanin/isobetanin, 17-decarboxy-betanin/-isobetanin, 2-decarboxy-betanin/-isobetanin, 2,17-bidecarboxy-betanin/-isobetanin pairs as well as neobetanin. The pigments were detected by LC-DAD and LC-MS. The results are crucial in the application of completely food-grade solvent systems in separation of food-grade compounds as well, and the systems can possibly be extended to other ionizable and polar compounds with potential health benefits. In particular, the method is applicable for the isolation and purification of betalains present in such rich sources as B. vulgaris L. roots as well as cacti fruits and Amaranthaceae flowering plants due to modification possibilities of the solvent systems polarity.	Journal of Chromatography A
78	Separating four diastereomeric pairs of dihydroflavonol glycosides from Engelhardia roxburghiana using HPCCC	Shi, H	2015	Four pairs of diastereomers were successfully isolated and separated from the water extract of Engelhardia roxburghiana by high performance counter-current chromatography (HPCCC) using a two-step procedure. The diastereomers were initially separated by a two-phase solvent system composed of n-hexane-n-butanol-0.1% trifluoroacetic acid (1:2:3, v/v/v) and followed by the same solvent system using hydroxypropyl-β-cyclodextrin (HP-β-CD) as an additive. The chromatographic conditions, elution mode, and concentrations of the additive were refined. The two-step HPCCC isolation yielded 43.7mg (2S, 3S)-astilbin, 27.6mg (2R, 3R)-astilbin, 5.9mg (2S, 3R)-astilbin, 4.8mg (2R, 3S)-astilbin, 6.9mg (2S, 3S)-engelitin, 3.1mg (2R, 3R)-engelitin, 8.2mg (2S, 3R)-engelitin, and 6.0mg (2R, 3S)-engelitin from 384mg crude extract in four runs with purities of 99.3%, 96.2%, 99.8%, 99.9%, 97.0%, 96.5%, 96.1%, and 96.8%, respectively. The present study revealed that HP-β-CD can be used as an additive in HPCCC to effectively improve the resolution of the diastereomers. The established HPCCC method may serve as an approach to obtain high purity diastereomers on a large scale.	Journal of Chromatography A
79	Preparative isolation of oleocanthal, tyrosol and hydroxytyrosol from olive oil by HPCCC	Adhami, H	2015	For the provision of oleocanthal (OLC), a phenolic compound with very promising pharmacological properties, isolation from olive oil is a very important option. Due to the compound's sensitivity to decomposition upon exposure to oxygen and light, a very gentle isolation method has been developed under use of high performance countercurrent chromatography (HPCCC). By partition of olive oil between hexane and methanol, an extract enriched in phenolics was prepared and subjected to a two-step HPCCC separation under use of heptane-EtOAc-MeOH-H2O mixtures in normal-phase and reverse phase mode, respectively. With this method, the isolation of tyrosol, hydroxytyrosol, and the mixture of (3S,4E)- and (3S,4Z)-OLC was achieved in approx. 70 min for each step. By one- and two-dimensional NMR-experiments and LC-MS, the equilibrium of (3S,4E)- and (3S,4Z)-OLC in such olive oil extracts has unambiguously been proven for the first time.	Journal of Food Chemistry
80	Application of HPCCC for the isolation of steroidal saponins from Liriope platyphylla	Choi, S	2015	High-performance countercurrent chromatography (HPCCC) with electrospray light-scattering detection was applied for the first time to isolate a spirostanol and a novel furostanol saponin from Liriope platyphylla. Due to the large differences in KD values between the two compounds, a two-step HPCCC method was applied in this study. The primary HPCCC employed methylene chloride/methanol/isopropanol/water (9:6:1:4 v/v, 4 mL/min, normal-phase mode) conditions to yield a spirostanol saponin (1). After the primary HPCCC run, the solute retained in the stationary phase (SP extract) in HPCCC column was recovered and subjected to the second HPCCC on the n-hexane/n-butanol/water system (1:9:10 v/v, 5 mL/min, reversed-phase mode) to yield a novel furostanol saponin (2). The isolated spirostanol saponin was determined to be 25(S)-ruscogenin 1-O-[β-D-glucopyranosyl (1→2)]-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside (spicatoside A), and the novel furostanol saponin was elucidated to be 26-O-β-D-glucopyranosyl-25(S)-furost-5(6)-ene-1β-3 β-22α-26-tetraol-1-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-fucopyranoside (spicatoside D).	Journal of Separation Science
81	Schinus terebinthifolius scale-up ccc part 1 HPCCC of triterpene acids with off-line detection using APCI-MS	Vieira, M	2015	Countercurrent chromatography (CCC) is an ideal technique for the recovery, purification and isolation of bioactive natural products, due to the liquid nature of the stationary phase, process predictability and the possibility of scale-up from analytical to preparative scale. In this work, a method developed for the fractionation of Schinus terebinthifolius Raddi berries dichloromethane extract was thoroughly optimized to achieve maximal throughput with minimal solvent and time consumption per gram of processed crude extract, using analytical, semi-preparative and preparative 'high performance countercurrent chromatography' (HPCCC) instruments. The method using the biphasic solvent system composed of n-heptane-ethyl acetate-methanol-water (6:1:6:1, v/v/v/v) was volumetrically scaled up to increase sample throughput up to 120 times, while maintaining separation efficiency and time. As a fast and specific detection alternative, the fractions collected from the CCC-separations were injected to an 'atmospheric pressure chemical ionization-mass-spectrometer' (APCI-MS/MS) and reconstituted molecular weight MS-chromatograms of the APCI-ionizable compounds from S. terebinthifolius were obtained. This procedure led to the direct isolation of tirucallane type triterpenes such as masticadienoic and 3β-masticadienolic acids. Also oleanonic and moronic acids have been identified for the first time in the species. In summary, this approach can be used for other CCC scale-up processes, enabling MS-target-guided isolation procedures.	Journal of Chromatography A
82	Comparison of three strategies for the isolation of black tea thea rubigins with a focus on CCC	Stodt, U	2015	Three different methods from the literature for the fractionation and isolation of thearubigins in black tea (Roberts' fractionation via liquid-liquid extraction, caffeine precipitation and high-speed countercurrent chromatography) were compared. The fractions were analyzed by both normal phase high-performance liquid chromatography (HPLC) on a diol-column and reverse phase HPLC. The methods were compared with regard to time consumption, yield and purity, showing that Roberts' fractionation yielded the largest fractions while the caffeine precipitation demanded the least time. The purest TR fraction was obtained via high-speed countercurrent chromatography. Based on these results the isolation of thearubigins via countercurrent chromatography was selected for further investigations. Different techniques (high-speed-, spiral-coil- and high-performance-countercurrent chromatography) were applied, the established method was modified and an additional solvent system was developed to receive well-purified thearubigin fractions.	Journal of Food Composition and Analysis
83	Bioactivity guided isolation of antimicrobial coumarins from Heracleum mantegazzianum fruits by HPCCC	Walasek, M	2015	An efficient strategy, based on bioassay-guided fractionation, high-performance liquid chromatography (HPLC), and high-performance counter-current chromatography (HPCCC), was established to purify and evaluate the bioactive compounds from the dichloromethane extract of the fruits of Heracleum mantegazzianum Sommier & Levier (Apiaceae). The quaternary solvent system n-heptane-ethyl acetate-methanol-water (6:5:6:5 v/v) was used in the reversed phase mode. Using this method, in a single run, seven fractions were isolated, among them three were the pure furanocoumarins: pimpinellin, imperatorin, and phelloptern. In order to purify xanthotoxin a more polar system (1:1:1:1 v/v) was further applied. The antimicrobial activity of extract, chromatographic fractions, and single compounds were in the range of MIC = 0.03-1 mg mL(-1). Xanthotoxin may have priority as a compound of further interest based on its antimicrobial activity. For the first time, an extensive antimicrobial study was performed for pimpinellin and phellopterin.	Journal of Food Chemistry
84	Divide and Conquer May Not Be The Optimal Approach to Retain the Desirable Estrogenic Attributes of the Cyclopia Nutriceutical Extract, SM6Met	Mortimer, M	2015	The genus Cyclopia, an indigenous South African fynbos plant used to prepare honeybush tea, contains phytoestrogenic compounds. An extract from C. subternata, SM6Met, displays three desirable estrogenic attributes for future development of a phytoestrogenic nutraceutical, namely, ER α antagonism, ERβ agonism, and also antagonism of E2-induced breast cancer cell proliferation. Activity-guided fractionation of SM6Met was used in an attempt to isolate and identify compounds conferring the desirable estrogenic profile to SM6Met. Initial liquid-liquid fractionation of SM6Met yielded a polar fraction (PF) and a non-polar fraction (NPF), with the desirable estrogenic attributes retained in the NPF. Subsequent high performance counter-current chromatography (HPCCC) fractionation of the NPF yielded three fractions (F1-F3). Interestingly, the fractions revealed separation of the previously demonstrated positive estrogenic attributes of the NPF into separate fractions, with F1 and F2 acting as ERα antagonists, only F2 inducing antagonism of E2-induced breast cancer cell proliferation and only F3 retaining robust ERβ agonist activity. In terms of major polyphenols, quantitative HPLC and liquid chromatography tandem mass spectrometry (LC-MS/MS) indicated that HPCCC fractionation resulted in a divergence of polyphenolic classes, with F1 emerging as the dihydrochalcone-rich fraction and F2 as the flavanone- and benzophenone-rich fraction, while the xanthones, flavones and phenolic acids were retained in F3. F3 was re-engineered into F3R by reassembling the major polyphenols identified in the fraction. F3R could, however, not replicate the effect of F3. In conclusion, although activity-guided fractionation results suggest that retention of all the desirable estrogenic attributes of the original SM6Met in one fraction is not an attainable goal, fractionation is a useful tool to enhance specific desirable estrogenic attributes.	Public Library of Science
85	Preparative isolation of biomarkers from the leaf exudate of Aloe ferox by HPCCC	Adhami, H	2015	One of the most crucial factors determining the safety and efficacy of any herbal medicine or natural product-based formulation is the quality of the raw material. The absence of readily available bio-markers (standards) is one of the hurdles which need to be overcome to develop robust and effective quality control protocols.Aloe ferox Mill. is a most coveted ethnomedicinally import plant indigenous to South Africa. A. ferox has been used since ancient times in folk medicine and recently it has gained popularity as an ingredient in cosmetic formulations and food supplements. This study aimed to develop a superior method for the isolation of bio-markers from "aloe bitters" (exudate) obtained from A. ferox.For separation by HPCCC the solvent system comprising of EtOAc/n-BuOH/H 2 O (3.5:1.5:5, v/v/v) was used in reversed phase mode. By this method, and only in one run, eight bio-markers were separated and isolated on semi-preparative scale including aloesin, aloeresin C, aloeresin A, 5-hydroxyaloin, aloin B, aloinoside B, aloin A and aloinoside A. The isolation of bio-active molecules from A. ferox (Cape aloes) is presented to illustrate the efficiency and advantages of high performance counter-current chromatography (HPCCC).	Phytochemistry Letters
86	Scale-up protein separation on stainless steel wide bore toroidal columns in the type-J CCC	Guan, Y	2015	Manufacturing high-value added biotech biopharmaceutical products (e.g. therapeutic proteins) requires quick-to-develop, GMP-compliant, easy-to-scale and cost effective preparatory chromatography technologies. In this work, we describe the construction and testing of a set of 5-mm inner diameter stainless steel toroidal columns for use on commercially available preparatory scale synchronous J-type counter-current chromatography (CCC) machinery. We used a 20.2m long column with an aqueous two-phase system containing 14% (w/w) PEG1000 and 14% (w/w) potassium phosphate at pH 7, and tested a sample loading of 5% column volume and a mobile phase flow rate of 20ml/min. We then satisfactorily demonstrated the potential for a weekly protein separation and preparation throughput of ca. 11g based on a normal weekly routine for separating a pair of model proteins by making five stacked injections on a single portion of stationary phase with no stripping. Compared to our previous 1.6mm bore PTFE toroidal column, the present columns enlarged the nominal column processing throughput by nearly 10. For an ideal model protein injection modality, we observed a scaling up factor of at least 21. The 2 scales of protein separation and purification steps were realized on the same commercial CCC device.	Journal of Chromatography A
87	Rapid separation of cyanidin-3-glucoside and cyanidin-3-rutinosid e from crude mulberry extract using high-performance countercurrent chromatography and establishment of a	Choi, S	2015	This study describes the rapid separation of mulberry anthocyanins; namely, cyanidin-3-glucoside and cyanidin-3-rutinoside, using high-performance countercurrent chromatography, and the establishment of a volumetric scale-up process from semi-preparative to preparative-scale. To optimize the separation parameters, biphasic solvent systems composed of tert-butyl methyl ether/n-butanol/acetonitrile/0.01% trifluoroacetic acid, flow rate, sample amount and rotation speed were evaluated for the semi-preparative-scale high-performance countercurrent chromatography. The optimized semi-preparative-scale high-performance countercurrent chromatography parameters (tert-butyl methyl ether/n-butanol/acetonitrile/0.01% trifluoroacetic acid, 1:3:1:5, v/v; flow rate, 4.0 mL/min; sample amount, 200–1000 mg; rotational speed, 1600 rpm) were transferred directly to a preparative-scale (tert-butyl methyl ether/n-butanol/acetonitrile/0.01% trifluoroacetic acid, 1:3:1:5, v/v; flow rate, 28 mL/min; sample amount, 5.0–10.0 g; rotational speed, 1400 rpm) to achieve separation results identical to cyanidin-3-glucoside and cyanidin-3-rutinoside. The separation of mulberry anthocyanins using semi-preparative high-performance countercurrent chromatography and its volumetric scale-up to preparative-scale was addressed for the first time in this report.	Journal of Separation Science
88	Separation of Polyphenols and Caffeine from the acetone extract of fermented tea leaves (Camellia sinensis) using high-performance countercurrent chromatography	Choi, S	2015	Leaves from Camellia sienensis are a popular natural source of various beverage worldwide, and contain caffeine and polyphenols derived from catechin analogues. In the current study, caffeine (CAF, 1) and three tea polyphenols including (−)-epigallocatechin 3-O-gallate (EGCg, 2), (−)-gallocatechin 3-O-gallate (GCg, 3), and (−)-epicatechin 3-O-gallate (ECg, 4) were isolated and purified by flow-rate gradient high-performance countercurrent chromatography (HPCCC) using a two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (1:9:1:9, v/v). Two hundred milligrams of acetone-soluble extract from fermented C. sinensis leaves was separated by HPCCC to give 1 (25.4 mg), 2 (16.3 mg), 3 (11.1 mg) and 4 (4.4 mg) with purities over 98%. The structures of 1–4 were elucidated by QTOF-MS, as well as 1H- and 13C-NMR, and the obtained data were compared to the previously reported values.	Journal of Molecules
89	Isolation and evaluation of the myorelaxant effect of bergapten on isolated rat jejunum	Skalicka-Wozniak, K	2016	Plants of the genus Heracleum L. (Apiaceae) have a long history of being used in traditional medicines for the treatment of alimentary tract disorders, and these biological effects have been ascribed to the presence of furanocoumarins (including bergapten).OBJECTIVES: This study aimed to develop an efficient, preparative, counter-current chromatographic separation of bergapten in order to characterize its spasmolytic activity in isolated rat jejunum strips. MATERIALS AND METHODS: Successful separation of the dichloromethane extract of the fruits of Heracleum leskovii Grossh. was achieved by high-performance counter-current chromatography (HPCCC) using a two-phase solvent system composed of n-heptane/EtOAc/MeOH/H2O (6:5:6:5, v/v/v/v). The pharmacological assessment of bergapten (0.0001–50 μM) on jejunum smooth muscle strips isolated from rats was conducted under isotonic conditions, following up to three hours of incubation. RESULTS: The separation method was scaled up six-fold from analytical to semi-preparative conditions, affording bergapten of >99% purity in less than 30 min. This permitted bergapten to be available in quantity for spasmolytic tests on isolated jejunum strips from rats. Bergapten caused myorelaxation of the intestine preparations in the concentration range of 0.0001–1 μM. At higher doses, bergapten caused either relaxation or contraction of the smooth muscle. DISCUSSION AND CONCLUSION: Bergapten was successfully isolated by rapid HPCCC and its spasmolytic activity was confirmed, thereby providing a preliminary evidence base for the traditional medicine application. The data suggest that bergapten causes no irreversible changes to intestinal tissue.	Pharmaceutical Biology
90	Flavonoids from the flowers of Impatiens glandulifera Royle isolated by HPCCC	Vieira, M	2016	Impatiens glandulifera Royle (Balsaminaceae) is an annual herb from the Himalaya region, currently widespread along European river systems and one of the most important neophyte invading plants in Germany. Exploring the effects of allelopathic plant chemicals is important for the understanding of its ecological impacts in the process of suppression of indigenous plant species. OBJECTIVE: To investigate the chemical composition of Impatiens glandulifera flowers (IGFs) using high-performance countercurrent chromatography (HPCCC). METHODS: The flowers of Impatiens glandulifera were manually separated and extracted with ethanol. LC-ESI-MS/MS was used to characterise the crude extract of IGF. The various flavonoids detected were isolated by HPCCC using of methyl tert-butyl ether-acetonitrile-water (2:2:3, v/v/v). The combination of the data provided by preparative ESI-MS/MS metabolite profiling, LC-ESI-MS/MS, UV-vis and 1D/2D-NMR spectroscopic analysis was used to elucidate the structures of the isolated compounds. RESULTS: HPCCC runs led to the direct isolation of pure dihydromyricetin (ampelopsin), eriodictyol-7-O-glucoside, kaempferol-3-O-glucoside (astragalin) and kaempferol-3-O-6''-malonyl-glucoside, as well as the pre-purification of kaempferol-3-O-rhamno-rhamnosyldiglucoside, quercetin-3-O-galactoside (hyperoside), quercetin and kaempferol in a single step. CONCLUSION: This is the first report on the flavonoid composition of the species Impatiens glandulifera. The developed protocol was successfully used to isolate the main flavonoids from the crude extract of IGFs. This combined HPCCC and HPLC procedure could be applied to the fast fractionation and recovery of flavonoid derivatives of other plant extracts.	Phytochemical Analysis
91	Simple quantitative method for low molecular weight dissolved organic matter extracted from natural waters based upon HPCCC	Rojas, A	2016	A simple, high-performance counter-current chromatography method with sequential UV absorbance (254 nm) and evaporative light scattering detection (ELSD) was developed for the quantification of pre-extracted low molecular weight dissolved organic matter (DOM) extracted from natural waters. The method requires solid-phase extraction (SPE) extraction of only small volumes of water samples, here using poly(styrenedivinylbenzene)-based extraction cartridges (Varian PPL). The extracted and concentrated DOM was quantified using reversed-phase high-performance counter-current chromatography (HPCCC), with a water/methanol (5:5) mobile phase and hexane/ethyl acetate (3:7) stationary phase. The critical chromatographic parameters were optimised, applying a revolution speed of 1900 rpm and a flow-rate of 1 mL min(-1). Under these conditions, 50 μL of extracted DOM solution could be injected and quantified using calibration against a reference natural dissolved material (Suwannee River), based upon UV absorbance at 254 nm and ELSD detection. Both detection methods provided excellent linearity (R(2) > 0.995) for DOM across the concentration ranges of interest, with limits of detection of 4 μg ml(-1) and 7 μg ml(-1) for ELSD and UV absorbance, respectively. The method was validated for peak area precision (<5%), and accuracy and recovery based upon spiking seawater samples prior to extraction, together with DOM solutions post-extraction (>95% recovery). The developed method was applied to the determination of the concentration of DOM in seawater, based upon initial sample volumes as small as 20 mL.	Analytica Chimica Acta
92	Schinus terebinthifolius countercurrent chromatography (Part II): Intra-apparatus scale-up and inter-apparatus method transfer	Das Neves Costa, F	2016	Countercurrent chromatography (CCC) is being widely used across the world for purification of various materials, especially in natural product research. The predictability of CCC scale-up has been successfully demonstrated using specially designed instruments of the same manufacturer. The reality is that the most of CCC users do not have access to such instruments and do not have enough experience to transfer methods from one CCC column to another. This unique study of three international teams is based on innovative approach to simplify the scale-up between different CCC machines using fractionation of Schinus terebinthifolius berries dichloromethane extract as a case study. The optimized separation methodology, recently developed by the authors (Part I), was repeatedly performed on CCC columns of different design available at most research laboratories across the world. Hexane – ethyl acetate – methanol – water (6:1:6:1, v/v/v/v) was used as solvent system with masticadienonic and 3β-masticadienolic acids as target compounds to monitor stationary phase retention and calculate peak resolution. It has been demonstrated that volumetric, linear and length scale-up transfer factors based on column characteristics can be directly applied to different i.d., volume and length columns independently on instrument make in an intra-apparatus scale-up and inter-apparatus method transfer.	Journal of Chemistry A
93	Application of HPCCC, UHPLC and HPLC for a rapid, one-step prep separation and quantification of rutin in Forsythia flowers	Kicel, A	2015	In this study, the flowers of Forsythia × intermedia, Forsythia suspensa and Forsythiaovata are presented as valued raw materials for isolation of rutin, an industrial and pharmacologically active flavonoid. An efficient strategy based on three high-performance chromatographic techniques was established to screen and purify the target compound from the plant matrices. In the first step, an UHPLC-PDA-ESI-MS3 assay documented the relatively simple phytochemical profiles of the flower extracts and led to the identification of ten polyphenols classified as caffeoyl acid derivatives, flavonoids, phenylethanoids and lignans, with rutin as the major constituent. Significant levels of rutin (20.0–57.6 mg/g dw) were found in the flowers by the validated HPLC-PDA method, with the highest values observed for F. suspensa. Finally, a novel, fast, single-step HPCCC method was developed and successfully applied for the separation of rutin from the plant samples. The ethyl acetate-n-butanol-water (4:1:5, v/v/v) was selected as the optimum two-phase solvent system for both analytical and preparative normal (NP) and reversed-phase (RP) HPCCC separations. Under optimal RP-HPCCC conditions, 155 mg of the crude methanol extract was separated in a single 50 min run, yielding 14.6 mg of rutin with 97.2% HPLC purity and 95.9% recovery. The process efficiency of the HPCCC rutin isolation was demonstrated to be superior to the existing HSCCC methods. The results indicated the usefulness of Forsythia flowers and the developed RP-HPCCC methodology for practical and commercial applications.	Industrial Crops and Products
94	Isolation and purification of resveratrol from a grape twig extract using HPCCC	Shin, H	2008	Resveratrol, a polyphenolic compound with antioxidative property, was purified from the grape's twig to be used as functional additives of food and/or cosmetics. Extraction of the grape's twig was performed using 80% ethanol in ultrasonic extractor for 60 min. The crude extract was purified up to 99% after elution through silica gel open column chromatography. The stability of the purified resveratrol was as follows: a half life of 90 days at 4.0 and 60 days at 25. A sensory test of the commercial grape juice including the 1-10 ppm of purified resveratrol showed better preference than the grape juice without purified resveratrol additive. Color and smell test showed no difference between the samples. The grape twig can be used as a valuable resource for the extraction of resveratrol, which would be added to nutraceutical and cosmetic products.	Advanced Engineering and Technology
95	Development of a Scalable and Sustainable High Performance CounterCurrent Chromatography (HPCCC) Purification for Spinosyn A and Spinosyn D from Spinosad	DeAmicis, C	2017	A high performance countercurrent chromatography (HPCCC) process was developed as a more efficient and sustainable alternative to reverse phase high performance liquid chromatography (RP-HPLC) for the pilot scale purification of the naturally occurring fermentation-derived insecticides, spinosyn A and spinosyn D, the major components of spinosad insecticide. While on pilot scale HPCCC and RP-HPLC both gave >99% purities and comparable combined recoveries of 77% and 83%, respectively, HPCCC was more efficient and sustainable by producing a 60% higher productivity, 11 times higher solute loading, 96% savings in stationary phase costs, and 42% reduction in solvent usage. The increase in productivity and reduction in solvent usage further reduced waste recycle and disposal costs, thus presenting significantly less environmental impact compared to RP-HPLC separations. Use of mixing on demand for solvent system at a preparative scale allowed a complete automation with minimized solvent consumption.	Organic Process Research & Development
96	Synthesis of medronic acid monoesters and their purification by HPCCC or by hydroxyapatite	Puljula, E	2016	We achieved the synthesis of important medronic acid monoalkyl esters via the dealkylation of mixed trimethyl monoalkyl esters of medronic acid. Two methods were developed for the purification of medronic acid monoesters: 1) small scale (10-20mg) purification by using hydroxyapatite and 2) large scale (tested up to 140mg) by high-performance countercurrent chromatography (HPCCC).	Beilstein Journal of Organic Chemistry
97	Phenolics from the Patagonian currants Ribes spp. Isolation, characterization and cytoprotective effects in the human AGS cells	Jimenez-Aspee, F	2016	The South American currants (Ribes spp.) are native species occurring in southern Chile and Argentina. Ripe fruits from Ribes cucullatum, Ribes magellanicum, Ribes punctatum and Ribes trilobum were investigated for antioxidant activity and phenolic constituents. The fruit extracts were submitted to membrane chromatography to separate the anthocyanins and copigments. Individual anthocyanins were isolated by high-performance counter-current chromatography and were identified as cyanidin-3-rutinoside, cyanidin-3-glucoside, delphinidin-3-glucoside and delphinidin-3-rutinoside. The main compound in the copigment fraction was 3-caffeoylquinic acid. Around 60 compounds were tentatively identified by HPLCDAD-MS/MSn. The fruit phenolics comprise 23 anthocyanins, 13 hydroxycinnamic acids (HCA) and 23 flavonols. From the polymeric fraction, (epi)-gallocatechin and (epi)catechin tetramers were identified after thiolytic depolymerization. Significant cytoprotection was exhibited by the extracts, anthocyanins and copigments against oxidative and dicarbonyl-induced stress in human gastric AGS cells. This study provides evidence on the potential of native Chilean currants as functional foods.	Journal of Functional Foods
98	Biological activity and safety profile of the essential oil from fruits of Heracleum mantegazzianum Sommier & Levier (Apiaceae)	Skalicka-Wozniak, K	2017	A composition of essential oils obtained from Heracleum mantegazzianum (Apiaceae) was examined using a GC-MS method. n-Octyl acetate (19.92%), n-hexyl-2-methylbutanoate (10.84%), n-octanol (10.13%), n-octyl butanoate (8.88%), n-octyl-2-methylbutanoate (8.01%), n-hexyl acetate (7.11%), n-octyl isobutanoate (5.5%) and n-hexyl isobutanoate (5.43%) were the main compounds. The high-performance counter-current chromatography was applied for purification of aliphatic alcohols and esters. A mixture of n-hexane, acetonitrile and tert-butyl methyl ether (1:1:0.1, v/v) allowed to obtain n-octanol, n-octyl acetate, n-hexyl-2- methylbutanoate, n-octyl isobutanoate and n-octyl-2-methylbutanoate, with the purity range of 94e99%, in one single 74 min run. The antimicrobial activity was also determined against plant and foodborne pathogens. While n-octanol shares responsibility for the antibacterial activity of the essential oil, n-octyl acetate determines its antifungal action. The cytotoxic activity assessed on two normal kidney fibroblast cell lines: Vero (animal) and HEK-293 (human embryonic), and two human cancer cell lines: FaDu (squamous cell carcinoma of the pharynx) and SCC25 (squamous cell carcinoma of the tongue), showed a moderate cytotoxicity with CC50 values ranging from 262.3 to 567.8 mg/mL. Results indicate that normal cell lines were more sensitive to the tested essential oil than cancer cell lines. The antioxidant activity of oil and pure compounds was not significant.	Food and Chemical Toxicology
99	Antiviral effect of compounds derived from Angelica archangelica L. on Herpes simplex virus-1 and Coxsackievirus B3 infections	Rajtar, B	2017	The dichloromethane extract from fruits of Angelica archangelica L. was separated by the modern highperformance countercurrent chromatography (HPCCC). The extract and five pure compounds: xanthotoxin, bergapten, imperatorin, phellopterin and isoimperatorin, and the mixture of imperatorin and phellopterin, have been studied as the potential antiviral agents against Herpes simplex virus type I and Coxsackievirus B3. The cytotoxicity was measured using the MTT method. Compounds were tested for the in vitro antiviral activity using the cytopathic effect (CPE) inhibitory assay and by the virus titre reduction assay. Real-time PCR was used to quantify the relative inhibition of the HSV-1 replication. The results indicate that the highest activity was demonstrated by the extract, imperatorin, phellopterin and the mixture of imperatorin and phellopterin, reducing the HSV-1 replication by 5.61 log, 4.7 log, 3.01 log and 3.73 log, respectively. The influence of isolated compounds on the CVB3 replication was not significant. Only the extract caused the decrease in the titre of virus in relation to the virus control. Our results show that coumarins of A. archangelica L. might be a potential candidate for the development of the alternative natural anti- HSV-1 compound. Moreover, the presence of isopentenyloxy moiety at C-8 position significantly improves their activity.	Food and Chemical Toxicology
100	Carrot seed essential oil - source of carotol and cytotoxicity study	Sieniawska, E	2016	Carrot seed essential oil is a common fragrance component in cosmetics and perfumes and a flavor ingredient in different categories of food products. It is also a major source of sesquiterpene alcohol carotol. In this work we aimed to compare carotol content in commercially available (Moroccan and French) and hydrodistilled (Polish) wild carrot seed essential oils (Daucus carota L. ssp. carota) and to evaluate cytotoxicity of these essential oils, as well as isolated carotol. The chemical composition of studied essential oils was analysed by gas chromatography- mass spectrometry. Cytotoxicity tests were performed on green monkey kidney (VERO) and human pharynx squamous cell carcinoma (FaDu) cell lines. Carotol and other terpenoids were isolated using high performance counter current chromatography technique in the reversed phase mode, with a mixtures of n-hexane/acetonitrile/tert-butyl methyl ether (1:1:0.1 and 2:1:0.1 v/v). Carotol was the main constituent in three carrot seed essential oils of different origin amounting 19–33% of the sum of compounds. -Pinene, sabinene, myrcene, limonene, geranyl acetate, bisabolene, cayophyllene oxide and daucol were identified as other main compounds. Separation of 280 mg of carrot seed essential oil yielded carotol (20 mg) and other terpenoids: -pinene, sabinene, limonene, geranyl acetate, caryophyllene oxide, and daucol with the purity in the range of 80–99%. The applied method enabled isolation of all main constituents of essential oil in the single run. The comparison of cytotoxicity of tested essential oils on VERO and FaDu cell lines indicated that Moroccan and French essential oils have similar cytotoxicity (35.3–46.1 g/mL), while Polish essential oil showed lower cytotoxic effect (70.9–96.3 g/mL). Carotol being the main constituent of all tested essential oils showed moderate cytotoxicity on both tested cell lines without any selectivity. ⊠	Industrial Crops and Products
101	Separation of the potential G-quadruplex ligands from the butanol extract of Zanthoxylum ailanthoides Sieb. & Zucc. by countercurrent chromatography and preparative high	Han, T	2017	G-quadruplex DNA structure is considered to be a very attractive target for antitumor drug design due to its unique role in maintaining telomerase activities. Therefore, discovering ligands with high stability of G-quadruplex structure is of great interest. In this paper, pH-zone refining counter current chromatography (CCC) and preparative high performance liquid chromatography (HPLC) were employed for the separation of potent G-quadruplex ligands from the n-butanol fraction of the crude extract of Zanthoxylum ailanthoides, which is a traditional Chinese medicine recently found to display high inhibitory activity against several human cancer cells. The 75% aqueous ethanol extract of the stem bark of Z. ailanthoides and its fractions with petroleum ether, ethyl acetate and n-butanol displayed almost the same G-quadruplex stabilization ability. Here, pH-zone refining CCC was used for the separation ofthe alkaloids from the n-butanol fraction by a seldom used solvent system composed of dichloromethane-methanolwater (4:1:2.5) with 10 mM TEA in the organic stationary phase as retainer and 10 mM HCl in the aqueous mobile phase as eluter. Compounds I, II and III were obtained, with purity greater than 95%, in the quantities of 31.2, 94.0, and 26.4 mg respectively from 300 mg of lipophilic fraction within 80 min, which were identified as three tetrahydroprotoberberines isolated for the firsttime in this plant. In addition, a phenylpropanoid glycoside compound IV (Syringin), an isoquinoline (Magnoflorine, V), and two lignin isomers (+)-lyoniresiol-3-O--d-glucopyranoside (VI) and (−)-lyoniresinol -3-O--D -glucopyranoside (VII) were isolated by traditional CCC together with preparative HPLC. Compounds IV, V, VI and VII were obtained, with purity greater than 95%, in the quantities of 4.0, 13.2, 6.7, and 6.5 mg respectively from 960 mg of hydrophilic fraction. Among the seven isolated compounds, tetrahydroprotoberberine I, II and III were found to display remarkable stabilization effects on G-quadruplex by increasing G-quadruplex's Tm approximately 10 ⊠C, which may be the most potent G-quadruplex ligands in Z. ailanthoides. ⊠	Journal of Chromatography A
102	Automated countercurrent chromatography method development and process scale-up at GlaxoSmithKline	Thornton, D	2017	The choice of an appropriate combination of stationary phase and mobile phase is essential in any purification technique. To expedite this task, we use automated method screening, typically with high performance liquid chromatography (HPLC),to reduce the time to develop a scalable purification process. We have extended this approach to countercurrent chromatography (CCC) and describe a customconfigured system using automation to execute the solvent screening process. We also present results from three examples of method transfer from analytical to preparative scale using CCC systems from two different manufacturers.	Journal of Chromatography A
103	Schinus terebinthifolius countercurrent chromatography (Part III): Method transfer from small countercurrent chromatography column to preparative centrifugal partition	Das Neves Costa, F	2016	Countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC) are support free liquid-liquid chromatography techniques sharing the same basic principles and features. Method transfer has previously been demonstrated for both techniques but never from one to another. This study aimed to show a feasibility using fractionation of Schinus terebinthifolius berries dichloromethane extract as a case study. Heptane – ethyl acetate – methanol –water (6:1:6:1, v/v/v/v) was used as solvent system with masticadienonic and 3-masticadienolic acids as target compounds. The optimized separation methodology previously described in Part I and II, was scaled up from an analytical hydrodynamic CCC column (17.4 mL) to preparative hydrostatic CPC instruments (250 mL and 303 mL) as a part of method development. Flow-rate and sample loading were further optimized on CPC. Mobile phase linear velocity is suggested as a transfer invariant parameter if the CPC column contains sufficient number of partition cells.	Journal of Chromatography A
104	Schinus terebinthifolius countercurrent chromatography (Part II): Intra-apparatus scale-up and inter-apparatus method transfer	Das Neves Costa, F	2016	Countercurrent chromatography (CCC) is being widely used across the world for purification of various materials, especially in natural product research. The predictability of CCC scale-up has been successfully demonstrated using specially designed instruments of the same manufacturer. The reality is that the most of CCC users do not have access to such instruments and do not have enough experience to transfer methods fromone CCCcolumnto another. This unique study ofthree internationalteams is based oninnovative approach to simplify the scale-up between different CCC machines using fractionation of Schinus terebinthifolius berries dichloromethane extract as a case study. The optimized separation methodology, recently developed by the authors (Part I), was repeatedly performed on CCC columns of different design available at most research laboratories across the world. Hexane – ethyl acetate – methanol – water (6:1:6:1, v/v/v/v) was used as solvent system with masticadienonic and 3-masticadienolic acids as target compounds to monitor stationary phase retention and calculate peak resolution. It has been demonstrated that volumetric, linear and length scale-up transfer factors based on column characteristics can be directly applied to different i.d., volume and length columns independently on instrument make in an intra-apparatus scale-up and inter-apparatus method transfer.	Journal of Chromatography A
105	Preparative separation of menthol and pulegone from peppermint oil (Mentha piperita L.) by high-performance counter-current chromatography	Skalicka-Wozniak, K	2014	The present paper describes a very convenient separation method of the six terpenoids: menthol and its isomers including neomenthol, isomenthone, and menthone, as well as terpinen-4-ol and pulegone, from peppermint oil (Mentha piperita L.) by high-performance counter-current chromatography (HPCCC). A two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (4:1:4:1, v/v) was applied. All compounds were separated with purity in a range between 94 and 99%, as determined by GC-MS. A novel method for the fast purification of active compounds, such as menthol and its derivatives, and detoxification, through the removal of pulegone, of one of the most popular essential oils was proposed for the first time. The method can be easily scaled-up for industrial purposes.	Phytochemistry Letters
106	Preparative separation of triterpene alcohol ferulates from rice bran oil using a high performance counter-current chromatography	Man, L	2013	A novel method for the separation of two major triterpene alcohol ferulates from rice bran oil (RBO) was developed using a high performance counter-current chromatography (HPCCC). A two-phase solvent system of n-hexane-acetonitrile (1:1, v/v) was applied to purify cycloartenyl ferulate (CAF) and 24-methylene cycloartanyl ferulate (24-mCAF) from RBO. The yields were 20.50 ± 2.60 mg CAF and 12.62 ± 1.15 mg 24-mCAF from 390 mg RBO through a two-step separation procedure. The purities of the two compounds were 97.97 ± 0.90% and 95.50 ± 0.75%, respectively, as determined by high performance liquid chromatography (HPLC). Their chemical structures were confirmed by ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS), and 1 H, 13C and 2D nuclear magnetic resonance (NMR). This represents the first report on direct separation of CAF and 24-mCAF from RBO by HPCCC.	Food Chemistry
107	Fast high resolution Orbitrap MS fingerprinting of the resin of Heliotropium taltalense Phil. from the Atacama Desert	Simirgiotis, M	2016	Mass spectrometry has become the preferred analytical technique to characterize organic compounds in biological samples because of its sensitivity and selectivity. Hybrid UHPLC coupled to Orbitrap mass analyzer is an innovative state of the art technology that allows fast and accurate metabolomic analyses. In this work, Several flavanones, flavonols, and benzoic acid geranyl derivatives were rapidly identified in the resinous exudate of Heliotropium taltalense Phil., an endemic species growing in Paposo valley northern Chile, by means of ultrahigh resolution liquid chromatography Orbitrap MS-MS analysis (UHPLC-PDA-OT-MS). The relationship between the compounds detected and some biosynthetic pathways or mechanisms of formation of the geranyl derivatives are proposed. Furthermore, some common flavonoids and the new compound 3,4-dihydroxy-5-geranyl-benzoic acid were isolated from the plant resin using liquid-liquid high speed countercurrent chromatography (HPCCC). The new structure was elucidated by spectroscopic means (NMR).	Industrial Crops and Products
108	Semi-preparative high-performance countercurrent chromatography method for the purification of chemically synthesized ATP analogue, Appl	Puljula, E	2017	An efficient high-performance countercurrent chromatography (HPCCC) based method has been developed for the purification of chemically synthesized 1-adenosin-5⊠-yl 3-(3-methylbut-3-enyl)triphosphoric acid diester (ApppI). ApppI is an adenosine triphosphate (ATP) analogue with biological significance due to its varied actions in the body. ApppI was synthesized and purified as its tetrabutylammonium (TBA) salt and converted successfully into its more practical sodium salt form after purification. The amount of TBA hydroxide (2.0, 2.5 and 3.0 eq) used in the synthesis of ApppI was shown to exert an effect on the purification process with HPCCC and on the overall yield (8%, 16% and 22%, respectively). 1-Adenosin-5⊠-yl 3-(3-methylbut-3-enyl)diphosphoric acid diester (AppI) was also isolated as a side product.	Journal of Chromatography B
109	Intermittent counter-current extraction–Equilibrium cell model, scaling and an improved bobbin design	Hewitson, P	2013	This paper describes an equilibrium cell model for intermittent counter-current extraction that is analytically solved for the first time for continuous sample injection between a pair of columns. The model is compared with practice for injections of a model mixture of compounds on a standard high-performance counter-current chromatography instrument giving good agreement for compound elution order and the times to maximum concentration for the eluted components. An improved design of end fittings for the counter-current chromatography bobbins is described which permits on-column switching of the mobile and stationary phases. This on-column switching successfully eliminates the displaced stationary phase seen in fractions when operating ICcE with standard flying leads and gives a 6% reduction in the retention time of compounds and improved resolution due to the elimination of the time delay required to pump the previous mobile phase from standard flying leads.	Journal of Chromatography A

www.dynamicextractions.com
info@dynamicextractions.com
+44(0) 1495 726 626