Review Article

High Speed Counter-Current Chromatography: A Modern Tool for Selective and Greener Extraction of Bioactive Phytochemicals from Rhubarb

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Received: 08-09-2022; **Revised:** 24-10-2022; **Accepted:** 08-11-2022; **Published on:** 15-11-2022.

ABSTRACT

Plants are important source of structurally diverse compounds for drug development. But the amounts of these bioactive phytochemicals in herbal medicines are very low. So, effective and specific methods should be developed for the selective extraction, isolation and purification of those active constituents. Besides the conventional methods, such as maceration, percolation, reflux and solvent extraction, a variety of modern techniques are also available in literature which offer more rapid and selective extraction of a particular compound with lesser consumption of organic solvents. These include pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), ultrasound assisted extraction (UAE) etc. For purification and separation of important components from medicinal plants, several chromatographic and electrochemical processes are available. In recent years, high speed counter current chromatography (HSCCC) has been a subject of increased attention for a green separation technique for selective natural products with high extraction yields. It involves the partition of a particular solute between two immiscible solvents. Some aspects of HSCCC in the field of isolation of bioactive molecules from plants are described in this mini review.

Keywords: Phytochemical screening, HSCCC, Green extraction, Bioactive secondary metabolites, Rhubarb.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v77i01.028

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v77i01.028

INTRODUCTION

Plants are considered as a unique reservoir of structurally diverse compounds which are proved to be versatile resource for the development of novel therapeutics. [1,2] In a recent report by World Health Organization (WHO) it was estimated that ~80% people in the world rely almost entirely on herbal medicines for their healthcare requirements. [3] In USA, ~12% population used plant-based drugs worth nearly five billion dollars and during last two decades in Europe, the consumption of botanical drugs is almost doubled. [4] In order to get the bioactive phytochemicals, which might provide novel drug leads, plant extracts should be simultaneously subjected to phytochemical analysis and screening against various pharmacological targets. [5,6] In fact, isolation, purification and finally structural elucidation of particular biologically active compounds from plants are big challenge for phytochemists. In majority of the cases, active ingredients are present in very minute amounts, so appropriate strategies should be developed for the phytochemical screening of a crude plant extract. Different methods (maceration, percolation, digestion, soxhlet extraction, supercritical fluid extraction, microwave-assisted, ultrasonic extraction) are available in literature for the extraction of biologically active components depending upon the nature of compounds which would be extracted from the medicinal plants. [7] For separation and purification of those important constituents, different chromatographic techniques viz. column chromatography (CC), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), counter current chromatography (CCC) etc. are employed, in which adsorption chromatography is most conveniently used. [8-11] High speed counter current chromatography (HSCCC) is fast - growing, partition chromatographic technique extensively used for the detection, separation and estimation of pharmacologically active phytochemicals. [12-17]

Rhubarb (Da-huang, Fig. 1) is one of the best-known herbal medicine used for thousands of years in Chinese pharmacopoeia. [18] It is mainly dried roots and rhizomes of different *Rheum species* (*R. palmatum*, *R. officinale*, *R. emodi*, *R tanguticum* etc.). [19] In traditional Oriental and some Western medicinal systems, it is well documented for the treatment of various ailments. [20-22] Modern researches showed that hydroxy anthraquinonoids are the main bioactive compounds in rhubarb and *Rheum* containing herbal preparations. [23,24] These are very popular purgative and laxative botanical remedies and used indiscriminately in almost every country across the world. To check the safety, authenticity and efficacy of these phytomedicines, evaluation of the quality control is very much needed. [25,26] Several analytical tools have been developed for the quantification and standardization of these marker constituents for overall assessments

warranting the reliability of these plant derived drugs. [27,28] Including different chromatographic methods, HSCCC is also efficiently applied for separation and estimation of anthraquinonoids in rhubarb samples. In this brief review, some of the reports on analysis of these therapeutically active compounds by HSCCC techniques have been described.

Figure 1: Rhubarb **(a)** plant; **(b)** roots.

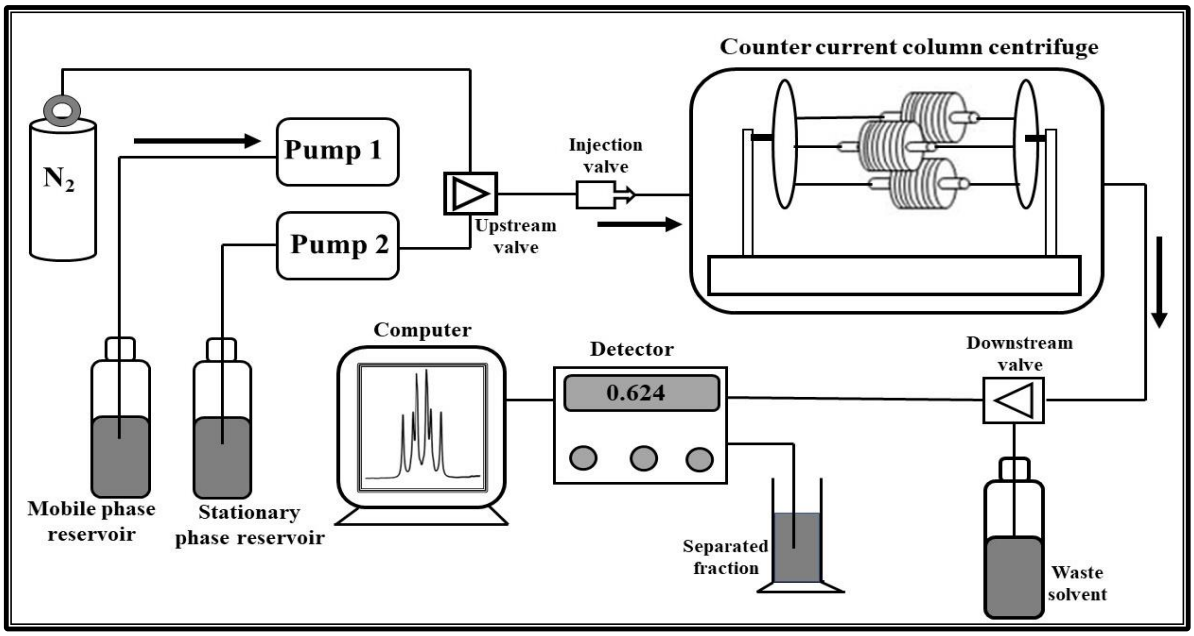


Figure 2: Schematic diagram of HSCCC instrumentation.

HSCCC: A CONVENIENT ANALYTICAL TOOL

High speed counter current chromatography is a solid support free liquid-liquid partition chromatographic technique, first introduced by Ito et al. in the early 80s. [29-31] and subsequently developed by several group of researchers. Two immiscible liquids are taken in this process - one retained in the column (*i.e.*, in the spinning coil by centrifugal partition forces) is the stationary phase and the other which is in mobile phase is pumped through it. [32] In fact, various natures of components presents in a crude material which are subjected to be separated by this method have differences in solubilities between the stationary and mobile phases, so appropriate selection of two-phase solvent system is very crucial. [33] Minute changes in the composition of the mobile phase can disturb the stationary phase composition also. Two solvents must be inert and innocuous. In a specific two-phase solvent system, compounds having almost similar polarities can have different partition coefficients, which results in the separation of baseline by counter current chromatography. Most commonly used solvents are *n*-hexane, ethyl acetate, methanol, water etc. Like high performance liquid chromatography, here also two modes of operations (normal phase as well as reversed phase chromatography) are available. [34]

Briefly, it is a three-stage process involving mixing, settling and separation of two-solvent phases (Fig. 2). [35] In the counter current column centrifuge, two liquid phases moving in opposite directions (thus called counter current) first come in contact with each other, two solvents must be

properly mixed together to achieve effective partitioning of analytes. [36,37] Then settling action allows the components to be distributed in two phases by their respective solubilities and finally, the separated compounds are detected by using various detectors like ultra violet or mass spectrometrically.[38,39]

Advantages and Disadvantages of HSCCC over the other conventional analytical methods

A broad range of molecules have been successfully extracted and purified by HSCCC. [40] Compared to conventional solid-liquid separation methods, here the stationary phase is liquid, so there is no scope for irreversible adsorption of the sample on the solid support matrix, which minimises the contamination and denaturation of the sample. Various polar and non-polar solvent systems can be used and the solutes have distributed to the whole volume of the liquid stationary phase resulting high extraction efficiency. Since both the phases are liquids, column regeneration is not needed, same column can be repeatedly used for separation with different stationary phases, and also no sample is lost during chromatographic separation. [41-43] Consequently, excellent recovery of the sample load could be obtained. Low solvent consumption, rapid separation, high resolution of the components is also achieved in this process. Usage of toxic chemicals (like chloroform, carbon tetrachloride) is generally avoided in this method. Low chemical and instrumental costs make this technique more economic than other chromatographic systems.[44]

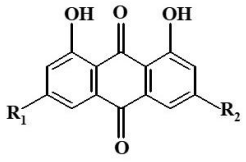
Both stationary and mobile phase are very much sensitive to the solution composition. Minute change in the composition ratio of any liquid phase may directly induce a variation in the same of the other liquid phase. It is very difficult to hold the liquid stationary phase in a steady state for a long time while the immiscible mobile phase is pushed through it. Sometimes low peak resolution is resulted. [45,46] During the separation process by HSCCC, only milligram - sized sample injections are available, so development in the field of small-scale research is somewhat faltered. [47,48]

Some applications

The counter current chromatography has been extensively used in detection, separation and purification of a wide variety of substances including various primary and secondary metabolites.[49-52] By using this method, natural or synthetic materials, heavy metal ions, antibodies, hydrocarbons from environmental samples etc. can also be analysed.[53-60] Most commonly, this method is used for the estimation, extraction and preparative-scale separation of phytochemicals having commercial importance. This can be exemplified by the analysis of the active constituents of a popular herbal drug, viz. rhubarb, which is discussed in the next section.

ANALYSIS OF BIOACTIVE COMPONENTS OF RHUBARB BY HSCCC

Five hydroxy anthraquinonoids viz. emodin, aloe-emodin, physcion, rhein and chrysophanol (Fig. 3) are the main active constituents of rhubarb which are also referred as 'taxonomic markers' of the respective plants. [23] To assess the quality control of this herbal medicine, several analytical techniques have been conveniently employed for the estimation and standardization of these marker compounds. [25]



	R_1	R_2
Emodin	-OH	$-CH_3$
Aloe-emodin	-H	$-CH_2OH$
Physcion	$-OCH_3$	$-CH_3$
Rhein	-H	$-CO_2H$
Chrysophanol	-H	$-CH_3$

Figure 3: Major bioactive hydroxy anthraquinonoids present in rhubarb

During the chromatographic separation of hydroxy quinonoids, it has been found that, these compounds often tend to remain strongly adsorbed on the solid stationary phase (generally silica gel).[61] This problem can be successfully overcome by using high speed counter current chromatographic technique. Since, it is a solid support free liquid-liquid partition chromatography, so it can easily eliminate the irreversible binding of those type of compounds onto the solid support.[33]

Separation of five hydroxy anthraquinonoid derivatives from the rhizomes of *R. palmatum* by analytical HSCCC was first reported by Zhang and his colleagues in 1988.[62] For rapid separation and high yield of the separated materials, they used combined modes of normal and reversed elution of n-hexane-ethyl acetate-methanol-water (9:1:5:5, v/v/v/v) as two-phase solvent at a flow rate of 60mL/hour followed by UV detection at 278 nm. Within 70 minutes, 1 mg of crude sample was separated and purified into its major components.

A pH modulated stepwise elution in HSCCC was performed by Yang et al. for analytical as well as preparative isolation and purification of these five anthraquinones (Fig. 4) from the crude ethanolic extract of Chinese rhubarb (*Rheum officinale* Baill).[63] The biphasic solvent system was optimized by mixing diethyl ether as stationary phase and basic water as mobile phase. A portion of the aqueous phase was basified by adding 4% $NaHCO_3$, 0.7% Na_2CO_3, and 0.2% NaOH, thereby three mobile phases with different pH values were obtained which were successively eluted through the column maintaining in an increasing order of pH. Except rhein, rest four major hydroxyanthraquinonoids were well resolved and in preparative HSCCC, more than 98% purity of these compounds was found.

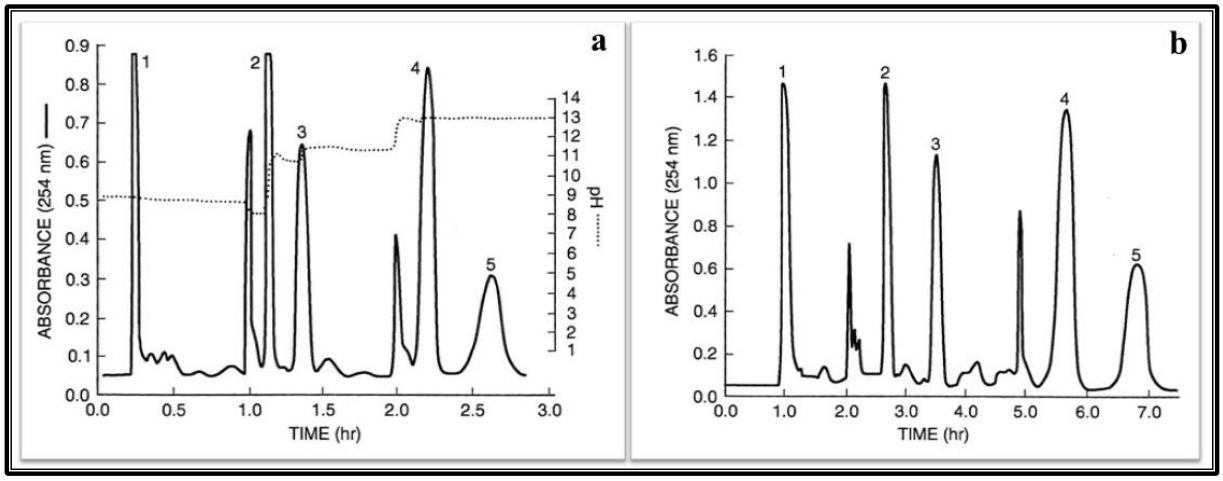


Figure 4: Chromatogram of the crude sample of hydroxyanthraquinones from *Rheum officinale* Baill by **(a)** analytical HSCCC and **(b)** preparative HSCCC. Solvent system for **(a)** analytical HSCCC: stationary phase (upper organic phase): diethyl ether-basic water; mobile phase: 35 mL of 4.0% $NaHCO_3$ and 55 mL of 0.7% Na_2CO_3 and 80 mL of 0.2% NaOH, flow-rate: 1.0 mL/min; revolution speed: 1500 rpm; sample size: 10 mg dissolved in 1 mL stationary phase; Solvent system for **(b)** preparative HSCCC: stationary phase (upper organic phase): diethyl ether-basic water; mobile phase: 120 mL of 4.0% $NaHCO_3$ and 240 mL of 0.7% Na_2CO_3 and 480 mL of 0.2% NaOH, flow-rate: 2.0 mL/min; revolution speed: 800 rpm; sample size: 300 mg dissolved in 20 mL stationary phase; retention of the stationary phase: 50%. Peaks: 1 = rhein + unknown; 2 = emodin; 3 = aloe-emodin; 4 = chrysophanol; 5 = physcion. Reproduced from Yang et al. [63] with permission from Elsevier.

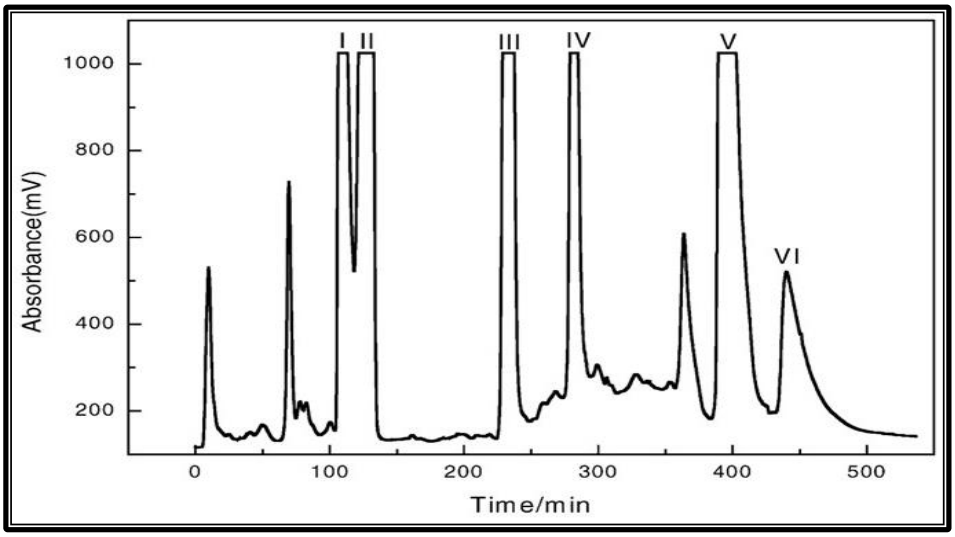


Figure 5: HSCCC chromatogram of crude extract from R. officinale Baill. Stationary phase: ether; mobile phase: 1% NaH2PO4 and 1% NaOH to perform pH-gradient elution (1% NaH_2PO_4:1% NaOH = 100:0–0:100 in 500 min); flow rate: 2.0 mL /min; revolution speed: 800 rpm; sample size: 120 mg crude extract dissolved in 20 mL of ether; temperature: 25°C; retention of the stationary phase: 40%. I: Rhein; II: cinnamic acid; III: emodin; IV: aloe-emodin; V: chrysophanol; VI: physcion. Reproduced from Liu et al. [64] with permission from Elsevier.

A continuous pH gradient elution in HSCCC was also established by Liu et al., in which preparative isolation of these five anthraquinonoid compounds in the root extract of *Rheum officinale* Baill was carried out by using ether as stationary phase and 1% aqueous solution of NaH_2PO_4 and 1% aqueous solution of NaOH as mobile phase in gradient mode (1% NaH_2PO_4 : 1% NaOH = 100:0 to 0:100 for 500 minutes). Including rhein, all the constituent quinonoids along with cinnamic acid were well separated under this optimum condition (Fig. 5). [64]

Wei et al. developed a repeated HSCCC system for preparative separation and purification of rhein from the crude ethanolic extract of *R. officinale*. [65] Under optimum condition, efficient resolution of rhein was achieved by analytical HSCCC using the two-phase solvent system composed of n-hexane-ethyl acetate- methanol-water = 3:7:5:5 (v/v/v/v) at a flow rate of 1.0 mL/min, revolution speed 1800 rpm followed by continuous monitoring with UV at 254 nm. Preparative HSCCC was also carried out by using the same solvent ratio at a flow rate of 2.0 mL/min with revolution speed of 800 rpm yielding 6.7 mg of rhein

(purity over 97% as determined by HPLC) from 500 mg the crude plant extract.

Ma et al. described a method for the separation and purification of rhein and emodin along with other five compounds from *R. palmatum* by using HSCCC combined with rapid preparative chromatography followed by LC-ESI-MS analysis. [66] From HSCCC separation, the purity of rhein was over 98.07% and that for emodin was found to be 94.76% from RPC separation.

CONCLUSION

High speed counter current chromatography is versatile and dynamic method for quantitative analysis, systematic purification and separation of a wide variety of materials including both organic and inorganic substances. It is a cost-effective technique with high resolution and separation efficiency of the components of the sample, particularly in the field of analytical phytochemistry. In fact, bioactive phytochemicals are present in very little amount in plants. By future development in machineries and protocols of HSCCC, there will be a tendency to affluence the quantity of the analytes from milligrams to grams and then further scale-up to kilograms level, and that will make this tool an excellent weapon for the separation of those active constituents.

REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products. 2020; 83: 770-803. Doi: 10.1021/acs.jnatprod.9b01285
2. Gu J, Gui Y, Chen L, Yuan G, Lu H-Z, Xu X. Use of natural products as chemical library for drug discovery and network pharmacology. PLos One. 2013; 8(4): e62839. doi: 10.1371/journal.pone.0062839
3. World Health Organization: WHO global report on traditional and complementary medicine 2019. Geneva; 2019. ISBN 978-92-4-151543-6
4. Aslam MS, Ahmed MS. Worldwide importance of medicinal plants: Current and historical perspectives. 2016 Recent Advances in Biology and Medicine. 2016; 2: 88-93. doi:10.18639/RABM.2016.02.338811
5. Mera IFG, Falconí DEG, Morera V. Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities. Revista Bionatura. 2019; 4: 1000-9. Doi: 10.21931/RB/2019.04.04.11
6. Nyiredy S. Separation strategies of plant constituents-current status. Journal of Chromatography B. 2004; 812: 35-51. doi: 10.1016/j.jchromb.2004.08.046
7. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020; 12: 1-10. doi: 10.4103/jpbs.JPBS_175_19
8. Marston A. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry. 2007; 68: 2786-2798. doi: 10.1016/j.phytochem.2007.08.004
9. Tugizimana F, Piater L, Dubery I. Plant metabolomics: A new frontier in phytochemical analysis. South African Journal of Sciences. 2013; 109(5/6): 1-11. doi: 10.1590/sajs.2013/20120005
10. Wu H, Guo J, Chen S, Liu X, Zhou Y, Zhang X, Xu X. Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography-mass spectrometry. Journal of Pharmaceutical Biomedical Analysis. 2013; 72: 267-291. Doi: 10.1016/j.jpba.2012.09.004
11. Oladimeji AV, Valan MF. HPLC techniques for phytochemistry. 2020 International Journal of Chemical Studies.2020; 8: 2590-2596. Doi: 10.22271/chemi.2020.v8.i6ak.11174
12. Sponrna-Kucab AE, Wybraniec S. High-speed counter-current chromatography in separation and identification of saponins from *Beta vulgaris* L. Cultivar Red Sphere. Polish Journal of Food and Nutrition Sciences. 2020; 70: 67-74. Doi: 10.31883/pjfns/116416
13. Slavik B, Roehrer S, Loos HM, Minceva M, Buettner A. Isolation of sesquiterpenoids from *Matricaria chamomilla* by means of solvent assisted flavor evaporation and centrifugal partition chromatography. Analytical and Bioanalytical Chemistry. 2021; 413: 4387–4396. Doi;10.1007/s00216-021-03400-w
14. Marston A, Hostettman K. Developments in the application of counter-current chromatography to plant analysis. Journal of Chromatography A. 2006; 1112: 181-194. doi: 10.1016/j.chroma.2005.10.018
15. Berthod A, Ruiz-Angel MJ, Carda-Broch S. Countercurrent chromatography: People and applications. Journal of Chromatography A. 2009; 1216: 4206-421. doi: 10.1016/j.chroma.2008.10.071
16. Costa FN, Leitao GG. Strategies of solvent system selection for the isolation of flavonoids by countercurrent chromatography. Journal of Separation Science. 2010; 33: 336-347. Doi: 10.1002/jssc.200900632
17. Fang L, Liu Y, Yang B, Wang X, Huang L. Separation of alkaloids from herbs using high-speed counter-current chromatography. Journal of Separation Science. 2011; 34: 2545-2558. doi: 10.1002/jssc.201000857
18. State Pharmacopoeia Committee. Pharmacopoeia of the People's Republic of China 2012. Beijing: Medical Science and Technology Press 22.
19. Fu XS, Cheng F, Liu XH, Xu H, Zhou YZ. Progress in research of chemical constituents and pharmacological actions of Rhubarb.Chinese Journal of New Drugs. 2011; 20: 1534-1539.
20. Rehman H, Begum W, Anjum F, Tabasum H. *Rheum emodi* (Rhubarb) : A fascinating herb. Journal of Pharmacognosy Phytochemistry. 2014; 3: 89-94.
21. National Pharmacopoeia Committee. Pharmacopoeia of Peoples Republic of China Part 1. 2020 : China Medical Science Press 24-25.
22. Rokaya M B, Münzbergová Z, Timsina B and Bhattarai K R. Rheum australe D. Don: a review of its botany, ethnobotany, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2012; 141; 761-774. doi: 10.1016/j.jep.2012.03.048

23. Cao Y J, Pu Z J, Tang Y P, Shen J, Chen Y-Y, Kang A, Zhou G-S and Duan J-A. Advances in bio-active constituents, pharmacology and clinical applications of rhubarb. Chinese Medicine. 2017; 12: 36. doi: 10.1186/s13020-017-0158-5.

24. Huang Q, Lu G, Shen H, Chung MCM, Choon NO. Anti-cancer properties of anthraquinones from rhubarb. *Medicinal Research Reviews.* 2007; 27: 609-630. doi: 10.1002/med.20094.

25. Chen Sun L, Yuan H, Wu A, Lu J, Ma S. A holistic strategy for quality and safety control of traditional Chinese medicines by the "iVarious" standard system. Journal of Pharmaceutical Analysis. 2017;7 : 271-279. doi: 10.1016/j.jpha.2017.07.008

26. Indrayanto G. Recent Development of Quality Control Methods for Herbal Derived Drug Preparations. Natural Product Communications. 2018; 13: 1599-1606. doi:10.1177/1934578X1801301208

27. Zhang H-X, Liu M-C. Separation procedures for the pharmacologically active components of rhubarb. Journal of Chromatography B. 2004; 812: 175-181. https://doi.org/10.1016/j.jchromb.2004.08.010

28. Xiang H, Zuo J, Guo F, Dong D. What we already know about rhubarb: a comprehensive review. Chinese Medicine. 2020;15; 88. https://doi.org/10.1186/s13020-020-00370-6

29. Ito Y, and Bowman RL. Countercurrent chromatography: liquid-liquid partition chromatography without solid support. Science. 1970; 167: 281-283. doi:10.1126/science.167.3916.281

30. Ito Y, Conway WD. Experimental observations of the hydrodynamic behavior of solvent systems in high-speed counter-current chromatography: III. Effects of physical properties of the solvent systems and operating temperature on the distribution of two-phase solvent systems. Journal of Chromatography. 1984; 301; 405-414. Doi:10.1016/S0021-9673(01)89214-1

31. Ito Y, Conway WD. High-speed countercurrent chromatography. CRC Critical Reviews in Analalytical Chemistry. 1986; 17: 65-143. Doi: 10.1080/10408348608542792

32. Han Q-B, Wong L, Yang N-Y, Song J-Z, Q C-F, Yiu Hillary, Ito Y, Xu H-X. A simple method to optimize the HSCCC two-phase solvent system by predicting the partition coefficient for target compound. Journal of Separation Science. 2008; 31: 1189-1194. Doi:10.1002/jssc.200700582

33. Sethi N, Anand A, Sharma A, Chandrul K K, Jain G, Srinivasa K S. High speed counter current chromatography: A support-free LC technique. Journal of Pharmacy and Bioallied Sciences. 2009; 1; 8-15. Doi: 10.4103/0975-7406.62680

34. Ito Y, Clary R, Powell J, Knight M, Finn TM. Spiral Tube Assembly for High-Speed Countercurrent Chromatography: Choice of Elution Modes for Four Typical Two-Phase Solvent Systems. Journal of Liquid Chromatography and Related Technologies. 2009; 32: 2013-2029. Doi; 10.1080/10826070903126823

35. Wood PL, Sutherland IA. Mixing, settling, and the movement of the interface between the mobile anf stationary phases in CCC. Journal of Liquid Chromatography and Related Technologies. 2001; 24: 1699-1710. Doi: 10.1081/JLC-100104372.

36. Conway WD. Pseudo-two-Component Notation for Heptane/Alcohol/Water Systems in Countercurrent Chromatography. Journal of Liquid Chromatography. 1990; **13:** 2409-2419. Doi:10.1080/01483919008049042

37. Berthod A, Maryutina T, Spivakov B, Shpigun O, Sutherland IA. Counter current chromatography in analytical chemistry(IUPAC technical report). Pure Applied Chemistry. 2009; 81: 355-387. doi:10.1351/PAC-REP-08-06-05

38. Zhou T, Chen B, Fan G, Chai Y and Wu Y. Application of high-speed counter-current chromatography coupled with high-performance liquid chromatography–diode array detection for the preparative isolation and purification of hyperoside from *Hypericum perforatum* with online purity monitoring. Journal of Chromatography A. 2006; 1116: 99-100. Doi: 10.1016/j.chroma.2006.03.041

39. Lee YW, Voyksner RD, Pack TW, Cook CE, Fang QC, Ito Y. Application of countercurrent chromatography/thermospray mass spectrometry for the identification of bioactive lignans from plant natural products. Analytical Chemistry. 1990; 62: 244-248. Doi: 10.1021/ac00202a004

40. Berthod A. Countercurrent chromatography and the Journal of liquid chromatography: A love story. Journal of Liquid Chromatography and Related Technologies. 2007; 30: 1447-1463. Doi: 10.1080/10826070701277067

41. Abe H, Usuda S, Takeishi H, Tachimori S. Separation of light lanthanoids by centrifugal partition chromatography in 30% TBP extraction system. Journal of Chromatography A. 1993; 16: 2661-2672. Doi; 10.1080/10826079308019601

42. Conway WD. An indexing scheme for optimizing the choice of biphasic system for CCC. Journal of Liquid Chromatography and Related Technologies. 2001: 24 1555-1573. Doi: 10.1081/JLC-100104363

43. Freisen JB, Pauli GF. Rational development of solvent system families in counter-current chromatography. Journal of Chromatography A. 2005; 1151: 51-59. Doi: 10.1016/j.chroma.2007.01.126

44. Gu M, Ouyang F, Su Z. Comparison of high-speed counter-current chromatography and high-performance liquid chromatography on fingerprinting of Chinese traditional medicine. Journal of Chromatography A. 2004;1022: 139-144. Doi: 10.1016/j.chroma.2003.09.038

45. Keay D, Wood P. http://www.iscnewsletter.com/newsletter/2008/nov/lin-2008/art4.pdf

46. Ito Y. Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography. Journal of Chromatography A. 2005; 1065: 145-168. Doi: 10.1016/j.chroma.2004.12.044

47. Sutherland I A, Hawes D, van den Heuvel R, Janaway L, Tinnion E. Journal of Liquid Chromatography and Related Technologies. 2003; 26: 1555-1573. Doi: 10.1016/j.chroma.2004.12.044

48. Wood P, Ignatova S, Janaway L. Keay D, Hawes D, Garrard I, Sutherland IA. Journal of Chromatography A. 2007; 1151; 25-30. Doi:10.1016/j.chroma.2004.12.044

49. Rahman M. Computational phytochemistry. In Application of Computational Methods in Isolation of Plant Secondary Metabolites eds Sarker S D, Nahar L. Amsterdam: Elsevier;

2018. chapter 4: p. 107-139. ISBN 9780128123645

50. Li H, Chen F. Bioactive natural products: Detection, isolation, and structural determination in Application of high speed countercurrent chromatography to the isolation of bioactive natural products (vol 2) eds Colegate S M and Molyneux R J. New York: CRC Press; 2007. chapter 10: p. 267-298.

51. McAlpine J B, Morris P. Separation by high speed Counter current chromatography. In: Methods in Biotechnology - Natural products isolation.Eds Sarker D S, Latif Z and Gray A I. Totowa,New Jersey: Humana Press; 2006. chapter 7, p. 185-211. eISBN 1-59259-955-9

52. Berthod A, Billardello B. in Counter current chromatography: Fundamentally a preparative tool. In Advances in chromatography. Eds Brown P, Grushka E. New York: CRC Press; 2000; Vol. 40 chapter 10, p. 503-538. PMID: 10740748.

53. Fang L, Liu Y, Yang B Wang X and Huang L. Separation of alkaloids from herbs using high-speed counter-current chromatography. Journal of Separation Science. 2011; 34: 2545-2558. Doi: 10.1002/jssc.201000857

54. Duan W-D, Huang X-Y and Di D-L. Chiral counter-current chromatography: A survey of its instrument, mechanism, procedure, and applications. Chirality. 2020; 32: 1264-1283. Doi: 10.1002/chir.23262.

55. Gong Y, Huang X-Y, Pei W, Duan W-D, Zhang X, Sun X, Di D-L. The applicability of high-speed counter current chromatography to the separation of natural antioxidants. Journal of Chromatography A. 2020;1623: 461150. Doi: 10.1016/j.chroma.2020.461150

56. Nakamura S, Hashimoto H, Akiba K. Enrichment separation of rare earth elements by high-speed countercurrent chromatography in a multilayer coiled column. Journal of Chromatography A. 1997; 789: 381-387. Doi: 10.1016/S0021-9673997)00782-6

57. Al-Shammary FJ, Mian NA, Mian SM. The rôle of countercurrent chromatography in the fractionation of complex mixtures. Journal of Separation Science. 2005; 14: 230-234. doi.:10.1002/jhrc.1240140405

58. Winterhalter P. Application of countercurrent chromatography (CCC) to the analysis of natural pigments. Trends in Food Science and Technology. 2007; 18: 507-513. doi:10.1016/j.tifs.2007.04.013

59. Yang Y, Khan BM, Zhang X, Zhao Y, Cheong KL, Liu Y. Advances in separation and purification of bioactive polysaccharides through high-speed counter-current chromatography. Journal of Chromatographic Science. 2020; 58: 992-1000. doi:10.1093/chromsci/bmaa063

60. Shibusawa Y, Hagiwara Y, Chao Z, Ma Y and Ito Y. Application of high-speed counter-current chromatography to the separation of coumarin and related compounds. Journal of Chromatography A 1997; 759: 47-53. Doi: 10.1016/S0021-9673(96)00762-5

61. Stensen W, Jensen E. High-performance liquid chromatographic separations of naphthoquinones and their derivatives: Effect of hydrogen bonding on retention. Journal of Chromatography A 1994; 659: 87-93. Doi: 10.1016/0021-9673(94)85009-7

62. Zhang T-Y, Pannell LK, Pu Q-L, Cai DG, Ito Y. Separation of hydroxyanthraquinone derivatives extracted from rheum from analytical high-speed counter-current chromatography. Journal of Chromatography, 1988; 442:445-458.

63. Yang F, Zhang T, Tian G, Cao H, Liu Q and Ito Y. Preparative isolation and purification of hydroxyanthraquinones from *Rheum officinale* Baill by high-speed counter-current chromatography using pH-modulated stepwise elution. Journal of Chromatography. A, 1999; 858: 103-107. doi.org/10.1016/S0021-9673(99)00827-4

64. Liu R, Li A and Sun A. Preparative isolation and purification of hydroxyanthraquinones and cinnamic acid from the Chinese medicinal herb Rheum officinale Baill. by high-speed counter-current chromatography. Journal of Chromatography A. 2004; 1052: 217-221. doi.org/10.1016/j.chroma.2004.08.101

65. Wei Y, Zhang T, Ito Y. Preparative separation of rhein from Chinese traditional herb by repeated high-speed counter-current chromatography. Journal of Chromatography A, 2003; 1017:125-130. doi: 10.1016/j.chroma.2003.08.015

66. Ma B, Wang J, Liu C-M and Wang Q. Preparative separation of rhein from Chinese traditional herb by repeated high-speed counter-current chromatography. Journal of Liquid Chromatography and Related Technologies. 2014; 37: 2546-2557. doi: 10.1016/j.chroma.2003.08.015

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.