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# Different qualitative and quantitative analytical techniques for determination of major anthraquinonoids in Rhubarb

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ABSTRACT

The dried roots and rhizomes of various *Rheum spp* (*R. officinale* Baill, *R. palmatum* L., *R. tanguticum* Maxim, *R. undulatum* L, *R. emodi* Wall. Ex Mesin. etc.) of Polygonaceae family are collectively known as Rhubarb. It is one of the popular herbs used in Chinese medicinal system. Rhubarb (Da-Huang) is not only used as purgative drug in Chinese pharmacopoeia since ancient time, but also well-documented in Korean and Japanese ethnomedical preparations for its various applications. The current research works on Rhubarb elucidated the various pharmacological activities including anticancer, antimicrobial, anti-infammatory, hepatoprotective, gastrointestinal regulating, cardiovascular protecting etc. Some hydroxy anthraquinonoids viz., aloe - emodin, emodin, physcion, rhein, chrysophanol and their glycosides are the mainly responsible for the versatile bio-activities of rhubarb. In fact, these constituents are referred as the 'taxonomic markers' for the respective plants. In this regard, multidisciplinary approach for rapid and simultaneous phytochemical analysis and biological screening of these plants should be adopted. Several extraction and analytical (chromatographic as well as electrochemical) techniques are reported in literature for the separation, identification and estimation of these plant secondary metabolites. Some of these methods may provide novel approach for the quality assessment of this widespread herbal drug. In this review article, some recent reports on various qualitative and quantitative methods for detection and estimation of rhubarb anthraquinonoids are summarized which may provide a novel pathway for the study of these active quality markers in this traditional Chinese medicine.

*Keywords:*
Rhubarb; *Rheum spp*, traditional medicines, bioactive anthraquinonoids; quality control, HPTLC, HPLC, UPLC, HSCCC, MEC,CZE

## 1. Introduction

Plants are considered as natural storehouse of enormous variety of bioactive compounds with medicinal significance [1]. More than 40% of modern drugs are originated from plants [2]. Different parts of plants have been widely used as traditional medicines for the treatments of various ailments since antiquity [3]. All over the world, the demand of botanical drugs is increased many folds in last few decades. According to a recent report of World Health Organisation, plant-based medicines are still serving the primary health care requirements of nearly 80% of the world's population [4, 5].

One such herbal preparation is 'rhubarb', popularly used in Chinese pharmacopoeia since thousands of years [6, 7]. The dried roots and rhizomes of various *Rheum species* (*R. officinale*, *R. palmatum*, *R.*

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*tanguticum*, *R. undulatum* etc.) are collectively known as rhubarb or Da-Huang (Figure 1) used mainly as purgative and laxative drug [8]. It is also well documented in Japanese and Korean and other Oriental traditional medicinal systems for the treatment of jaundice, constipation, ulcer, gastro-intestinal haemorrhage etc [9-12].

Rhubarb is a rich source of different classes of secondary metabolites [13-16], they are anthraquinonoids and their glycosides, anthrones, stilbenes, flavonoids, tannins, chromones, polysaccharides etc. The major bioactive components of rhubarb are five hydroxy anthraquinonoids, viz. aloe-emodin, emodin, physcion, chrysophanol and rhein (Figure 2). These compounds exhibited broad spectrum of pharmacological properties including antitumour, anti-inflammatory, antioxidant, antimicrobial, antiulcer, hepatoprotective activities etc [13-30]. In fact, these anthraquinones are considered as 'taxonomic markers' of the respective plants [13, 31].

Rhubarb itself and rhubarb containing herbal preparations having these five anthraquinonoids are consumed indiscriminately as febrifugal, cathartic and antidotal purposes in all over the world. The safety, reliability and efficacy of these botanical drugs are largely depended on the quality control of their active constituents [31 -36]. But it is difficult to identify and also quantify the active compounds in a particular herbal drug due to the complex nature of chemical ingredients in it. So multivariate analytical techniques have been developed for the standardization and quantification of these marker compounds for holistic evaluations ensuring the authenticity and stability of these phytomedicines. Several chromatographic and electrochemical methods are conveniently applied for the detection, separation and estimation of these hydroxyanthraquinones in rhubarb samples [37-42]. Some of the recent research works on the different techniques of analysis of these medicinally important anthraquinonoids have been highlighted in this review.

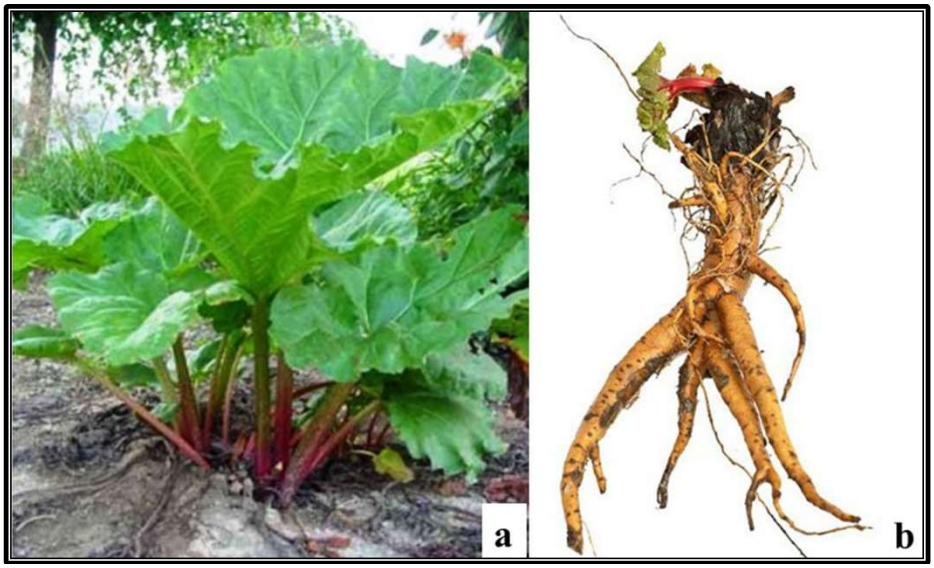


**Fig. 1.** Rhubarb (a) plant; (b) roots

## 2. Analysis of Anthraquinonoids in Rhubarb

### 2.1. Analysis by TLC Method

TLC is the simplest method to detect hydroxyquinones in rhubarb [43]. On silica gel bed, five anthraquinones were simultaneously determined by using hexane: acetone: tert-butanol (85:10:5, v/v/v) [44]. A two-dimensional TLC system was developed to analyse the above quinones in several *Rheum* species [38]. Two developing solvents were ethyl acetate–methanol-$H_2O$ (100:16.5:13.5, v/v/v) and petroleum ether-hexane-ethyl formate-formate acid (1:3:1.5:0.2, v/v/v/v). A reversed phase TLC on polyamide plate was carried out by Wang et al. to study the interaction between aloe-emodin and emodin with six cyclodextrins by using the mobile phase as $NH_4OH$-$NH_4Cl$ buffer (pH 9.7) containing different cyclodextrins at 20°C [45].

### 2.2. Analysis by HPTLC Method

Singh and his co-workers developed a convenient HPTLC method for rapid quantification of four major anthraquinones (emodin, physcion, chrysophanol and chrysophanol glycoside) in the methanolic extract of *Rheum emodi* (Indian rhubarb) collected from three different Himalayan regions in India [46]. The chromatography was performed on precoated RP-18 $F_{254S}$ HPTLC plates using the mobile phase of methanol: water: formic acid = 80:19:1 (v/v/v) ratio followed by detection at 445 nm in the reflectance/absorbance mode. All of the compounds were well separated on HPTLC plate with very good LOD and LOQ values.

Similar results were also obtained when HPTLC study was performed on a silica gel 60 $F_{254}$ plates, using hexane - ethyl acetate = 45: 5 *(*v/v*)* as mobile phase and quantitative detection was done densitometrically at $\lambda_{max}$ = 366 nm [47]. Recently, a hyphenated HPTLC and $^1$H-NMR-based metabolomics tool was developed by Ge and his colleagues to evaluate the quality control of the roots of *R. palmatum* amd *R. tanguticum* obtained from five different geographical regions with varied altitudes of China [48]. Chromatographic separation was performed on the silica gel 60 $F_{254}$ plates using three mobile phases $^n$PrOH-EtOAc-water (4:4:3, v/v/v, saturation time 30 mins), EtOAc-MeOH-water (100:17:13, v/v/v, saturation time 25 mins) and finally cyclohexane-EtOAc-MeOH-HCOOH-water (3:1:2:0.1:2, v/v/v/v/v, saturation time 30 mins) followed by UV detection at 366 nm. Total 125 numbers of rhubarb samples were studied and it was showed that the differentiation in the growth altitude was the most significant factor of quality control assessment and catechin, aloe-emodin and rhein were identified as the specific markers of those plant samples.

### 2.3. Analysis by HPLC Method

HPLC is the most extensively used robust method for the rapid analysis of different phytochemicals in rhubarb [38, 39]. Various HPLC techniques coupled with UV, DAD, MS, CE, FL detectors were developed for the simultaneous detection, quantitative and qualitative estimations and separation of five most abundant bioactive anthraquinonoids in rhubarb. Few of them are summarised in Table 1.

**Table 1.** Different HPLC Techniques for detection, quantification and separation of major anthraquinonoids (aloe-emodin, emodin, physcion, chrysophanol and rhein) in rhubarb

| | Analytical Technique | Column | Mobile phase | Remarks | Ref |
|---|---|---|---|---|---|
| 1. | LC-UV | Dimethylamino bonded Senshu Pak SN-352N column (15 cm × 4.6 mm ID) | 15% Acetic acid - tetrahydrofuran in gradient mode, flow rate 1.0 mL/min, UV detection at 361 nm | [3D UV absorbance] $t_R$ (min) physcion + chrysophanol: 3.52 emodin: 3.80 aloe-emodin: 4.18 | [49] |
| 2. | LC-UV | Nucleosil $5C_{18}$ column (250 mm × 4 mm ID) | Increasing amount of acetonitrile in 0.05 M $H_3PO_4$ in gradient mode, flow rate 0.75 mL/min, UV detection at 280 nm | $t_R$ of aloe-emodin, emodin and rhein 60-70 minutes | [50] |
| 3. | LC-UV | Normal phase column of Spherisorb-CN (250 mm × 4 mm ID, particle size 5 μm) | $CHCl_3$ - 96%AcOH = 95:5, v/v in isocratic mode, flow rate 0.7 mL/min, UV detection at 254 nm | $t_R$ (min) chrysophanol: 4.10 rhein: 5.80 emodin: 8.50 | [51] |
| 4. | LC-UV | Intertsil ODS column (250 mm × 4 mm ID, particle size 5 μm) | 0.05 M $H_3PO_4$ solution – acetonitrile – methanol in gradient mode, detection at 280 nm | $t_R$ of five anthraquinones 60 - 70 minutes with low detection limits 0.1 μg/mL | [52] |
| 5. | LC-DAD | Zorbax SB-C18 column (250 mm × 4 mm ID, particle size 5 μm) | MeOH – 0.5%AcOH = 85:15, v/v in isocratic mode, flow rate 0.6 mL/min, UV detection at 254 nm | $t_R$ of five anthraquinones 7 - 21 minutes | [53] |

| | | | | | |
|---|---|---|---|---|---|
| 6. | LC-DAD | Zorbax SB-C18 column (150 mm × 4 mm ID, particle size 5 μm) | 0.1% $H_3PO_4$ in water – acetonitrile in gradient mode, flow rate 1.0 mL/min, UV detection at 280 nm | $t_R$ of five anthraquinones 52 - 70 minutes, low LODs (0.18 - 1.40 ng) and LOQs (0.48 - 3.80 ng) for all five anthraquinones | [54] |
| 7. | LC-UV | Zorbax RX-$C_{18}$ column (15 cm × 0.46 cm ID, particle size 5 μm) | 36mM triethylamine phosphate (pH 2.5) - acetonitrile in gradient mode, flow rate 1.0 mL/min, UV detection at 254 nm | $t_R$ (min)<br>aloe-emodin: 11.20<br>rhein: 12.10<br>emodin: 18.20<br>chrysophanol: 26.00<br>physcion: 28.80 | [55] |
| 8. | LC-MS | Hypersil-ODS column (150 mm × 4.6 mm ID, particle size 5 μm) | $CH_3CN$-water (pH 3.0, adjusted by formic acid) in gradient mode, APCI-MS detector in negative ion detection mode with 1800 V probe voltage, scan rate 2 s/scan, scan range 100-800 | $t_R$ of aloe-emodin, emodin and rhein 34 -48 minutes | [56] |
| 9. | LC-Q-HR/MS | XBridge™ $C_{18}$ column (150 mm × 2.1 mm, particle size 5 μm) | 3 mM ammonium acetate - methanol in gradient mode, flow rate 0.3 mL/min, ESI in negative full scan mode, spray voltage 2.8 kV(-), scan range m/z 100-1500 | $t_R$ (min)<br>rhein: 3.54<br>emodin: 5.40<br>chrysophanol: 8.85<br>aloe-emodin: 7.99<br>physcion: 10.10<br>low LOD (1.8 -2.97 ng/mL) and LOQ (5.4 -8.90 ng/mL) for all five anthraquinones | [57] |
| 10. | LC-ESI-MS | LiChroCART RP-18e column (125 mm × 3 mm, particle size 5 μm) | 0.015% AcOH - $CH_3CN$ containing 0.015% AcOH in gradient mode, flow rate 0.5 mL/min, ESI in negative ion mode, capillary entrance voltage 3500 V, scanning from 50 to 1000 in 0.5 seconds | $t_R$ of aloe-emodin, emodin and rhein 23 -35 minutes | [58] |
| 11. | LC-ESI-$MS^n$ (n = 2-4) | Zorbax Eclipse XDB-$C_{18}$ column (250 mm × 4.6 mm, particle size 5 μm) | $CH_3CN$-water containing 0.5% (v/v) AcOH in gradient mode, flow rate 0.8 mL/min, ESI in negative ion mode, ion spray voltage 4.5 kV, scan range m/z 120-1000 | Each of five anthraquinonoids can be easily identified by their ESI-MS/MS spectra | [59] |
| 12. | LC-UV-ESI-MS | Cosmosil 5$C_{18}$-MS column (250 mm × 4.6 mm, particle size 5 μm) | {($H_2O$-MeOH-AcOH) = 475: 25: 5, v/v, pH 2.5} and MeOH in gradient mode, flow rate 0.9 mL/min, UV detection at 254 nm, ESI in negative ion mode, ion spray voltage 4.5 kV | $t_R$ of five anthraquinones 57 - 70 minutes, detection limit 6 - 20 ng for all five anthraquinones | [60] |
| 13. | LC-DAD-ESI-MS | Kromasil 100A C18 column (250 mm × 4.6 mm, particle size 5 μm) | 0.05% AcOH in water and acetonitrile in gradient mode, flow rate 1.0 mL/min, diode array detector set at 268 nm, ESI in negative ion mode, capillary voltage 3.5 kV, scan range m/z 100-1000 | $t_R$ (min)<br>aloe-emodin: 106.60<br>rhein: 106.81<br>emodin: 138.98<br>chrysophanol: 139.67<br>physcion: 142.33 | [61] |

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|---|---|---|---|---|---|
| 14. | LC-DAD-ESI-MS | Symmetry Shield RP18 column (250 mm × 4.6 mm, particle size 5 μm) | MeOH and 0.1% $H_3PO_4$ in water in gradient mode, flow rate 1.0 mL/min, detection at 280 nm for fingerprinting analysis and 254 nm for quantitative analysis | LOD (0.01 -1.45 ng) and LOQ (0.02 - 6.15ng) for all five anthraquinones | [62] |
| 15. | LC-FL | Hypersil C18 column (200 mm × 4.6 mm, particle size 5 μm) | MeOH - 0.1% formic acid (85:15, v/v) in isocratic mode, flow rate 1.0 ml/min, excitation at 440 nm and emission at 540 nm | The total analysis time =15 minutes, lower LODs (2.20 – 12.50 ng/mL) and LOQs (70 - 140 ng/mL) for all five anthraquinones were obtained as compared to LC-UV method under similar LC conditions. | [63] |
| 16. | LC-UV and CE | *LC system*<br>Cosmosil 5C18-AR column (250 mm × 4.6 mm, particle size 5 μm)<br><br>*CE system*<br>Crystal 310 CE system with fused silica capillary tube (90 cm × 75 μm ID, applied voltage 23kV | *LC condition*<br>20 mM aqueous $KH_2PO_4$ (pH 2.9 adjusted with $H_3PO_4$) and MeOH in gradient mode, flow rate 0.8 mL/min, UV detection at 260 nm<br><br>*CE condition*<br>30 mM sodium borate (pH 10.56 adjusted with 0.05 N NaOH) and acetonitrile = 9:1 (v/v) | Chrysophanol, rhein, emodin and aloe-emodin were detected within 63 minutes by LC-UV and 39 minutes by CE | [64] |
| 17. | LC-DAD and CE | *LC system*<br>Cosmosil 5C18-AR-II column (150 mm × 4.6 mm, particle size 5 μm)<br><br>*CE system*<br>Beckman MDQ and P/ACE System 5000 apparatus with fused silica capillary tube (57 cm × 75 μm ID, applied voltage 20 kV | *LC condition*<br>$CH_3CN$ - water in gradient mode, flow rate 1.0 mL/min, detection at 254 nm<br><br>*CE condition*<br>0.03 M sodium tetraborate (pH 10.0 adjusted with 10% w/v NaOH) and additions of 0.002 M 2,6-di-O-methyl-β-cyclodextrin, 0.005 M α-cyclodextrin, and 25% (v/v) acetonitrile | chrysophanol, rhein, emodin and aloe-emodin were detected within 25 minutes by CE<br>*LOD in LC-DAD* (μg/mL)<br>emodin: 0.04<br>aloe-emodin: 0.02<br>chrysophanol: 0.03<br>rhein: 0.1<br>*LOD in CE* (μg/mL)<br>emodin: 0.1<br>aloe-emodin: 0.3<br>chrysophanol: 0.4<br>rhein: - | [65] |
| 18. | LC-DAD | Kromasil 100 Å $C_{18}$ column (250 mm × 4.6 mm ID, particle size 5 μm) | 0.05% $H_3PO_4$ in water – acetonitrile in gradient mode, flow rate 1.0 mL/min, UV detection at 268 nm | Applying this HPLC fingerprint method quality control of 21 raw samples of *R. tanguticum* from various sources were evaluated | [66] |

Analysis of chromatographic fingerprint has been widely used to assess the integrity and stability of the herbal medicines and HPLC fingerprint should be representative of authentic plant [67]. Recently, Sun et al. developed bioactivities-based estimation of quality control of rhubarb by using HPLC fingerprint analysis and delayed luminescence measurements. Characteristic chromatogram combined with luminescence study may provide rapid, sensitive,

highly accurate and inexpensive technique to the overall quality evaluation of herbal drugs [68].

### 2.4. Analysis by CZE Method

Among all the capillary electrophoresis methods, capillary zone electrophoresis is considered as simplest, most rapid and reproducible technique for the separation of the target compounds.

Xiaoyu and Zhuobin analysed two commercial rhubarb samples by a cyclodextrin modified capillary zone electrophoresis method [69]. The study was performed in a 1229 HPCE analyser system coupled with a UV detector at 254 nm. In an uncoated fused silica capillary, the separation was made by using the running buffer of 50 mM NaOH - $H_3BO_3$ (pH = 10.7) containing 10 mM β-cyclodextrin and 12.5% aqueous ethanol at an applied voltage of 15 -18 kV. Four components, viz. physcion, emodin, aloe-emodin and rhein in rhubarb were easily separated within 12 minutes under this optimum condition.

Similar studies reported by Li et al., where five bioactive components (aloe-emodin, emodin, physcion, chrysophanol and rhein) were simultaneously determined in pulverised rheum powder and rheum containing preparations [70]. The analysis was performed on a Beckman MDQ capillary electrophoresis system equipped with a PDA detector operating at 254 nm and a uncoated fused capillary. The separations were achieved within 20 minutes at 20kV applied voltage by using a buffer solution containing 15 mM borax, 30 mM β-cyclodextrin, 20% acetonitrile and 1.0% ethanediol.

### 2.5. Analysis by CEC, MEC, MEEKC methods

Various electro-chromatographic methods were established for the detection and separation of therapeutically important anthraquinone components of rhubarb.

Capillary electrochromatography is a microcolumn separation technique combining HPLC and capillary electrophoresis. Li et al. developed a capillary electro-chromatographic method connected with diode array detection for rapid separation of anthraquinones in rhubarb [71]. The study was performed on a C18 column (100 μm ID × 375 μm OD, packed bed length 40 cm, 3 μm particle size) using polyimide coated fused silica as stationary phase and 5mM acetic acid (pH 4.5) with 80% acetonitrile as mobile phase at 30kV applied voltage, 40°C and 5 bar pressure with UV detection at 214 and 254 nm. Four anthraquinones, viz. aloe-emodin, emodin, chrysophanol and physcion were eluted within 12 minutes.

A more rapid (within 5 minutes) separation of five anthraquinonoids was achieved by pressurized capillary electrochromatography using a specially designed polymeric monolithic column with the mobile phase 10 mM phosphate buffer containing 65% acetonitrile (pH 6.2) [72].

A cyclodextrin modified micellar electrokinetic chromatographic method was reported by Shang and Yuan [73] for the determination of active constituents of rhubarb in less than 20 minutes. They used the mixed micellar system containing 20 mM sodium cholate and 20 mM sodium taurocholate with 15 mM β-cyclodextrin and 20 mM borax buffer (pH 11).

Microemulsion electrokinetic chromatography is easier and more efficient technique for the determination and separation of these five anthraquinones in commercial *Rheum* plants [74]. Microemulsion consisted of ethyl acetate (0.64%, v/v), sodium dodecyl sulphate (0.7%, m/v) and 1- butanol (0.16%, v/v) with 5mM phosphate buffer (pH 7.2) and different concentrations of acetonitrile as additive were used for this study and at 25 ± 1°C, 15 kV applied voltage, all five anthraquinones in the plant sample were migrated within 9 minutes.

### 2.6. Analysis by UPLC method

Besides pressurised capillary electrochromatography, the different methods discussed above are very much time-consuming for the analysis of major bioactive constituents in rhubarb. They required almost 15 -70 minutes for a single run. In fact, a specially designed polymeric monolithic column was used in pressurised capillary electro-chromatographic study for the separation of five major anthquinonoids in rhubarb within 5 minutes [72]. For rapid and more efficient quantification of those five derivatives in rhubarb, Wang et al. developed a convenient reversed phase ultra-performance liquid chromatographic technique in which those components were simultaneously determined within 3 minutes [75]. They used commercially available Acquity BEH $C_{18}$ column, consisting the stationary phase of sub 2 μm particles and the mobile phase of 1% aqueous $H_3PO_4$: methanol = 31: 69, (v/v) in isocratic mode with a flow rate of 750 μL/minute at 35°C followed by UV detection at 254 nm. This UPLC system provided equivalent resolution, but more rapid and sensitive quantification of the plant sample. In this study, the

elution time of UPLC is almost eight-fold reduced as compared to conventional HPLC method.
Gao et al. established a fast separation and simultaneous identification of 30 compounds including emodin, aloe-emodin, rhein, chrysophanol and their glycosides from the methanol extract of *R. tanguticum* powder by using by UPLC/Q-TOF-MS/MS [76]. This technique was also successfully applied by Wang and his co-workers to explore the marker constituents of raw and processed samples of *R. palmatum* [77].

### 2.7. Analysis by UHPLC method

A highly sensitive solid phase extraction of the important anthraquinones in rhubarb and urine of rat fed with rhubarb extract was carried out based on UHPLC coupled with Q-TOF/MS [78]. Reduced graphene oxide with iron oxide ($Fe_3O_4$) were chosen as sorbent in magnetic SFE in this study. Under the optimised condition, the compounds were extracted within 5 minutes with very low LOD values (0.28 - 58.99 pg/mL). Compared with other conventional extraction techniques, this method showed much better results with respect to extraction time, good recovery and lower LODs. Similar UHPLC-Q-TOF/MS experiments associated with multivariate data processing approach were also performed by Yang et al. for the pharmacokinetic studies of rhubarb extract and its metabolites in rat plasma [79]. About 80 compounds including the five important anthraquinones were rapidly identified in this high-throughput screening assay. In another report, taking aloe-emodin 8-O-β-D-glucopyranoside and chrysophanol as references, twenty batches of rhubarb samples were analysed by UHPLC- DAD method [80]. Chromatographic studies were carried out in eight different $C_{18}$ columns (100 mm × 2.1 mm ID) with varying particle sizes (1.7 – 2.7 μm), where eleven bioactive compounds (free anthraquinones, anthraquinone glycosides, sennosides) were simultaneously determined both qualitatively and quantitatively within 25 minutes.

### 2.8. Analysis by HSCCC method

During conventional thin layer and column chromatography, hydroxy quinonoid compounds have a tendency to remain adsorbed strongly on the silica gel, alumina etc. stationary phases, which causes adsorptive loss and deactivation of the sample [81]. High speed counter current chromatography can successfully solve this problem. By employing HSCCC, the chance of irreversible binding between these type of compounds and the stationary phase can easily be excluded because this is a liquid-liquid partition chromatographic technique which is free from any solid support system [82]. Zhang and his co-workers first reported an analytical HSCCC method for separation of these five anthraquinones from *Rheum palmatum* rhizomes [83]. They used the bi-phasic solvent *n*-hexane-ethyl acetate-methanol-water (9:1:5:5, v/v/v/v) as normal and reversed elution modes at a flow rate of 60 mL/hr and UV detection at 278 nm. 1 mg of complex mixture of the sample was purified into its major quinonoid components within 70 minutes. The method was rapid, reproducible and requires small amount of solvent for separation. Later on, several group of workers developed analytical and preparative HSCCC methods which can rapidly and efficiently determine and purify these analogous compounds by using two-phase solvent system at an optimum pH. Some reports are summarised in Table 2.

**Table 2.** Different HSCCC Techniques for separation and purification of major anthraquinonoids (aloe-emodin, emodin, physcion, chrysophanol and rhein) in rhubarb

| | Sample | Bi-phasic solvent | HSCCC condition | Remarks | Ref |
|---|---|---|---|---|---|
| 1. | Ethanolic extract of *Rheum officinale* Baill | Stationary phase: Diethyl ether<br>Mobile phase: Basic water<br>(a) *Analytical HSCCC :* 35 mL aqueous solution of 4.0% $NaHCO_3$ and 55 mL of 0.7% $Na_2CO_3$ and 80 mLof 0.2% NaOH<br>(b) *Preparative HSCCC :* 120 mL aqueous solution of 4.0% $NaHCO_3$ and | (a) *Analytical HSCCC*: flow-rate: 1.0 mL/min; revolution speed: 1500 rpm; sample: 10 mg dissolved in 1 mL stationary phase<br>UV detection : 254 nm<br>(b) *Preparative HSCCC :* flow-rate: 2.0 mL/min; revolution speed: 800 rpm; sample: 300 mg dissolved in 20 mL | Except Rhein, rest four quinones viz., emodin, aloe-emodin, chrysophanol and physcion were separated in preparative HSCCC with more than 98% purity | [84] |

| | | | | | |
|---|---|---|---|---|---|
| | | 240 mL of 0.7% $Na_2CO_3$ and 480 mL of 0.2% NaOH | stationary phase; retention of the stationary phase: 50%. UV detection : 254 nm | | |
| 2. | Ethanolic extract of *Rheum officinale* Baill (Dahuang) | (a) *Analytical HSCCC :* i) *n*-hexane–ethyl acetate-methanol-water (9:1:5:5, v/v); ii) *n*-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v); (iii) *n*-hexane–ethyl acetate–methanol–water (3:7:5:5, v/v)<br>(b) *Preparative HSCCC :* *n*-hexane–ethyl acetate–methanol–water (3:7:5:5, v/v) | (a) *Analytical HSCCC*: flow-rate: 1.0 mL/min; revolution speed: 1800 rpm; sample: 15 mg dissolved in 1 mL lower aqueous phase UV detection : 254 nm<br>(b) *Preparative HSCCC :* flow-rate: 2.0 mL/min; revolution speed: 800 rpm; sample: 500 mg dissolved in 20 mL lower aqueous phase; UV detection : 254 nm | 500 mg crude extract of Dahuang yields 6.7 mg of Rhein (purity over 97%) | [85] |
| 3. | Root extract (20% $H_2SO_4$:benzene = 1:5, v/v) of *Rheum officinale* Baill | Stationary phase: ether Mobile phase: 1% aqueous $NaH_2PO_4$ :1% aqueous NaOH in gradient elution (100:0 to 0:100 for 500 minutes) | flow-rate: 2.0 mL/min; revolution speed: 800 rpm; sample: 120 mg dissolved in 20 mL ether at 25 ºC; retention of the stationary phase: 40%. UV detection : 254 nm | Purification of rhein, emodin, aloe-emodin, chrysophanol, physcion along with cinnamic acid (purity over 98%) | [86] |
| 4. | Ethanolic extract of *Rheum palmatum* Linn | n-hexane-ethyl acetate–*n*-butanol-water (1:2:1:4, v/v/v/v) | flow-rate: 2.0 mL/min; revolution speed: 1400 rpm; sample: 14.97 mg of extract/mL of each phase retention of the stationary phase: ~ 80.6% UV detection : 280 nm | Analysis of fraction: LC-ESI-$MS^n$ in negative ion mode Rhein (purity over 98.07%) Emodin (purity over 94.76%) | [87] |

### 2.9. Analysis by a SFC method

A highly selective and rapid supercritical chromatographic technique was established by Aichner and Ganzera for the analysis of five marker anthraquinonoids from rhubarb [88]. The assay was performed on $UPC^2$ HSS C18 SB column (100 mm × 3.0 mm ID, particle size 1.8 μm) with the mobile phase of liquid carbon dioxide and 0.05% diethyl amine in methanol (as mobile phase modifiers) in gradient elution mode, at 30°C, 2mL/min flow rate followed by UV detection at 254 nm. Within less than 5 minutes, all five compounds were separated. While comparing analysis time, LOD values with other reported HPLC, CE and UPLC methods, this technique showed excellent results (LOD: 0.26 -0.43 μg/mL).

### 2.10. Extraction by a modified solid-phase dispersion method

A simple, cost effective and rapid tool was developed by Hong and Chen for the extraction of anthraquinonoids from rhubarb by using molecularly imprinted polymer matrix solid-phase dispersion technique [89]. The extracted compounds were detected by LC-UV method. During the preparation of molecularly imprinted polymer, emodin was chosen as template molecule, methacrylic acid and ethylene glycol dimethacrylate were taken as functional monomer and cross-linking agent respectively. The optimized condition of extraction was achieved at 1:1 sample and molecularly imprinted polymer, with a dispersion time of 5 minutes with eluent as methanol-acetic acid (99:1, v/v). The range of LODs for four anthraquinones (emodin, aloe-emodin, physcion and chrysophanol) in rhubarb sample (0.23 -.027 μg/mL) was much higher in this method as compared with the

other commonly used soxhlet and ultrasonic extraction methods followed by various detection (HPLC, CE, CEC, MEKC, MEEKC etc.) techniques.

## 3. Conclusion

Rhubarb is widely distributed in all over the world and it is one of the most ancient herbal drug used in many traditional Oriental medicines. In recent years, a great progress has been observed on rhubarb research due to its immense pharmacological actions. Five hydroxy anthraquinonoids have been identified as the active constituents responsible for the diverse biological activities of rhubarb and these compounds also have been recognized as the quality markers of the drug.

Quality control and finger print of herbal formulation can easily be done by the analysis of its different marker constituents and sensitive analytical method helps to identify the same herbs in combination. Several chromatographic and electrochemical techniques (HPTLC, HPLC, UPLC, HSCCC, *p*-CEC, MEC, MEKC, CZE) are being successfully implemented for detection, separation, estimation and standardisation of major bioactive anthraquinonoid compounds in rhubarb which may provide insights into the further development of rhubarb in future. To improve the authenticity, safety and stability of multicomponent phytomedicines, some modern integrated technologies should also be explored which will ensure the high-throughput screening of large number of botanical samples. This may open up a new wing to the overall quality assessment of herbal medicines.

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## Abbreviations

| | | | | | |
|---|---|---|---|---|---|
| CE | : | capillary electrophoresis | MS | : | mass spectrometry |
| CZE | : | capillary zone electrophoresis | NMR | : | nuclear magnetic resonance |
| DAD | : | diode array detection | OD | : | Outer diameter |
| ESI-MS | : | electro-spray ionisation mass spectrometry | *p*CEC | : | pressurised capillary electro-chromatography |
| FL | : | fluorescence | PDA | : | photo diode array |
| HR/MS | | High resolution mass spectrometry | Q-TOF-MS | : | quadrupole time-of-flight mass spectrometry |
| HPLC | : | high performance liquid chromatography | RP | : | reversed phase |
| HPTLC | : | high performance thin layer chromatography | SFC | : | supercritical fluid chromatography |
| HSCCC | : | high speed counter current chromatography | TEAP | : | triethyl amine phosphate |
| ID | : | Internal diameter | TLC | : | thin layer chromatography |
| LC | : | liquid chromatography | $t_R$ | : | time of retention |
| LOD | : | limit of detection | UHPLC | : | ultra -high performance liquid chromatography |
| LOQ | : | limit of quantification | UPLC | : | ultra -performance liquid chromatography |
| MEC | : | micellar electro-chromatography | UV | : | ultra violet |
| MEEKC | : | microemulsion electro kinetic chromatography | | | |

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