Annals of Phytomedicine 4(1): 68-73, 2015

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN : 2393-9885

Validated UPLC-Q-TOF-MS method for quantitative determination of emodin in rhizome of *Rheum emodi* Wall. ex Meissn. and its traditional polyherbal formulation using three different extraction techniques

Wasim Ahmad, Syed Mohammad Arif Zaidi* and Sayeed Ahmad

Bioactive Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-110062, India

**Department of Surgery, Faculty of Medicine, Jamia Hamdard (Hamdard University), New Delhi-110062, India*

Received February 6, 2015: Revised March 10, 2015: Accepted March 20, 2015: Published online June 30, 2015

Abstract

A new method using high throughput ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC/Q-TOF-MS) was developed and validated for the quantitative analysis of emodin in the *Rheum emodi* Wall. ex Meissn. and Safoof-e-pathar phori (SPP), using three different extracts. The extraction technique has been optimized for maximum yield using conventional, ultrasonic and matrix solid phase dispersion (MSPD) extraction, with an objective of reducing analysis time and sustaining good efficiency. The chromatographic separation was achieved on a Waters ACQUITY UPLC™ BEH C_{18} (100.0 mm × 2.1 mm; 1.7 µm) column packing, using isocratic mobile phase, consisting of acetonitrile : water (90:10) at a flow rate of 0.2 mL min^{-1}. The Waters Q-TOF premier was operated in multiple reactions monitoring mode *via* negative ionization interface, using the transitions 268.9 *m/z* for emodin. The total run time was 3.2 min and the elution of emodin occured at 1.55 min. The method was proved to be accurate and precise at a linearity range of 1.0-1000.00 ng mL^{-1} with a correlation coefficient (r^2) of ≥ 0.998. The lower limit of quantitation was 1.0 ng mL^{1}. The intra-and inter-day precision and accuracy values were found to be within the assay variability limits as per the ICH guidelines. The developed method was found rapid, accurate, reliable and highly sensitive for the quantitative analysis of emodin in the rhizome of *R. emodi* and formulation in different extracts. MSPD technique was found most suitable for the extraction of emodin as compare to other techniques.

Key words: UPLC/Q-TOF-MS, *Rheum emodi*, emodin, safoof-e-pathar phori, validation, MSPD

1. Introduction

"Rhubarb", this word originates from its Latin name rhabarbarum (Wright, 2001). The botanical name of Himalayan rhubarb is *Rheum emodi* Wall. ex Meissn., is commonly known as revand chini (Anonymous, 1972, 1990; Prasad and Purohit, 2001). It is the Himalayan species of Indian rhubarb, distributed in the alpine and sub-alpine zones of the Western Himalaya (Zargar *et al.*, 2011).

Rhubarb is a storehouse of a large number of anthraquinone derivatives such as physcion, chrysophanol, emodin, aloe emodin, rhein, *etc*. which are reportedly known for a large number of biological activities including antioxidant (Yen *et al.*, 2000), antimicrobial (Babu *et al.*, 2003), antifungal (Agarwal *et al.*, 2000), cytotoxic (Kubo *et al.*, 1992), larvicidal (Yang *et al.*, 2003), casein kinase II inhibitory (Yim *et al.*, 1999) and antiviral (Semple *et al.*, 2001). Besides, these anthraquinone derivatives, have a great potential for dyeing textile (Novotna *et al.*, 1999) and food stuffs (Muller *et al.*, 1999) as safe and eco-friendly natural dyes. It has also been used as a purgative, stomachic, astringent and natural cure for the treatment of chronic bronchitis, asthma and certain skin diseases (Ahmad *et al.*, 2014).

There are various analytical methods, reported for determination of emodin (the major hydroxyanthraquinones of *R. emodi*) (Figure 1) in plant extracts such as high-speed counter-current chromatography, capillary zone electrophoresis, high-performance thin layer chromatography and most commonly used high-performance liquid chromatography, out of which HPLC method utilizing a Merck Germany C_{18} reverse phase column (25 × 4.6 mm I.D. 5.0 µm) at a flow rate of 1.0 mL min^{-1} found inadequate sensitivity and has the disadvantage of being time consuming because of lengthy processing steps and in HPTLC methods inadequate sensitivity, lacking proper validation and time consuming (Singh *et al.*, 2005; Verma *et al.*, 2005). However, there has been no report till date for the determination of emodin content in traditional Unani/Ayurvedic formulations by using UPLC/Q-TOF-MS. All these studies have further emphasized the need to perform rapid and sensitive quality-control analysis of emodin with low retention time and improved sensitivity. Moreover, in recent years, LC-MS/MS has proved to be an extremely sensitive and specific technique for the analysis of basic drugs. Therefore, an attempt has been made for quantitative characterization of traditional Unani formulation for their emodin content, using developed and validated UPLC/Q-TOF-MS method,

Author for correspondence: Dr. Sayeed Ahmad
Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India
E-mail: sahmad_jh@yahoo.co.in
Tel.: +91-9891374647

as per the ICH guidelines, which is rapid and highly sensitive for determination of emodin content of multi-component traditional formulations.

In this paper for the first time, a rapid, sensitive and high throughput ultra-performance liquid chromatography/quadrupole time-of-flight mass spectroscopy (UPLC/Q-TOF-MS) method was developed and validated for the determination of emodin in the polyherbal formulation (Safoof-e-Pathar phori) and rhizome of *R. emodi* for the analytical investigations, using three different extraction techniques. The extraction technique was optimized by using conventional extraction, ultrasonic and a new advanced extraction technique Matrix solid-phase dispersion (MSPD). MSPD extraction is a cleanup and sample preparation technique from complex matrix like from plants. It simplifies the process and reduces time of extraction, utilizing lesser solvent through dissolution and dispersion of organic phase bond to sorbent (Yin *et al.*, 2013).

2. Materials and Methods

2.1 Chemicals, standards and samples

Emodin ($C_{15}H_{10}O_5$; assigned purity >98%; M.P. 212ºC) was purchased from Sigma Aldrich chemicals private Ltd (Bangalore, India). Chemical structure of emodin is shown in Figure 1. LC-MS grade acetonitrile Lot No: CX038 was purchased from Honeywell B&J Brand, USA. HPLC grade water was obtained from Thomas Baker (Chemicals) Private Limited, Mumbai. Water used in the entire analysis was of LC-MS grade. Other chemicals used were of analytical grade from commercial sources.

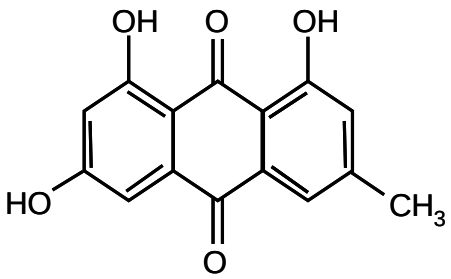


Figure 1: Chemical structure of emdoin

2.2 Plant material and polyherbal formulation

Rhizome of *R. emodi* purchased from Kharibawli local market of Delhi and authenticated by Dr. H. B. Singh, Ref. NISCAIR/RHMD/ 1327/129, New Delhi. The rhizomes were dried, powdered in an electric grinder and passed through sieve # 60 so as to obtain uniform powder.

Safoof-e-Pathar phori (SPP) is an Unani polyherbo-mineral formulation and has been used in Unani system of medicine for its antiurolithiatic activity (Anonymous, 1986). It is a powdered formulation, which contains six different plant/mineral constituents: Pathar phori (*Didymocarpous pedicellata*) (Anonymous, 2006), kulthi (*Dolichous biflorus*) (Anonymous, 2007), revand chini (*Rheum emodi*) (Anonymous, 2007), namak turb (*Raphanus sativus*), jawakhar (Potassium carbonate) and shora qalmi (Potassium nitrate). The formulation was prepared using authenticated constituents as per standard traditional protocol by a Unani Physician in the Department of Surgery, Faculty of Medicine, Jamia Hamdard, and supplied to us for analysis. Voucher Specimen Number : JH/FP/BNPL/WA/SAF 03-2010 was also deposited in laboratory for future reference.

2.3 Sample preparations

2.3.1 *Conventional extraction (CE)*

Accurately weighed 1.0 g of *R. emodi* rhizome powder, was taken in a 250 mL of round bottom flask. It was extracted below 50°C, using reflux condenser, using 50 mL of LC-MS grade methanol for three times to ensure complete extraction. The filtered extracts obtained were pooled and evaporated under vacuum to dryness, then reconstituted in LC-MS grade methanol and volume was adjusted to 10 mL, further it was diluted with solvent to get the desired concentration of emodin in sample, which was filtered through 0.22 µm syringe filter before chromatographic analysis. The samples were kept in auto sampler and 10 µL of the sample were injected for LC-MS analysis. Same method was followed for the extraction of emodin from formulation.

2.3.2 *Ultrasonic extraction (USE)*

Accurately weighed 100 mg of *R. emodi* rhizome powdered were taken in a 10 mL of volumetric flask. It was kept in a sonicator for 20 min by adding 7.5 mL of LC-MS grade methanol. The filtered extract obtained was pooled and evaporated under the current of nitrogen to dryness and then reconstituted in methanol and volume was adjusted to 10 mL, finally diluted to make the desired concentration emodin in sample, which was filtered through 0.22 µm syringe filter before chromatographic analysis. The samples were kept in auto sampler and 10 µL of the sample were injected for LC-MS analysis. Similarly, formulation sample was processed for extraction of emodin.

2.3.3 *MSPD extraction*

100 mg of *R. emodi* rhizome powder blended with 400 mg of C_{18} using in a glass mortar pestle to produce homogeneous mixture. It was packed in a 6 mL SPE tube (Agilent Technologies) with filter disc on both sides after compression injection syringe plunger and attached with SPE- vacuum manifold (Agilent Technologies). The column was washed with 5% methanol to remove very polar matrix, followed by drying under the current of nitrogen. The 70% methanol (3 mL) was added to macerate the mixture in column, which was ultrasonicated for 5 min at 45 KH_3 after closing the column with propylene cap. The sample was eluted with 5 mL of solvent (70%, Methanol) and elute was dried undercurrent of nitrogen. The residue obtained was redissolved in 10 mL of LC-MS grade methanol; it was diluted to make the desired concentration of emodin in sample, which was filtered through 0.22 µm syringe filter before chromatographic analysis. The samples were kept in auto-sampler and 10 µL of the sample were injected for LC-MS analysis. Same method was repeated for sample preparation using traditional Unani formulation.

2.4 UPLC conditions

UPLC was performed with a Waters ACQUITY UPLC™ system (Serial No# F09 UPB 920M; Model Code# UPB; Waters Corp., MA, USA) equipped with a binary solvent delivery system, an auto-sampler, column manager and a tunable MS detector (Serial

No# JAA 272; Synapt; Waters, Manchester, UK). Chromatographic separation was performed on a Waters ACQUITY UPLC™ BEH C_{18} (100.0 mm × 2.1 mm; 1.7 µm) column at 40ºC. The mobile phase for UPLC analysis consisted acetonitrile and water which was degassed. For isocratic elution, the flow rate of the mobile phase was kept at 0.2 mL min^{-1} and 10 µL of sample solution was injected in each run. The retention time of emodin was 1.55 min and total chromatographic run time was 3.2 min. The column and auto-sampler were maintained at 40ºC, respectively and the pressure of the system was set to 15000 psi.

2.5 Q-TOF-MS conditions

The mass spectrometry was performed on a quadrupole orthogonal acceleration time- of- flight tandem mass spectrometer (Waters Q-TOF Premier™). The nebulizer gas was set to 500 L h^{-1}, the cone gas set to 50 L h^{-1} and the source temperature set to 100 ºC. The capillary voltages were set to 2.50 KV and sample cone voltages were set to 40 V, respectively. Argon was employed as the collision gas at a pressure of 5.3×10^{-5} Torr. Quantitation was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 268.9 225 for emodin with a scan time of 1.0 s scan time, and 0.02 s inter-scan per transition. The optimum values for compound-dependent parameters like trap collision energy (Trap CE) and transfer collision energy (Tran CE) were set to and 34 and 1.0 V, respectively for fragmentation information.

The Q-TOF Premier™ was operated in V mode with resolution over 8500 mass with 1.0 min scan time, and 0.02 s inter-scan delay. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the Mass Lynx V 4.1 software incorporated in the instrument.

2.6 Calibration standards and quality control (QC) sample preparation

The standard stock solution containing 1000 µg mL^{-1} of emodin was prepared by dissolving requisite amount in LC-MS grade Methanol (sonicated: 44 kHz, 250W at 25 ºC for 15 min). The stock solutions were appropriately diluted to prepare a series of standard working solutions, and then stored at 4ºC. The solutions were brought to room temperature and filtered through 0.22 µm membrane filter before UPLC/Q-TOF-MS analysis.

Calibration curve standards consisting of a set of twelve non-zero concentrations (A-L) in methanol yielding concentration range from 1-1000 ng mL^{-1} was plotted. The final concentrations for each analyte were prepared to be 1, 2, 5, 10, 20, 50, 100, 200, 400, 600, 800 and 1000 ng mL^{-1}. However, QC samples were prepared at three levels; 800 ng mL^{-1} (HQC, high quality control), 400 ng mL^{-1} (MQC, middle quality control) and 10 ng mL^{-1} (LQC, low quality control). All the solutions were stored at 2-8ºC until use.

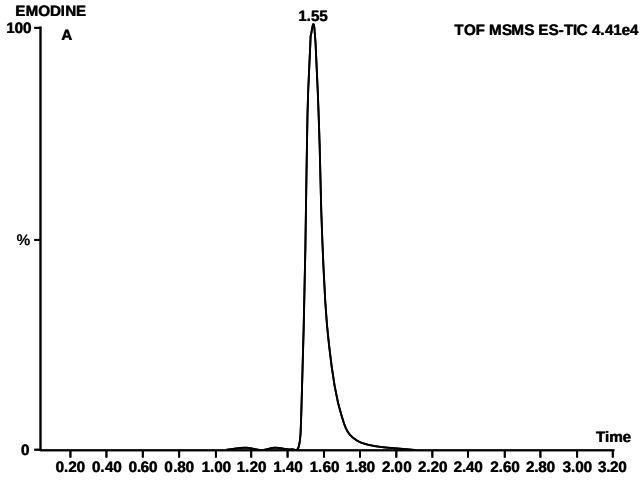
2.7 Validation of method

The method validation of emodin was performed as per ICH guidelines (ICH, 1997) as well as similar to the methods reported by Garg *et al*. (2014), Rehman *et al*. (2014) and Singh *et al*. (2014). The linearity of the method was determined by analysis of six standard plots containing twelve non-zero concentrations. Peak area of standard emodin *vs* concentration of emodin was utilized for the construction of calibration curves, using linear least squares regression of the emodin. The limit of quantification (LOQ) is the lowest concentration of the calibration curve, which could be measured with acceptable accuracy and precision. The LOQ was determined based on the signal-to noise ratio of 10:1. For determining the intra-day precision, replicate analysis of all the samples of emodin was performed on the same day. The run consisted of a calibration curve and six replicates of LOQ, LQC, MQC and HQC samples. The inter-day precision was assessed by analysis of sample on three consecutive validation days. The accuracy was analyzed as recovery by standard addition method at 0, 50, 100 and 150% levels by adding standard analyte to pre-analysed samples and measurement were done for six times. For evaluating the ruggedness of the method, one sample of precision and accuracy was run using a different column (same type) by a different analyst employing the same instrument.

3. Results and Discussion

3.1 Selection of column and optimization of chromatographic conditions

The objective of this study was to chromatographically quantify emodin in the rhizome of *R. emodi* and its traditional formulation. For the analysis, two analytical columns, Chirobiotic V2 (25 cm × 4.6 mm, 5 μm) and UPLC™ BEH C_8 (100.0 mm × 2.1 mm; 1.7 μm), were shortlisted for method development. Baseline chromatographic resolution could not be achieved on the Chirobiotic V2 column with a mobile phase ammonium formate buffer (10mM, pH 4.00 - 5.50): Acetonitrile (70:30, v/v). However, a good resolution was observed on the UPLC™ BEH C_{18} column, using mobile phase-Acotonitrite:Water (90:10, v/v). Baseline separation of emodin was obtained within runtime of 3.2 min without any interference. Methanol, acetone and isopropyl alcohol have been also tried for instant elution but they did not afford chromatographic separation. In order to undertake successful quantification of emodin, tuning parameters for ESI^+ were optimized for the protonated precursors and product ions of analytes. Finally a sharp peak of emodin was found at 1.55 retention time. The MS full scan spectra for emodin showed protonated precursor $[M+H]^+$ ions at 268.9 *m/z* and most abundant product ions at 225.2 *m/z* (Figure 2). The optimum collision energies employed were 34.0 eV for emodin. Quantification was done on the basis of main product ions by keeping the identical capillary voltage of 3.0KV for monitoring the product ions.



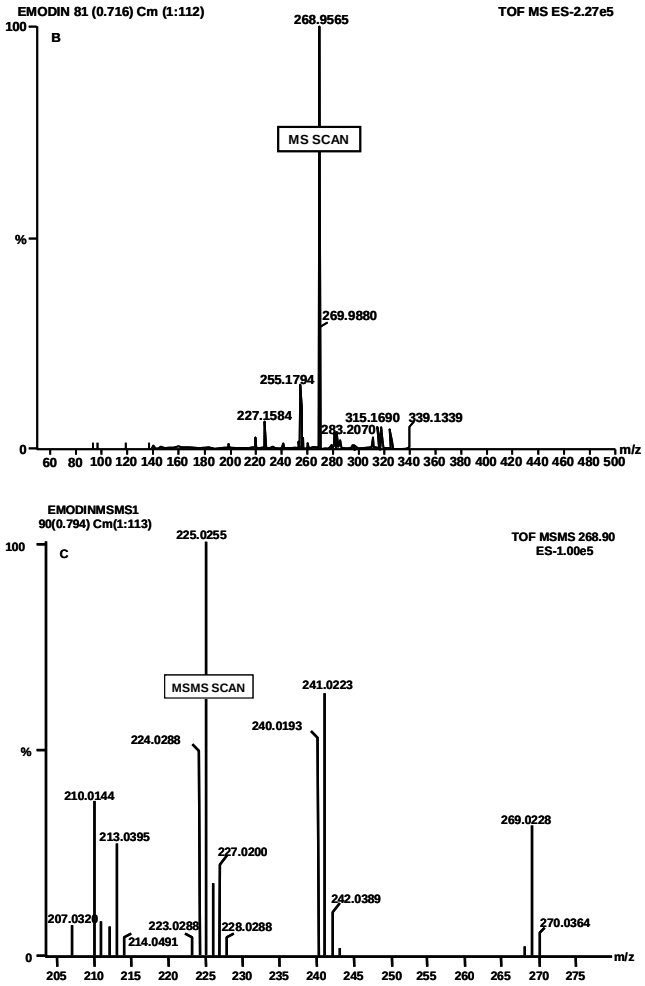


Figure 2: (A) The chromatogram of emodin showing RT at 1.55 min., (B) Precursor ion spectra (protonated precursor $[M+H]^+$ ions at *m/z* 268.9); and (C) product ion spectra (major fragmentated product ion mass spectra at *m/z* 225.2) showing fragmentation transitions.

3.2 Linearity, limit of quantification, accuracy, precision and recovery

Linearity of emodin was established over a concentration range of 1-1000 ng mL^{-1}. Linear coefficient of regression (r^2 >0.998) was obtained using least squares linear regression model using peak area *vs* concentration. The limit of quantification in the present method was 1.0 ng mL^{-1} (Table 1). The results of precision are summarized in Table 2. The % RSD ranged from 1.09 to 1.70 for intra-day and from 1.09 to 1.85 and to % for inter-day precision.

Table 1: Linearity and limits of detection and quantification of UPLC/Q-TOF-MS analysis of Emodin

Parametrs	Emodin
Regression equation	Y= 9.002x+32.68
Linear range	1-1000 ng mL^{-1}
Correlation coefficient (*r*)	0.998
LOD	0.36 ng mL^{-1}
LOQ	1.0 ng mL^{-1}

Table 2: Precision of the method

Con	Inter-day (n=6) precision		Intra-day (n=6) precision	
cng mL^{-1}	Mean peak area ± S.D.	% RSD	Mean peak area ± S.D.	% RSD
10	82.36 ± 0.90	1.09	83.92 ± 1.27	1.56
400	3328.26 ± 56.71	1.70	3363.23 ± 62.31	1.85
800	6541.41 ± 79.80	1.21	6562.46 ± 71.91	1.09

The recovery for emodin was calculated by comparing the peak areas of samples which were pre-spiked with analytes at 50, 100 and 150% levels, the extraction recovery of emodin was noted in the range of 97.93 to 101.56%. (Table 3.)

Table 3: Accuracy of the method (n = 6)

Excess spike standard concentration added (%)	Theoretical content (ng mL^{-1})	Amount of drug recovered (ng ± S.D.)	% of drug recoverd	% RSD
0	20.44	20.05 ± 0.27	98.1	1.36
50	30.66	30.02 ± 0.40	97.93	1.34
100	40.88	41.21 ± 0.68	100.8	1.67
150	51.1	51.90 ± 0.30	101.56	0.58

3.3 Selectivity

From the chromatogram shown in Figure 3, it is evident, that under the chosen chromatographic conditions, emodin (RT-1.55) was completely separated from sample, which indicated that the method is selective and could be used for their identification and quantification.

3.4 Specificity

The specificity of the method was confirmed by injecting blank sample. No other peaks were observed at the retention times of emodin, indicating that interfering substances were not present, and MSMS spectrum showing the specificity of the method.

3.5 Analysis of emodin content in the polyherbal formulation and rhizome of *R. emodi*

The analysis of emodin in polyherbal formulation namely; Safoof-e-Pathar phori and *R. emodi* samples showed higher content in MSPD extraction as compared to conventional extraction and ultrasonication (Figure 4). The content of emodin was found 427 and 3490 mg kg^{-1} by conventional extraction, 488 and 3970 mg kg^{-1} by ultrasonic extraction and 535 and 4350 mg kg^{-1} by MSPD extraction, respectively in polyherbal formulation of *R. emodi* rhizome. The trueness of content of emodin in formulation with respect to the ratio of drug added was found 97.87%, 98.33% and 98.39% using conventional, ultrasonic and MSPD extraction techniques, respectively.

4. Conclusion

UPLC with QTOF-MS offers improved quality data in terms of increased detection limits, and chromatographic resolution with greater sensitivity. In the present investigation, a validated UPLC/Q-TOF-MS/MS method for the determination of emodin in the rhizome *R. emodi* and its traditional formulation were optimized. This method was found sensitive enough to monitor the lower concentrations of emodin in the samples. The advantages of our

developed method are the short analysis time (RT 1.55 min), high sensitivity (LOQ: 1.0 ng mL^{-1}) and simple extraction procedure. The assay was successfully employed for the quantification of emodin in the rhizome *R. emodi* and its traditional formulation using different extraction techniques with acceptable precision, adequate sensitivity and accuracy. MSPD method was found most suitable for extraction of emodin from complex matrix.

Acknowledgments

Authors are thankful to the CCRUM, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, New Delhi, India, for the financial support and CSIR for fellowship.

Conflict of interest

We declare that we have no conflict of interest.

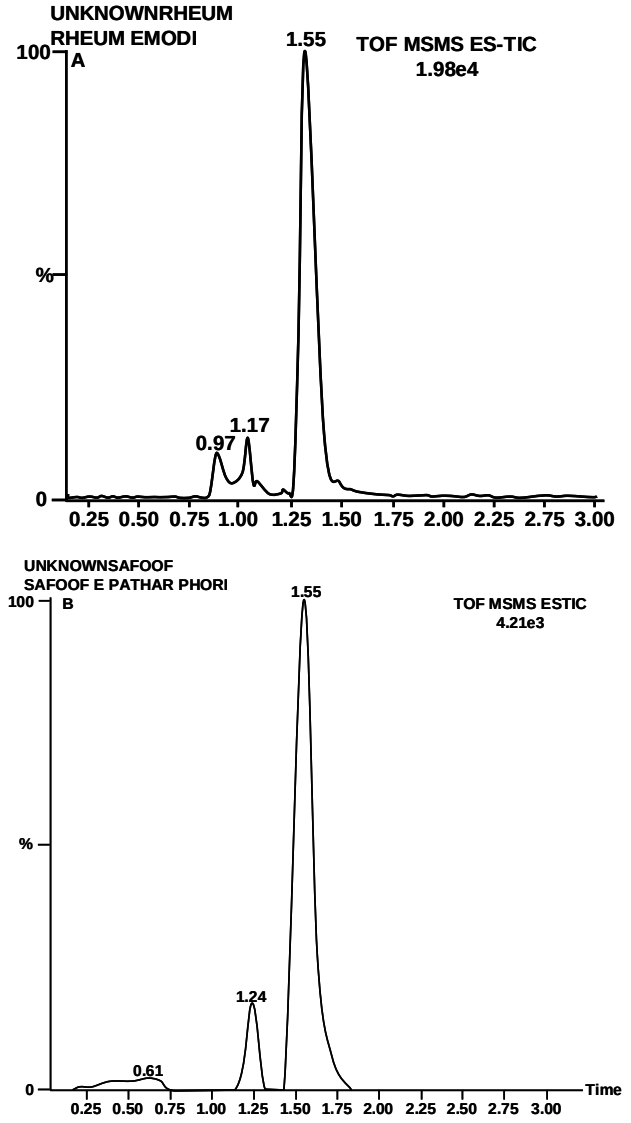


Figure 3: Typical chromatograms of sample showing R_T 1.55 min by selective reaction monitoring scan mode. (A) *R. emodi* (B) Safoof-e-Pathar phori

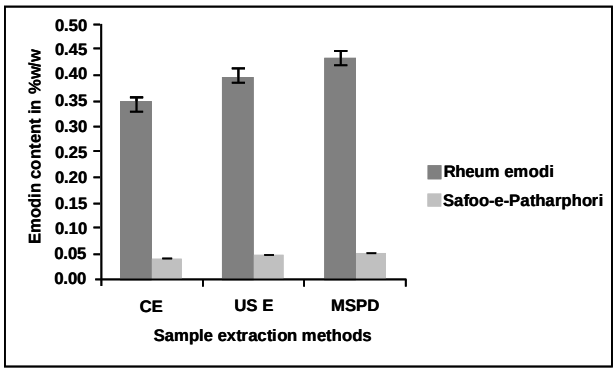


Figure 4: Bar diagram showing comparative content of emodin in samples by using different extraction techniques

Refrences

Agarwal, S.K.; Singh, S.S.; Verma, S. and Kumar, S. (2000). Antifungal activity of anthraquinone derivatives from *Rheum emodi*. J. Ethnopharmacol., **72**:43-46.

Ahmad, W.; Zaidi, S.M.A.; Mujeeb, M.; Ansari, S.H. and Ahmad, S. (2014). HPLC and HPTLC methods by design for quantitative characterization and *in vitro* antioxidant activity of polyherbal formulation containing *Rheum emodi*. J. Chromatogr. Sci., **52**:911-918.

Anonymous. (1972). Wealth of India, Raw Material, CSIR, New Delhi, **9**:3.

Anonymous. (1986). Qarabadeen Majeedi. All India Unani Tibbi Conference, Delhi, **9th** Edition.

Anonymous. (1990). Handbook of Ayurvedic Medicinal Plants, CRC Press, Boca Raton, FL and London, pp:487.

Anonymous. (2006). The Wealth of India, Second Supplement Series Raw Material, **3**:58.

Anonymous. (2007). The Unani Pharmacopoeia of India, **1**:58.

Anonymous. (2007). The Unani Pharmacopoeia of India, **1**:91.

Babu, K.S.; Srinivas, P.V.; Praveen, B.; Kishore, K.H.; Murty, U.S. and Rao, J.M. (2003). Antimicrobial constituents from the rhizomes of *Rheum emodi*. Phytochemistry, **62**:203-207.

Garg, M.; Tamboli, E.T.; Singh, M.; Kamal, Y.T.; Parveen, R. and Ahmad, S. (2014). Rapid, sensitive and validated UPLC/Q-TOF-MS method for quantitative determination of vasicine in *Adhatoda vasica* and its *in vitro* culture. Pharmacogn. Mag., **10**:S198-205.

ICH. (1997).Validation of Analytical Procedures, Methodology, ICH harmonized tripartite guidelines.

Kubo, I.; Murai, Y.; Soediro, I.; Soeetarno, S. and Sastrodihardjo, S. (1992). Cytotoxic anthraquinones from *Rheum palmatum*. Phytochemistry, **31**:1063-1065.

Muller, S.O.; Schmitt, M.; Dekant, W.; Stopper, H.; Schlatter, J. and Schreier, P. (1999). Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. Food Chem. Toxicol., **37**:481-491.

Novotna, P.; Pacakova, V.; Bosakova, Z. and Stulik, K. (1999). High-performance liquid chromatographic determination of some anthraquinone and naphthoquinone dyes occurring in historical textiles. J. Chromatogr. A., **863**:235-241.

Prasad, P. and Purohit M.C. (2001). Altitude acclimatization and concentration of active constituents and calorific value of two medicinal plant species *Rheum emodi* and *R. Nobile* (Rhubarb) in Sikkim Himalaya. Curr. Sci., **80**:734-736.

Rehman, U.H.; Ahmad, J.; Chester, K.; Tajuddin, E.T.; Ahmad, S. (2014). Chemoprofiling and tissue culture studies on *Picrorhiza kurroa* Royale ex Benth. for production of picroside II. Ann. Phytomed., **3**:70-77.

Semple, S.J.; Pyke, S.M.; Reynolds, G.D. and Flower, R.L.P. (2001). *In vitro* antiviral activity of the anthraquinone chrysophanic acid against poliovirus. Antiviral Res., **49**:169-178.

Singh, M.; Ansari, S.H. and Ahmad, S. (2014). Production and estimation of asarone in *in vitro* cultures of *Acorus calamus* Linn. Ann. Phytomed., **3**:82-86.

Singh, N.P.; Gupta, A.P.; Sinha, A.K. and Ahuja, P.S. (2005). High-performance thin layer chromatography method for quantitative determination of four major anthraquinone derivatives in *Rheum emodi*. J. Chromatogr. A., **1077**:202-206.

Verma, S.C.; Singh, N.P. and Sinha, A.K. (2005). Determination and locational variations in the quantity of hydroxyanthraquinones and their glycosides in rhizomes of *Rheum emodi* using high-performance liquid chromatography. J. Chromatogr. A., **1097**:59-65.

Wright, C.A. (2001). Mediterranean vegetables: A cook's ABC of vegetables and their preparation. Albany Street, Boston, Massachusetts: The Harvard Common Press.

Yang, Y.C.; Lim, M.Y. and Lee, H.S. (2003). Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed shows larvicidal activity against three mosquito species. J. Agric. Food Chem., **51**:7629-7631.

Yen, G.C.; Duh, P.D. and Chuang, D.Y. (2000). Antioxidant activity of anthraquinones and anthrone. Food Chem., **70**:437-441.

Yim, H.; Lee, Y.H.; Lee, C.L. and Lee, S.K. (1999). Emodin, an anthraquinone derivative isolated from the rhizomes of *Rheum palmatum*, selectively inhibits the activity of casein kinase II as a competitive inhibitor. Planta Med., **65**:9-13.

Yin, J.; Wang, Y.; Tan, B.; Kang, Y.; Xie, D. and Tian, L. (2013). Matrix solid-phase dispersion extraction for chromatographic analysis of labdane diterpenoids in *Coleus forskohlii*. Phytochem Anal., **24**:117-123.

Zargar, B.A.; Masoodi, M.H.; Ahmad, B. and Ganie, S.A. (2011). Phytoconstituents and therapeutic uses of *Rheum emodi* wall. ex Meissn. Food Chem., **128**:585-589.