Hygeia:: journal for drugs and medicines
October 2014 –March 2015
OPEN ACCESS
Research article section: Medicinal natural products / Pharm.Analysis
A Half Yearly Scientific, International, Open Access Journal for Drugs and Medicines

Research article
DOI: 10.15254 / H.J.D.Med.6.2014.134

DEVELOPMENT AND VALIDATION OF AN ANTIDIABETIC POLYHERBAL FORMULATION CONTAINING CURCUMIN USING RP-UFLC METHOD.

Rashmi N G, Gurupadayya B M, Sirisha Tadiboyina, Jinesh B Nagavi, Chetan G Shinde

Department of Pharma. Chemistry, JSS College of Pharmacy, JSS University, Mysore, Karnataka, India.

Keywords: Curcumin, Mehagni, RP-UFLC, Anti- Diabetic Herbal Formulation.

Correspondence:

Rashmi N G, Department of Pharma. Chemistry, JSS College of Pharmacy, JSS University, Mysore, Karnataka, India.

Email: ng.rashmi85@gmail.com

Received: 5 July 2014,
Revised: 20 July 2014,
Accepted: 30 July 2014,
Available online: 10 October 2014

ABSTRACT

Plan: *The principle destination of our work was to develop a simple, rapid and sensitive reverse phase ultra-fast liquid chromatographic (RP-UFLC) method for estimation of curcumin in anti-diabetic poly herbal formulation (Mehagni).*

Methodology: *Chromatography was carried on a reverse phase C_{18} column (250 x 4.6 mm) with the mixture of methanol and 2% acetic acid as a mobile phase at the proportion of 70:30 v/v with the flow rate of l.2 ml/min. The absorbance measured at 420 nm by PDA detector.*

Outcome: *Optimized chromatographic conditions were achieved and results showed good peak resolution. The retention time was found at 5.02 min. The method was validated as indicated by International Conference on Harmonization guidelines. The parameters, such as specificity, sensitivity, linearity, precision, accuracy, ruggedness, robustness and system suitability were performed. The framework was linear with a correlation co-efficient of 0.9945. %RSD of system and method precision were found to be 1.14 and 1.13. The LOD & LOQ for curcumin was found to be 0.2 µg/ml and 0.65 µg/ml.*

INTRODUCTION

Standard pharmaceutical "Ayurveda" since extraordinary oldness, has been giving the human kind to act against diseases. Mehagni is a polyherbal formulation[1] holding curcumin[2], amalaki, madhunasini and ekanayakam, used as anti-diabetic. Curcumin is a polyphenol derived from the herbal remedy and dietary spice turmeric. Chemically it is (1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione. Curcumin is an ancient medicine, having various remedial properties it might be utilized for the medication of chronic disorder like diabetes, cancer, arthritis, HIV infections. It possess diverse anti-diabetic[3-4], anti-inflammatory[6], anti-oxidant[6], anti-microbial[7], anti-cancer[8-9] properties following oral or topical administration.

Hygeia.J.D.Med. Vol.6 (2), October 2014,

Hygeia journal for drugs and medicines 2229 3590, 0975 6221
Researcher ID: J-1912-2014

The numerous spectrophotometric, Thin layer chromatograpy[10-11], High performance thin layer chromatography [12-14] , High performance thin liquid chromatograpy[15-16], ultra-fast liquid chromatography method were developed for curcumin[17]and advance methods like LC-MS and LC-ES-MS method[18-19] were also developed. The principle target of this study was to develop a simple, economic, rapid, precise, and validated technique for quantitative estimation active ingredients in commercially available poly herbal formulation.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Standard curcumin (purity>99%) was purchased from lobal chime, Mumbai. HPLC grade Methanol, acetic acid were obtained from Merck, Germany. Triple distilled water was obtained from the Milli Q unit. Mehagni poly herbal formulation from SNA Oushadhasala Pvt. Ltd., India.

2.1.1. Instrumentation

The Ultra-Fast Liquid Chromatography consists of Shimadzu LC-20AD solvent delivery system (pump), Photodiode Array Detector (PDA) with a 7725i rheodyne injector with 20µL loop volume (Kyoto, Japan). 20µl of injection volume was used for injection. The LC Solution software was used for integration.

2.1.2. Chromatography conditions

Chromatographic separation was done using Phenomenex C_{18} column (250 X 4.6 mm, 5µ ID). The mobile phase consists of methanol and 2% acetic acid (70:30, v/v). The flow rate was adjusted to 1.2 ml/min and run time was adjusted to 10 min. Curcumin was detected at wavelength of 420 nm using a PDA detector with retention time 5.03 min. 20µl of injection volume was used for injection.

2.1.3. Preparation of curcumin standard solution

100 mg standard curcumin was weighed accurately and transferred to a 100 ml volumetric flask and 70 ml of methanol was added and dissolved and the above solution was again made up to volume with methanol to produce 1000µg/ml solution. From the stock solution final concentration (10 µg/ml) of the individual working standards was prepared with methanol.

2.1.4. Preparation of sample solution

Twenty tablets (Mehagni) were weighed accurately and the average weight was determined and then grounded to fine powder. Quantity equivalents to 0.1 g were transferred to 100 mL of volumetric flask and volume was adjusted with 100ml with methanol. The solution was centrifuged for 15 minutes at 3000 rpm. Centrifugation was found to be faster and more effective than filtration. Centrifugation forms a cake of excipients at the bottom of the test tube, which is not disturbed while drawing out the supernatant solution.

This supernatant solution was pipette out and diluted appropriately with methanol to obtain the concentration of 10µg/mL concentration of curcumin.

2.1.5. Mobile phase preparation

HPLC grade methanol (solution A) and acetic acid (solution B). 2% of acetic acid was prepared by dissolving 2ml of acetic acid in 100ml HPLC grade water.

2.1.6. Development of calibration curve of curcumin Standards

Working solutions for the calibration study were prepared from the stock solution by an adequate dilution using Methanol. Calibration standards of concentrations 5- 25 µg/ml were prepared for curcumin.

2.1.7. Method Validation

The validation parameters such as accuracy, precision, linearity, limit of detection and limit of quantification, Robustness, specificity and system suitability has been evaluated as per ICH guidelines[20].

2.1.8. Accuracy

Accuracy is expressed as the closeness of agreement of trueness[20]. It was carried out by recovery studies by adding the known concentration of the standard solution of curcumin to the samples of known percentage recovery. The results were shown in the (Table 1).

2.1.9. Precision

Precision studies were carried out in different ways such as system precision, method precision and intraday precision, interday precision[20]. In system precision same concentration was injected six times and method precision same concentration were injected in different six vials of curcumin (20 µg/ml). In intraday precision (10, 15, 20 µg/ml) of curcumin was carried out to check the repeatability, interday variation for intermediate precision (10, 15, 20 µg/ml) and at same prescribed conditions.

2.1.10. Linearity

Linearity for curcumin was plotted from the standard solution from the concentration of 5µg/ml to 25µg/ml which wa analyzed to check the linearity response.

2.1.11. Ruggedness and Robustness

The ruggedness of the proposed method was carried out by changing the different instrument, different operators and different column of similar model of C_{18}. Robustness of the method were carried out by small change in flow rate ± 0.1, column temperature ± 5, % organic strength ± 2%. . Peak area was not affected by small variation in parameters.

2.1.12. Limit of detection (LOD)

Limit of detection of the method was determined by measuring the signal to noise ratio. LOD for curcumin was found to be 0.2µg/ml. Whereas LOD was obtained by the formula.

$$\mathbf{LOD} = 3.3\sigma/m$$

Where 'σ' is the standard deviation of intercept of regression line and 'm' is slope of calibration curve.

2.1.13. Limit of Quantification (LOQ)

The smallest concentration of the analyte which can be quantified based on the signal to noise ratio. Limit of quantification for curcumin was found to be 0.6µg/ml. whereas LOQ was obtained by the formula.

$$\mathbf{LOQ} = 10\sigma/m$$

Where 'σ' is the standard deviation of intercept of regression line and 'm' is slope of calibration curve.

2.1.14. Specificity

Specificity is the method's ability to shows good separation of analyte in the presence of compounds which may not be affected by the matrix[20], degradants, impurities or any other plant matrix. The specificity of the method was carried out by comparing the standard retention time spectra and the sample retention time spectra. No endogenous substance were interfered with the drug response. Delegate run of the chromatograph were shown in (Figure 3 and 4).

3. RESULTS & DISCUSSION

3.1. Method development

Optimization of the chromatographic condition was carried out by changing the methanol concentration, strength and acetic acid concentration. Improvement of the chromatographic conditions revealed good separation of curcumin with the mobile phase of methanol: 2%acetic acid (70:30) v/v. Retention time for curcumin was found to be 5.02 min (Figure 3). Increase in acetic acid concentration shifts the peak to the void volume side. Decrease in the acetic acid leads to the loss of resolution. Increase or decrease in methanol concentration leads to the peak splitting. The calibration curve for the standard curcumin was plotted from 5-25µg /ml (Figure 1) and it was found to be linear in range. The regression equation for curcumin was found to be 0.9945 respectively.

3.2. Accuracy

The results indicate that the recovery of curcumin (Table 1) was found to be accurate, the % nominal mean was found to be 99 – 100%, percentage of Standard Deviation (SD) and Relative Standard Deviation (RSD) were found to be within the limits which proves the method was accurate.

3.3. Precision

Precision studies were carried out by methods such as system precision, method precision and intraday precision, interday precision. In system precision injecting same concentration six times and method precision injecting same concentration in different six vials of curcumin (20 µg/ml). In intraday precicision (10, 15, 20 µg/ml) of curcumin was carried out to check the repeatability, interday variation for intermediate precision (10, 15, 20 µg/ml) and at same prescribed conditions. The percentage Standars Deviation (SD) and Relative Standard Deviation (RSD) were calculated and found to be within the limits (Table 2 & 3).

3.4. Ruggedness and Robustness

The method was discovered to be rugged and robust, since there were no changes in the chromatogram by changing the optimized chromatographic conditions, instruments, operator and column.

3.5. LOD and LOQ

The LOD & LOQ are calculated as given in the above formula and found to be 0.2 µg/ml & 0.65 µg/ml respectively.

3.6. Specificity

The Specificity of the method showed good separation of the standard curcumin and it is not affected by the other plant constituents and matrix.

4. CONCLUSION

A simple, sensitive and rapid method for estimation of curcumin which is present in Mehagni anti-diabetic formulation have been developed and validated as per the ICH guidelines. This system is specific, sensitive, accurate, precise, robust and reproducible. The developed method may be used for the quantitative and qualitative estimation of curcumin in commercially available herbal formulations.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, JSS college of Pharmacy, Mysore for providing necessary facilities.

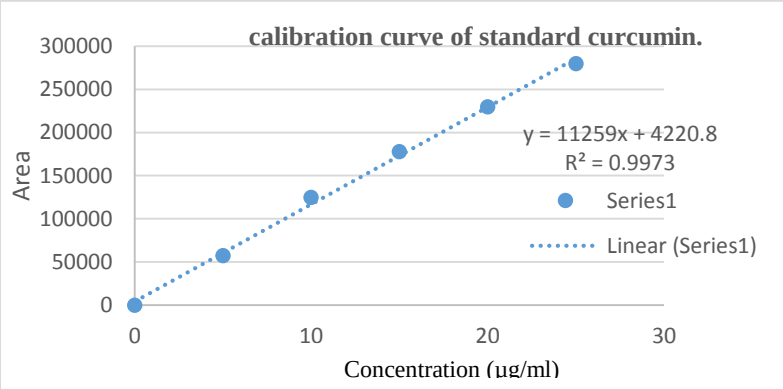

Figure.1. Calibration Curve of Standard Curcumin.

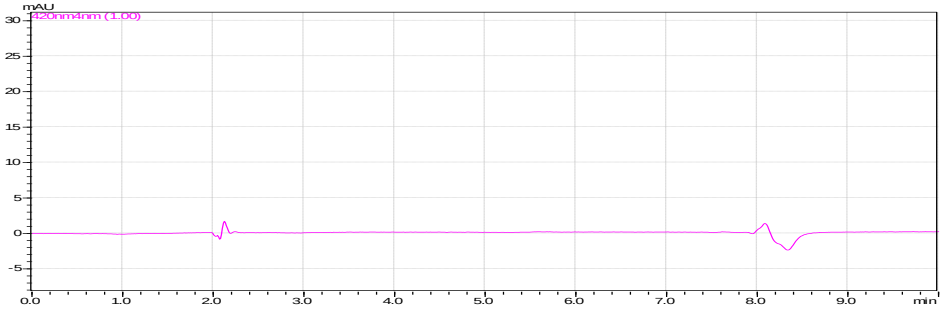

Figure.2. Typical UFLC chromatogram of Blank.

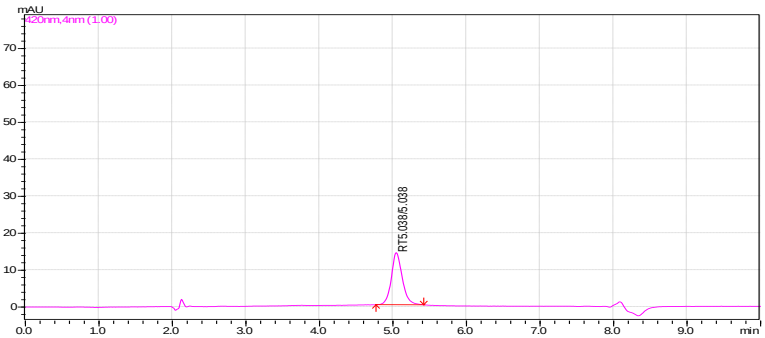

Figure.3. Typical UFLC chromatogram of standard curcumin.

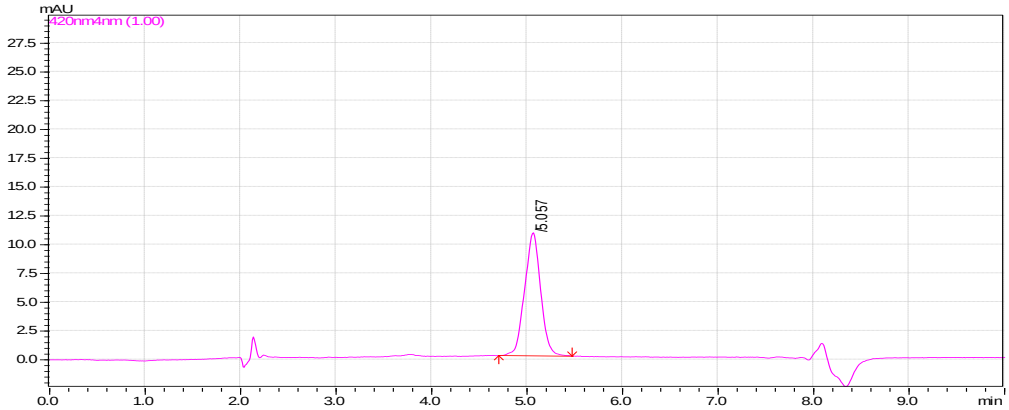

Figure.4. Typical UFLC chromatogram of sample (Mehagni).

Table.1. Accuracy (% Recovery Data)

S.No	*% of drug added*	*Amount of drug taken(µg/ml) (STD)*	*Amount of drug added (µg/ml) (sample)*	*Total amount of drug*	*Total Amount of drug found*	*% recovery*	*Mean*	*% SD*	*% RSD*
1	50%	10	5	15	14.9	99	100	0.8	0.8
					15	100			
					15	100			
2	100%	10	10	20	20	100	100	1	1
					19.8	98			
					20	100			
3	150%	10	15	25	25	100	100	0.6	0.6
					24.9	99			
					25	100			

Table.2. Intraday and Interday Precision

Parameter	*Value*	
	Intraday	*Interday*
Standard deviation	0.9	1.2
%RSD	1	1.3

Table.3. System and Method Precision

Parameter	*Value*	
	System	*Method*
Standard deviation	1.1	1.2
%RSD	1.1	1.2

REFERENCES

1. Chandel HS, Pathak A, Tailag M. Polyherbal formulation for Anti-diabetic therapy. *International journal of pharmacy and pharmaceutical science* **2011**; 3, 3: 226-228.
2. Sharma RA, Gescher AJ, Steward WP, Curcumin: The story so far, *European journal of cancer* **2005**; 41, 13: 1955-1968.
3. Halim Esher M, Ali Hussain, hypoglycemic, hypolipidemic and antioxidant properties of combination of curcumin from curcuma longa, Linn, and Partially purified product from abroma augusta, Linn, In streptozotocin induced diabeties. *Indian journal of clinical Biochemistry* **2002**; 17, 2: 33-43.
4. Sabu MC., Smitha K., Ramadasan Kuttan, Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes, *Journal of Ethnopharmacology* **2002**; 83: 109-116.
5. Satoskar RR, Shah SJ, Shenoy SG, Evalution of anti-inflammatory property of curcumin (diferuloyl methane) in patient with postoperative inflammation. *International journal of clinical pharmacology, Therapy, and toxicology* **1986**; 24, 12: 651-654.
6. Haseeb Ahsan, Nazneen Parveen, Nizam U. Khan, Hadi SM., Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. *Chemico-Biological Interactions* **1999**; 121: 161–175
7. Kruti pandya, Bhavana Solanki, Bijal Shah, Diksha Parekh, Hardik Soni, Phyto-chemical screening &evalution of antibacterial activity of polyhebal formulation, *indo-global journal of pharmaceutical sciences* **2011**; 1, 3: 206-218.
8. Ali Mohammad Alizadeha,n, Mahmood Khaniki, Saleh Aziziana, Mohammad Ali Mohaghgheghi, Chemoprevention of azoxymethane-initiated colon cancer in rat by using a novel polymeric nanocarrier–curcumin. *European Journal of Pharmacology* **2012**; 689: 226–232.
9. Rubesh Kumar S, Ram Kishan J, Venkateshwar Roa KN, Duganath N, Simultaneous Spectrophotometric Estimation of Curcuminoids and Gallic Acid in Bulk Drug and Ayurvedic Polyherbal Tablet Dosage Form, *International Journal of Pharmaceutical Quality Assurance* **2010**; 2, 1: 56-59.
10. Patil snehal J, Salunkhe Vijay R, Simulataneous Estimation of curcumin and quercetin in Ayurvedic proprietary medicine by U.V, Spectrophotometry, *International Journal of Research in Ayurveda and Pharmacy* **2012**; 3, 2:267-271.
11. Y. Thejeswari S. Rubesh Kumar N. Duganath, N. Devanna, Assimilation of Curcuminoids from Curcuma longa by IR, UV and TLC by using Different Solvent System, *International Journal of Universal Pharmacy and Bio Sciences* **2013**; 2, 4:140-148.
12. Ansari MJ, Ahemad S, Kohil K, Ali J. stability indicating HPTLC determination of curcumin bulk drug and pharmaceutical formulation. *Jounal of pharmaceutical and Biomedical Analysis* **2005**; 39, 1-2:132-138.
13. Vinitkumar Y. Thakker*1,Vaishali N. Shah1, Uravashi D. Shah1, Manish P. Suthar, Simultaneous estimation of Gallic acid, Curcumin and Quercetin by HPTLC method, *Journal of Advanced Pharmacy Education & Research* **2011**; 1:70-80.
14. Sonawane SD, Nirmal.SA, Patil AN, Development and validation of HPTLC Method to detect Curcumin and Gallic acid in polyherbal formulation, *Journal of liquid chromatography & amp Related Technologies* **2011**; 34:2664-2673.
15. Taylor SJ, McDowell IJ, Determination of the curcuminod pigments in turmeric (curcuma domestica Val) by RP-HPLC. *Chromatographia* **1993**; 34, 1-2: 73-77.
16. JiLi, Yunyun Jiang, Jun Wen, Guorong Fan, Yutian Wn and Chunm Zhang. A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, Validation and application to a pharmacokinetic study of curcumin lipsome. *Biomedical chromatography* **2009**; 23, 11: 1201-1207.
17. Shanmugam.R, Gowthamarajan.K, Priyanka DL, Madhuri.K, Dhanabal. SP, Development and Validation of a RP-UFLC Method for Simultaneous Estimation of Quercetin and Rutin, *Hygeia: Journal for drug and medicines* **2013**; 5, 1: 113-120.

18. Anchang Liu, Hongxiang Lou, Lixia Zhab, Peihang Fan. Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. *Journal of pharmaceutical and Biomedical Analysis* **2006**; 40, 3: 720-727.
19. Kuo-Yi Yang, Lei-Chewen Lin, Ting-Yu. Tseng, Shau-Chun Wang, Oral bioavailability of curcumin in rat and the herbal analysis from curcuma long a by LC-MS/MS. *Journal of chromatography B* **2007**; 853, 1-2: 183-189.
20. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology **2005**, Available at:http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html, (accessed Jan 18, 2011).

Rashmi N G, Gurupadayya B M, Sirisha Tadiboyina, Jinesh B Nagavi, Chetan G Shinde. Development and validation of an antidiabetic polyherbal formulation containing curcumin using RP-UFLC method. *Hygeia.J.D.Med* **201**4; 6(2):36-44. Available from http://www.hygeiajournal.com / Article ID-Hygeia.J.D.Med/134/14. doi:10.15254/H.J.D.Med.6.2014.134.