Journal of Pharmaceutical Research Vol. 13, No. 1, January - March 2014 : 16-19.

DEVELOPMENT AND VALIDATION OF RP - HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DICYCLOMINE AND MEFANAMIC ACID

Baokar Shrikrishna[1]*, Mulgund Sugandha[2], Ranpise Nisharani[2]

*[1]Department of Pharmaceutical Chemistry, Shivnagar Vidya Prasarak Mandal's College of Pharmacy, Malegaon (Bk.), Tal- Baramati, Dist- Pune- 413115, Maharashtra, India.

[2]Sinhgad College of Pharmacy, Sy.No. 44/1, Off Sinhgad Road, Vadgaon (Bk.), Pune- 411041, Maharashtra, India.

Received on : 01.10.2013 Revised : 03.03.2014 Accepted : 13.03.2014

ABSTRACT

Objective: The aim of present work was to develop a RP-HPLC method for simultaneous analysis of Dicyclomine (DCL) and Mefanamic acid (MFA) in a tablet dosage form. **Method:** Waters Chromatographic system was optimized using a Lichrocart C18 column (250 x 4.60 x 5ìm) with mobile phase comprising of 50 mM KH_2P0_4: Acetonitrile in the ratio of 75:25. The flow rate was adjusted to 1.0 ml/min with UV detection at 256 nm. **Result:** DCL and MFA were eluted with retention times of 7.213± 0.3 min and 11.102± 0.3 min respectively. Beer's Lambert's Law was obeyed over the concentration ranges of 5-25 ìg/ml, 50-250 ìg/ml for DCL and MFA respectively. **Conclusion:** The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of both drugs in a tablet dosage form. Statistical analysis proves that the method is sensitive and significant for the analysis of DCL and MFA in pure and in pharmaceutical dosage form without any interference from the excipients. The method was validated in accordance with ICH guidelines.

Keywords – *Dicyclomine; Mefanamic acid; Simultaneous estimation; HPLC.*

INTRODUCTION

Dicyclomine hydrochloride (DCL) is 2-(diethylamino) ethyl bicyclohexyl-1-carboxylate hydrochloride[1]. It binds more firmly to M_1 and M_3 than to M_2 and M_4 receptors. It has one-eighth the neurotropic activity of atropine and approximately twice the musculotropic activity of papaverine. It is used for its spasmolytic effect on various smooth muscle spams, particularly those associated with the gastrointestinal tract. It is also useful in dysmenorrheal, pylorospam and biliary dysfunction[2]. It is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. This medication works by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines. MFA [N-(2,3-xylyl) anthranilic acid] is a white to off white crystalline solid with a bitter aftertaste. It will darken if exposed to light for long periods but is otherwise stable at room temperature. It is virtually water insoluble except at an alkaline pH. It is synthesized from o-chlorobenzoic acid and 2,3-dimethylaniline under catalytic conditions. MFA is the only fenamic acid derivative which produces analgesia centrally and peripherally[3,4] . It is COX-1 inhibitor as well as PG receptor antagonist action. It inhibits leukotriene levels by inhibiting phospholipase-A_2. It is very effective in dysmenorrhoea besides osteoarthritis[5]. Combination of MFA and DCL has a Synergistic effect[6].

EXPERIMENTAL

Materials and Methods

Reference standard of DCL and MFA gifted by Fortune Health Care Pvt. Ltd., Vadodara (Gujarat), India and Novartis Pharmaceuticals Pvt. Ltd., Hyderabad, (A.P.) India respectively and they were used as such without further purification. Water HPLC grade was obtained from a Milli-Q RO water purification system. All the chemicals and reagents used were of analytical reagent grade.

Instrumentation

Analysis was performed on HPLC system equipped with waters pump, UV-Visible detector, Lichrocart C18 column (250 x 4.60 x 5ìm) was used for separation.

Preparation of standard stock solution

10 mg of DCL and 10 mg of MFA were weighed accurately and transferred to separate 10 ml volumetric flask and volume was adjusted to the mark with the mobile phase to give a stock solution of 1000 ìg/ml.

Preparation of working standard solution

From stock solution of DCL 05-25 µg/ml and from stock solution of MFA 50-250 µg/ml concentrations were prepared.

***Correspondence :** krishnabaokar@gmail.com Mob : 9960225455.

System Suitability

According to United State Pharmacopoeia 2007 system suitability tests are an integral part of LC method in the course of optimizing the conditions of the proposed method[7,8]. The system suitability test solution was injected and chromatographic parameters for DCL and MFA were evaluated for proving the system suitability. Results are given in Table 1.

Table 1: *System Suitability Parameters*

Parameters	DCL*	MFA*
Retention Time	7.214 + 0.3 min	11.214 + 0.3 min
Area Under Curve	1425.25	20985.11
Theoretical Plates	2574	2768
Tailing Factor	1.20	1.173

(Results are average of 3 readings)*

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Finally optimized parameters for method development are presented in Table 2.

Table 2: *Selection of Separation Variable*

Variable	Condition
Column	C_{18}, 250mm x 4.60mm, 5µ
Mobile Phase	50 mM KH_2PO_4 : Acetonitrile = 75 : 25
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	256 nm
Retention time-	
Dicyclomine	7.213 ± 0.3 min
Mefenamic acid	11.102 ± 0.3 min

Quantification of drugs present in marketed formulation

The chromatograms of mixture showed complete separation of two drugs. The chromatograms of individual components were also obtained. The ingredients were also quantified with respect to the standards. The results obtained are presented in Table 3 and HPLC Chromatogram for DCL and MFA in Fig. 1.

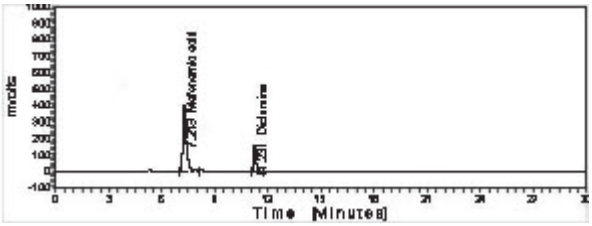

Fig. 1 : *Chromatogram of DCL and MFA*

Method validation for HPLC

The method was validated according to ICH guidelines for linearity, selectivity, precision, accuracy, robustness. Selectivity was checked using drug sample and mixture of standards in order to optimize separation and detection[9].

Table 3: *Analysis for DCL and MFA in Tablet Formulation*

Std. Conc. µg/ml	DCL		MFA	
	10	20	100	200
1	10.01	19.48	100.05	200.21
2	9.9	20.43	99.95	199.93
3	9.9	19.82	99.12	200.12
Mean	9.93	19.91	99.70	200.08
%found*	99.36	99.55	99.70	100.04
SD	0.063	0.481	0.510	0.142
%RSD	0.639	2.417	0.512	0.071

**Each reading is mean reading of three batch of formulation*

Linearity

Linearity of method was performed by analyzing a standard solution of drugs by the method in the selected concentration range for both drugs[10,11] (Table 4).

Table 4: *Linearity for DCL and MFA*

DCL (µg/ml)		MFA (µg/ml)	
5	1425.258	50	20985.14
10	2827.380	100	33477.25
15	4175.327	150	48756.39
20	5749.173	200	60534.61
25	7056.784	250	71245.55
Slope	0.0035	Slope	0.0039
Corrl. Coeff.	0.999	Corrl. Coeff	0.998
Equation of Line	y=283.4x - 4.132	Equation of Line	y=280.0x + 4155

Accuracy

The accuracy of the proposed method was determined by a recovery study, carried out by adding standard in drugs[12] (Table 5).

Table 5: *Recovery Study*

Level of Recovery	80%		100%		120%	
	DCL	MFA	DCL	MFA	DCL	MFA
Amount present (mg)	10	50	10	50	10	50
Amount of Std. added (mg)	8	40	10	50	12	60
Amount recovered (mg)	8.56	39.96	10.03	50	11.93	59.93
% Recovery	98.75	99.58	100.33	100	99.44	99.44

Precision

Precision was determined by repeatability, inter day and intraday reproducibility experiments[13,14] (Table 4). A standard solution containing drugs were injected six times. % Relative standard deviation of all the parameters was less than 3.5% for the degree of repeatability of the developed method. The low coefficient of variation values of intraday and inter day precision revealed that the method is precise (Table 6).

Table 6: *Precision for DCL and MFA*

Precision (%RSD)	DCL	MFA
Intraday (n=3)	0.653	0.810
Interday (n=3)	1.155	1.662
Repeatability (n=6)	0.154	0.125

Ruggedness

The ruggedness of analytical method is degree of reproducibility of test results by the analysis of same sample under varieties of normal test conditions such as different laboratories, different analyst, different day, different instrument, different column[15]. Results are presented in Table 7.

Table 7: *Ruggedness for DCL and MFA*

Ruggedness (%RSD)	DCL	MFA
Analyst 1 (n=3)	0.110	0.115
Analyst 2 (n=3)	0.225	0.220

Robustness

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust for changes in mobile phase flow rate, mobile phase ratio[16]. The analysis data are presented in (Table 8).

Table 8: *Robustness for DCL and MFA*

Robustness (%RSD)	DCL	MFA
Flow rate (-10%)	0.123	0.141
Flow rate (+10%)	0.114	0.145
Mobile phase ratio (- 2 %)	0.880	0.457
Mobile phase ratio (+ 2 %)	0.250	0.445

Therefore this HPLC method can be regarded as selective, accurate and precise.

CONCLUSION

The developed method describes in detail the steps necessary to perform each parameter for validation. Interpretation of results of validation parameters study shows that results of method is directly proportional to the concentration of analyte within a given range shows linearity of method. Different environmental condition and minor change in chromatographic condition doesn't cause any significant change in results shows stability and reproducibility of developed method. There was no interference by excipients with analyte peak shows proposed method is specific for analyte. As well as recovery study shows developed method is highly accurate. Hence the proposed HPLC method has been evaluated and validated for the accuracy, precision, and linearity and found to be convenient, sensitive and specific for the quality control of DCL and MFA in tablet dosage form.

ACKNOWLEDGEMENT

The authors wish to thank Principal and Management of Shivnagar Vidya Prasarak Mandals College of Pharmacy, Malegaon (Bk), Tal- Baramati, Dist-Pune and Scan Research Bioanalytical Laboratories, Bhopal, for providing required lab facilities with enthusiastic environment.

REFERENCES

1. Lokhande SR, Mhetre SM, Pekamwar SS, Kalyankar TM. Development and validation of reverse phase HPLC method for simultaneous estimation of dicyclomine hydrochloride, mefenamic acid and paracetamol in tablet dosage form. World J of Pharmacy and Pharmaceutical Sci. 2012; 1(3): 968-980.
2. Carolyn JF, Matthias CL. *Analgesics*. In *Wilson and Gisvolds text book of organic medicinal and pharmaceutical chemistry*. John MB, John HB, eds, 10th Ed. Lippincott-Raven publishers, 1998, p 536.
3. Ronald FB. *Nonsteroidal anti-inflammatory drugs*. In *Principles of medicinal chemistry.* Foye WO, eds, 4th Ed. B.I. Waverly Pvt Ltd, 1995, p 566.
4. Wahab SU, *et al.* Development of RP-HPLC method for the simultaneous determination of mefenamic acid and drotaverine hydrochloride combined tablet dosage form. Int J of Pharmaceutical Sci. 2011; 3(1): 115.
5. Sharma HL, Sharma KK, *Principles of pharmacology*. 2nd Ed. Paras medical publisher, 2007, p 368.
6. Dipal P, *et al.* Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by spectroscopic method. Int J Pharmaceutical Sci and Res. 2012; 3(10): 3766.
7. Anonymous, United State Pharmacopoeia, USP 30 NF25. 2007.
8. Zarna D, *et al.* RP-HPLC method for simultaneous estimation of omeprazole and ondansetron in combined dosage forms. Asian J Res Chem. 2009; 2(2): 108.
9. Ranjith A, *et al.* Simultaneous estimation of phenolic acids in sea buckthorn using RP-HPLC with DAD. J of Pharmaceutical and Biomed Anal. 2008; 47: 31.

10. Panjagala S, *et al.* Validated HPLC method for simultaneous estimation of zinc carnosine and aceclofenac in bulk and tablet dosage form. Int. J. Pharm & Ind. Res. 2011; 1(2): 109.

11. Pattan SR, *et al.* RP- HPLC method for simultaneous estimation of paracetamol and etoricoxib from bulk and tablets. Journal of Chemical and Pharmaceutical Research. J of Chem and Pharmaceutical Res. 2009; 1(1): 329.

12. Shaikh KA, *et al.* Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form. J of Pharmaceutical and Biomed Anal. 2008; 48: 1481.

13. Dewani AP, *et al.* RP-HPLC-DAD method for the determination of phenylepherine, paracetamol, caffeine and chlorpheniramine in bulk and marketed formulation. Arabian J of Chem. 2012.

14. Gopala ST, *et al.* RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in pharmaceutical dosage form. Int J Drug Dev Res. 2011; 3(4): 362.

15. Baokar SB, *et al.* Analytical method development and validation for estimation of sildenafil citrate from tablet dosage form by using RP-HPLC. Int J Res Pharm Sci. 2011; 2(2): 130.

16. Arun R, *et al.* Development and validation of analytical method for lopinavir and ritonavir by HPLC. Int J of Drug Dev Res. 2013; 5(2): 151.