Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MESALAMINE AND PREDNISOLONE IN BULK AND FORMULATION

N. K. Drisya[*1], S. S. Prasanth[1], V. Akhila[1], K. Sruthi[1]

[1*]Department of Pharmaceutical Analysis, Al Shifa College of Pharmacy, Perinthalmanna, Kerala, 679325, India.

ABSTRACT

A simple, precise, accurate, sensitive and economical reversed phase high performance liquid chromatographic method was developed and validated for the simultaneous determination of Mesalamine (MSL) and Prednisolone (PRD) in combined dosage forms in accordance with the analytical parameter mentioned in the ICH guidelines. Chromatographic separation of the drugs was achieved on C-18 Phenomenex column(250mm × 4.6mm i.d) and a mobile phase composed of Acetonitrile: phosphate buffer(p^H adjusted to 6 with Orthophosphoric acid) (20:80 v/v). The detection was carried out at 330nm. The retention times of Mesalamine and Prednisolone were found to be 4.373 min and 1.589min, respectively. Linearity was established for Meselamine in the range of 5-25µg/ml and Prednisolone in the range of 2-10µg/ml. The percentage recoveries of Mesalamine and Prednisolone were found to be 99.57% and 100.63% respectively. Correlation coefficient of Mesalamine was found to be 0.9973 and for Prednisolone it is 0.9984. The method showed adequate precision with smaller RSD (less than 1%).

KEYWORDS

Mesalamine, Prednisolone, HPLC and Method validation.

Author for Correspondence:

Drisya N K,
Department of Pharmaceutical Analysis,
Al Shifa College of pharmacy,
Perinthalmanna, Kerala, 679325, India.

Email: drisyank1996@gmail.com

INTRODUCTION

Intra bowel disorder or inflammatory bowel disease (IBD) is a group of idiopathic chronic inflammatory intestinal conditions. The two main disease categories are Cohn's disease (CD) IU and Ulcerative colitis (UC), which have both overlapping and distinct clinical and pathological features. Ulcerative Colitis is characterized by diffuse mucosal inflammation limited to the colon. Cohn's disease is characterized by patchy,

transmural inflammation, which may affect any part of the gastro intestinal tract.

IBD is a mild to moderate disease, but when no care is given properly it may lead to life threatening complications IBD is major risk factor and continues to be a major health problem in many areas of the world. Amino salicylates (MSL) and corticosteroids (PRD) are the effective and well tolerated first line therapy of this disease.

Mesalamine belongs to a group of amino salicylates or 5-ASA group and Prednisolone comes under corticosteroids. MSL and PRD have major role in maintaining the re-emission of intra bowel disease. They act on the inflamed lining of the gut (intestine) to prevent the formation of substance that causes inflammation. Therefore different preparations of this two are combined are used depending on the pattern of inflammation. Higher doses are prescribed in acute attacks and lower doses used to maintain re-emission. The research work done with a lower dose of MSL (250mg) and high dose of PRD (100mg) in a combination therapy.

MATERIAL AND METHODS

MSL and PRD pharmaceutical grade were procured from Mylan laboratories Pvt. Ltd. Hyderabad and Yarrow chem. Products, Dombivalli, Mumbai respectively. Hydrochloric acid, Sodium hydroxide and Disodium hydrogen phosphate were purchased from nice chemicals Pvt. Ltd, Mumbai. The HPLC grade water procured from Research lab fine chem. industries, Mumbai. The LC system includes two LC-20 AD vp solvent delivery module, SPD-M 20A vp UV-visible PDA detector, software CLASS-VP and column(Phenomenex C-18 Chromosil, 5µ particle size, 250mm × 4.6mm i.d), injection through Rheodyne injector port by Hamilton syringe. An electronic balance (Shimadzu BL 220H, 0.001, 220G), UV Spectrophotometer (Shimadzu UV-1700, Pharmaspec, Japan), P^H Meter (Systroniks), Vaccum filter (Tarsons rockyvac 300), a probe sonicator (Amplitude, model SPS200) were used in this study.

Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on a C-18, column on working standard solution of MSL (25µg/ml) and PRD (10µg/ml). Initially trials were carried out using Acetonitrile: Phosphate buffer in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials Acetonitrile: Phosphate buffer (pH adjusted to 6 with Orthophosphoric acid) (20:80v/v), at 1.5ml/min flow rate was chosen as the mobile phase, which have good resolution and acceptable peak parameters.

Preparation of standard stock solution

50mg of MSL and PRD weighed accurately and transferred them separately in to 50 ml volumetric flask, dissolved and made up to the mark with M/P (80:20v/v). From the stock solution (1000µg/ml), diluted further to obtain final concentrations of MSL (25µg/ml) and PRD (10µg/ml) were prepared using the same solvent.

Preparation of Mobile phase

Mobile phase was prepared by mixing 5.6ml of 0.2M NaOH in 50ml of 0.2M potassium di hydrogen phosphate made up the volume with water, the pH was adjust to 6 by adding Orthophosphoric acid, filtered through 0.45µ membrane filter paper and degassed. Buffer and ACN are adjusted in the ratio of 80:20 v/v. The mobile phase was pumped at 1.5ml/min and the injection volume for both standard and sample are 20µl.

Selection of detection wavelength

From the standard stock solution further dilutions were done using the same solvent and scanned over the range of 200- 400 nm and the spectra was obtained and it shows maximum absorbance at 330nm (MSL) and 246nm (PRD).

Preparation of sample solution

A quantity of powder equivalent to 50mg MSL and 20mg of PRD from synthetic mixture were transferred in to 50 ml volumetric flask containing the solvent, sonicated, filtered and diluted to obtain a final concentration in the ratio of 2:5. This test solution was injected and chromatogram was

recorded, thus results calculated to determine amount of drug present in the formulations.

Method Development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for MSL and PRD was obtained with a mobile phase Acetonitrile: Phosphate buffer, pH 3.5 (20:80 v/v) at a flow rate of 1.5ml/min to get better reproducibility and repeatability. Quantitation was carried out at 330nm based on peak area. Complete resolution of the peaks with clear base line was obtained (Figure No.1). System suitability test parameters for MSL and PRD for the proposed method are reported in Table No.1.

Validation of the Proposed Method

The proposed method has been validated for the simultaneous determination of MSL and PRD in synthetic mixture as per ICH guidelines.

RESULTS AND DISCUSSION

Linearity

Linear correlation was obtained between peak area Vs concentrations of MSL and PRD in the concentration ranges of 5-25µg/ml and 2-10µg/ml.

Range

Range is the interval between upper and lower concentration (amount) of analyte. The linear response was observed over a range of 5-25µg/ml for MSL and 2-10µg/ml PRD and the calibration curves of these two drugs at 330nm.

Method precision (Repeatability)

The RSD values for MSL and PRD were found to be 0.32 and 0.30 %, respectively (Table No.3). The RSD values were found to be < 2%, which indicates that the proposed method is repeatable.

Intermediate precision (Reproducibility)

The low RSD values of interday (0.41-0.85% and 0.35-0.93%) and intraday (0.30-0.80% and 0.32-0.64%) for MSL and PRD, respectively, reveals that the proposed method is precise (Table No.2).

LOD and LOQ

LOD values for MSL and PRD were found to be 7.4676µg/ml and 0.6280µg/ml, respectively and LOQ values for EPE and PCM were found to be 22.628µg/ml and 1.903µg/ml respectively (Table No.2). These data show that the proposed method is sensitive for the determination of MSL and PRD.

Accuracy

The recovery experiment was performed by the standard addition method. The low value of standard deviation indicates that the proposed method is accurate.

Assay

The proposed validated method was successfully applied to determine MSL and PRD in synthetic mixture. The result obtained for MSL and PRD was comparable with the Corresponding labeled amounts (Table No.5). The RP-HPLC chromatogram for MSL and PRD in sample was recorded and is shown in Figure.

Table No.1: System suitability parameters of chromatogram for MSL and PRD

S.No	Parameters	MSL ±RSD (n=6)	PRD± RSD (n=6)
1	Retention time	4.3704±0.1150	1.5808±0.4205
2	Tailing factor	1.382 ±0.9110	1.07 ±1.56
3	Theoretical plates	3487±1.80	1754±1.47

Table No.2: Regression analysis data and summary of validation parameters for proposed method

S.No	Parameters	RP-HPLC METHOD	
		MSL	PRD
1	Concentration range (µg/ml)	5-25µg/ml	2-10µg/ml
2	Regression equation Y= mx + c	2184.7840x + 40565.4000	1125.9480x + 10962.2000
3	Correlation coefficient (r^2)	0.9973	0.9984
4	LOD(µg/ml)	7.4676	0.6280
5	LOQ(µg/ml)	22.629	1.903
6	% Recovery (n=3)	99.57±0.107	100.63±0.620
7	Repeatability (% RSD) (n=6)	0.32	0.30
8	Precision(% RSD) (n=3) Interday Intraday	0.41-0.85 0.30-0.80	0.35-0.93 0.32-0.64

Table No.3: Precision data for MSL and PRD

S.No	MSL and PRD (5 and 2µg/ml)	Retention time (min)		Peak area		Tailing factor	
		MSL	PRD	MSL	PRD	MSL	PRD
1	1	4.363	1.589	154331	70428	1.216	1.770
2	2	4.373	1.577	155774	70488	1.244	1.731
3	3	4.368	1.572	156032	70635	1.230	1.724
4	4	4.372	1.581	154400	70787	1.232	1.732
5	5	4.376	1.585	154719	70220	1.219	1.750
6	Mean	4.3704	1.5808	155051.2	70511.6	1.2282	1.7414
7	SD	0.00502	0.00664	494.9966	214.2715	0.01118	0.01864
8	%RSD	0.1150	0.4205	0.32	0.30	0.9110	1.070

Table No.4: Recovery data for the proposed method (n = 3)

S.No	Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Mean % Recovery ± SD
1	MSL	I	15	14.92	99.45
		II	20	19.93	99.65
		III	25	24.9	99.62
2	PRD	I	6	5.97	99.92
		II	8	8.09	101.07
		III	10	10.09	100.9

Table No.5: Analysis of MSL and PRD in synthetic mixture by proposed method (n= 6)

S.No	Sample No	Label claim (mg)	Amount found (mg)	%Label claim
1	MSL	250	248.9	99.6
2	PRD	100	99.09	99.14

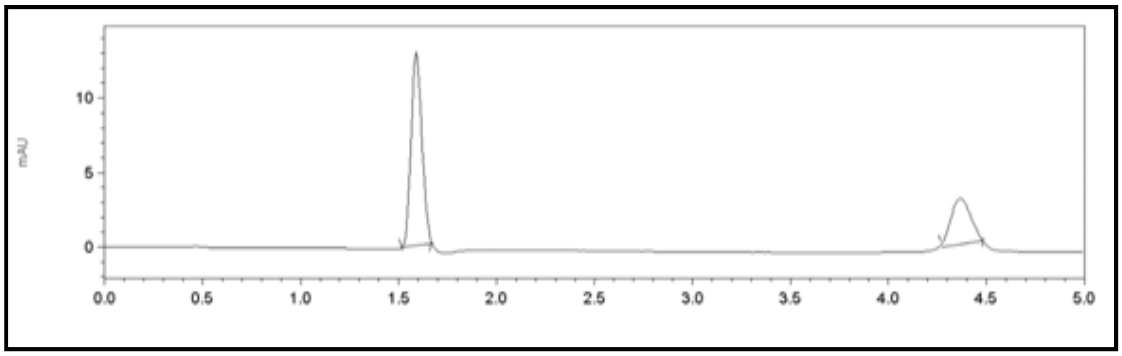

Figure No.1: Chromatogram of standard solution of MSL (10µg/ml) and PRD (4µg/ml) at 330nm

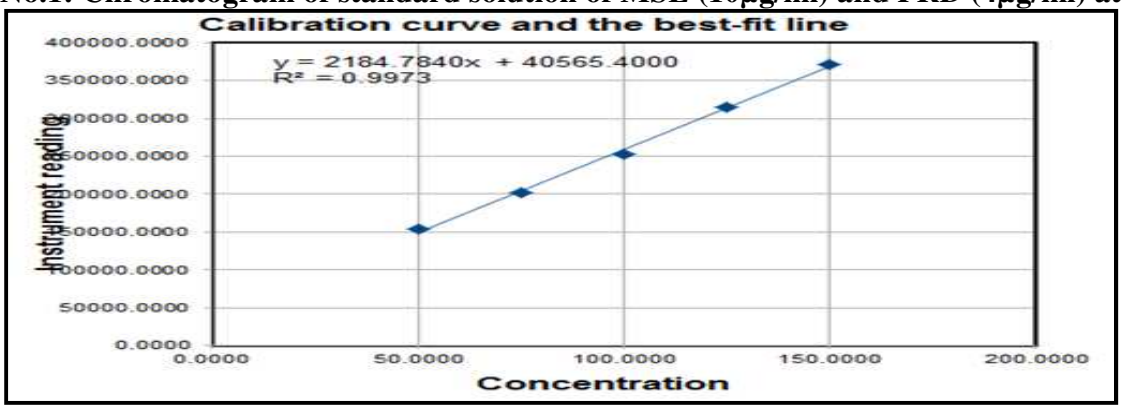

Figure No.2: Calibration standard graph of MSL at 330nm

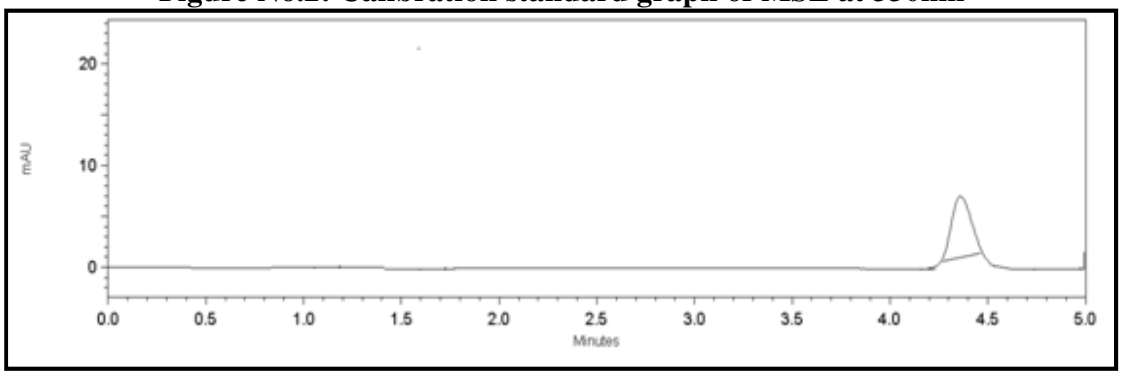

Figure No.3: Chromatogram of standard solution of MSL at 330nm

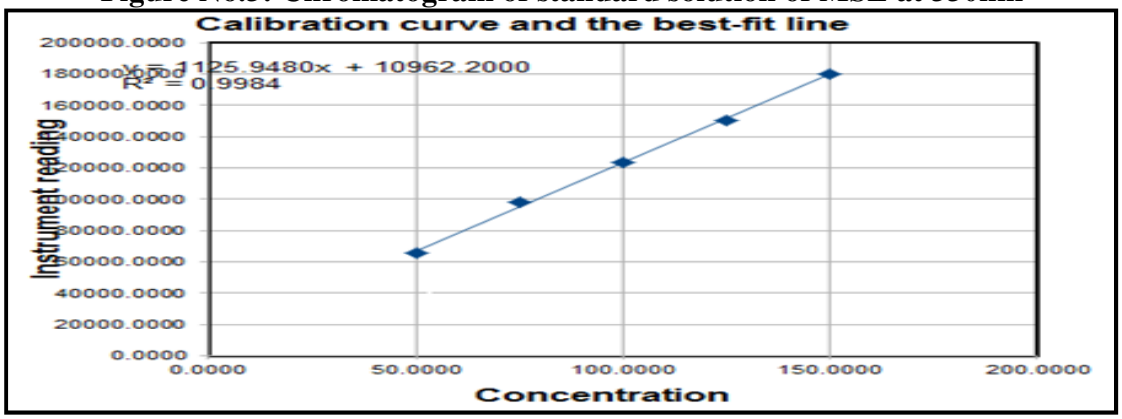

Figure No.4: Calibration standard graph of PRD at 330 nm

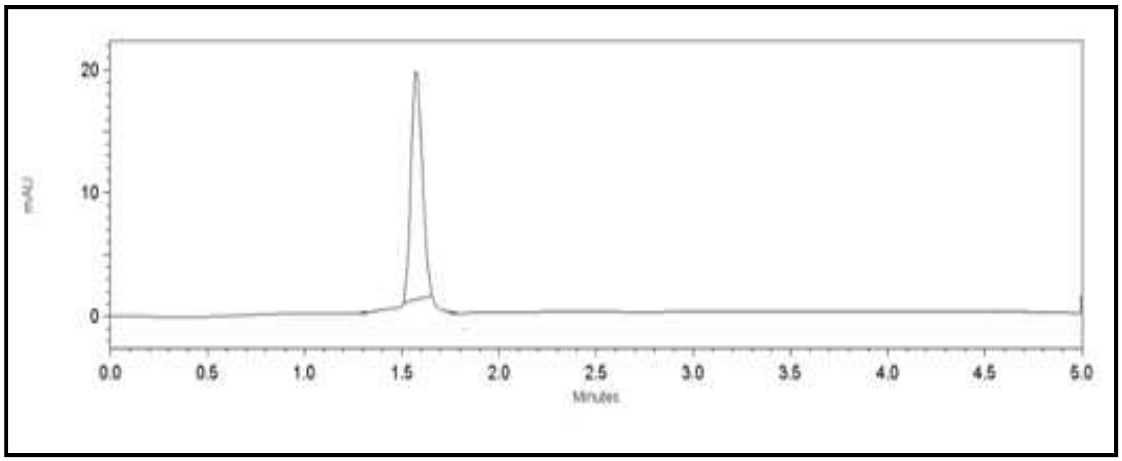

Figure No.5: Chromatogram of standard solution of PRD at 330 nm

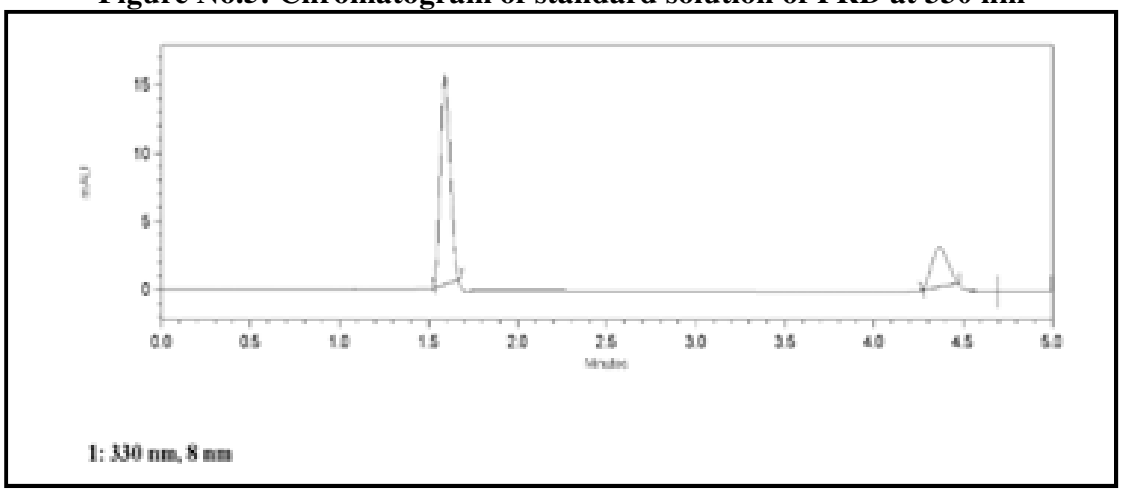

Figure No.6: Chromatogram of sample solution of MSL (10µg/ml) and PRD (4µg/ml) at 330 nm

CONCLUSION

RP-HPLC method was developed for the simultaneous estimation of MSL and PRD in bulk and in pharmaceutical preparations. The developed method was validated for linearity, accuracy, method precision, intra-day and inter-day precision, limit of detection and limit of quantification. The method is simple, linear, precise, accurate and suitable for simultaneous estimation of MSL and PRD in their combined dosage form. Validation parameters and Assay results for proposed method are listed on table Based on the results, obtained from the analysis using proposed method, it can be concluded that the method has linear response in the range of 5-25µg/ml and 2-10µg/ml for MSL and PRD, respectively.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutical Analysis, Al Shifa College of Pharmacy, Perinthalmanna, Kerala, 679325, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Gary R. Lichtenstein. Novel Formulations and Dosing Strategies for 5 ASA, *Gastroenterology and Hepatology,* 4(12), 2008, 1-13.

2. James Swarbrick, Boylan J C. Encyclopedia of Pharmaceutical Technology, *Marcel Decker Inc,* 20(3), 1998, 217-224.
3. Beckett A H, Stenlake J B. Practical Pharmaceutical Chemistry, *CBS Publishers and Distributors, New Delhi,* 4th Edition Part 2, 2002, 602.
4. Paul A Feldman, Daniel Wolfson and James S Barkin. Medical Management of Cohn's Disease, *Clinics in Colon and Rectal Surgery,* 20(4), 2007, 269-281.
5. Carter M J, Lobo A J and Travis S P L. Guidelines for the Management of Inflammatory Bowel Disease in Adults, *Gut,* 53(6), 2004, 1-6.
6. Frieri G, Pimpo M, Galletti B, Palumbo G. Corrao G, Latella G. *et al*. Long-term oral plus topical Meselamine in frequently relapsing ulcerative colitis, *Dig Liver Dis,* 37(2), 2005, 92-96.
7. Desreumaux P, Ghosh S. Review article: mode of action and delivery of 5-aminosalicylic acid - new evidence, *Aliment Pharmacological Therapy,* 24(1), 2006, 2-9.
8. Ford A, Bernstein C, Khan K, Abreu M, Marshall J, Talley N. *et al*. Glucocorticosteroid therapy in inflammatory bowel disease: systematic review and meta-analysis, *Am J Gastroenterol,* 106(4), 2011, 590-599.
9. ICH/CPMP guidelines Q2A, *Text on validation of Analytical procedures,* 1994.
10. ICH/CPMP guidelines Q2B, *Validation of Analytical procedures methodology,* 1996.
11. Validation of Analytical procedure, methodology as per ICH harmonized tripartite guidelines 1996, *Q2A having reached step 4 of the ICH process at the ICH steering meeting,* 1994, 1-8.
12. Rao K H, Rao A L, Sekhar K C. Validated RPHPLC Method for the Estimation of Mesalamine in Bulk and Tablet Dosage Form, *International Journal of Research in Pharmacy and Chem,* 3(2), 2013, 472-476.
13. Reddy K S, Ramachandra B, Naidu N V S. Development and Validation of HPLC Assay Method for Determination of Mesalamine in Bulk Drug and Tablet Formulation, *International Journal of Scientific Engineering and Research,* 2(6), 2014, 52-56.
14. Prakash A, Lone K D, Shukla A, Mandloi R and Ghosh V. Spectrophotometric Estimation of Mesalamine in tablet dosage forms, *Asian Journal of Research in Chemistry,* 1(2), 2008, 80-82.
15. Nobilis M, Vybiralova Z, Sladkova K, Lisa M, Holcapek M and Kvetina J. High Performance Liquid Chromatographic Determination of 5- Amino salicylic Acid and it's metabolites in blood plasma, *Journal of Chromatography,* 1119(1-2), 2006, 299-308.
16. Lakshman Rao A, Hanumantha Rao K and Chandra Sekhar K B. Validated RP-HPLC Method for the Estimation of Mesalamine in bulk and tablet dosage forms, *International Journal of Research in Pharmacy and Chemistry,* 3(2), 2013, 472-476.
17. Trivedi R K, Patel M C and Kharkar A R. Determination of Mesalamine related impurities from drug product reversed phase validated UPLC method, *European Journal of Chemistry,* 8(1), 2011, 131-148.
18. Palumbo G, Bachhi S, Primavera L, Palumbo P and Carlucci G. Validated HPLC method with electrochemical detection for simultaneous assay of 5-ASA and its metabolite in human plasma, *Biomedical Chromatography,* 19(5), 2004, 350-354.
19. Nalini Kanta Sahoo, Madhusmita Sahu, Podilapu Srinivasa Rao and Goutam Ghosh. Validation of Stability indicating RP-HPLC Method for the estimation of Mesalamine in bulk and tablet dosage form, *Pharmaceutical Methods,* 4(1), 2013, 56-61.
20. Ruby Bonfilo, Magali Benjamim De Arajuo, Herida R N. Recent Applications of Analytical Techniques for Quantitative Pharmaceutical Analysis: A Review, *Brazilian Journal of Transactions on Biogy and Biomed,* 7(4), 2010, 316-338.

21. Naganuma M, Iwao Y, Ogata H, Inoue N *et al*. Measurement of Colonic Mucosal Concentrations of 5-ASA is useful for estimating it's therapeutic efficacy in distal ulcerative colitis: Comparison of orally administered Mesalamine and Sulphasalazine, *Inflammatory Bowel Disorder,* 7(3), 2001, 221-228.
22. Sonia K and Nappinai M. Development and Validation of HPLC and UV Visible Spectrophotometric method for the pharmaceutical dosage forms and biological fluid-Review, *European Journal of Biomedical and Pharmaceutical Sciences,* 3(3), 2016, 382-391.

Please cite this article in press as: Drisya N K et al. Development and validation of RP-HPLC method for simultaneous estimation of mesalamine and prednisolone in bulk and formulation, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 7(3), 2019, 108-115.