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Original Article

SIMULTANEOUS ESTIMATION OF FEBUXOSTAT AND NAPROXEN IN SYNTHETIC MIXTURE BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

Objective: To develop simple, precise, rapid, and accurate RP-HPLC method for the simultaneous estimation of Febuxostat and Naproxen in synthetic mixture. To perform complete validation of newly developed analytical methods as per ICH(Q2) R1 [32] Guideline.

Methods: In RP-HPLC method for Febuxostat and Naproxen, chromatographic separation was carried out on Shimandzu LC-20AT, Hypersil BDS column (250mm x 4.6mm, 5 µm) using mobile phase ACN: Methanol: Phosphate Buffer (50:35:15 v/v) pH 5, detected at 288 nm, with flow rate 1.0ml/min and injection volume 20 µL.

Results: For RP- HPLC Linearity of Febuxostat and Naproxen were found to be 4 – 12 µg/ml and 25 - 75 µg/ml respectively. For both the developed and validated methods the %RSD was found to be less than 2% and the % recovery was found to between 98-102 %.

Conclusion: The developed and validated method was found to be simple, accurate, economical, robust and reproducible. There was no interference of any diluent and excipient in the determination of drugs from synthetic mixture. So, the method can be successfully applied for routine Quality control analysis.

Keywords: Febuxostat, Naproxen, RP-HPLC, Synthetic mixture.

INTRODUCTION

Febuxostat (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid) (ULORIC) (Figure 1) is a xanathine oxidase (XO) inhibitor indicated in patients with gout suffering from hyperuricemia and is used in its chronic management. Febuxostat received marketing approval by the European Medicines Agency on April 21, 2008 and was approved by the U.S. Food and Drug Administration on February 16, 2009. [1] It is freely soluble in Dimethylformamide; soluble in Ethanol; soluble in Methanol and Acetonitrile and practically insoluble in Water. [2] Naproxen(+)-(*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid (figure 2) is an anti-inflammatory agent with analgesic and antipyretic properties. Naproxen is commonly used for the reduction of pain, fever, inflammation and stiffness caused by conditions including migraine, osteoarthritis, kidney stones, rheumatoidarthritis,gout, ankylosingspondylitis, tendinitis.[1]
Soluble in Water, Methanol and Ethanol, slightly soluble in Acetone, practically insoluble in Chloroform and Toluene. [2]

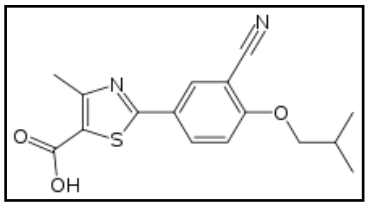

Fig. 1: Febuxostat [3]

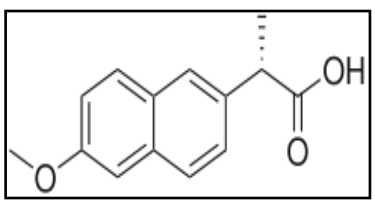

Fig. 2: Naproxen [3]

Febuxostat is used as anti-gout.Naproxen is used as gout suppressant and pain killer. Combination of Febuxostat and Naproxen increases the Cmax and AUC Absorption of Febuxostat increases by 40%. Literature review revealed that there are several methods like U.V. Spectrophotometry [4-8], HPLC[9-31] avalilabe for estimation of Febuxostat and Naproxen as single formulation and in combination with other drugs. But literature review revealed that there is no method available for Simultaneous estimation of Febuxostat and Naproxen in combination. Thus, this article presents the simultaneous estimation of Febuxostat and Naproxen in synthetic mixture.

MATERIALS AND METHODS

Methanol HPLC grade and Acetonitrile HPLC grade was procured from E. Merck Ltd, Mumbai. Orthophosphoric acid, Potassium Dihydrogen phosphate was procured from Astron chemicals, Ahmedabad. The analysis of drug was carried out on a SHIMADZU LC2010 series HPLC system equipped with a reverse phase Hypersil C18 column (250×4.6mm, 5µm n particle size) a LC 20 AT isocratic pump, a 20µl injection loop and a SPD- 20 A Promenence UV- Visible Detector and running on Spinchrom Chromatographic Software version. Isocratic elution with 0.5 mM Phosphate buffer: Methanol: acetonitrile (ACN) (15: 35: 50 v/v) is used at a flow rate of 1 ml/ min. Milli-Q water is used for buffers and other reagents preparation.

HPLC conditions

The contents of mobile phase were Methanol, ACN, and phosphate buffer adjusted to pH 5 with 5% Orthophosphoric acid. These were filtered through 0.45µ membrane filter and degassed by sonication for use. The flow rate for mobile phase was optimized to 1ml/min.

The run time was set at 20 min and column temperature was maintain at ambient (room temperature). The volume of injection was 20 µL, and eluent was detected at 288 nm.

Method

The RP-HPLC method of Febuxostat and Naproxen were achieved by isocratic elution technique with UV-VIS Detector. Febuxostat and Naproxen were determined at 288nm respectively with the

concentration range of 4-12 µg/ml for Febuxostat and 25-70 µg/ml for Naproxen respectively. For analysis of synthetic mixture, the powder of 10 mg of Febuxostat and 62.5 mg of Naproxen was taken, dissolved in 100 ml volumetric flask and made up to 100 ml with Methanol. The solution was sonicated for 15min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made to get 10 µg/ml of Febuxostat and Naproxen.

Standard Stocks Solutions of binary mixture

About 10 mg of Febuxostat and 62.5 mg Naproxen weighed accurately and transferred to 100 ml volumetric flask and dissolved in methanol. This solution was sonicated for about 15 min and make up to the mark with methanol to get the concentration of 100 µg/ml Febuxostat and 625 µg/ml Naproxen.

Method validation

Specificity

A blank solution (mobile phase) and Common excipients like starch, lactose, magnesium stearate were dispersed in methanol, filtered and injected. The chromatogram showed no inferring peaks at retention time of the two drugs which indicates specificity of method.

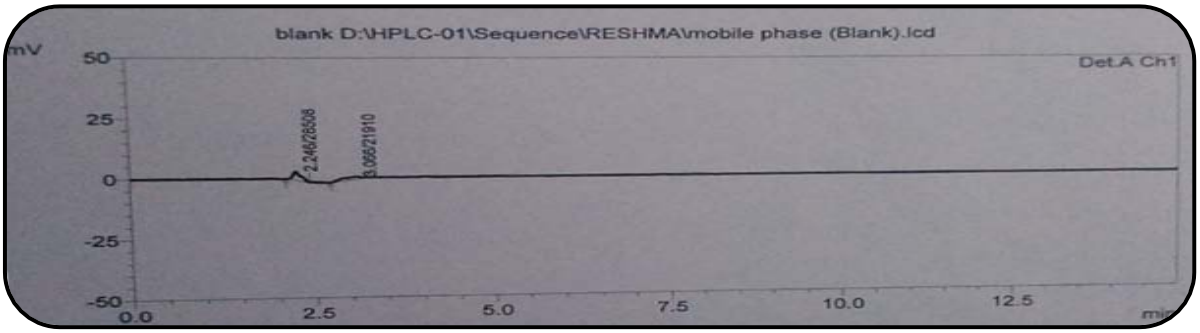

Fig. 3: Chromatogram of mobile phase

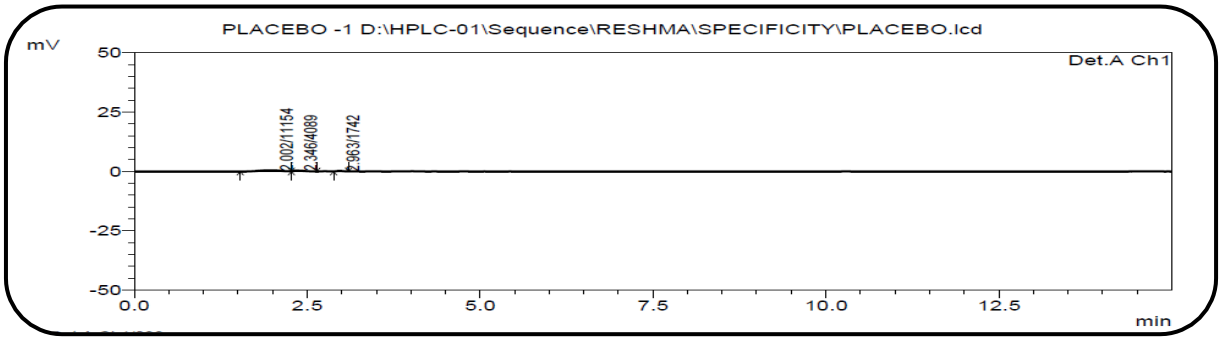

Fig. 4: Chromatogram of excipients

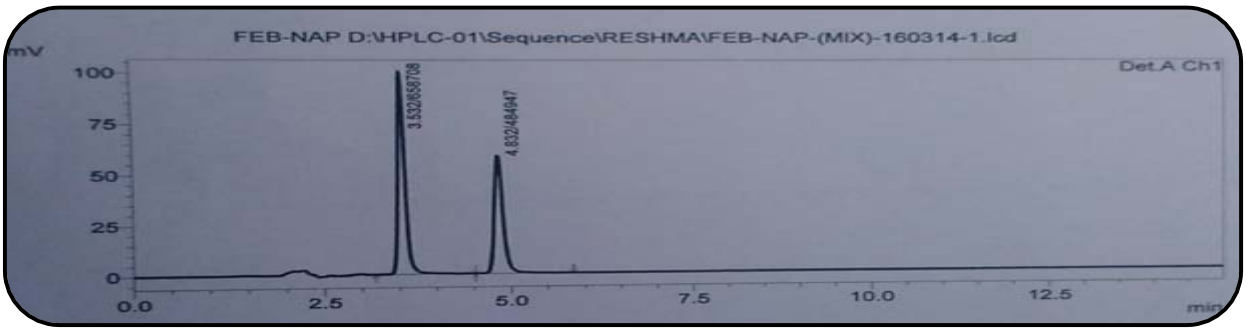

Fig. 5: Chromatogram of binary mixture (nap-3.53 min and feb-4.83 min)

Table 1: Linearity data

Febuxostat			Naproxen		
Conc.(µg/ml)	**Mean Area± SD**	**%RSD**	**Conc.(µg/ml)**	**Mean Area± SD**	**% RSD**
4	368594.3 ± 525.696	0.14	25	511140.3 ± 1090.314	0.21
6	421046.3±1161.009	0.27	32.5	572052.3 ± 3107.962	0.54
8	473462.3 ± 1077.19	0.22	50	638483.7 ± 5802.145	0.90
10	527129.3 ±1776.04	0.33	62.5	705566.3 ± 5342.425	0.75
12	579406 ±1620.786	0.27	75.0	771264.7 ± 7397.649	0.95

Linearity and range

Aliquots of standard stock solutions of Febuxostat and Naproxen were taken in 10 ml volumetric flasks and diluted with mobile phase to get final concentrations in range of 4-12µg/ml for Febuxostat and 25-75µg/ml for Naproxen. Triplicate injections were made for each concentration.

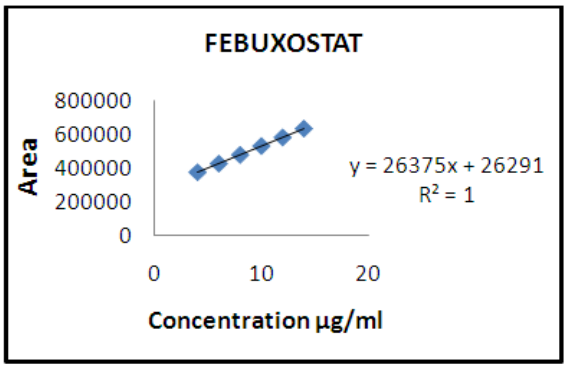

Fig. 7: Febuxostat (4-12 µg/ml)

The plots of peak area versus respective concentrations of Febuxostat and Naproxen were found to be linear in the concentration range of 4-12µg/ml and 25-75µg/ml respectively. The linear regression equations of the lines are:

For Febuxostat -y = 26375x + 262916, (r^2 = 1)

For Naproxen -y = 5250.8x + 377336, (r^2 = 0.9998)

For Febuxostat

Precision

Precision study was performed as per ICH Q2 (R1)[32] to find out repeatability, intra-day and inter-day variations. For the precision, three replicates of each concentration are injected into the system and results are reported in terms of relative standard deviation as given in table No. 2,3and 4 respectively.

Table 2: Repeatability data

Drug Name	Febuxostat	Naproxen
Wavelength	288 nm	
Concentration	8 µg/ml	50 µg/ml
MEAN±SD(n=3)	550955.8 ± 940.8129	723179.3 ± 1961.269
%RSD	0.17	0.27

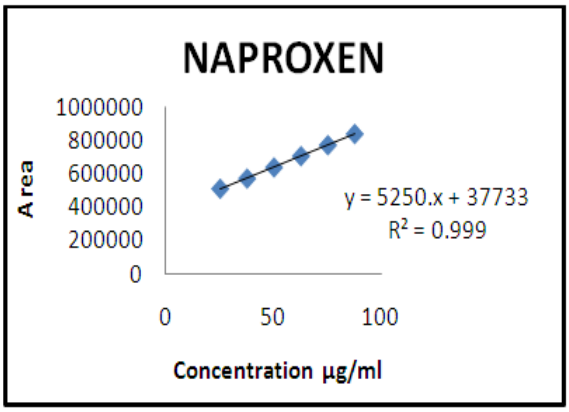

Fig. 8: Naproxen (25-75 µg/ml)

Accuracy

The accuracy was determined by recovery studies. The recovery studies were performed by standard addition method, at 80%, 100%, 120% level. Percent recovered was calculated using regression equation.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

LOD = 3.3 × Standard deviation of y intercept /Slope of calibration curve

Febuxostat – 0.105µg/ml

Naproxen – 1.93µg/ml

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

LOQ = 10 × Standard deviation of y intercept/ Slope of calibration curve

Febuxostat – 0.316µg/ml

Naproxen – 5.86µg/ml

Applicability to synthetic mixture

Applicability of proposed method was tested by analyzing the synthetic mixture.

Table 3: Intraday precision data

Febuxostat Wavelength = 288 nm			**Naproxen Wavelength = 288 nm**		
Conc. (µg/ml)	Mean Area ± SD	% RSD	Conc. (µg/ml)	Mean Area ± SD	% RSD
6	441926.3 ± 619.1545	0.14	37.5	591421.3 ± 282.2788	0.04
10	552418.3 ± 711.5703	0.12	62.5	725942 ± 233.8269	0.03
14	661356.7 ± 807.8814	0.12	87.5	858148.3 ± 2791.556	0.3

Table 4: Interday precision data

Febuxostat Wavelength = 288 nm			**Naproxen Wavelength = 288 nm**		
Conc. (µg/ml)	Mean Area ± SD	% RSD	Conc. (µg/ml)	Mean Area ± SD	% RSD
6	435200.3 ± 11232.98	1.39	37.5	584190.7 ± 2263.98	1.46
10	543938 ± 14944.42	0.72	62.5	717163.7 ± 5088.96	0.69
14	651883 ± 16758.17	0.99	87.5	851619.3 ± 8517.86	0.81

Table 5: Accuracy data

Drug name	% Level of Recovery	Total amount (µg/ml)	Total amount obtained (n=3) ± SD	Area ± SD	% Recovery ± SD
Feb	80 %	7.2	7.1±0.03	409975±139.0	99.5 %±0.0577
	100 %	8.0	8.0±0.04	457843±724.6	100.8 %±0.12
	120 %	8.8	8.7±0.01	494441±1460	99.59 % ±0.31
Nap	80 %	45	44.7±0.43	464680±3766	99.41 % ±0.91
	100 %	50	50.9±0.51	516868±4662	100 %±1.02
	120 %	55	55.26±0.80	555129.3±731	99.52%±1.46

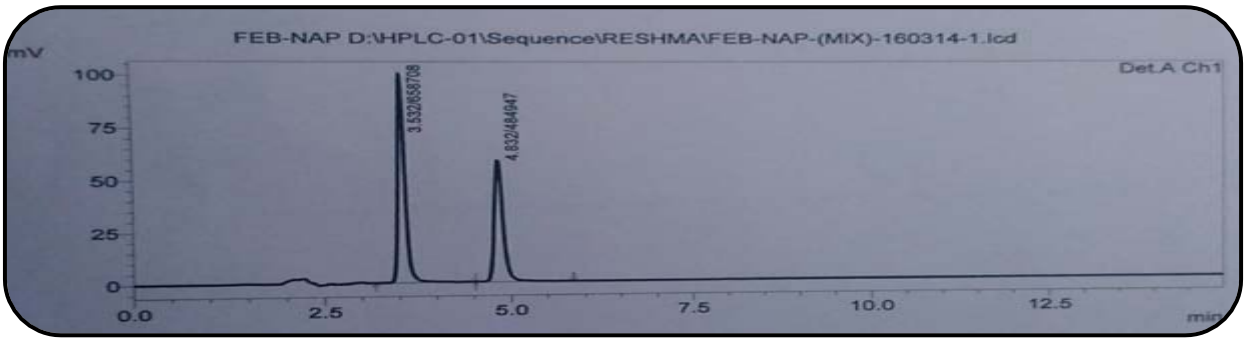

Fig. 6: Chromatogram of synthetic mixture

Table 7: Applicability to synthetic mixture Data

Synthetic mixture	Drug	Amount taken in synthetic mixture	Amount found (mg) (n=3) ± SD.	%Amount obtained ± SD.
	Febuxostat	40 mg	39.8 **±** 0.52	99.5% ± 0.125
	Naproxen	250 mg	251.0 **±** 0.763	100.4% ± 1.014

RESULT

Table 8: Optical regression characteristics and validation parameters

S. No.	Parameters	Febuxostat	Naproxen
1	Wavelength (nm)	288 nm	
2	Beer's Law Limit (µg/ml)	4 – 12	25 – 75
3	Regression equation (y = mx +c)	y = 26375x + 262916	y = 5250.8x + 377336
4	Slope (m)	26375	5250.8
5	Intercept (c)	262916	377336
6	Correlation Coefficient (r^2)	1.0	0.999
7	Repeatability (% RSD, n=6)	0.17	0.27
8	Interday (n=3) (% RSD) (µg/ml)	1.5 – 1.7	1.0 – 2.0
9	Intraday(n=3) (% RSD) (µg/ml)	0.12 – 0.14	0.03 – 0.3
10	LOD(µg/ml)	0.105	1.93
11	LOQ(µg/ml)	0.319	5.86
12	Accuracy	99.5-100.8 %	99.4-100 %

CONCLUSION

A Simple, specific, accurate, and precise RP-HPLC method has been developed and validated for simultaneous estimation of both these drugs. The chromatographic separation was achieved on Hypersil BDS coloumn (250 mm x 4.6mm, 5µm) in size column using ACN: Methanol: Phosphate Buffer (50:35:15 v/v) as mobile phase at 288 nm.

The plot of area versus respective concentration of Febuxostat and Naproxen was found to be linear in the concentration range of 4-12 µg/ml for Febuxostat and 25- 75 µg/ml for Naproxen with $r^2 = 1$ for Feb and $r^2 = 0.998$ for Nap respectively.

Precision was calculated as repeatability, interday and intraday variations and % RSD was found to be less than 2% for both the drugs.

Mean % recovery for Febuxostat and Naproxen were found to be 100.8 % and 99.52 % respectively. Recovery is in the range of 98-102 %, indicates that method is accurate.

System suitability test reveals that all system suitability parameters complies standard values.

CONFLICT OF INTERESTS

Declared None

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REFERENCES

1. Tripathi K. D, Essentials of Medical Pharmacology, sixth edition, Jaypee Publications, p. Biotechnol Lett;2006:351-64.
2. Pharmacopoeia NF. United State The Standard of quality, The official compendia of standard, Asian edition, volume no. p124volume no301133904 2009;32(1).
3. Indian Pharmacopoeia, volume I,II,III, Government of India, Ministry of Health and family welfare.
4. Rajan D, Srinivasan R, Kumarappan C, Shamala N, V. U. Senthil "Development And Validation Of Method For The estimation Of Naproxen In Bulk And Semi-Solid Formulation", International Journal of Analytical, Pharmaceutical, and Bio-medical Sciences, Volume:Issue-1:Jan-Mar-Copyrights@ ISSN: Received: 15th Feb-Revised:20th Feb-Accepted:28th Feb-Coden:IJAPBS www. ijapbscom 2013;2:2278-0246.
5. Nisha C, Inamullah S, Jyoti R, Sunil S, Sharma S, Hemendar G. Simultaneous estimation of Lansoprazole and Naproxen", Scholars Research Library Der Pharma Chemica,,:(http:// derpharmachemica.com/archive.html)2013. 67-74 p.
6. Chandrakant S, Abhishek P, Sadhana R, ChemTech USA, Research C. Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Naproxen and Esomeprazole Magnesiumtrihydrate in Combined Pharmaceutical Formulation", International Journal of:IJCRGG ISSN:Vol. No.2, pp 602-608. Biotechnol Lett;4:0974-4290.
7. Tasnuva H, Mesbah K, Fatema L, Uddin T, Susmita L, Abul K, et al. Md. Kabir;" Simultaneous Estimation of Naproxen and Ranitidine HCl by Using, Stamford Journal of Pharmaceutical Pharm. Sci;1(1&2):18-24.
8. Paramdeep B, Abdul A, Tariq M, Salman HH, Kuldeep S, A., et al. Mohd Siddiqui, and " Method For The Determination Of Febuxostat In Bulk And Pharmaceutical Formulations", International journal of Pharmaceutical Sciences and, Vol. Issue 10, 28 September. Biotechnol Lett 2011;2.
9. Sudhir M, Rama R, Venkata R, A., Hplc, Research C. RP-for the Estimation of Febuxostat in Bulk Drugs", International Journal of PharmTech (USA):IJPRIF ISSN:Vol. No.4, pp 1358-1366. Biotechnol Lett;4:0974-4304.
10. Snyder LR, Kirkland JJ, Dolan JW. and Introduction to Modern Liquid Chromatography;3rd Edn;p. Biotechnol Lett:516-7.
11. Naresh M, Chandra R, Chandra K, Tablets H, By RP. Estimation Of Related Substances Of Febuxostat In Bulk & 40/80/120mg, International Journal of Pharmaceutical Biological and Chemical Sciences. Biotechnol Lett.
12. Rahul S, Ravi K, Patnaik K, Hplc. A RP-Development And Validation For The Estimation Of Febuxostat In Bulk And Pharmaceutical Dosage Form", International Research Journal of Pharmacy. Biotechnol Lett.
13. Kumaraswamy G, J., Venkatesh G, Spandana R, A., Hplc, et al. D. Bhikshapathi, "RP-For Simultaneous Estimation Of Febuxostat And Ketorolac Tromethamine in Pharmaceutical Formulations", Journal of Drug Delivery & Therapeutics;,:173 ©, JDDT. All Rights 2250-1177 CODEN:online at http://jddtonline.info2011. 173-6 p.
14. Frida D, Volume A. Lotfi Monser, "Simultaneous determination of naproxen and related compounds by HPLC using porous graphitic carbon column";Journal of Pharmaceutical and Biomedical,August,pp no. 10871092 2003;32:4-5.
15. Udo H "Analysis of anti-inflammatory drugs by HPLC";*Agilent technologies* [Accessed:feb 2014].
16. Pakhuri M, Chandra S. Development and validation of related substances method by HPLC for analysis of Naproxen in Naproxen tablet formulations", International journal of Pharmaceutical Sciences and drug research. Biotechnol Lett 2012.
17. Parvin Z, Mohammad B, Hosniyeh T, Yadollah A, Hadi V. Simultaneous determination of Naproxen, Ketoprofen and Phenol Red in samples from rat intestinal permeability studies:HPLC method development and validation". Biotechnol Lett.
18. Minakshi P, Pooja C, Shubhini S. Simultaneous Estimation of Sumatriptan Succinate, Naproxen and Domperidone by Reverse Phase High Performance Liquid Chromatography", Asian Journal of Pharmaceutical and Clinical ResearchVol Suppl 3, ISSN. Biotechnol Lett 2012;5:0974-2441.
19. Rajnish KN, Asraf SAKS, Manju N, Gunasekaran P. Functional characterization of a putative β-lactamase gene in the genome of Zymomonas mobilis. Biotechnol Lett 2011;33(12):2425-30.
20. Mahesh N, Harshini A, Sciences I, Chemical P. S. Manikantar and V. Prakash Diwan Reverse Phase High Performance Liquid Chromatographic Estimation Of AntiGout In Pharmaceutical Dosage Form International Journal Of Biological Available online at wwwijpcbscom 2012;3(1):137-42.
21. Nageswara R, Lakshmana R, Of RP, Hplc, Chemistry I. S. Ganapaty and "Development And Validation For Estimation Of Febuxostat In Bulk And Tablet Dosage Form", International Journal Of Research In Pharmacy And,, Available online at www. ijrpccom 2012;2(4).
22. Sudhir M, Rama R, Venkata R, A., Hplc, Research C. RP-for the Estimation of Febuxostat in Bulk Drugs", International Journal of PharmTech (USA):IJPRIF ISSN:Vol. No.4, pp 1358-1366, Oct-Dec. Biotechnol Lett 2012;4:0974-4304.
23. Bhalekar R, Sciences I. Sameer Lakade, Minal. H;"Developed And Validated Reverse Phase Hplc Method For The Determinationof Febuxostat In Bulk And Formulations", International Journal of Pharmacy and Pharmaceutical Vol Issue 4. Biotechnol Lett 2012;4:0975-1491.
24. Ashwini G, Karnaker R, Anand K. N.Aravindsai, " Estimation of Febuxostat drug present in formulation by RP-HPLC", Journal of Pharmacy Research,,-1227Research ArticleISSN:0974-6943 Available online through www. jpronlineinfo Received on1011 Revised on 1512 Accepted on1201 2012;5(2):1224-12271224.
25. Babu R, Chandu K, Prakash K, Mukkanti K, Nagiat T. Bioequivalance and pharmacokinetic study of Febuxostat in human plasma by using LC-MS/MSwith liquid liquid extraction method", Springer Plus, 194 http:// www.springerplus.com/ content/2/1/1942013.
26. Haidong W, Pan D, Xiaoyan C, Lixia G, Dafang Z. Development and validation of a liquid chromatography-tandem mass spectrometry method for the determination of Febuxostatin human plasma", Received:16 February Revised:11 March, Accepted:11 March Published online in Wiley Online Library:24 May (wileyonlinelibrary.com) DOI 102012.
27. Naresh M, Chandra R, Chandra K, Tablets H, By RP. Estimation Of Related Substances Of Febuxostat In Bulk & 40/80/120mg, International Journal of Pharmaceutical Biological and Chemical Sciences [Accessed:Oct]. Biotechnol Lett 2013.
28. Rahul S, Ravi K, Patnaik K, Hplc. A RP-Development And Validation For The Estimation Of Febuxostat In Bulk And Pharmaceutical Dosage Form", International Research Journal of Pharmacy [Accessed:Oct]. Biotechnol Lett 2013.
29. Kumaraswamy G, J., Venkatesh G, Spandana R, A., Hplc, et al. D. Bhikshapathi, "RP-For Simultaneous Estimation Of Febuxostat And Ketorolac Tromethamine in Pharmaceutical Formulations", Journal of Drug Delivery & Therapeutics;,:173 ©, JDDT. All Rights 2250-1177 CODEN:online at http:// jddtonline.info2011. 173-6 p.
30. HardikSinh P, Hardik M, Priyanka P, Sanjay P, Kruanl C, Of RP, et al. B.Priyanka, "Development And Validation For Simultaneous Estimation Of Febuxostat And Diclofenac Potassium In Bulk And Tablet Dosage Form", Inventi Rapid:Pharm Analysis & Quality Assurance publication date:/1/7. Biotechnol Lett 2013.
31. Vaibhav M, Mohit R, Of RP, Hplc. S. Sadhana, "Validation For Simultaneous Estimation Of Febuxostat And Diclofenac Potassium In Bulk Drug And In Bi-Layer Tablet Formulation", International Research Journal of Pharmacy. Biotechnol Lett.
32. International M, Harmonization I. ICH, Q2B(R1) Validation of Analytical Procedure;on Geneva, Switzerland. Biotechnol Lett 2005.