Research Article

Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Thiocolchicoside and Flupirtine Maleate in Pharmaceutical Formulation

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Thiocolchicoside and Flupirtine maleate in pharmaceutical formulation. The column used was Inertsil ODS, C18, (150 X 4.6mm, 5µ). in isocratic mode, with mobile phase containing Acetonitrile and potassium dihydrogen phosphate buffer (55:45) v/v, pH was adjusted to 4.5, with a flow rate of 0.8ml/min. and effluents were monitored at 255nm. The retention times of Thiocolchicoside and Flupirtine maleate were 2.5 min and 4.9.min, respectively. The linearity for Thiocolchicoside and Flupirtine maleate were in the range of 1-6 µg/ml and 100 to 600 µg/ml respectively with correlation coefficient of r2=0.999 for both the drugs. The recoveries of Thiocolchicoside and Flupirtine maleate were found to be 99.89% and 100.03% respectively. The % RSD from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Thiocolchicoside and Flupirtine maleate in pharmaceutical formulation.

Keywords: Thiocolchicoside, flupirtine maleate, RP-HPLC, Inertsil ODS, Validation, Forced degradation studies.

INTRODUCTION

Thiocolchicoside[1] (TCC) is a muscle relaxant with antiinflammatory and analgesic effects. It acts as a competitive $GABA_A$ receptor antagonist and also glycine receptor antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It has powerful convulsant activity and should not be used in seizure-prone individuals. Chemically it is known as (s)-N-[3-D-glucopyranoxyloxy)-5, 6, 7, 9 tetrahydro-1,2-dimethoxy-10-(methylthio- 9-oxobenzo(a) heptalen- 7yl]acetamido. Flupirtine[2] (FLU) is an aminopyridine that functions as a centrally acting non-opioid analgesic. It is unique among analgesics in that it is a non-opioid, non-NSAID, non-steroidal centrally acting analgesic. Its muscle relaxant properties make it popular for back pain and other orthopedic uses, but it is also used for migraines, in oncology, postoperative care, andgynecology. It is chemically Ethyl {2-amino-6-[(4- fluorobenzyl) amino] pyridin-3-yl} carbonate. Literature survey revealed that there are few analytical methods[3-12] reported for the determination of TCC and FLU in single or in combination with other drugs. However, no RP-HPLC method was reported for the simultaneous estimation of TCC and FLU.

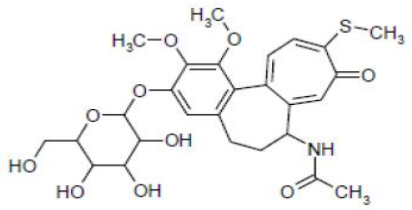

Fig. 1: Structure of Thiocolchicoside

Fig. 2: Structure of flupirtine maleate

Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the simultaneous determination of TCC and FLU in bulk and in tablet dosage form.

MATERIALS AND METHODS

Waters HPLC 2695 series consisting 4 pumps, Auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature.

CHEMICALS AND REAGENTS

TCC and FLU pure drug was obtained as gift sample from Bio-leo analytical labs, Hyderabad. KETOFLAM T4 is the dosage form purchased from local pharmacy. Other chemicals all are of HPLC grade.

Standard stock solution preparation

Accurately weigh and transfer 100 mg of FLU working standard and 4 mg of TCC working standard in to 100 mL volumetric flask, add 50 mL of mobile phase and sonicate to dissolve and dilute to volume with mobile phase. Transfer 1 mL of standard stock solution into 10 mL volumetric flask and to volume with mobile phase.

Sample Preparation

Finely grind pre weighed 20 tablets. Accurately weighed and transfer grinded sample quantitatively equivalent to 100 mg of FLU and 4 mg of TCC in to 100 mL volumetric flask add 25 mL of mobile phase, sonicate to dissolve for 10 minutes and dilute to volume with mobile phase. Further filter the solution through filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

RESULTS

Method Validation

Specificity: Specificity is the ability of analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample. The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample.

Linearity: Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with given range. The linearity of TCC was in the concentration range of 1-6 µg/ml, for FLU 100 - 600 µg/ml .From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient was found to be 0.999 and shows good linearity for both the drugs.

Precision: The precision of the method was demonstrated by system and method precision studies. All the solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated.

Accuracy: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Accuracy of the method was evaluated by standard addition method. Recovery of the method was determined by spiking an amount of the pure drug (80%,100% ,120%) at three different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug.

LOD& LOQ: LOD is the lowest concentration of analyte in a sample that can be detected but not quantified under experimental conditions. The LOD values were determined by the formulae LOD=3.3σ/s (where σ is the standard deviation of the responses and s is the mean of the slopes of the calibration curves). LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under experimental conditions. It is a parameter of the quantitative determination of compounds in the mixtures. The LOQ values were determined by the formulae LOD=10σ/s.

Robustness: The robustness study was performed by slight modification in flow rate and temperature. Mixed samples of TCC and FLU at a concentration 4µg/mL and 100µg/mL respectively were analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

Acid Hydrolysis: Sample quantity equivalent to 4 mg of TCC and 100 mg of FLU in to 200 ml was transferred in to RB flask. 100 ml of freshly prepared 0.1 Hydrochloric acid was added and refluxed for 30mins at 60^0c Leave it for 12 Hrs. After 12 hrs filter the solution through filter paper and neutralize the solution with suitable Base. Dilute 1 ml of filtrate to 10 ml with mobile phase).

Base Hydrolysis: sample quantity equivalent to 4 mg of TCC and 100 mg of FLU into 200 ml was transferred into a RB flask add 100 ml of freshly prepared 0.1 N sodium hydroxide was added and refluxed for 30mins at 60^0c. Leave it for 12 Hrs. After 12 hrs filter the solution through filter paper and neutralize the solution with suitable acid. Dilute 1 ml of filtrate to 10 ml with mobile phase.

Oxidation (Peroxide): Transfer sample quantitatively equivalent to 4 mg of TCC and 100 mg of FLU in to 200 ml RB flask add 100 ml of freshly prepared 10% Hydrogen peroxide solution. The solutions were kept for 30 min at 60^0c. Leave it for 10 Hrs. After 10 hrs filter the solution through filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

UV Exposure: Sample quantity equivalent to 4 mg of thiocolchicoside and 100 mg of Flupirtine was transferred on to clean and dry petri dish. Place the petri dish in UV chamber by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. After exposure transfer the contents in to 100 ml volumetric flask and add 25 ml of mobile phase and sonicate to dissolve. Then dilute to volume with mobile phase. Further filter the solution through filter paper. Dilute 1 ml of filtrate to 10 ml with mobile phase.

DISCUSSION

Several trials has made until getting good peak resolution, acceptable plate count and tailing factor. Method was optimized and the retention times of TCC and FLU maleate was reported as 2.5 and 4.9 mins.

Specificity: The chromatograms of standard and sample are identical with nearly same retention time. There is no interference with blank and placebo to the drugs. Hence the proposed method was found to be specific.

Linearity: From the Linearity data it was observed that the method was showing linearity in the concentration range of 1-6 µg/ml for TCC and 100-600 µg/ml for FLU. Correlation coefficient was found to be 0.999 for both the compounds.

Accuracy: The recoveries of pure drug from the analyzed solution of formulation were 99.89% and for TCC and 100.03% for FLU, which shows that the method was accurate.

Precision: The %RSD for the sample chromatograms of system precision was found to be 0.084 & 0.260 (Rt & Area) for TCC and 0.057 & 0.103 (Rt & Area) for FLU. The %RSD for the sample chromatograms of method precision was found to be 0.103 & 0.203 (Rt & Area) TCC and 0.035 & 0.058 (Rt & Area) for FLU. Hence it passes method precision.

Robustness: All the system suitability parameters are within limits for variation in flow rate (±0.2 ml). Hence the allowable flow rate should be within 0.6 ml to 1.0 ml. All the system suitability parameters are within limits for variation (±2nm) in wavelength. Hence the allowable variation in wavelength is ± 2nm.

LOD & LOQ: LOD & LOQ of TCC was found to be 0.259, 0.758and for FLU was found to be 0.751, 0.227 respectively. All the system suitability parameters are within in the limits when the drugs are subjected to stress conditions like acid, base peroxide, thermal and photolysis. The results obtained were satisfactory and good agreement as per the ICH guidelines.

Table 1: Details of marketed Formulation

S. No.	Brand name	formulation	Available strength		Address of manufacturer
1	KETOFLAM T4	Tablet	Thiocolchicoside Flupirtine maleate	4mg 100mg	Lupin laboratories Ltd.
2	KETOFLAM T8	Tablet	Thiocolchicoside Flupirtine maleate	8mg 100mg	Lupin laboratories ltd.

Table 2: Optimized chromatogram conditions

Instrument	**High Performance Liquid Chromatography Waters HPLC 2 2695**
Stationary phase	**Inertsil ODS, C18, 150 X 4.6mm, 5µ.**
Flow rate	**0.8 ml/min**
Operating temperature	**30^0c**
Selected wave length	**255 nm.**
Mobile phase ratio	**ACN : potassium di hydrogen phosphate buffer (55:45) v/v P^H- 4.5**
Injection volume	**20 µL**
Run time	**10 min.**

Table 3: Specificity Data

Name of solution	Retention time (min)
Blank	No peaks
Thiocolchicoside	2.5
Flupirtine	4.9

Table 4: Linearity study

Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1	94295	100	328490
2	187360	200	651770
3	280856	300	973266
4	366504	400	1259013
5	459825	500	1574373
6	556396	600	1931704
Correlation coefficient	0.999	Correlation coefficient	0.9995

Table 5: Precision Data (System Precision)

S. No.	Thiocolchicoside		Flupirtine maleate	
	RT	Area	RT	Area
1	2.532	355354	4.980	1245462
2	2.535	355642	4.978	1245715
3	2.531	356505	4.977	1247928
4	2.536	356188	4.979	1248015
5	2.531	357121	4.976	1245545
6	2.532	354490	4.984	1247749
Avg	2.533	355883	4.979	1246736
Std Dev	0.0021	926.38	0.0028	1278.04
RSD	0.084	0.260	0.057	0.103

Table 6: Precision Data (Method Precision)

S. No.	Thiocolchicoside		Flupirtine maleate	
	RT	Area	RT	Area
1	2.532	355795	4.977	1247451
2	2.536	356105	4.979	1246545
3	2.532	357312	4.980	1245465
4	2.531	356651	4.981	1246754
5	2.528	355345	4.980	1245725
6	2.533	355675	4.982	1246245
Avg	2.532	356147	4.980	1246364
Std Dev	0.0026	721.51	0.0017	720.71
RSD	0.103	0.203	0.035	0.058

Table 7: Robustness study for Thiocolchicoside

Condition	Mean area
Unaltered	355883
Flow rate at 0.6ml/min Flow rate at 1.0ml/min	324352 396701
Temperature at 25^{0}C Temperature at 35^{0}C	346733 367295

Table 8: Robustness study for Flupirtine maleate

Condition	Mean area
Unaltered	1243736
Flow rate at 0.6ml/min Flow rate at 1.2ml/min	1138904 1397212
Temperature at 25^{0}C Temperature at 35^{0}C	1227250 1318462

Table 9: Summary of validation parameters

PARAMETERS	Thiocolchicoside		Flupirtine maleate	
Linearity	**1-6** µg/ml		**100 – 600** µg/ml	
Precision (% RSD)	0.084 (Rt)	0.260(Area)	0.057(Rt)	0.103 Area)
Accuracy	99.89%		100.03%	
LOD & LOQ	0.259, 0.758		0.751, 0.227	
Assay	99.80%		100.72%.	

Table 10: Summary of Forced degradation data

Stressed Condition	R_t in min Thiocolchicoside	R_t in min Flupirtine maleate	Peak area Thiocolchicoside	Peak area Flupirtine maleate
Acid degradation	2.538	4.994	318158	998038
Alkali degradation	2.539	4.989	327954	1063303
Oxidative degradation	2.536	4.998	327954	1075121
Uv degradation	2.537	4.993	326522	1115535

CONCLUSION

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust, rugged and economical. Hence this method is validated and can be used for routine and stability sample analysis.

REFERENCES:

1. http://en.wikipedia.org/wiki/Thiocolchicoside
2. http://en.wikipedia.org/wiki/Flupirtine
3. Ramakrishna M, Venkata S Rao Somisetty, Rambabu P, Sudhakarbabu AMS and Ramana MV. Development of new analytical methods for the estimation

of flupirtine maleate in bulk and in formulations. World Journal of Pharmaceutical Research. 2014;3(4):1902.
4. Mohan Gandhi B, Lakshmana Rao A and Venkateswara Rao J. A novel stability-indicating RP-HPLC method for the estimation of flupirtine in pharmaceutical dosage forms. IJPCBS. 2014;4(2):208-218.
5. Haritha P, Sreenivasa Rao B and Sunandamma Y. Method development and validation for simultaneous determination of flupirtine maleate and paracetamol by RP – HPLC technique. International journal of pharmaceutical sciences and research. 2014;5(2):463-472.
6. Lalitha KV, Golla Murali Mohan J, Ravindra Reddy, Vinod Kumar K and Aliekya A. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Paracetamol and Flupiritine Maleate in Pharmaceutical Dosage. Journal of Scientific and Innovative Research. 2013;2(3):634-641.
7. Sanjeev Kumar, Amith Joshi, Rahul Singh Thakur, Anupam K Pathak and Kamal Shah. Simultaneous estimation of etoricoxib and thiocolchicoside by RP-HPLC method in combined dosage forms. Acta poloniae pharmaceutica. 2011;68(6):839-43.
8. Rajan V. Rele and Swapnil A. Sawant. Simultaneous Determination of Aceclofenac and Thiocolchicoside in Formulation by Reversed Phase High Performance Liquid Chromatography. American journal of pharmaceutical research. 2012;2(4):332.
9. Umarkar AR, Rewatkar NS, Charde MS, Charde RM and Kasture AV. RP-HPLC method development and validation for estimation of thiocolchicoside and diclofenac potassium in bulk and capsule dosage forms. Journal of pharmacy research. 2011;4(5):1307.
10. Satyanarayana MV, Satyadev TNVSS, Ganji Ramanaiah and Anuradha V. Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of thiocolchicoside and diclofenac in bulk and its pharmaceutical formulations. Indo American Journal of Pharmaceutical Research. 2014;4(3).
11. Bhavin P Marolia , Divyesh J Vanparia, Bhavik H Satani, Pintu B Prajapati, Shailesh A Shah and Dinesh R Shah. Application of RP-HPLC method for simultaneous estimation of Thiocolchicoside and flupirtine maleate. American journal of pharmaceutical research. 2012;2(3):244.
12. Satheesh BS, Ree Ganesh KK, Saravanan D, Gundu Ramakant K, Sivaraman and Sivananthan. Quantification of Flupirtine and its related compounds in pharmaceutical dosage forms by UPLC. Journal of Pharmacy Research. 2012;5(5):2694.