ISSN 2394-7446

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICAL SCIENCES

DEVELOPMENT AND VALIDATION RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION HYDROCHLORTHIAZIDE AND THIOCOLCHICOSIDE IN BULK AND IN SYNTHETIC MIXTURE

GURUMURTHY.T*, NAGUNATH .S
Smt. Sarojini Ramulamma College of Pharmacy, MBNR , T.S, India
Mail id:gurumurthy.telugu@gmail.com

RESEARCH ARTICLE

Abstract

A simple, economic, selective, precise, and accurate Reverse phase High Performance Liquid Chromatography method for analysis of Hydrochlorothiozide and Thiocolchlcoside was developed and validated according to ICH guidelines. The Chromotography was performed with mobilephase containing a mixture of (Phosphate) buffer: Acetonitrile (20:80) with flowrate 1 ml/min and detectron carried at 267nm. The proposed method determining sensitivity,accuracy precesion,robustness,stability,specificity, selectivity & system suitability parameter

Key Words: Hydrochlorthiazide, Thiocolchicoside, Rp-HPLC,Estimation validation

Introduction:-

Hydrochlorthiazide is chemically 6-chloro-1-1dioxo-3,4-dihydro-24-1,2,4-benzothiadiazine-7-sulfonamide.
Hydrochlorothiazide a thiazide dilutic lumibits water reabsorption in nephron by lubibiting the sodium-chloride symptor in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption throughout the nephron.

Thiocochicosidine is chemically N [(7s)-3-(D-Glucopyranosyloxy)1,2,-dimethoxy-10-(methoyl sulfanyl)-9-oxo-5,6,7,9 tetra hydroben (a) heptalen-7-yl)]autamide. This is chemically related to colchicines this musule relaxant is believed to act as GABA and glycinergic drug, it boind to GABA-A and struchine sensitive glycine receptors and act as GABA-A autagonist.

Experimental material & methods:

Hydrochlorothiazide &Thiocochicosidine were obtained as gift samples from Alzant pvt ltd., Hyderabad, we used HPLC grade acetonitrile, water & GR grade ammonium dihydrogen phosphate.

Instrumentation:

A-HPLC(schimadzu,LL2010 GAT)with UV/VIS detector/PDA detector UV(shimadzu, 1700 series)& zodiac c 18(250mm× 4.6mm × 5 µm column was used. HPLC system was equipped with LC solution software for data processing.

Chromatographic Condition

The mobile phase containing Buffer: Acetonitrile ph =4 ratio of (20:80)V/V. the contest of MP were filtered before use through 0.45µ mentrane feltor& sonicated for 15 mins. The flow rate of MP was maintened at 1ml/min. the colour temperature was set at 25 degrees .the detection was carried out by UV detector wave lenth was set 267nm the run time set at 10 mins on the volume of the injection 100P was 10ml, prior to the dry solution, the colour was equilibrated at fast 30 mins with the MP flow in through the system. The data wave acquired stored and analyzed.

Buffer Preparation

Accurately weighed and transferred 2072gm & potassium dihydrogen orthophosphate was taken into 1000 ml of distilled water and adjust P^H with orthophospic acid .

Preparation of Mobile Phase

Mobile phase was prepared and degassed the mixture 20 volves of the buffer and 80 volves of acetonitrile .

Diluent solution

MP was used as diluents.

Preparation of Standard solution:

Accurately weighed & transferred about 50mg of hydrochlorothiazide 4mg of thiocochicoside working standerd into 50ml VF add 50 ml of MP sonicate for 15 mins and make upto the mark with MP.(diluents)

Preparation of Sample solution:

Weigh accurately a quantity of the powdered tablets equivalent to about 50mg of hydrochlorothiazide & 4mg of thiocochicoside sample into 50 ml VF and make volume mark with diluents sonicate for about 20mins to dissolve and make upto the volume with mobile phase.

Filter through 0.45 µ filter.transfer 25ml of the above solution into 25ml volumetric flask & dilute upto mark into mobile phase.

METHOD VALIDATION

1) System Suitability:

System Suitability was performed by injecting five replicate injections of standard solutions of Hydrochlorothiazide and Thiocolchiside at 100% & measured retention time, theoretical plates and tailing factor.

2) System Precision:

To assess the system precise for conducting validation inject five replicates of standard preparations of 100% level for of Hydrochlorothiazide & Thiocolchiside and expressed as %RSD of peak area

3) Specificity:

To demonstrate that diluents and placebo are not interfering with analytic peak Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system

4) Method Precision:

Method Precision was measured in terms of repeatability of application and application was carried out using five measurement. Repeatability of sample replicates of the same sample concentration.

5) Linearity[10]:

The linearity of the HPLC method was demonstrated for Niacin and Simvastatin solutions ranging from 20% to 120% of standard concentrations

6) Accuracy (%Recovery)[11]:

%Recovery studies were carried out at three different levels of 80%, 100% and 120% of standard solution (i.e. Niacin and Simvastatin API spiked to the placebo) in triplicate in each level

7) Robustness:

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing

physical parameters like flow rate and wave length which may differ but the responses were still within the specified limits.

8) **Ruggedness:**

The variability of the results obtained with the analysis of Hydrochlorothiazide & Thiocolchiside sample five times by two different analysts, two different reagents, two different columns different instruments on two different days to assess the method hydrochlorothiazide and thiocochicosidine.

The percentage relative standard deviation for peak area in Hydrochlorothiazide and thiacolchicoside in system precision was found

Good resolution obtained between analytes hydrochlorothiazide and thicochicoside peaks and no interferena of placebo observed at the retension time of hydrochlorothiazide & thiacochicoside and chromatograms were shown in fig 3& 4 Precision was determined &

The correlation coefficient (r^2) for hydrochlorothiazide was found to be 0.999&0.998 and shows good linearity data of calliberation curve was given in Table no:4

As part of the Robost Ness, deliberate changes in the flow rate and wave length was made to impact on the method.RT & Peak area were

Conclusion:-

It can be concluded that the proposed RP – HPLC Method is accurate , precise, sensitive, Specific, robust and reproducible for the simultaneous analysis of Hydrochlorothiazide & Thiacolchicoside with less tailing and is also economical . The proposed method can be used

ruggedness

RESULTS AND DISCUSSION:

Optimization of the mobile phase was performed based on resolution , asymmetric factor and peak area obtained for hydrochlorothiazide and thiocochicosidine.

The mobile phase acetonitrile: buffer (80:20) was found to be satisfactory & gave symmetric & well resolved peak for Results were summerised in table no1

to be 0.45 and 1.67 which indicates that the method meets the acceptance criteria. Results were summerised in table no .2

the results are represented in the form of %RSD for peak area of hydrochlorothiazide & thiacolchicoside were found to be below 2% shows that the test method was precise &results in the table no.3

The mean recovery for Hydrochlorothiazide & Thiacolchicoside were found to be in the range of 98.05% -99.96% and 98.31-99.52-99.52%. The result were summarized in the table no:5

significantly changed but within the acceptance limit and results given in the Table No 6

for routine analysis of both drugs in the process in the control of Bulk drugs and function products with out any interferience. Thus experiments in Labaratories and Phrmacuticals

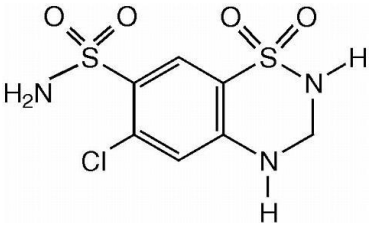

Fig:- 1 Chemical Structure of Hydrochlorthizade

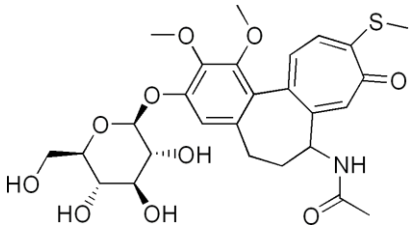

Fig :2 Chemical Structure of Thiocolcoside

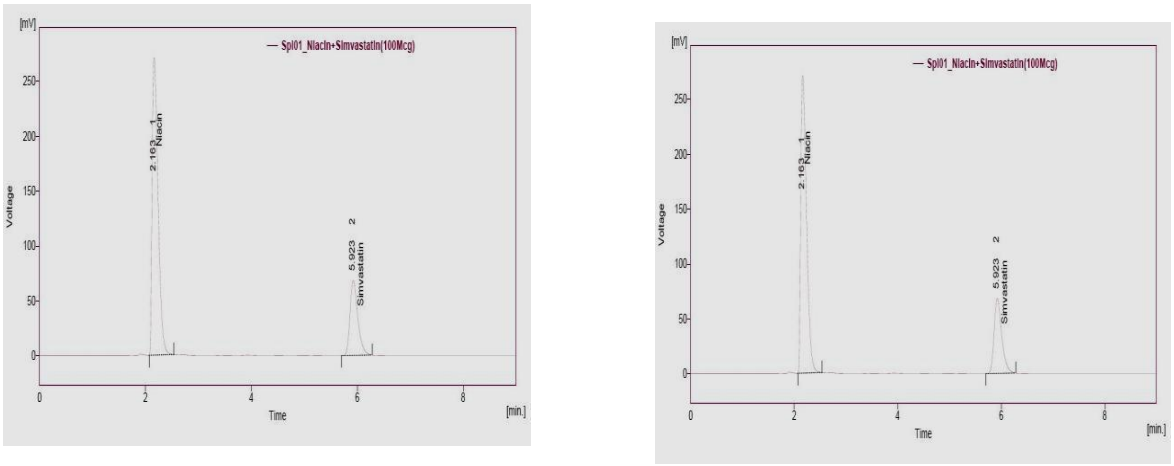

Fig No:-3 Typical chromatograms

Table-1: System Suitability Data

Parameter	Hydrochlorothiazide	thiacolchicoside
Tailing factor	1.339	1.295
Theritical plates	2924	2698
%RSD	1.45	1.67

Table.2: System Precision Data

Parameter	Hydrochlorothiazide	Thiacolchicoside
Mean peak area	4374.497	87.230
SD	63.377	1.455
%RSD of peak	1.45	1.67

Table.3: Repeatability data

Parameter	Hydrochlorothiazide	Thiacolchicoside
Mean peak area	4874.497	87.230
SD	63.377	1.455
% RSD	1.45	1.67

Table :4 Linearity Data

Level	**Concentration (µg/ml)**		**Peak Area**	
	Hydrochlorothiazide	Thiacolchicoside	Hydrochlorothiazide	Thiacolchicoside
60	60.0	2.4	2994.136	45.993
80	80.0	3.2	3968.002	68.958
100	100.0	4.0	4651.352	89.445
120	120.0	4.8	5600.904	113.979
140	140.0	5.6	6519.719	140.311
Slope	-	-	43.4	27.805
Intercept	-	-	40.94	20.597
Corelation cofficient	-	-	0.999	0.998

Table.5: Accuracy data

Drug	%Level	Mean Peak Area	% Mean recovery	SD
Hydrochlorothiazide	80%	3968.00	98.05	0.321
	100%	4651.353	99.43	0.147
	120%	5600.404	99.6	0.202
Thiacolchicoside	80%	68.958	100.31	1.673
	100%	89.445	101.5	0.496
	120%	113.979	97.08	0.457

TABLE No 6:- Robutness Data

Sl No	Parameter Flow Rate (ml/min)	Platelet Count		Tailing Factor	
		Hydrochlorothiazide	Thiacolchicoside	Hydrochlorothiazide	Thiacolchicoside
1	1.0ml/min	2897	2976	2.309	1.658
2	1.2ml/min	2935	3858	1.175	1.111
3	1.4ml/min	2950	2979	1.396	1.323

References:

1) Hemamrutha,s; development and validation of RP-HPLC method for simultaneous estimation of Irbesartan and Hydrochlorthiazide in bulk & pharmaceuticals dosage forms int J phar2013;3(2):P,360-363.
2) Ramprasad reddy , GVS kumar development and validation of stability indicating PR –HPCL method for simultaneous estimation of Irbesartan and Hydrochlorthiazide in bulk drug tablet dosage form IJPCbs 2012,2(4), P.696-703
3) Sunitha T patil ,vidhya K Bhusari and Sunil R Dhaneshwar validated HPTLC method for simultaneous estimation of Thiocolthiaside and Acelofenac in bulk drug & formulation Int j pharm & Biosciences,Vol 12 issues 2 April-June 2011. P 482-490.
4) Abhijit D etal RP HPL method for simultaneous estimation of Etodolac & thiacolchicoside in combined tablet dosage form int J pharm vol4 supply 4 P 214-216.
5) Kiran rathod etal RP-HPLC method for simultaneous estimation of Etodolac & thiocolchiaside in their combined marketed formulation Int Journ of pharmtech research vol4,No 4,P,1513-1519 Oct-Dec 2012.
6) R.A Mhaske D.J Garole etal RP-HPLC method for simultaneous estimation of Amlodipile B, Valsartan , Telmisartan,Hydrochorthiazide & Chlorthilidone,. Application to commercially drug products IJPSR 2012 vol3(1): P, 141-149.
7) Chaudhari, Sunil B, etal simultaneous estimation of thiocolchiaside & Aceclofenac by RP-HPLC in research, Oct 2011, vol 4 issue 10, P-3638.