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Development and validation of RP-HPLC method for simultaneous estimation of hydrochlorothiazide and thiocolchicoside in bulk and in a synthetic mixture

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ABSTRACT

A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for simultaneous estimation of Hydrochloro thiazide (HCT) and Thiocolchicoside (TCS) in bulk drug and in a synthetic mixture. The analysis was carried out using Zodiac ODS C18 (4.6 x 250mm, 5µm, Make: Zodiac Life Sciences), pre-packed column. The separation was carried out using a mobile phase containing a buffer of pH 4.0, Acetonitrile (80:20 v/v), was pumped at a flow rate of 1.2 mL/min with UV-detection at 267 nm. Both the drugs were well resolved on the stationary phase and the retention times were around 5.170 minute for Hydrochloro thiazide and 7.477 minute for Thiocolchicoside. The method was validated and shown to be linear for Hydrochloro thiazide and Thiocolchicoside. The correlation coefficients for Hydrochloro thiazide and Thiocolchicoside are 0.997 and 0.999 respectively. The relative standard deviations for five replicate measurements in two sets of each drug in the tablets is always less than 2% and mean % error of active recovery not more than ±1.5%. The method was validated for precision and accuracy. The proposed method was successfully applied to the synthetic mixture containing the above-mentioned drug combination without any interference by the excipients.

Keywords: Hydrochloro thiazide, Thiocolchicoside, HPLC, Validation.

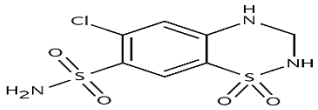
INTRODUCTION

HYDROCHLOROTHIAZIDE [10-13]

IUPAC Name

6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide

STRUCTURAL FORMULA



CHEMICAL FORMULA

$C_7H_8ClN_3O_4S_2$

MOLECULAR MASS

297.739gms.

DESCRIPTION

A White or almost white, crystalline powder; odourless.

SOLUBILITY

Soluble in *acetone;* sparingly soluble in *ethanol;* very slightly soluble in *water*.

MELTING POINT

266-268 °C

LOSS ON DRYING

Not more than 0.5 per cent, determined on 1.0 g drying in an oven at 105˚

CATEGORY

Antihypertensive Agents, Diuretics and Sodium Chloride Symporter Inhibitors

STORAGE

Store protected from light and moisture.

MECHANISM OF ACTION

Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. Normally, the sodium-chloride symporter transports sodium and chloride from the lumen into the epithelial cell lining the distal convoluted tubule. The energy for this is provided by a sodium gradient established by sodium-potassium ATPases on the basolateral membrane. Once sodium has entered the cell, it is transported out into the basolateralinterstitium via the sodium-potassium ATPase, causing an increase in the osmolarity of the interstitium, thereby establishing an osmotic gradient for water reabsorption. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

DOSAGE FORMS

Tablet, Extended release

BRAND NAMES

Hydrazide®,Bpzide, Xenia.

USES

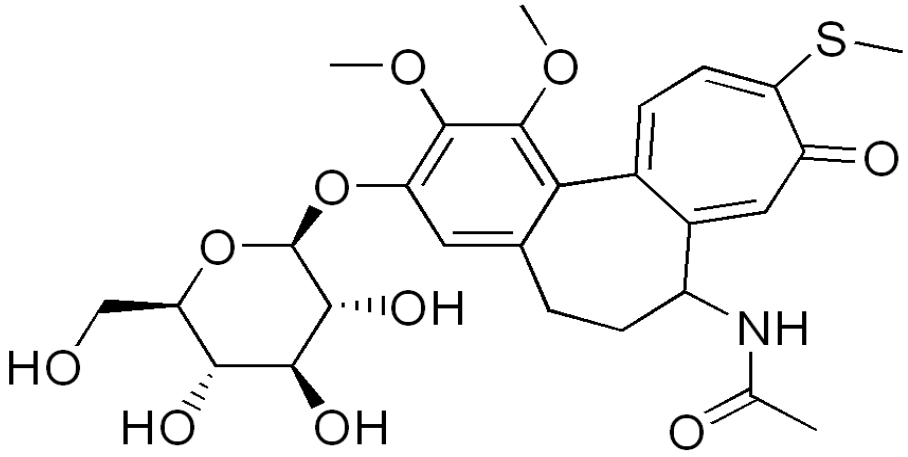
For the treatment of high blood pressure and management of edema. Thiazides such as hydrochlorothiazide promote water loss from the body (diuretics). They inhibit Na^+/Cl^- reabsorption from the distal convoluted tubules in the kidneys. Thiazides also cause loss of potassium and an increase in serum uric acid. Thiazides are often used to treat hypertension, but their hypotensive effects are not necessarily due to their diuretic activity. Thiazides have been shown to prevent hypertension-related morbidity and mortality although the mechanism is not fully understood.

THIOCOLCHICOSIDE: [14-17]

IUPAC NAME

N-[(7*S*)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide

STRUCTURAL FORMULA



CHEMICAL FORMULA

$C_{27}H_{33}NO_{10}S$

MOLECULAR MASS

563.618 g/mol

DESCRIPTION

A yellow crystalline powder.

Soluble in *water* and in *ethanol*

MELTING POINT

208-213 °C

LOSS ON DRYING

Not more than 0.5 per cent, determined on 1g by drying in oven at 105˚

CATEGORY

Anti-Anxiety Agents, Hypnotics and Sedatives

STORAGE

Store protected from light and moisture

MECHANISM OF ACTION

Chemically related to colchicine, this muscle relaxant is believed to act as a GABA mimetic and glycinergic drug. Combined with $NSAID_S$, it is being used for painful muscle spasms, such as torticollis, sprains, backache, etc. Side effects are gastric upset and photosensitivity reactions. It binds to GABA-A and strychnine sensitive glycine receptors and act as a GABA-A antagonist.

DOSAGE FORMS

Capsule, Extended release

BRAND NAMES

Myoril®, Zyflex, Neorelax.

USES

Thiocolchicoside is a muscle relaxant. (Thiocolchicoside), a muscle relaxant agent with anti-inflammatory and analgesic actions, also is used topically for the treatment of muscular spasms and for rheumatologic, orthopedic, and traumatologic disorders. Thiocolchicoside is broken down in the body to a metabolite called 3-demethylthiocochicoside (also known as SL59.0955 or M2) that could damage dividing cells therefore inducing toxicity in the embryo, neoplastic changes and fertility reduction in males.Therefore recommended oral dose should not exceed 7 days and intramuscular dose duration should not exceed 5 days. Local skin preparations are less toxic.

MATERIALS AND METHODS

CHEMICALS

Hydrochlorothiazide, Thiocolchicoside was gift Samples obtained from Chandra labs, Hyd , TS . Triethly amine ,HPLC grade water ,Methanol (HPLC grade), Acetonitrile (HPLC grade), Ortho Phosphoric Acid (HPLC grade) Potassium dihydrogen Ortho phosphate buffer (HPLC grade), Ammonium Acetate buffer (HPLC grade), were purchased from Standard Reagents Pvt Ltd.

INSTRUMENTATION

Analysis was performed on Shimadzu HPLC with UV Detector and Zodiac **C_{18}** column compartment with Spinchrome software. Other equipment used in the study was Analytical Balance (Shimadzu AY220) and Ultra Sonic bath.

CHROMATOGRAPHIC CONDITIONS

Zodiac C_{18} column (50mm x 4.6mm, 5µm) was used for chromatographic separation.The mobile phase composed of 2.72 mg of KH2PO4 was dissolved in 100ml water pH adjusted with Orthophosphoric Acid Ortho phosphoric acid buffer of pH is 4.0 and Acetonitrile in the ratio (80:20v/v); at a flow rate of 1.2ml/min with run time 10mins.The detection of drugs was carried out at 267nm.

METHOD DEVELOPMENT

PREPARATION OF BUFFER AND MOBILE PHASE

PREPARATION OF PHOSPHATE BUFFER (0.02M KH_2PO_4)

Accurately weighed 2.72 gm of Potassium dihydrogen orthophosphate was taken into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and adjusted to pH 4.0 with orthophosphoric acid.

PREPARATION OF MOBILE PHASE

Accurately measured 800ml (80%) of above buffer and 200ml (20%) of HPLC Acetonitrile were mixed and degassed in an ultrasonic water bath for 5 minutes and then filtered through 0.45µ filter under vacuum filtration.

DILUENT PREPARATION

The Mobile phase was used as a diluent.

PREPARATION STANDARD SOLUTION

Accurately weigh and transfer 50mg of Hydrochloro thiazide and 4mg of Thiocolchicoside working standard into a 50ml clean dry volumetric flask and make up to the mark with the diluent.

Further pipette 2.5 ml of Hydrochloro thiazide and Thiocolchicoside of the above stock solution into a 25ml volumetric flask and dilute up to the mark with diluent.

PREPARATION SAMPLE SOLUTION

Accurately weigh and transfer equivalent to 50 mg of Hydrochloro thiazide and 4 mg of Thiocolchicoside sample into a 50ml clean dry volumetric flask and make volume up to the mark with the diluent. Further pipette 2.5 ml of Hydrochloro thiazide and Thiocolchicoside of the above stock solution into a 25ml volumetric flask and dilute up to the mark with diluent.

VALIDATION METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing HCT and TCS. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of HCT and TCS. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analyzed sample solution of HCT and TCS and a known amount of standard drug powders of HCT and TCS were added at 80, 100, 120% level. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line. The robustness of the method was studied for the sample. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by 100µg/ml. The system suitability parameters such as number of theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at 25^0c for 24hr and sample was reanalyzed after 24 hr. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for these Drug samples.

ANALYSIS OF FORMULATION

100mg of Tablet (ACENAC-MR) was weighed accurately in a 25ml volumetric flask. To this 15ml of methanol was added and sonicated to dissolve, and then make up to the mark. The obtained solutions were filtered through 0.45µ Nylon syringe filter. Further the 5ml was transferred into a 50ml calibrated flask and diluted to volume with Methanol and volume was made up to the mark with diluent to obtain a concentration of HCT (10 µg/ml), TCS (10 µg/ml) which was then subjected to proposed method and the amounts of HCT and TCS were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantitation of HCT (271nm) and TCS (257nm) with good resolution, peak shapes and minimal tailing. The overlay UV spectra was shown in figure 1. The typical chromatogram for HCT and TCS were shown in Figure 2. The drugs was found to give linear detector response in the concentration range under study with correlation coefficient of 0.997 - 0.999.The samples had followed linearity in the concentration range of HCT(99.43 µg/ml), TCS(4.06 µg/ml) were shown in Figure 3, 4. Percent recoveries for HCT (99.52%), TCS (101.5%). The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 1.45,1.67 respectively. As the RSD values were < 2%, the developed method was found to be precise(Table 1).The accuracy of the method studied at three different concentration levels i.e. 80, 100, 120% showed acceptable recoveries in the range of 97.08-101.5% (Table 2).The LOD for HCT (2.40 µg/mL), TCS (0.16 µg/mL). Further the LOQ for HCT (7.28 µg/mL), TCS (0.48 µg/mL) respectively. Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 mL/min), wave length (± 2nm) and mobile phase composition (± 5%). The validation parameters were summarized in (Table 3). The results of robustness study of the developed method was validated by change in flow rate ,change in wave length and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4). When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method. The system suitability parameters such as number of theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at 25^0 c for 24hr and sample was reanalyzed after 24 hr and assay was determined.

The results were shown in (Table 6). Six replicates of sample solutions containing of HCT (10 µg/ml), TCS (10 µg/ml) were injected for quantitative analysis. The amounts of HCT, TCS were found to be 98.97% and 99.47% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

DISCUSSION

The developed method was found suitable for simultaneous estimation of HCT and TCS with good peak shapes and minimal tailing. The peak area of the drugs was reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.998 showed the stable linear detector response in different concentration range. The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for HCT (99.52%), TCS (101.5%). LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate, wave length and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of HCT and TCS from the commercial Tablet dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used excipients and buffer used in analysis.

TABLE 1: PRECISION OF DEVELOPED METHOD

HYDROCHLOROTHIAZIDE			**THIOCOLCHICOSIDE**		
S.No.	Rt	Area	S.No.	Rt	Area
1	5.093	4435.754	1	7.430	86.157
2	5.097	4298.219	2	7.400	89.968
3	5.103	4371.170	3	7.410	87.635
4	5.147	4299.058	4	7.483	86.982
5	5.093	4435.754	5	7.430	86.157
6	5.113	4407.024	6	7.437	86.479
avg	5.1077	4374.497	**avg**	7.432	87.230
stdev	0.0207	63.377	**stdev**	0.029	1.455
%RSD	**0.41**	**1.45**	**%RSD**	**0.39**	**1.67**

TABLE 2: ACCURACY DATA

% Added	**Constant amount added[a] (µg/ml)**	**Amount added[b] (µg/ml)**	**Total amount found[c] (µg/ml)**	**Amount found[d] (µg/ml)**	**% Recovery[e]**	**%RSD**
80	20	80	98.44	78.44	98.05	0.321
	20	80				
	20	80				
100	20	100	119.43	99.43	99.43	0.147
	20	100				
	20	100				

120	20	120	138.96	119.96	99.96	0.202
	20	120				
	20	120				

% Added	Constant Amount added[a] (µg/ml)	Amount Added[b] (µg/ml)	Total Amount found[c] (µg/ml)	Amount found[d] (µg/ml)	% Recovery[e]	%RSD
80	0.8	3.2	4.01	3.21	100.31	1.073
	0.8	3.2				
	0.8	3.2				
100	0.8	4.0	4.86	4.06	101.5	0.496
	0.8	4.0				
	0.8	4.0				
120	0.8	4.8	5.46	4.66	97.08	0.457
	0.8	4.8				
	0.8	4.8				

TABLE 3: VALIDATION AND SYSTEMSUITABILITY PARAMETERS
PARAMETER

Parameters	Hydrochlorothiazide	Thiocolchicoside
Range(µg/ml))	10	10
Slope(m)	43.4	27.805
Intercept(c)	404.94	20.597
Correlation coefficient(R^2)	0.9975	0.9994
Retention time	5.017	7.393
Precision % RSD	<2	<2
Accuracy	99.52	101.5
LOD(µg/ml))	2.40	0.16
LOQ(µg/ml))	7.28	0.48
Theoretical Plates	2961	2672
Asymmetry	1.345	1.253
Resolution	-	4.636

TABLE 4: INFLUENCE OF FLOW RATE AND WAVELENGTH ON ANALYTICAL PARAMETERS

Parameter	HYDROCHLOROTHIAZIDE		THIOCOLCHICOSIDE	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow				
1.0 ml/min	6.107	1.309	8.807	1.658
1.2 ml/min	5.093	1.339	7.430	1.295
1.4ml/min	4.440	1.396	6.493	1.323

Wavelength				
265nm	5.113	1.382	7.433	1.089
267nm	5.093	1.339	7.430	1.295
269nm	5.087	1.339	7.377	1.644

TABLE 5: ASSAY OF COMMERCIAL FORMULATION

Active ingredient	**Hydrochlorothiazide**	**Thiocolchicoside**
Label claim(mg)	100	4
Standard % purity	99.2	99.3
Standard weight (mg)	100	4
Sample weight	175	175
Average weight (mg)	175	175
Area of Sample	4422.638	77.2326
Area of Std	4433.138	77.1006
% Assay	98.96	99.47

TABLE 6: STABILITY STUDIES

S.NO	**Peak name**	**Retention time**	**Area**	**Height**	**Plate count**	**USP Resolution**	**USP Tailing**
1	Hydrochlorothiazie	5.093	4435.754	234.369	2924	-	1.339
2	Thiocolchicoside	7.430	86.157	4.199	2698	4.508	1.295

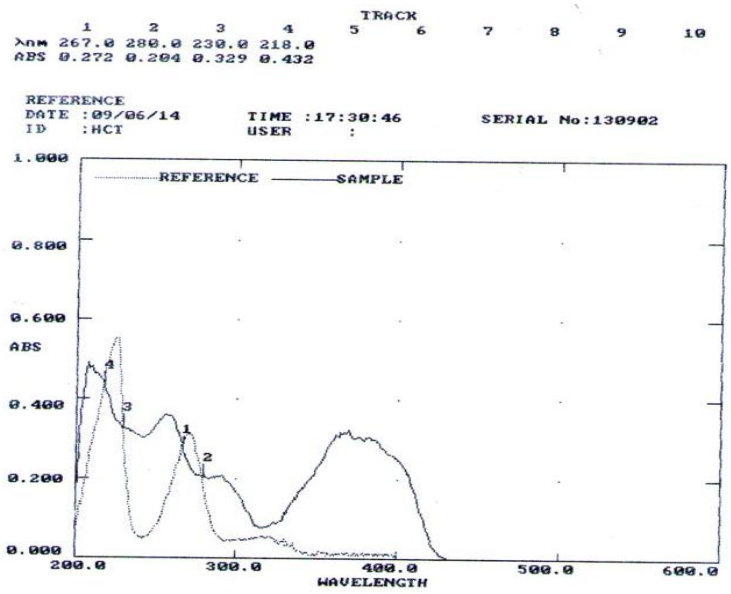


Figure 1: Overlay UV Spectra of Standard HCT and TCS

---------- **HCT**

_____ **TCS**

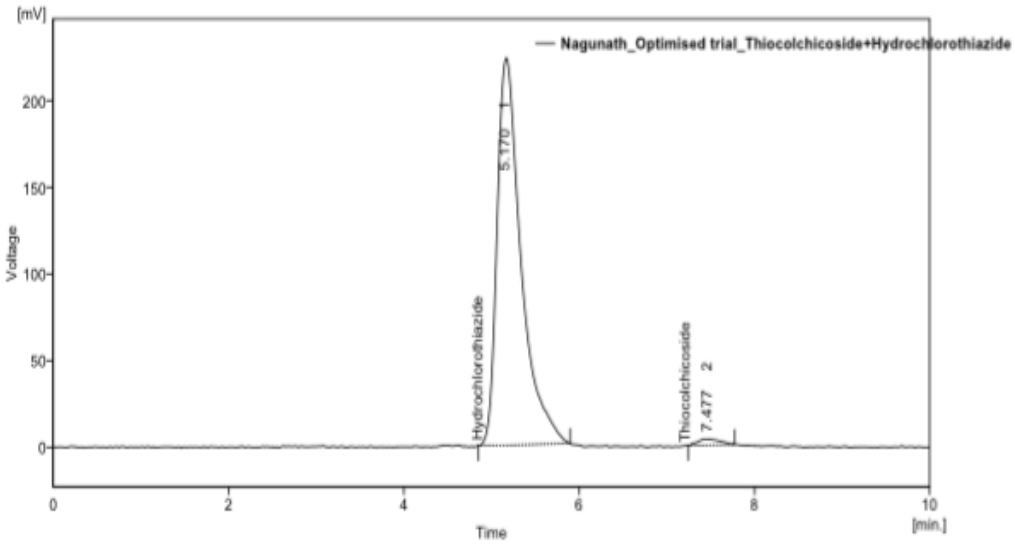


Figure 2: Typical HPLC chromatogram of HCT and TCS

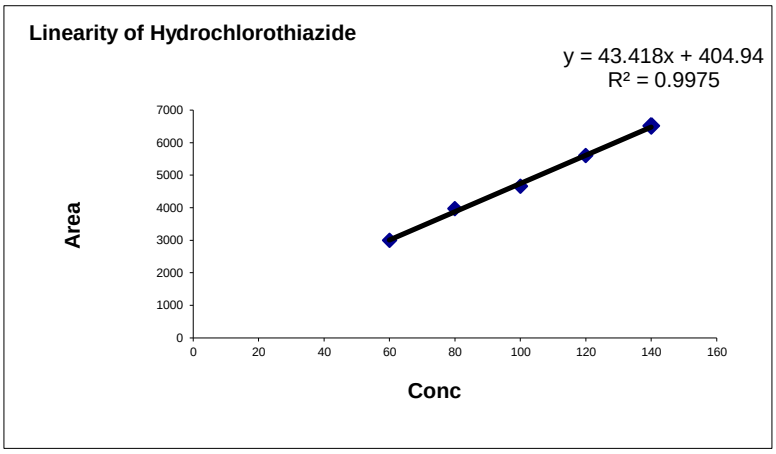


Figure 3: calibration curve for Hydrochlorothiazide at 267 nm

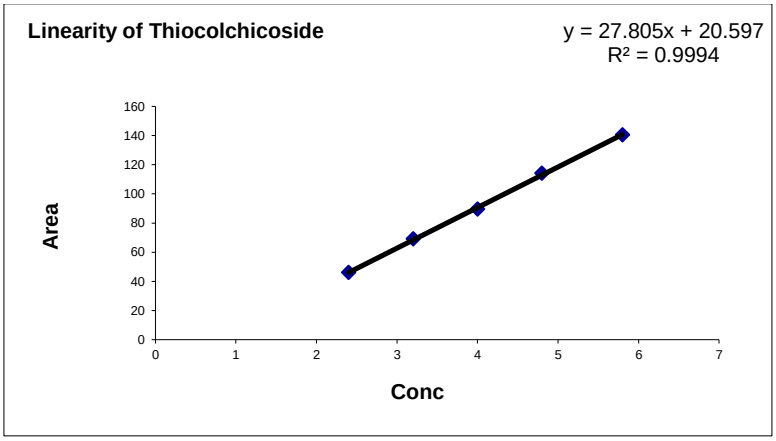


Figure 4: calibration curve for Thiocolchicoside at 267 nm

CONCLUSION

The RP-HPLC procedure was optimized with a view to develop an accurate and stable assay method with the pure drugs Hydrochloro thiazide and Thiocolchicoside, in a bulk drugs and in a synthetic mixture using Zodiac C18 (4.6×250mm, 5µm, Make: Zodiac life sciences) column in isocratic mode using a mobile phase containing Buffer of pH is 4.0 and Acetonitrile (80:20 v/v). The flow rate was 1.2 mL/min at 30 °C with UV detection at 267 nm. Linearity was assessed by plotting concentration versus area, and it is linear in the range of 60–140 µg/mL for Hydrochloro thiazide and 2.4-5.8 µg/mL for Thiocolchicoside with correlation coefficients of 0.997 and 0.999 respectively. The % recovery was found to be within limits of the acceptance criteria with a recovery range of 98.44%–99.25% for Hydrochloro thiazide and 100.37% –100.75% for Thiocolchicoside. The % RSD for precision was less than 2% for Hydrochloro thiazide and Thiocolchicoside. The detection limit of the proposed method was 2.40 µg/mL and 0.16 µg/mL, and the quantification limit was 7.28 µg/mL and 0.48 µg/mL for Hydrochloro thiazide and Thiocolchicoside respectively. The assay procedures were repeated five times and the results were found to give 98.97 % of Hydrochloro thiazide and 99.47% of Thiocolchicoside. The proposed study describes a new and simple RP-HPLC method for the estimation of Hydrochloro thiazide and Thiocolchicoside in bulk drug and in a synthetic mixture. The method has been validated and found to be simple, rapid, sensitive, accurate, and precise. Moreover, the lower solvent consumption along with the short analytical run time of 10.0 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, the proposed method can be used for routine analysis of both drugs in the process control of bulk drugs and formulation products without any interference from excipients in Laboratories and in the pharmaceutical industries.

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