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R E S E A R C H A R T I C L E

Simultaneous Estimation of Moxonidine and Hydrochlorothiazide in Tablets by RP-HPLC

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A B S T R A C T

A simple, precise, specific sensitive and accurate reverse phase high performance liquid chromatographic (HPLC) method was developed for the determination of Moxonidine and Hydrochlorothiazide in tablet dosage form. The separation was performed by phenomenex C18 column (250mm×4.6mm,5µ) using mobile phase consisting of sodium pentane sulfate buffer , pH3.5 with diluted sulphuric acid : acetonitrile (870:130v/v).The flow rate was 1ml/min and detection was monitored at 230nm.The column temperature was set at 40°c.The retention times were found to be 9.5 , 14.5mins for Hydrochlorothiazide and Moxonidine respectively. The calibration curves were found to be linear in the concentration range of 8-44µg/mL (r^2=0.9992) for Moxonidine and 18-93µg/mL(r^2=0.9993) for Hydrochlorothiazide. The percentage recoveries of Moxonidine at assay level were found to be in the range of 9.0% to 103.0% and for Hydrochlorothiazide in the range of 9.0% to 103.0%.The method was validated in the terms of reproducibility and recovery studies. The proposed method was found to be accurate, reproducible and economical for routine analysis of Moxonidine and Hydrochlorothiazide in tablet dosage form.

Keywords: Moxonidine, Hydrochlorothiazide, RP-HPLC, Tablets.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Moxonidine is chemically 4-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-6 Methoxy-2-methylpyrimidin-5-amine. It is used as an anti-hypertensive drug. Hydrochlorothiazide is chemically chloro-1-1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzo thiadiazine-7-sulfonamide. It is also an antihypertensive drugs belongs to thiazide class of diuretics. Both of them are very soluble in water and freely soluble in methanol. From the literature survey; it was found that there are many analytical methods reported for Moxonidine and Hydrochlorothiazide either individually or in combination with other drugs by LC-MS/MS, and HPLC methods. However no method is reported for simultaneous estimation of these two drugs in tablet dosage form by HPLC. Hence the present work was attempted to develop accurate, simple and sensitive method for simultaneous estimation of Moxonidine and Hydrochlorothiazide in tablet dosage form.

2. Materials and Methods

Reagents and chemicals:

HPLC grade Methanol and acetonitrile were procured from the E. Merk limited. Mumbai. Water is also obtained as HPLC grade. Moxonidine and Hydrochlorothiazide used in this study were gifted by Macleods pharmaceuticals limited, Chennai. The tablets used in this study were MOXOVAS (Macleods (procare AHT)) labelled to contain 0.2mg of Moxonidine and 12.5mg of Hydrochlorothiazide.

Chromatographic conditions: A Liquid chromatographic separation was performed on SHIMADZU-LC-2010CHT isocratic Liquid chromatographic system, equipped with Thermolabs-model-283 with 20µL fixed loop. UV-visible detector SHIMADZU- UV- 3600 was employed and separation achieved on C18 phenomenex (250mm×4.6mm, i.d.,5µ) column using a mixture of dilute sulphuric acid :acetonitrile in the ratio of (870:130v/v) and adjusted the pH3.5 using mobile phase. The elution was carried out at the flow rate of 1mL/minute. Detection was made and the obtained data were analyzed.

Preparation of standard stock solution:

Accurately weighed 30mg of Moxonidine and 62.5mg of Hydrochlorothiazide were transferred into a 50mL volumetric flask ,diluted with little amount of mobile phase and the contents were shaken thoroughly to dissolve and finally make up the volume to 50mL with diluent.5ml of the above solution is transferred to a 25mL volumetric flask; dilute it to 25mL with mobile phase.

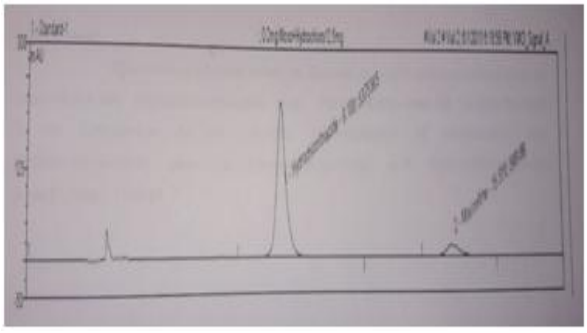

Fig 1: Typical standard chromatogram of Moxonidine and Hydrochlorothiazide

Preparation of working standard solution:

Working standard solution was prepared by diluting to 10mL of the stock solution to 100mL with mobile phase to achieve the concentration of 0.2mg/mL of Moxonidine and 12.5mg/mL of Hydrochlorothiazide.

Estimation of drug in commercial tablet formulation:

For the estimation of drugs in tablet formulation, twenty tablets were weighed and their average weights were determined. The tablets were finely powdered. Accurately weighed tablet powder equivalent to 30mg of Moxonidine and 62.5mg of Hydrochlorothiazide was transferred to a 50mL volumetric flask. Little amount of mobile phase was added and the contents were shaken well to dissolve and sonicated for 15minutes.The volume was made up to 50mL with mobile phase and filtered through 0.45µm nylon membrane filter.5mL of the above solution is taken and diluted to 25mL with mobile phase. Further this solution was diluted suitably to get the concentration of 0.2mg/mL of Moxonidine and 12.5mg/mL of Hydrochlorothiazide .Both the standard and sample preparations were injected separately and peak area responses were recorded. The percentage label claim was calculated and given in table-1.

Validation of the Method:

System suitability:

For system suitability, 5 replicates of standard solutions were injected and parameters studied were number of theoretical plates, peak area, resolution, retention time and tailing factor. The relevant data is shown in table-2.

Accuracy:

The accuracy of the experiment was established using recovery technique i.e. by external standard addition method. The result of recovery analysis is presented in table-3.The result of recovery was well within the acceptable limit.

Precision:

The validation of the proposed method was verified by system precision and method precision. The system precision was evaluated by measuring the peak area response of Moxonidine and Hydrochlorothiazide for six replicate injections of the standard solutions. The method precision was determined by quantifying the sample solution as per the proposed method, which yielded quite concurrent results, indicating the reliability of the method. The value of SD and RSD were within the prescribed limit of 2% showing high precision of the method.

Linearity and range:

During linearity study, it was observed that the absorbance values of Moxonidine and Hydrochlorothiazide in the marketed formulations were linear in the concentration of 8-44µg/mL for Moxonidine and 18-93µ/mL for Hydrochlorothiazide with R^2 close to one of this method of analysis.

Robustness and Ruggedness:

Robustness of the method is determined by analyzing the sample in duplicate with varying the method conditions i.e. very small changes in flow rate, showed there were no marked changes in chromatographic behaviour and content of the drug, as evident from the low value of RSD indicating the method was robust. The method was also confirmed by ruggedness study, analyzing the product day

to day, analyst to analyst and instrument to instrument. The results are shown in table-3, proved that the method was reproducible.

3. Results and Discussion

Estimation of Moxonidine and Hydrochlorothiazide in tablet dosage form by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of Moxonidine and Hydrochlorothiazide is shown in fig.1.The retention time was achieved at 9.5 and 14.5 for Hydrochlorothiazide and Moxonidine respectively. The total run time was 20 minutes. The theoretical plates were found to be 6053 and 13766 for Hydrochlorothiazide and Moxonidine respectively. The tailing factors were1.0 and 1.4 for both drugs and the method showed linear at a concentration of 8-44µg/ml for Moxonidine and 18-93µg/ml for Hydrochlorothiazide.The regression coefficient value for Moxonidine and Hydrochlorothiazide were found to be 0.9992 and 0.9993 respectively. The validation of the proposed method was verified by system precision and method precision. The %RSD was found to be less than 2 for both drugs indicates the proposed method is precise. The specificity of the method was confirmed by injecting the placebo and observed that there was no interference due to placebo. The data for Moxonidine and Hydrochlorothiazide were found to be within the acceptance limit. Different validation parameters for the proposed HPLC method for determining Moxonidine and Hydrochlorothiazide were summarized in table-3.The results of analysis showed that the amounts of drugs were in good agreement with the label claim of the formulation.

Table 1: Assay of tablets

Drug Name	Label claim mg/tab	Mean peak area		% Amount found ± SD (mg/tab)	% Label claim ± SD
		standard	Sample		
Moxonidine	0.2mg	605616	610569	98.2%±0.02	98.2%±0.01
Hydrochlorothiazide	12.5mg	530551	5456955	101.2%±0.05	101.2%±0.05

Table 2: System suitability parameters

S.No	Parameters	Obtained Values	
		Moxonidine	Hydrochlorothiazide
1	Theoretical plates(N)	13766	6053
2	Tailing factor(T)	1.1	1.0
3	Retention time(min)	14.5	9.5
4	%RSD of peak retention time	0.095	0.118

4. Conclusion

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Moxonidine and Hydrochlorothiazide as bulk drugs and combined dosage forms without any interference from the excipients. The developed method could be conveniently adopted for routine analysis in quality control laboratories.

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