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Analytical method development and validation for the simultaneous estimation of hydrochlorthiazide and reserpine by using rp-hplc technique

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ABSTRACT

A new method was established for simultaneous estimation of Reserpine and Hydrochlorothiazide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Reserpine and Hydrochlorothiazide by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) .

Keywords: RP-HPLC, Hydrochlorothiazide.

INTRODUCTION

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.

Structure of Hydrochlorothiazide

Reserpine's mechanism of action is through inhibition of the ATP/Mg^{2+} pump responsible for the sequestering of neurotransmitters into storage vesicles located in the presynaptic neuron.

The neurotransmitters that are not sequestered in the storage vesicle are readily metabolized by monoamine oxidize (MAO) causing a reduction in catecholamine's.

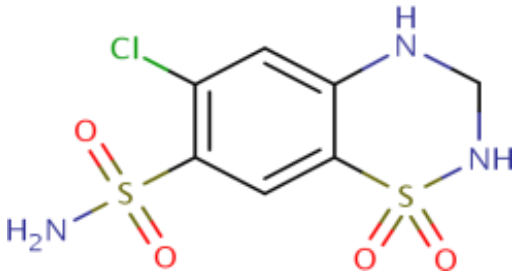

Fig 1: Structurure of Hydrochlorothiazide

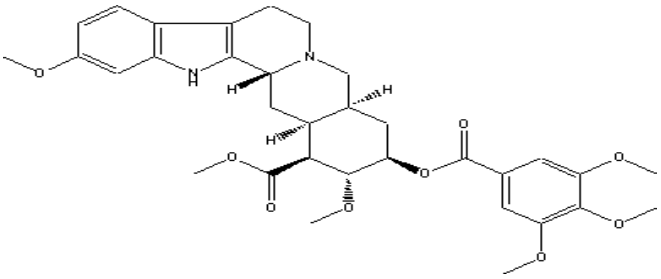

Fig 2: Structure of Reserpine

MATERIALS AND METHODS

Instruments used

Alliance, model No. BSA224SC LC+20AD HPLC, Software- UV WIN5, Solution, LABINDIA UV 3000+ UV/VIS spectrophotometer, Adwa – AD 102U pH meter

Drugs used

Hydrochlorothiazide and Reserpine Active pharma ingredients and marketed samples of hydrochlorothiazide and reserpine

Chemicals and reagent

Potassium di hydrogen ortho phosphate (Make: Merck and Grade: Empata ACS), Orthophosphoric acid (Make : Merck and Grade: Emparta ACS), Acetonitrile and Methanol (Make :Merckand Grade: HPLC)

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wavelength

10 mg of Reserpine and Hydrochlorothiazide was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Reserpine and Hydrochlorothiazide. The isobestic point was taken as detection wavelength.

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved, and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Sample solution preparation

Accurately weigh and transfer 59.8 mg of Hydrochlorothiazide and Reserpine Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock solution): Further pipette 0.6ml of Hydrochlorothiazide & Reserpine the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

Standard solution preparation

Accurately weigh and transfer 12.5 mg & 8 mg of Hydrochlorothiazide and Reserpine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock solution): Further pipette 0.6ml of Hydrochlorothiazide & Reserpine the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Optimized Chromatographic conditions

Column : Zodiac sil C18 column (4.6×150mm)5µ
Mobile phase ratio : Methanol:pH3phosphate buffer (70: 30 % v/v)

Detection wavelength	:	240 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	2.170 and 7.280 mins

Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Method validation

By using Optimized condition Analytical Method of Assay carried out by ICH Guideline Q2B.[3-4] The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

System suitability and system precision

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochlorothiazide and Reserpine. Working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.6ml of Hydrochlorothiazide & Reserpine the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

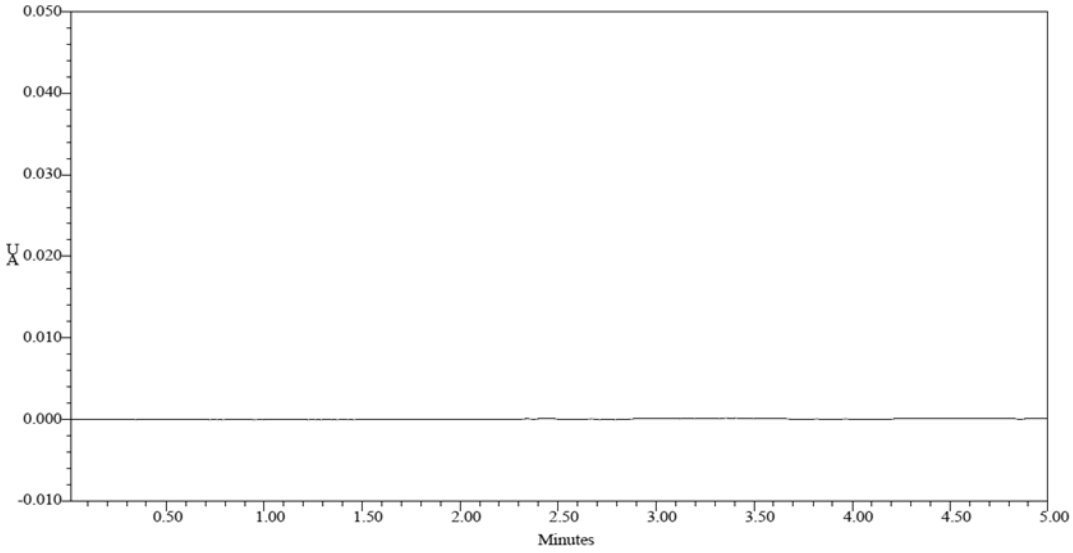

Fig 3: Chromatogram blank preparation

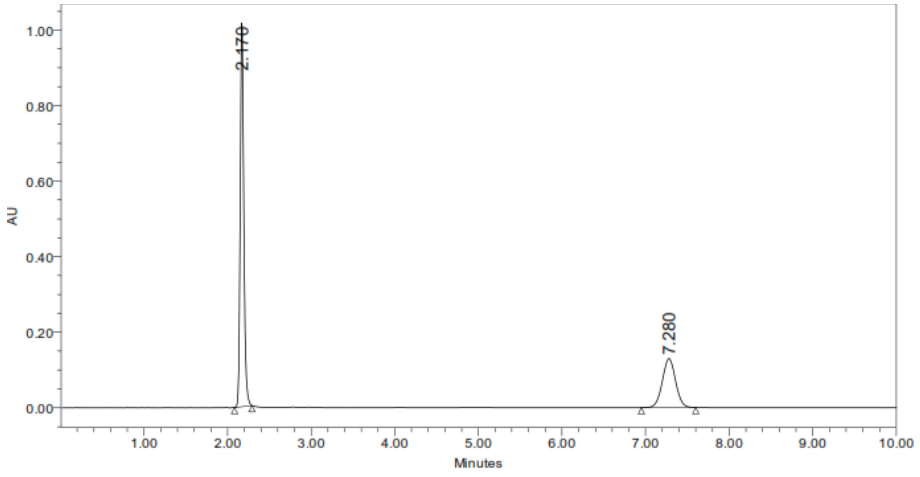

Fig 4: Chromatogram showing sample injection

Linearity

The linearity study was performed for the concentration of 25 ppm to 150 for Hydrochlorothiazide and 16ppm to 80ppm for Reserpine. Each level was injected into chromatographic system. The area of each level was used for of correlation coefficient.

Table 1: Linearity Results for Reserpine

S.No	Linearity Level	Concentration	Area
1	I	16ppm	1027461
2	II	32ppm	1233566
3	III	48ppm	1437030
4	IV	64ppm	1644336
5	V	80ppm	1880590
Correlation Coefficient			**0.999**

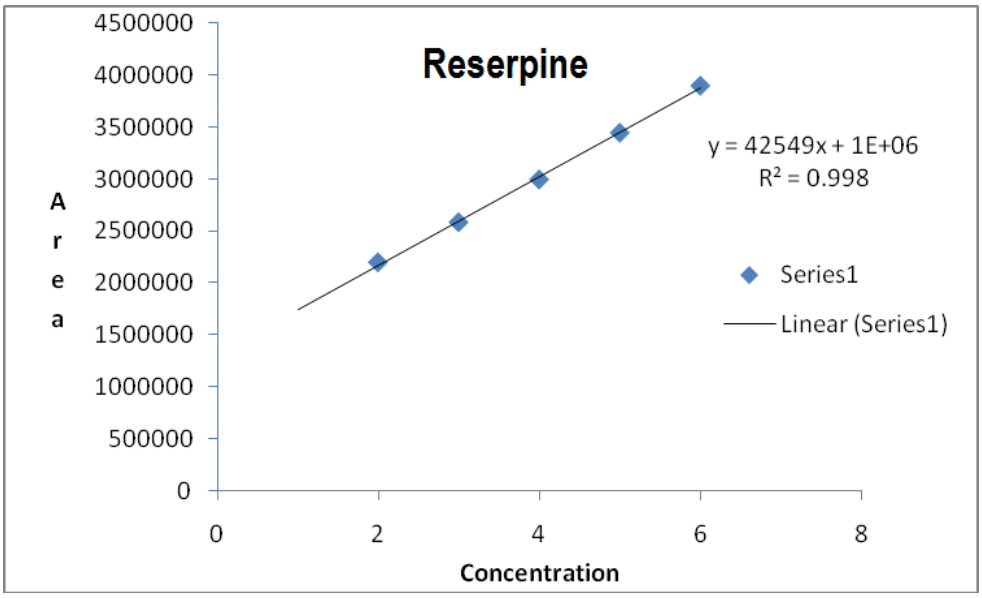

Fig 5: Reserpine Linearity Results for Hydrochlorothiazide

S.No	Linearity Level	Concentration	Area
1	I	25ppm	2201022
2	II	50ppm	2585033
3	III	75ppm	2996553
4	IV	100ppm	3446224
5	V	125ppm	3897922
Correlation Coefficient			0.999

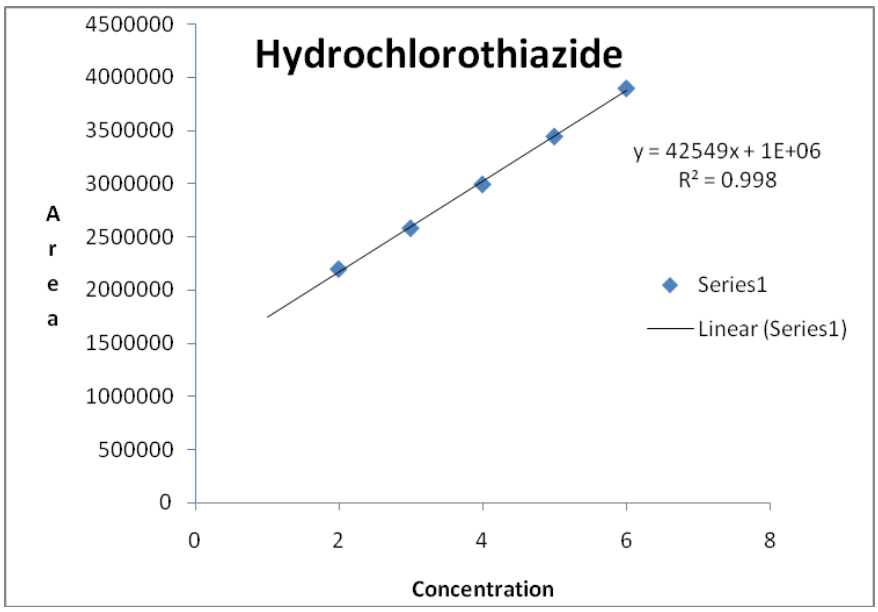

Fig 6: Calibration graph for Hydrochlorothiazide

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Reserpine and Hydrochlorothiazide.The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

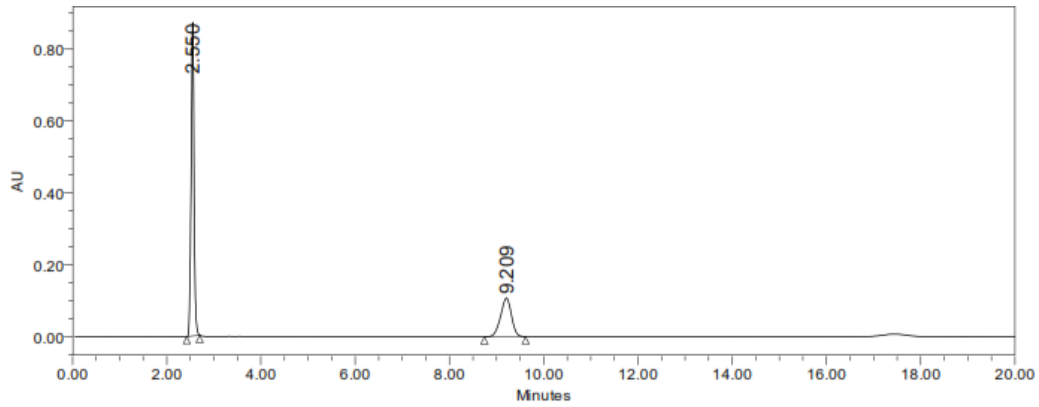

Fig 7: Chromatogram showing more flow rate 0.8ml/min

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

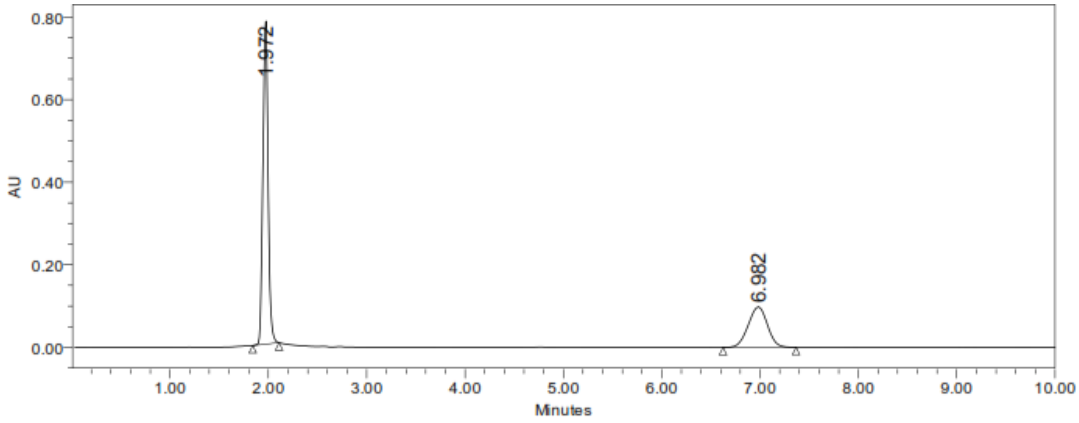

Fig 8: Chromatogram showing less flow rate 1.2 ml/min

Table 2: system suitability results for Reserpine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6953.5	1.0
2	*Actual	10026.7	1.0
3	10% more	6048.5	1.0

Table 3: system suitability results for Hydrochlorothiazide

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	7079.0	1.0
2	*Actual	12458.5	1.2
3	10% more	6228.5	1.1

CONCLUSION

A new method was established for simultaneous estimation of Reserpine and Hydrochlorothiazide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Reserpine and Hydrochlorothiazide by using Zodiac sill C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoric acid), detection wave length was 240nm.

REFERENCES

1. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi. 6th ed; 1986. p. 1-15.
2. Skoog Douglas A, Holler F James, Nieman Timothy A. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2ndedition; 1980. p. 725-60.
3. Watson David G. Pharmaceutical Analysis, A textbook for Pharmacy students and Pharmaceutical Chemists. 2nd ed. Harcourt Publishers Limited; 1999. p. 221-32, 267-311.
4. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC method development, Wiley inter science, New York. 2nd ed; 1997. p. 1-56, 234-89, 685-712.
5. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. C.B.S.Publications, Pg. No.53-62.
6. Remingtonn's the science and practice of pharmacy. 20th ed; 2000.
7. Connors KA. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc, New Delhi. 3rd ed; 1994. p. 373-421.
8. Rashmin, Patel B, Patel Mrunali R. An Introduction to Analytical method development for pharmaceutical formulations. Pharmainfo.net. 2008;17:19.
9. Sharma Bk. Instrumental methods of chemical analysis. 19th ed. Goel Publishing House; 2003.
10. Ewing Galen Wood. Instrumental methods of chemical analysis. p. 340-5.
11. United States of Pharmacopeia, USP30-NF25, The official compendia of standards, official May 1, 2007.
12. ICH topic Q2B, validation of analytical procedure & methodology, The European agency for evaluation of medicinal products, human medicines evaluation unit 1996.
13. ICH. Q2A, Text on validation of analytical procedure; October 1994.
14. Puranik Manisha P, Wadher Sailesh J, Kosarkar Ashish L, Yeole Pramod G et al. Method Development and Validation of candesartan cilexetil by RP-HPLC. Int J Res Pharm Biomed Sci;3(3) Jul – Sep2012.
15. Kalyani Gunjan, Deshmukh Vishal S, Kashyap Pranita, Bawankar Ram D, Yogesh, Tablet Release. Chromatogr Res Int. 2014;2014.