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Original Article

SIMULTANEOUS ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY HPTLC

MRUNALINI H. KULKARNI[1*], POONAM R. INAMDAR[1], PALLAVI DHEKALE[1], AMRITA THAKUR[1], SWATI MUTHA[1], NEETA RAI[1], VISHAL GALAVE[2]

[1]Department of Pharmacy, School of Pharmacy Vishwakarma University, Pune, [2]Navsahyadri Institute of Pharmacy, Pune, India
*Email: mrunalini.kulkarni@vupune.ac.in

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ABSTRACT

Objective: The prevalence of Hypertension in India is estimated to be greater than 45% in the middle age population; elevated hypertension is one of the known causes of CHD, strokes and death. The objective of this work was to develop a simple, accurate and robust HPTLC method was developed for simultaneous estimation of Valsartan and Hydrochlorothiazide in bulk and tablets.

Methods: The mobile phase optimized by HPTLC method consists of Toluene: Ethyl acetate: formic acid in the ratio of (3:7:0.3, v/v/v). The solvent front was a run-up to a distance of 80 cm; which took 15 min for the development of TLC plate. Analytical Wavelength for UV detection selected was at 225 nm.

Results: Retention factor was found to be 0.74±0.02 and 0.45±0.02 for VAL and HTZ, respectively. Developed chromatographic method was validated as per ICH, Q2(R1) guidelines. Linearity was found at concentration range 300-1800 ng/spot (r^2 = 0.999), 50-300ng/spot (r^2 = 0.9991) for VAL and HTZ respectively. LOD were found to be 23.02 ng/spot and 4.12ng/spot respectively for Valsartan and Hydrochlorothiazide. LOQ was found to be 69.76ng/spot and 12.5ng/spot for, Valsartan and Hydrochlorothiazide respectively.

Conclusion: Percentage RSD was found out to less than 2, indicating the developed method was precise and can be successively applied to pharmaceutical formulation. No interferences with the excipients were reported.

Keywords: Valsartan, Hydrochlorothiazide, HPTLC, Tablet dosage Form, ICH guidelines

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INTRODUCTION

Hypertension is a cause of the increased risk of cardiovascular disease. Hence antihypertensive drugs and their combination is essential to treat hypertension also to reduce side effects and enhance effectives. Angiotensin-converting enzyme inhibitors (ACE-II antagonists) are combined with thiazide diuretics to reduce the side effects and achieve better control on hypertension by combined therapy.

Valsartan belongs to ACE-II receptor antagonist used alone or in combination with other agents to treat hypertension its molecular formula is C24H29N5O3, it is soluble in ethanol, methanol, alkalies and slightly soluble in water. it is marketed alone or in combination with other drugs to treat elevated blood pressure.

Hydrochlorothiazide belongs to the class of thiazide diuretic and acts by it deceasing the reabsorption of electrolytes from the renal tubules. Therefore, rate of excretion of water and electrolytes (sodium), is increased. Its molecular formula is $C_7H_8ClN_3O_4S_2$. It is Soluble in methanol, and acetone, alkalies insoluble in cold water [1, 2].

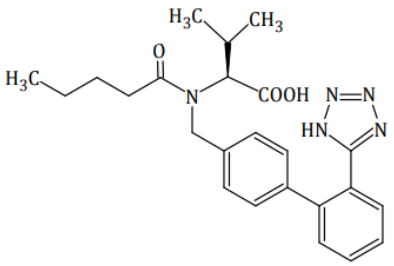

Fig. 1: Structure of Valsartan

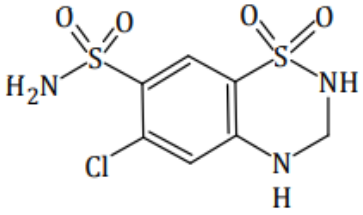

Fig. 2: Structure of hydrochlorothiazide

Extensive literature survey revealed that few spectroscopic and chromatographic methods were reported but with the combination of other drugs. Various discrepancies were observed in the reported methods Hence a need was felt to develop a novel HPTLC method for estimation of Valsartan and Hydrochlorothiazide in bulk and Tablet dosage form, which can be routinely opted for quality control analysis [3-11].

MATERIALS AND METHODS

Pharmaceutical grade Valsartan (VAL) and Hydrochlorothiazide (HTZ) were kindly supplied by Lupin Pharmaceuticals, Jammu and Kashmir, India. Both drugs were used without further purification. Toluene, Ethyl acetate, formic acid, methanol were purchased from Thomas Baker Chemicals (Mumbai, India). Pharmaceutical finished dosage form used in the present work was Valent-H® (Lupin Pharmaceuticals India. Ltd.), tablets containing 12.5 mg of HTZ and 80 mg of VAL.

Instrumentation

The chromatographic conditions and Instrumentation are formulated in table 1.

Table 1: Instrumentation and chromatographic conditions

Parameter	Specification
Stationary phase	Aluminum backed silica gel F254 TLC plates (10X10)cm
Slit dimensions	5 mm × 0.45 mm
Chamber Saturation and development time.	15 min
Migration distance	80 mm
Mode of application	Band
Band width	6 mm
Scanning wavelength	225 nm
Spraying rate	15s µl
Source of radiation	D2 lamp
Activation temperature	110°C for 20 min
Software version	WinCATS software version 1.4.2

Solubility studies

VAL and HTZ were found to be completely soluble in methanol; hence stock solution was prepared in methanol and dilutions were prepared in methanol for HPTLC studies.

Selection of analytical wavelength

From the overlaid spectra, analytical wavelength selected was 225 nm since considerable absorbance was seen at this wavelength (fig. 3).

HPTLC densitometry method

For quantitation of VAL and HTZ in Pharmaceutical formulation and Active Pharmaceutical Ingredient (API), Various solvent systems like ethyl acetate, chloroform, acetone, Toluene, methanol, formic acid was tried in different proportions for better separation and resolution of spots of VAL and HTZ from other excipients of formulations. The most satisfactory separation was obtained by using a mixture of Toluene: Ethyl acetate: formic acid (3:7:0.3, v/v/v) for VAL and HTZ with proper resolution. The R_f values found out to be 0.745±0.02 for VAL and 0.45±0.021 for HTZ.

Preparation of standard stock solutions

Standard stock solution (SSS) was prepared by solubilizing 10 mg of VAL and HTZ in 10 ml of methanol separately to get the concentration of 1 mg/ml. 3 ml of SSS was further diluted to 10 ml to get a stock solution of 300 ng/µl of VAL and 0.5 ml of SSS was further diluted to 10 ml to get a stock solution of 50 ng/µl of HTZ separately.

RESULTS AND DISCUSSION

Validation of the analytical methods

Linearity

For linearity studies, a mixture of VAL (50 ng/µl) and HTZ (300 ng/µl) were made. Different volumes (1-6 µl) for both the drugs were applied on the plate to furnish a concentration of 300-1800 ng/band for VAL and 50-300 ng/band HTZ, respectively. Sample determinations were done in 5 replicates and the regression equation was calculated. The results of validation parameters are formulated in table 2.

Precision

For Precision studies, samples were treated for repeatability and intermediate precision studies with the sample. Repeatability studies (intra-day) were performed with tablet samples by using analysis of 300 ng/band of VAL and 50 ng/band of HTZ, respectively. Intermediate precision (inter-day) of the method was checked by repeating analysis of 300 ng/band of VAL and 50 ng/band of HTZ, respectively on the three different days over a period of two weeks. Percentage RSD was computed. The results of validation parameters are formulated in table 2.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) for VAL and HTZ were computed using the formula as per ICH guidelines. The results of validation parameters are formulated in table 2.

Robustness

To assess the robustness study, small, deliberate variations were done on the optimized method. Factors varied were saturation time, change in ratio of mobile phase composition g change in time of development to scanning. An Each factor selected was changed at three levels (-1, 0,+1) Concentration level applied was 640 ng/band for VAL and 100 ng/band for HTZ in tablet solution. The results of validation parameters are formulated in table 2.

Recovery studies (Accuracy)

Recovery studies were carried out by the standard addition method. API was spiked with blend prepared for assay at three different levels 50, 100 and 150% of label claim. Sample concentration was analyzed at 640 ng/band of VAL and 100 ng/band of HTZ. The areas were noted after the development of plate. The drug concentrations of VAL and HTZ were calculated by using regression equations,3 concentrations and 3 replicates of the sample were analyzed. The results of validation parameters are formulated in table 2.

Specificity

Specificity studies were carried by analyzing API and sample solutions of tablet formulation. The R_f values of VAL and HTZ were confirmed by comparing with that of the R_f values of the standard drug to indicate that there is no interference of the excipients in the tablet formulation. The results of validation parameters are formulated in table 2 [12-14].

Analysis of marketed formulation

Tablet powder (of 20 tablets) equivalent to 10 mg of HTZ (64 mg of VAL) was weighed and transferred to a 100 ml volumetric flask separately containing approximately 100 ml of methanol, and applied to obtain final concentration of 100 ng/band forHTZ and 640 ng/band of VAL. The results of analysis of the marketed are formulated in the table 3.

Table 2: Validation parameters for VAL and HTZ

S. No.	Validation parameter	HTZ	VAL
1.	Linearity ng/spot	50-300ng/spot (r^2=.9991)	300-1800 ng/spot (r^2= 0.999)
2.	Precision (Intraday) %RSD	0.666	0.646
3.	Precision (Interday) %RSD	0.588	0.527
4.	LOD	4.127 ng/spot	23.02 ng/spot
5.	LOQ	12.50 ng/spot	69.76 ng/spot
6.	Accuracy	99.83-100.5	99.54-100.2
7.	Robustness %RSD	0.33-0.96	0.36-0.99

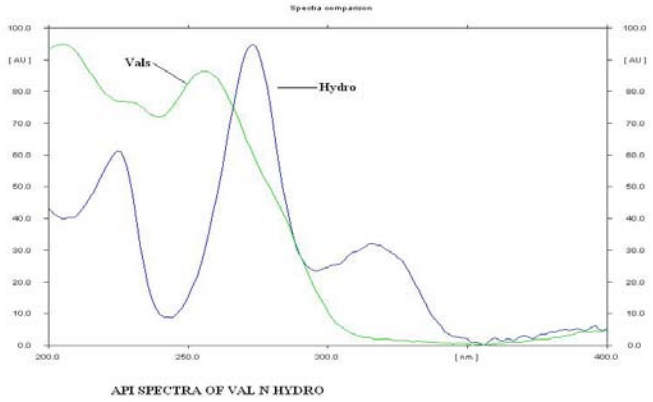

Fig. 3: Overlay of valsartan and hydrochlorothiazide

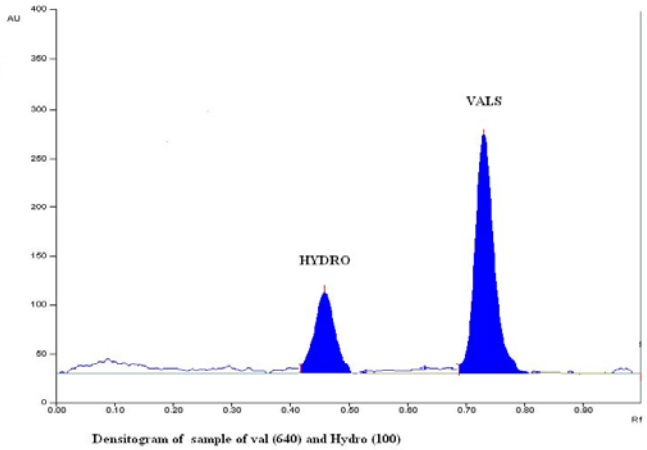

Fig. 4: Representative Densitogram of VAL (Rf= 0.74±0.02 and for HTZ (Rf=0.45±0.02).

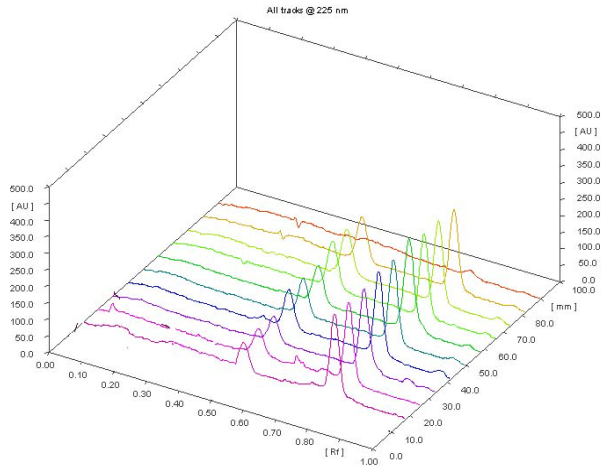

Fig. 5: 3D Linearity spectra of HTZ and VAL

Table 3: Results from analysis of marketed tablet formulation

Content	Label claim (mg)	Amount of drug estimated	% Label claim	SD	%RSD
VAL	80	79.84	99.8	21.62	1.05
HTZ	12.5	12.44	99.52	12.99	0.69

The primary objective of the reported work was to develop a simple, precise accurate and validated HPTLC method in bulk and tablet dosage form. Several reported methods had discrepancies viz include, N J Shah, *et al.* reported HPTLC method had reported Coefficient of variation, (r^2) value was not 0.999. Rasha A. Shaalan *et al.*, Manish Sharma, *et al.,* Jui J. Pandya, *et al.* reported HPTLC method

for various Anti-hypertensive drugs with poor resolution and peaks which were not properly differentiated, with R_f value difference between two or more drugs<0.2. Kadam, B. R. *et al.* didn't report chromatogram developmental time in the paper Hence a need was felt to develop a validated HPTLC method with well resolved peaks. Optimization was carried out by selecting a solvent system which would give dense and compact spots with well resolved and differentiated R_f values. Various solvent systems like ethyl acetate, chloroform, acetone, Toluene, methanol, formic acid was tried in different proportions to separate and resolve spots of VAL and HTZ from other excipients of formulations, after several trials combination of Toluene: Ethyl acetate: formic acid could resolve the peaks in satisfactorily. The optimized HPTLC method consists of the mobile phase Toluene: Ethyl acetate: formic acid in the ratio of (3:7:0.3, v/v/v). The solvent front was run up to distance of 80 cm; development time was 15 min approximately for TLC plate. Analytical wavelength selected for detection was 225 nm. R_f was found to be 0.74±0.02 and 0.45±0.02 for VAL and HTZ respectively. Linearity was found at concentration range 300-1800 ng/spot (r^2 = 0.999), 50-300ng/spot (r^2 = 0.9991) for VAL and HTZ respectively. LOD were found to be 23.02ng/spot, 4.12 ng/spot, for Valsartan and Hydrochlorothiazide respectively were found to be 69.76ng/spot,12.5ng/spot and respectively for, Valsartan and Hydrochlorothiazide.

CONCLUSION

The developed method was validated for analysis of Valsartan and Hydrochlorothiazide in Bulk and combination (Tablet dosage form) hence it can be concluded that the developed HPTLC method can be routinely applied without any interference from the excipients for bulk and pharmaceutical dosage form.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest was declared by the authors.

REFERENCES

1. PubChem. Bethesda: National Library of Medicine. PubChem Compound Summary for CID 60846, Valsartan. National Center for Biotechnology Information; 2014. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/valsartan. [Last accessed on 14 Mar 2022]
2. United State Pharmacopoeia, The U.S. Pharmacopeia convention, united state pharmacopoeia, Rockville, Md, USA. 30th ed; 2007.
3. Shah NJ, Suhagia BN, Shah RR, Patel NM. HPTLC method for the simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci. 2009 Jan;71(1):72-4. doi: 10.4103/0250-474X.51967, PMID 20177464, PMCID PMC2810056.
4. Shaalan RA, Belal TS, El Yazbi FA, Elonsy SM. Validated HPTLC methods for determination of some selected antihypertensive mixtures in their combined dosage forms. Bull Fac Pharm Cairo Univ. 2014 Dec;52(2):225-37. doi: 10.1016/j.bfopcu.2014.07.001.
5. Pandya JJ, Sanyal M, Shrivastav PS. Simultaneous densitometric analysis of amlodipine, hydrochlorothiazide, lisinopril, and valsartan by HPTLC in pharmaceutical formulations and human plasma. Journal of Liquid Chromatography and Related Technologies. 2017;40(9):467-78. doi: 10.1080/10826076.2017.1324482.
6. Jadhav ML, Girase MV, Tidme SK, Junagade MS. Development and validation of spectrophotometric methods for simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form. Int J Spectrosc. 2014. doi: 10.1155/2014/873819.
7. Sharma M, Kothari C, Sherikar O, Mehta P. Concurrent estimation of amlodipine besylate, hydrochlorothiazide and valsartan by RP-HPLC, HPTLC and UV–spectrophotometry. J Chromatogr Sci. 2014 Jan;52(1):27-35. doi: 10.1093/chromsci/bms200, PMID 23293040.
8. Kadam BR, Bari SB. Quantitative analysis of valsartan and hydrochlorothiazide in tablets by high performance thin-layer chromatography with ultraviolet absorption densitometry. Acta Chromatogrphica. 2007;18:260-9.
9. Deshpande MM, Mahajan MP, Sawant SD. Simultaneous estimation of valsartan and hydrochlorothiazide in fixed dose combination in UV spectrophotometry. Int J Pharm Sci Res. 2012;3(1):236-40.
10. Tian DF, Tian XL, Tian T, Wang ZY, Mo FK. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by RP-HPLC. Indian J Pharm Sci. 2008 May-Jun;70(3):372-4. doi: 10.4103/0250-474X.43006, PMID 20046750.
11. Singh S, Yadav AK, Gautam H. Simultaneous estimation of valsartan and hydrochlorothiazide in solid dosage form using UV spectroscopy. Bull Pharm Res. 2011;1(3):10-2.
12. Sethi PD. Quantitative analysis of pharmaceutical formulations. New Delhi: CBS Publications; 1996. p. 76-80.
13. ICH. (R1), Harmonized Tripartite Guideline, Validation of analytical Procedure Methodology, IFPMA. Proceedings of the international conference on harmonization, Geneva. Vol. Q2; Nov 2005.
14. ICH guidance on analytical method validation. In: Proceeding of the international convention on quality for the pharmaceutical industry. Toronto, Canada: September; 2002.