Asian Journal of Chemistry; Vol. 25, No. 8 (2013), 4267-4270

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13929

Quantitative Nuclear Magnetic Resonance Spectroscopic Analysis of Valsartan in Bulk and Solid Oral Dosage Form

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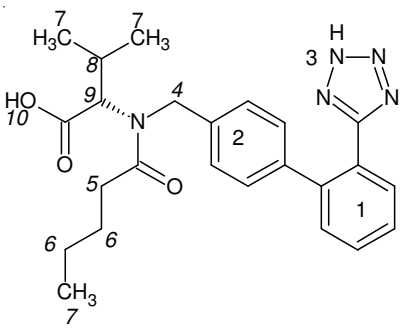
(*Received*: 18 April 2012; *Accepted*: 9 February 2013) AJC-12941

NMR spectroscopy has been applied to the quantification of valsartan in bulk and pharmaceutical dosage form. The estimation was carried out by proton nuclear magnetic resonance spectroscopy using tetra chloro nitro benzene as an internal standard and deuterated chloroform as the NMR solvent. NMR patterns were recorded for both valsartan and tetra chloro nitro benzene in deuterated chloroform. The signals corresponding to the chemical shifts of 1.73 and 4.95 ppm for valsartan and 7.75 ppm for tetra chloro nitro benzene were considered for quantification process.

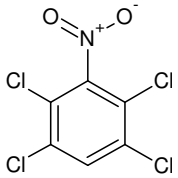
Key Words: Valsartan, Proton nuclear magnetic spectroscopy, Chemical shift.

INTRODUCTION

NMR spectroscopy is used for identification, structure elucidation, detection of impurities from synthetic pathway, to conduct degradation studies and to investigate metabolites of drugs in body fluids[1], *etc*. Currently, due to availability of highly sensitive and sophisticated NMR instrument, application of quantitative NMR (qNMR) has increased[2]. The qNMR is based on the direct proportionality of the analyte response and molar concentration of the analyte which is a major advantage of NMR over other quantitative spectroscopy[3]. The determination is based on the integral ratio between a specific proton of the analyte and the selected proton in NMR patterns of the internal standard. Valsartan[4] (VSN) is an angiotensin II receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease[5]. Valsartan is chemically described as N-(1-oxopentyl)-N-[[2'-(1*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine. Quantification of valsartan by various instrumental methods like UV[6], RP-HPLC[7], HPLC[8] has been developed. Stability indicating degradation studies of valsartan by UPLC[9] RP-HPLC, has been reported. Extensive literature survey reveals that determination of valsartan by NMR spectroscopy has not yet been reported. So an effort has been made to determine valsartan by NMR spectroscopy. The aim of this work is to develop a simple, precise, specific, accurate NMR method to quantify valsartan in bulk and pharmaceutical formulation and also to validate the developed method.



Valsartan
m.f.: $C_{24}H_{29}N_5O_3$
m.w.: 435.52



Tetra chloro nitro benzene
(internal standard)
m.f. $C_6HNO_2Cl_4$
m.w.: 260.89

EXPERIMENTAL

All the chemicals of the highest purity analytical grade were used throughout the experiment. Authentic sample of valsartan was procured from Madras Pharmaceuticals Ltd. Tetra chloro nitro benzene (TCNB) and deuterated chloroform ($CDCl_3$)was obtained from Merck India Ltd. Tablet dosage form of valsartan was obtained from the local market.

All ^{1}H NMR spectra were acquired using JEOL AL 300 FT-NMR spectrometer operating at 300.13 MHz. 32 free induction decays (FIDs) were collected for each sample into 32,768 data points using a spectral width of 6,001.50 Hz; digital resolution of 32768/6001.5 = 5.459 points/Hz and an acquisition of time of 5.46 sec. The chemical shifts were referenced internally to tetra methyl silane (TMS, δ = 0.0), The spectrometer is equipped with 5 mm Z-gradient BB probe using 1H 90º pulse width of about 11 µs. Relaxation delay of 60 s was applied during the analysis. The ^{1}H NMR spectra were processed and handled using AL 300 software.

Calibration of the standard: An aliquot weighed quantity of the internal standard TCNB was dissolved in $CDCl_3$ to obtain a concentration of 30 mg/mL. The standard drug valsartan was weighed and dissolved in the above prepared internal standard stock solution to obtain a concentration of 150.05 mg/mL. The standard stock solution was further diluted to obtain 12, 13.5, 15, 16.5 and 18 mg/mL of valsartan. The NMR spectra were recorded for each solution and calibration chart was plotted between integral value along Y-axis and concentration along X-axis. Two integral values one at 1.73 ppm and the other at 4.9 ppm were considered.

Assay: Weighed 20 tablets of valsartan and ground to fine powder. Accurately weighed tablet powder equivalent to 15 mg of valsartan was transferred to a 1 mL standard flask. Weighed accurately 15 mg of TCNB and transferred to the same standard flask. Added 1 mL of $CDCl_3$ and warmed gently for 5 min, the mixture was sonicated for 2 min and filtered through Whatmann filter paper No. 41. About 0.6 mL of the solution was transferred into a 5 mm NMR tube. The NMR patterns were then recorded under the given experimental condition and the signals corresponding to the chemical shift of 1.73, 4.95, 7.74 ppm were integrated and further used for quantification. Six replicate analysis were performed to assess the precision. The concentration of the analyte in the sample can be calculated using the given formula[10].

$$Ss = \frac{Is \times Wr \times Ms \times Nr \times Sr}{Ir \times Ws \times Mr \times Ns \times Ss}$$

where, Is and Ir are the integral value of the sample (valsartan) and internal stanadard, Ws and Wr are the weight taken, Ms and Mr are the molecular mass and Ns and Nr are the number of protons under consideration for sample (valsartan) and the internal standard respectively.

Accuracy and precision: The precision of the developed method was assessed by performing the assay of the formulation for about six times. Accuracy of the method was ascertained by the standard addition technique (recovery). Known amount of the standard valsartan was added to the previously analyzed sample. This was performed on three spiked levels and the NMR patterns were recorded. Six replicate analysis of above said procedure was performed.

RESULTS AND DISCUSSION

Assignment of ^{1}H NMR signals of valsartan: The ^{1}H NMR spectra showed the multiplets from 0.938 to 1.001 δ is attributed to the aliphatic ($-CH_3$) protons. The signals at 1.7 δ can be attributed to the H_4 ($-CH_2$) protons. The signal at 1.402 can be attributed to H_5 ($-CH_2$) protons. The singlets at 8.0 δ could assigned to the H_3 (-NH) proton of the tetrazole ring as they resonate downfield. The signals at 7.452 δ and 7.519 δ can be assigned to the aromatic protons H_1 and H_2. The signal at 2.5 δ could be assigned to the H_6 ($-CH_2$) protons. The signals at 4.2 δ and 4.9 δ are due to the resonance of the (-CH) protons. The -OH proton of the carboxylic acid group is off the chart to the left which is characteristic of carboxyl hydrogen[11]. The ^{1}H NMR spectra of sample valsartan (Figs. 1 and 2) was found to match with that of the standard valsartan. The signals and the integral value pertaining to the chemical shift of various protons of the standard and sample were similar confirming the identity and purity of the sample. The NMR spectrum of the blank *i.e.*, TCNB in $CDCl_3$ is represented by a sharp single signal at 7.74 δ due to the proton of TCNB at its 4th position.

Characteristics of the internal standard: 2,3,5,6-Tetra chloro nitro benzene was selected as the internal standard as it is chemically inert and is less volatile. The NMR studies of TCNB showed that it had a good acceptable relaxation time T_1 and produces a sharp single signal due to single proton at the 4th position at a chemical shift of 7.74 ppm which does not overlap with the signals of the analyte valsartan. Tetra chloro nitro benzene was soluble in $CDCl_3$ same as that of the analyte valsartan. Moreover TCNB is available in pure form and is a crystalline solid at room temperature and can be dried to remove water of hydration.

Calibration: The signals of the NMR pattern obtained for the drug were found to be symmetrical, well separated and were automatically integrated. Limit of detection[12] for valsartan was determined by the sensitivity of the NMR spectrometer. Limit of quantification was determined by the saturation limits of the drug into 1 mL of $CDCl_3$. A straight line linear graph was obtained on plotting the integral value (normalized signal area; $A_{drug}/A_{int\ std}$) along Y-axis and concentration of the drug in mgms along X-axis, using signals at 1.73 or 4.49 ppm of valsartan and 7.74 of TCNB. The linearity of the chart was assessed using the regression analysis (Fig. 3). The regression equation was determined to be Y = 0.06864X - 0.03800 with the correlation coefficient of 0.99911 for the signal at 1.73 ppm and Y = 0.03132X + 0.02400 with the correlation coefficient of 0.99932 for the signal at 4.92 ppm.

Formulation: The developed NMR methods for the standard drug valsartan was applied to the pharmaceutical formulation. The major advantage of NMR spectrometry is that it allows the identification and quantification of the analyte simultaneously. It allows the identification of the presence of impurities along with their estimation provided the impurities are detectable by NMR spectrometer. The result of the analysis of the formulation was appreciable and determined to be 98.02 %. The specificity of the method was assessed by the determination of the sample using two specific signals at two different chemical shifts (Fig. 4). The method precision was assessed by performing six replicate analysis. The amount

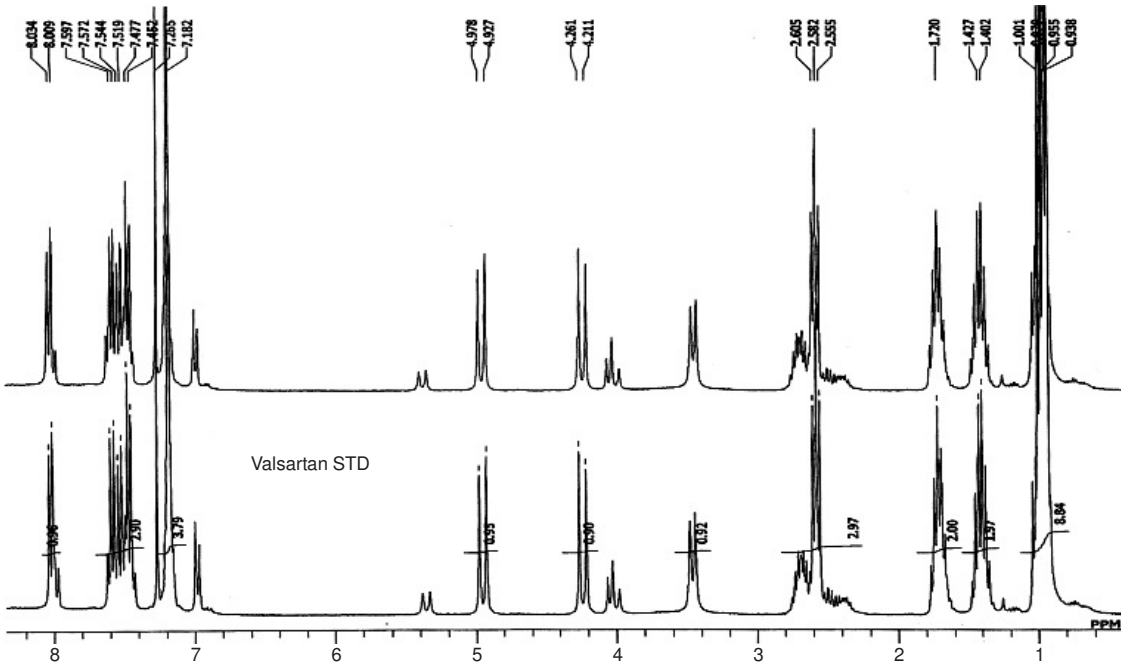


Fig. 1. Comparative NMR spectra of standard and sample valsartan

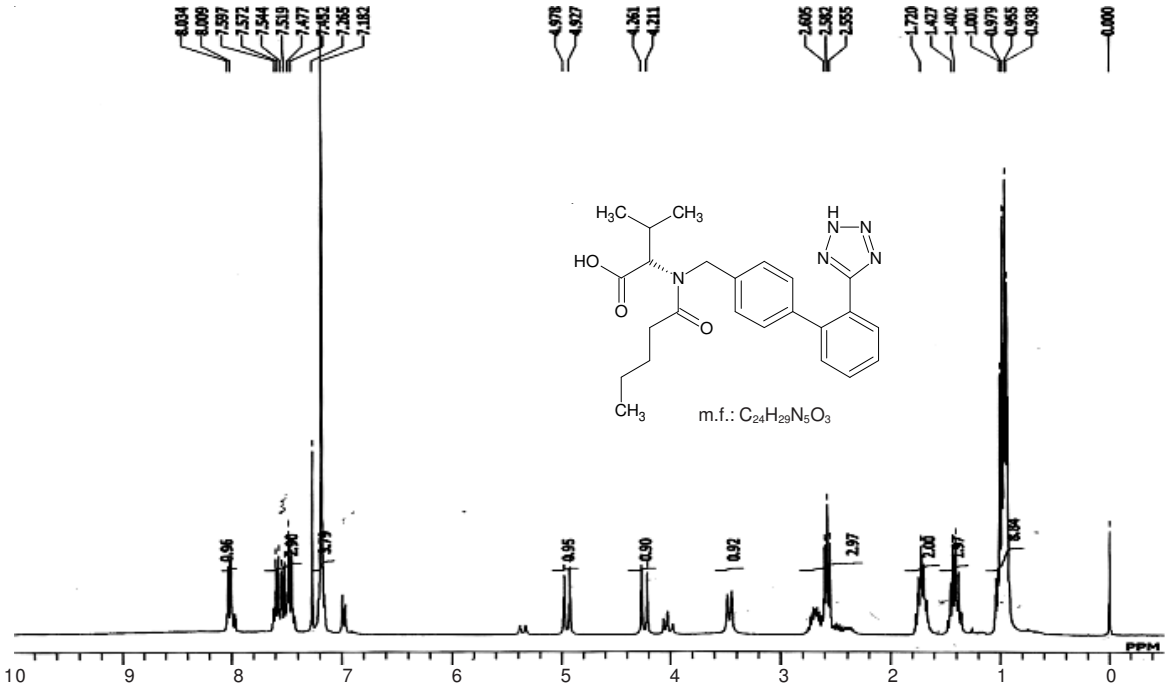


Fig. 2. NMR spectra of standard valsartan

present was found to be 39.19 mg ± 0.256 with the RSD of about 0.652 against the label claim of 40 mg. The percentage purity was determined to be 97.970 ± 0.586 % (Table-1).

The accuracy of the developed method was determined by the usual standard addition technique. It was performed at three different spiked levels 80, 100 and 120 %. The recovery was found to be 100 ± 1 % and RSD of 1. The results of the recovery are tabulated in Table-2. This shows that the developed method unaffected by the different matrix of the formulation.

Conclusion

A novel and modern NMR method has been developed for the estimation of the drug valsartan in bulk and pharmaceutical formulation using tetra chloro nitrobenzene as the internal standard. The developed method was found to be specific, precise and accurate. The LOD was found to be 1.2 mg/mL of $CDCl_3$. The proposed method could be applied for the analysis of valsartan.

TABLE-1
ASSAY AND PRECISION

Drug	Label claim	Amount present[a] ± SD	RSD	% Purity* ± SD	RSD
Valsartan	40 mg	39.19 ± 0.256	0.652	97.97±0.586	0.598

*Values are mean of six replicate analysis

TABLE-2
RECOVERY STUDIES

Amount of drug added (mg/mL)		Recovery using integral value at 1.73 ppm			Recovery using integral value at 4.9 ppm		
Tablet powder	Standard valsartan	Amount recovered* (mg/mL)	% Recovery ± SD	RSD	Amount recovered* (mg/mL)	% Recovery ± SD	RSD
15	12.00	12.05	100.35±1.44	1.44	12.05	100.38±1.55	1.55
	15.01	15.01	100.08±0.74	0.74	15.07	100.43±0.75	0.75
	18.01	18.04	100.23±1.36	1.36	18.05	100.28±1.83	1.83

*Values are mean of six determinations

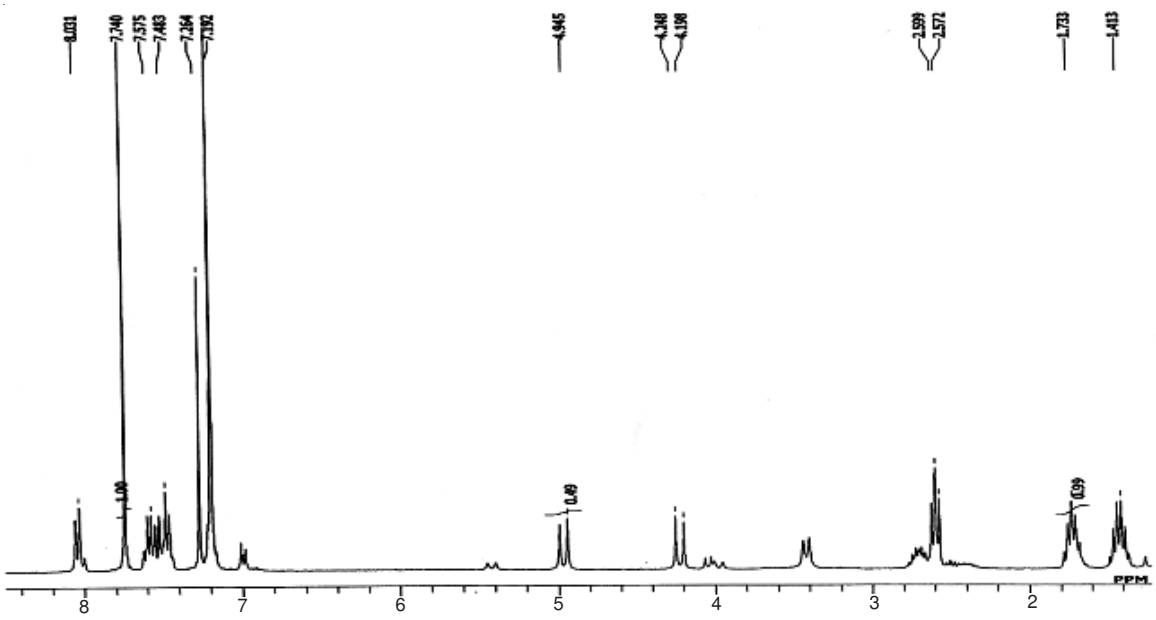


Fig. 4. NMR spectra of sample valsartan with internal standard (TCNB)

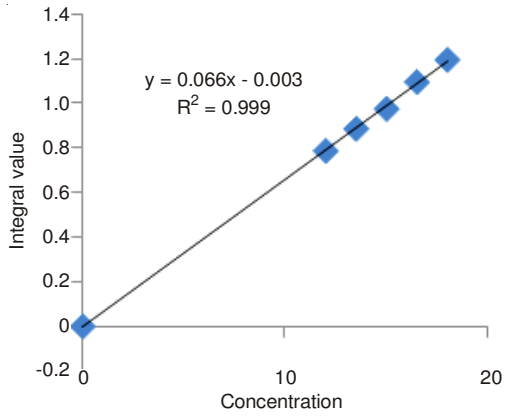


Fig. 3. Calibration chart for valsartan at chemical shift 1.73 ppm

ACKNOWLEDGEMENTS

The authors are thankful to M/s Piramal Health Care, Ennore, Chennai and Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, for rendering full support to carry out the study.

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