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Research Article

Simple and Sensitive Methods for the Determination of 2-(4′-Chloromethyl phenyl) Benzonitrile and 2-(4′-Bromomethyl phenyl) Benzonitrile Contents in Valsartan Drug Substance by Gas Chromatography

G. Amarnatha Reddy,[1] K. Hussain Reddy,[2] M. Narendra Kumar,[1] and Hemant kumar Sharma[1]

[1] *Aurobindo Pharma Limited Research Centre-II, Survey No. 71&72, Indrakaran Village, Sangareddy Mandal, Medak, Andhra Pradesh 502 329, India*
[2] *Department of Chemistry, Sri Krishnadevaraya University, Anantapur 515 003, India*

Correspondence should be addressed to K. Hussain Reddy; khussainreddy@yahoo.co.in

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Simple and reliable gas chromatographic methods were developed, optimized, and validated for the determination of 2-(4′-chloromethyl phenyl) benzonitrile (2-CMPB) and 2-(4′-bromomethyl phenyl) benzonitrile (2-BMPB) contents in valsartan drug substance, using benzophenone as internal standard (IS). Efficient chromatographic separations were achieved on DB-1, 30 m length with 0.53 mm i.d., and 3 μm particle diameter column consists of 100% dimethyl polysiloxane as a stationary phase by passing helium as a carrier gas. The analytes were extracted in dichloromethane and monitored by flame ionization detector. The performance of these methods was assessed by evaluating specificity, precision, sensitivity, linearity, and accuracy. The limits of detection (LOD) and limits of quantification (LOQ) established for 2-CMPB are 0.10 μg mL^{-1} and 0.32 μg mL^{-1}, respectively. For 2-BMPB, LOD is 0.31 μg mL^{-1} and LOQ is 0.95 μg mL^{-1}. The average recoveries for 2-CMPB are in the range of 96.8% to 106.7% and for 2-BMPB (LOQ level) are 99.3%. The methods can be successfully applied for the routine analysis of valsartan drug substance.

1. Introduction

Valsartan belongs to a class of medicines known as angiotensin II receptor antagonist, which helps to control high blood pressure and congestive heart failure or postmyocardial infarction [1–4]. Chemically, valsartan is N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-L-valine, having an empirical formula $C_{24}H_{29}N_5O_3$ with a molecular weight of 435.5. Valsartan is marketed under the trade name of Diovan [5] and is available as 40 mg, 80 mg, 160 mg, and 320 mg tablets for oral administration. In the synthesis process of valsartan drug substance, 2-(4′-chloromethyl phenyl) benzonitrile (2-CMPB) and 2-(4′-bromomethyl phenyl) benzonitrile (2-BMPB) were used as key raw materials. These residual organic raw materials may come through the manufacturing process. Based on structural alert, these raw materials come under genotoxic category. The maximum daily dosage of valsartan drug substance is 320 mg. As per threshold of toxicological concern (TTC) approach, these residual impurities should be less than 4.7 μg g^{-1} [6–8]. Moreover, there is no specific information that is available in the literature on toxicity of these residual impurities confirming their carcinogenicity or mutagenicity. In the available literature many analytical procedures have been reported for the estimation of valsartan and its related substances [9–14]. A good number of analytical methods also reported for the determination of valsartan in combination of other drugs such as

hydrochlorothiazide and amlodipine besylate in tablets [15–17]. To the best of our knowledge, no report has been published on the analysis of 2-CMPB and 2-BMPB contents in valsartan drug substance. Hence, we trialed and successfully developed simple and reliable gas chromatographic (GC) methods with flame ionization detector for the determination of 2-CMPB and 2-BMPB contents in valsartan drug substance with sufficiently low levels of detection.

2. Experimental

2.1. Chemicals, Reagents, Samples, and Gases. Valsartan drug substances, 2-(4′-chloromethyl phenyl) benzonitrile (2-CMPB) and 2-(4′-bromomethyl phenyl) benzonitrile (2-BMPB), are used. Analytical reagent (AR) grade benzophenone, dichloromethane, sodium hydroxide pellets, anhydrous sodium sulfate, and HPLC grade water were used. High purity gases, helium, hydrogen, zero air, and nitrogen, were used.

2.2. Gas Chromatography. All experiments were performed on Shimadzu GC 2010 gas chromatograph equipped with flame ionization detector and AOC-5000 autosampler with data handling system GC solutions/Empower Pro software. Agilent 7890A gas chromatograph aligned with flame ionization detector and CombiPal multipurpose autosampler with EZChrom software. Helium gas was used as a carrier gas. The analysis was carried on DB-1, 30 m long with 0.53 mm i.d., and 3.0 μm particle diameter column consists of 100% dimethyl polysiloxane as stationary phase (Agilent J&W GC columns).

2.2.1. The Chromatographic Conditions for the Determination of 2-CMPB Method as Follows. The initial oven temperature of 150°C is maintained for 4 min and then increased to 230°C at a rate of 10°C/min, followed by holding at 230°C for 18 min. The injection volume is 2 μL with a split ratio set at 1 : 1. Helium is used as the carrier gas with a constant pressure of 75 kpa. The injector and detector temperatures are set at 250°C and 260°C, respectively. The acquisition time was 30 min. The retention times of the benzophenone and 2-CMPB are about 10.0 and 16.0 minutes, respectively. Relative standard deviation for the ratio of peak area of 2-CMPB to the peak area of internal standard (benzophenone) for six injections of the standard solution is not more than 10.0%.

2.2.2. The Chromatographic Conditions for the Determination of 2-BMPB Method as Follows. The initial oven temperature of 180°C is maintained for 0 min and then increased to 220°C at a rate of 10°C/min, followed by holding at 220°C for 26 min. The injection volume is 2 μL with a split ratio set at 2 : 1. Helium is used as the carrier gas with a constant pressure of 70 kpa. The injector and detector temperatures are set at 200°C and 260°C, respectively. The acquisition time was 30 min. The retention times of the benzophenone and 2-BMPB are about 5.0 and 12.0, minutes respectively. Relative standard deviation for the ratio of peak area of 2-BMPB to the peak area of internal standard (benzophenone) for six injections of the standard solution is not more than 10.0%.

2.3. Standard and Sample Solutions

2.3.1. Preparation of 1.5 N Sodium Hydroxide Solution. Dissolve 6.0 g of sodium hydroxide pellets into a 100 mL of volumetric flask, add about 50 mL of water, dissolve by shaking, and then make up to volume with water.

2.3.2. Preparation of Internal Standard Solution (IS) for Determination of Both 2-CMPB and 2-BMPB. Accurately weigh and transfer about 0.024 g of benzophenone into a 50 mL volumetric flask, add 25 mL of dichloromethane to dissolve and make up to volume with dichloromethane. Dilute 1.0 mL of this solution to 500 mL with dichloromethane.

2.3.3. Preparation of Blank Solution. Into a clean and dry separating funnel, add 4 mL of 1.5 N sodium hydroxide solution and 3 mL of internal standard solution, and shake vigorously for 1 min. Allow the two phases to separate, and collect the lower layer (dichloromethane) passed through anhydrous sodium sulfate.

2.3.4. Standard Solutions

(1) Preparation of Standard Stock Solution for 2-CMPB. Accurately weigh and transfer about 0.0245 g of 2-CMPB reference standard into a 25 mL clean, dry volumetric flask, add 15 mL of internal standard solution, and dissolve and make up to volume with internal standard solution. Dilute 2 mL of this solution to 25 mL with internal standard solution. Further, dilute 1 mL of this solution to 50 mL with internal standard solution.

(2) Preparation of Standard Stock Solution for 2-BMPB. Accurately weigh and transfer about 0.0196 g of 2-BMPB reference standard into a 50 mL clean, dry volumetric flask, add 25 mL of internal standard solution, and dissolve and make up to volume with internal standard solution. Dilute 1 mL of this solution to 10 mL with internal standard solution. Further, dilute 2 mL of this solution to 50 mL with internal standard solution.

(3) Preparation of Standard Solution for Both 2-CMPB and 2-BMPB. Into a clean and dry separating funnel, add 4 mL of 1.5 N sodium hydroxide solution and 3 mL of 2-CMPB/2-BMPB standard stock solution and shake vigorously for 1 min. Allow the two phases to separate, and collect the lower layer (dichloromethane) passed through anhydrous sodium sulfate.

2.3.5. Preparation of Sample Solution. Accurately weigh and transfer about 1.0 g of sample into a clean, dry separating funnel, add 4 mL of 1.5 N sodium hydroxide solution, and shake to dissolve the sample. Added 3 mL of internal standard solution and shaken vigorously for 1 min. Allow the two phases to separate, and collect the lower layer (dichloromethane) passed through anhydrous sodium sulfate.

3. Results and Discussion

3.1. Method Development and Optimization. The objective of this work is to determine low-level concentrations of 2-CMPB and 2-BMPB in valsartan drug substance by using gas chromatography (GC) system. In the synthesis process of valsartan drug, 2-(4′-chloromethyl phenyl) benzonitrile (2-CMPB) and 2-(4′-bromomethyl phenyl) benzonitrile (2-BMPB) were used as key raw materials. Because of high boiling point of 2-CMPB and 2-BMPB, it is not easy to develop by head space gas chromatograph. Method development was initiated with valsartan, 2-CMPB and 2-BMPB solubility, miscibility, and extraction studies. Trial experiments were conducted to optimize the suitable solvent for extraction. Dichloromethane was finalized with 1.5 N sodium hydroxide solution. Hence, direct liquid injection method was adopted to develop 2-CMPB and 2-BMPB contents with liquid-liquid extraction. From few experimental trials, longer retention times, broad peak shapes, and interference were observed. These problems were rectified and optimized by using DB-1, 30 m long with 0.53 mm i.d., and 3.0 μm particle diameter column consisted of 100% dimethyl polysiloxane as stationary phase, and carrier gas was helium.

Finally, satisfactory separation with better peak shapes was achieved, on chromatographic conditions which have been mentioned in gas chromatography (GC), and was used for validation study to evaluate its performance characteristics.

3.2. Method Validation. In order to determine the contents of 2-CMPB and 2-BMPB in valsartan drug substance, the methods were validated as per the ICH guidelines [18].

3.2.1. Specificity. To establish the specificity of both methods, valsartan drug, valsartan drug spiked with 2-CMPB/2-BMPB, and valsartan drug spiked with 2-CMPB/2-BMPB including all other residual solvents which are used in the synthesis process of valsartan drug substance and individual RT solutions were subjected to GC with FID to their respective methods. No interference was found at the retention of benzophenone and 2-CMPB/2-BMPB. The identity and specificity of 2-CMPB/2-BMPB and benzophenone were demonstrated with better separation of all other impurities.

3.2.2. Limit of Detection and Limit of Quantification (LOD and LOQ). The LOD and LOQ values for 2-CMPB and 2-BMPB were determined by *S*/*N* ratio. The minimum concentration at 3.3 : 1 *S*/*N* was considered as detection limit, and the concentration at 10 : 1 *S*/*N* was established as quantification limit. A solution containing 2-CMPB and 2-BMPB was prepared around its quantification limit and detection limit concentrations, and it injected six replicates in their respective methods. The relative standard deviation [% RSD ($n = 6$)] for LOQ precision of 2-CMPB and 2-BMPB is 3.2 and 5.2 and for LOD precision 5.2 and 6.8, respectively. Overlay chromatograms of LOQ solution with standard solution are shown in Figure 1. The achieved precised values were shown in Table 1.

Table 1: Statistical data of linearity, LOD/LOQ for 2-CMPB and 2-BMPB.

Statistical parameters	2-CMPB	2-BMPB
Correlation coefficient	0.9993	0.9995
Intercept	−0.009	0.0170
Residual standard on deviation response	0.033	0.0101
Slope	1.065	0.4967
Concentration range (μg mL^{-1})	0.32–2.40	0.95–2.38
Limit of detection (μg mL^{-1})[a]	0.1	0.31
Limit of quantification (μg mL^{-1})[a]	0.32	0.95
Precision for limit of detection (% RSD)	5.2	6.8
Precision for limit of quantification (% RSD)	3.2	5.2

[a]Precised LOD and LOQ values.

3.2.3. Linearity. The linearity was evaluated by measuring area ratio for 2-CMPB with respect to benzophenone and 2-BMPB with respect to benzophenone over the range of 0.32 μg mL^{-1} to 2.40 μg mL^{-1} and 0.95 μg mL^{-1} to 2.38 μg mL^{-1}, respectively. Six concentrations for 2-CMPB and five concentrations for 2-BMPB were prepared across the range and injected each in single injection. The statistical parameters slope, intercept, residual standard on deviation response, and correlation coefficient values were calculated and shown in Table 1.

3.2.4. Accuracy. Accuracy of the method was validated through recovery experiments by spiking known amount of 2-CMPB at LOQ level, 50%, 100%, and 150% levels (with respect to test concentration). Each preparation was analyzed in triplicate, and % recovery was calculated. The % recovery for 2-CMPB ranged from 96.8 to 106.7, and the average % recovery of four levels (twelve determinations) was 102.3%, whereas the average recovery for 2-BMPB at LOQ level (with respect to test concentration) was 99.3%. The validated accuracy results are shown in Table 2.

3.2.5. Precision. 2-CMPB standard solution was injected in six replicates (system precision). The % RSD ($n = 6$) value obtained for the ratio of areas of 2-BMPB with respect to benzophenone was 0.9. The method precision and ruggedness of the method were evaluated by performing the valsartan drug analysis in six replicates by using two different columns, different instruments, and different analysts on different days. This study was done to prove the compliance of different gas chromatographs.

For 2-BMPB, standard solution was injected in six replicates (system precision). The % RSD ($n = 6$) value obtained for the ratio of areas of 2-BMPB with respect to benzophenone was 1.2. The precision results are shown in Table 3.

3.2.6. Robustness. This study was performed by making small but deliberate variations in the method parameters. These parameters are varied by ±10% of carrier gas flow, ±2°C of

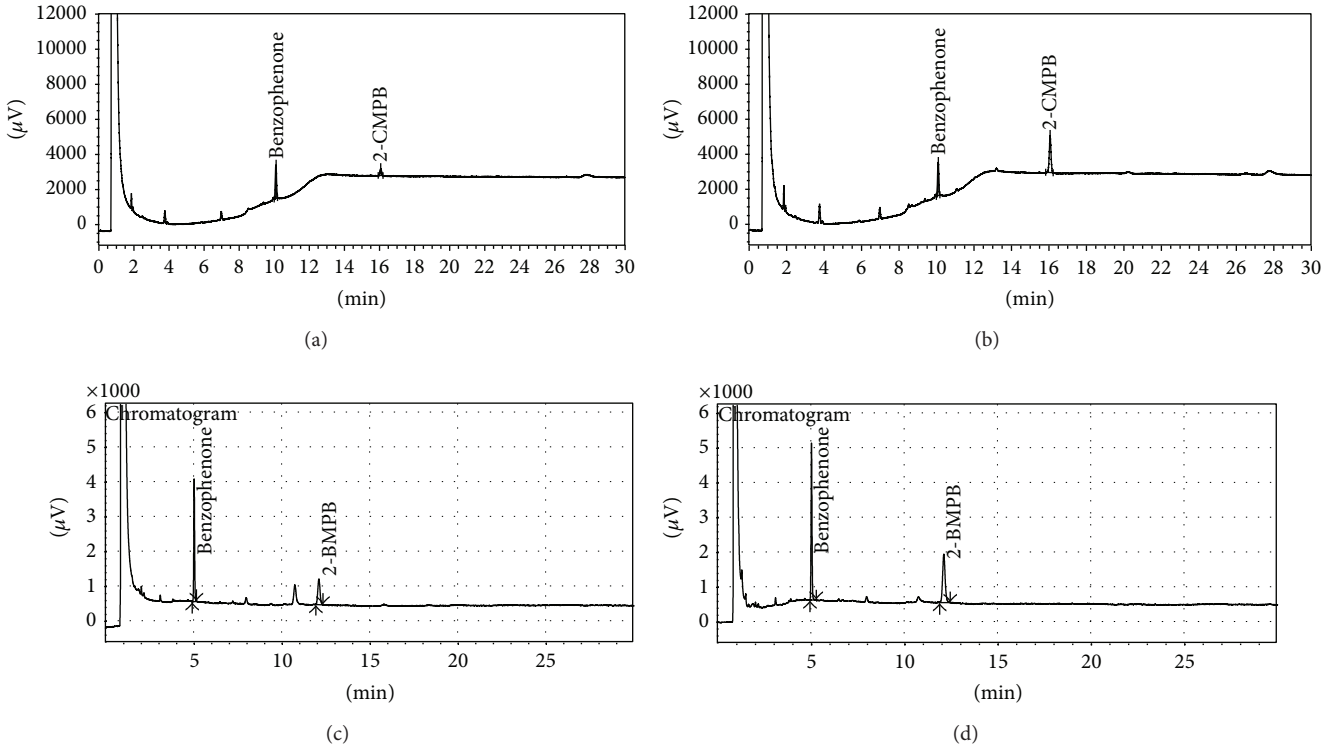

Figure 1: A typical representative overlaid GC chromatograms of (a) LOQ of 2-CMPB; (b) standard of 2-CMPB; (c) LOQ of 2-BMPB with (d) standard of 2-BMPB.

Table 2: Accuracy results of 2-CMPB and 2-BMPB.

S. no	2-CMPB				2-BMPB
	LOQ level	50% level	100% level	150% level	LOQ level
Added* (μg/g)	0.95	2.47	4.96	7.43	2.84
Found* (μg/g)	0.92	2.57	5.29	7.54	2.82
Recovery (%)	96.8	104.0	106.7	101.5	99.3
% RSD*	2.7	2.0	0.3	1.1	3.4
Average recovery**	102.3				—

*Average of 3 replicates.
**Average recovery of four levels.

Table 3: Precision results of 2-CMPB and 2-BMPB.

S. no.	2-CMPB		Ruggedness (μg/g)	2-BMPB	
	System precision (area ratio)	Method precision (μg/g)		System precision (area ratio)	Method precision (μg/g)
1	1.318	5.28	4.82	0.785	4.96
2	1.331	5.31	4.87	0.789	4.46
3	1.309	5.28	5.09	0.779	4.63
4	1.334	5.30	5.08	0.762	4.38
5	1.312	5.24	4.95	0.778	4.67
6	1.306	5.25	4.96	0.783	4.40
Avg.	1.318	5.28	4.96	0.779	4.58
SD	0.01	0.03	0.11	0.01	0.220
% RSD	0.9	0.5	2.2	1.2	4.8

initial column oven temperature and ±2°C/min of ramp column oven temperature for the determination of 2-CMPB. In each robustness condition, solutions of blank, standard, and valsartan drug spiked with 2-CMPB were prepared and injected into GC to confirm the retention times. There is no much variation in the relative retention time (RRT) of 2-CMPB obtained at different deliberately varied robustness conditions from the developed methodology. Hence, the proposed method is robust.

4. Conclusion

The static GC methods described in this investigation were proved to be an ideal tool for the determination of 2-CMPB and 2-BMPB in valsartan drug substance at lower levels. Method validation data demonstrated that the developed methods are simple, sensitive, cost-effective, and accurate for the estimation of 2-CMPB and 2-BMPB. The method for the determination of 2-CMPB is robust to minor variations in the chromatographic parameters. The identity and specificity of 2-CMPB and 2-BMPB were well established in this method by GC with FID with moderate run time at very low levels. Hence, the proposed GC methods can be employed conveniently in the pharmaceutical laboratory for the routine quality control of 2-CMPB and 2-BMPB in valsartan drug substance.

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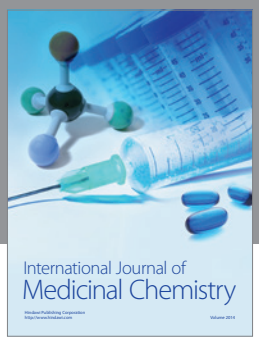

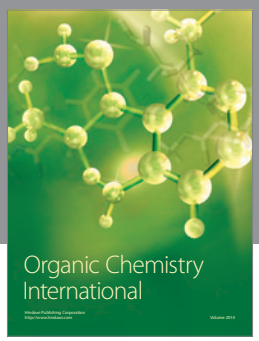

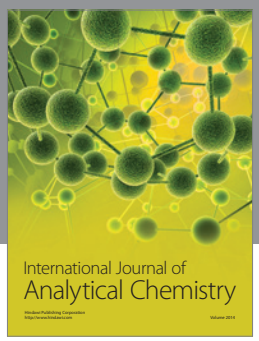

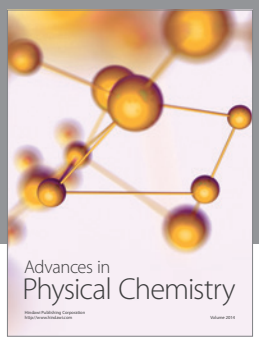

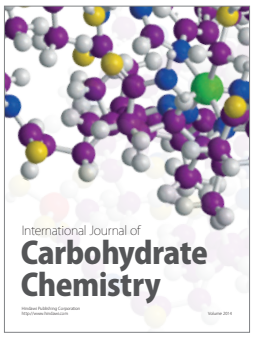

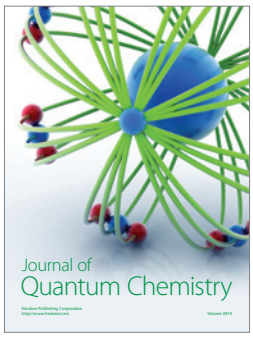

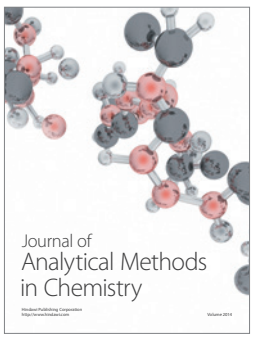

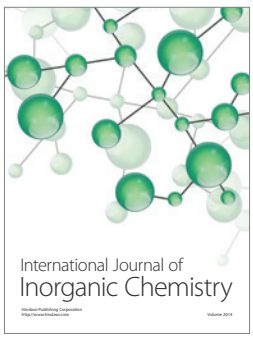

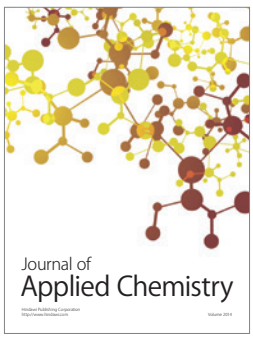

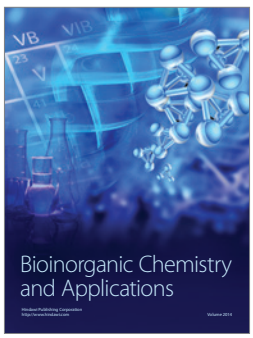

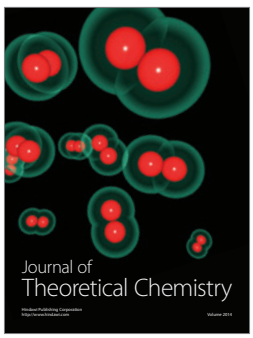

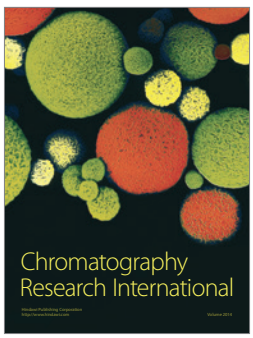

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