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Development and Validation of a Headspace Gas Chromatographic Method for determination of Residual Solvents in Bosentan Monohydrate.

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Abstract: A simple and sensitive method for the simultaneous determination of methanol, ethanol, acetone, acetonitrile and toluene in Bosentan monohydrate by headspace techniques with FID detection is described. Bosentan monohydrate is a dual endothelin receptor antagonist used in the treatment of pulmonary artery hypertension (PAH). In order to increase productivity of drug analysis in the pharmaceutical industry, an efficient and sensitive HSGC method was successfully developed and validated for the determination of 44 classes 2 and 3 above mentioned solvents of International Conference of Harmonization (ICH) guideline Q3C, as residual solvents in drug substance. Based on Good manufacturing practices, measuring residual solvents is mandatory for the release testing of all active pharmaceutical ingredients. The method was validated for repeatability, linearity, limit of detection, limit of quantification and recovery according to the International Conference on Harmonization guidelines. The method validation results indicate that the method is accurate, precise, linear and sensitive for solvents assessed. Excellent results were obtained, within the globally accepted validation reference values, particularly taking into account the low concentration levels investigated.
Key Words: Validation, Residual solvents, HS-GC and Bosentan monohydrate.

INTRODUCTION:

Residual solvents, or organic volatile impurities, are a potential toxic risk of pharmaceutical products and have been a concern of manufacturers for many years[1]. Moreover, residual solvents can also affect the quality and stability of not only drug substances but also drug products[2,3]. Thus, acceptable levels of many are included in regulatory guidance documents; in particular in guideline Q3C issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH)[4]. Residual

solvents are classified into four classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard[5]. Class 1 solvents are known carcinogens and are strongly suspected of being harmful to humans and the environment, so they should be avoided. Class 2 solvents are nongenotoxic animal carcinogens. Solvents of this class should be limited in pharmaceutical products because of their inherent toxicity. Class 3 solvents have low toxic potential to humans and should be used only where it would be impractical to remove them. Finally, Class 4 solvents are those for which no adequate toxicological data have been found. These last three classes of solvents are the ones most commonly analyzed. Residual solvents are typically determined using chromatographic techniques such as static headspace gas chromatography (HS-GC)[6]. Developing and validating an efficient and sensitive generic analytical method for the determination of residual solvents may significantly increase productivity of an analytical laboratory in the pharmaceutical industry. Determination of residual solvents using GC with a flame ionization detector (FID) is the most common technique in the pharmaceutical industry, because of its high separation efficiency and sensitivity for volatile organic compounds. Headspace gas chromatography (HS-GC) method has been used for the determination of residual solvents in pharmaceutical compounds[7,8]. Direct injection of analytes evaporated through equilibration between liquid (or solid) phase and gas phase to GC system minimized the contamination of GC system and the deterioration of GC column[9,10] . Volatile residual solvents are accumulated prior to analysis.

Here I report a full validation of a HS-GC analytical method for determination of five residual solvents (Class Bosentan monohydrate methnol, ethanol, acetone, acetonitrile and toluene) commonly used during the manufactories of drug substances and purification steps and its dosage forms.

MATERIALS AND METHODS

Instrumentation and Chromatographic conditions

The analysis was performed on Shimadzu Gas Chromatography; Japan equipped with model no Shimadzu-GC-2010 head-space AOC 5000 autosampler and a flame-ionization detector. The injector temperature was 100CC and detector temperature was 250^{o}C. Column was DB-624 with serial no-US7109941H (100% dimethylpolysiloxane 30.0 m × 0.53 mm ID, 3.0 µm d.f. Capillary). Split ratio of injection 5:1. Initially kept at 40°C for 6 min, raised to 130°C @ 10°C/min hold 8 min. Raised to 240°C @ 35°C/min hold 5 min. Total run time was 30 min. Helium was used as a carrier gas at a constant flow rate of 2.8 ml/min.

Material

Methanol, ethanol, acetone, acetonitril, toluene and dimethyl sulfoxide (DMSO) were obtained from Merck-Mumbai. Bosentan monohydrate API was obtained from Ranbaxy Laboratories Limited, gurgaon, India.

Preparation of standard solution:

Transfer 300mg of Methanol, 500mg of Ethanol, 500mg of Acetone, 41mg of Acetonitrile and 89mg of Toluene into 100ml volumetric flask containing 10ml of diluent and make upto the mark with diluent. Dilute 10mL of the above solution into a 100mL volumetric flask and make upto the mark with diluent.

Sample preparation:

Test Preparation: Weighed accurately 200 mg each of the test sample into two different HSS vials, and add 2 ml of DMSO solvent and seal the vials with aluminum closure.

Procedure:

Transfer the above prepared standard solutions each 2 ml into six different HSS vials and sealed with aluminum closure. Each of the vials contains 500 ppm of methanol, 500 ppm of ethanol, 500 ppm of acetone, 500 ppm of acetonitrile and 100 ppm of toluene with respect to the sample. The vials have a DMSO solution containing solvents at different concentrations, the vials are kept at 40^{o}C the headspace sampler was equipped with a 1-mL sample loop. Since a sufficient flow must be maintained through the system to avoid excessive peak broadening.

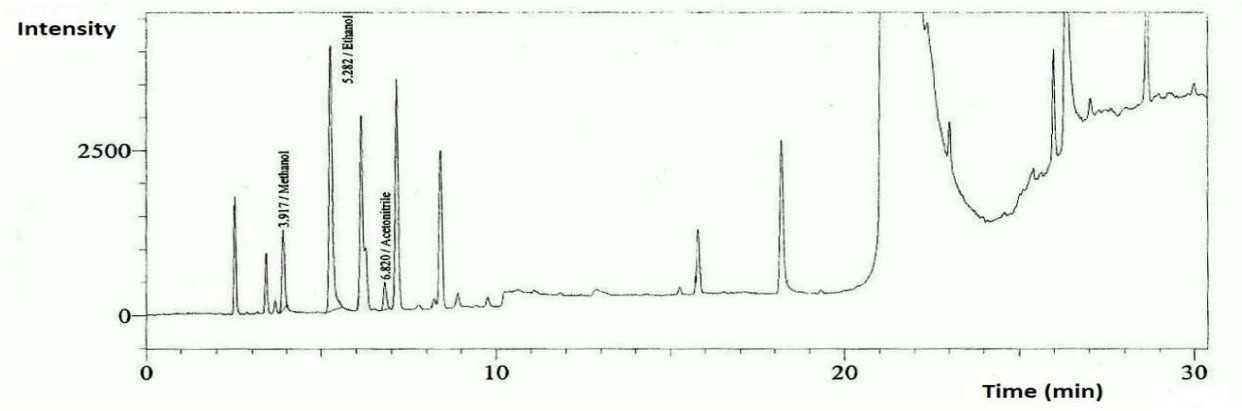

Figure 1: Chromatogram for Sample solution of Bosentan monohydrate (100mg/ml)

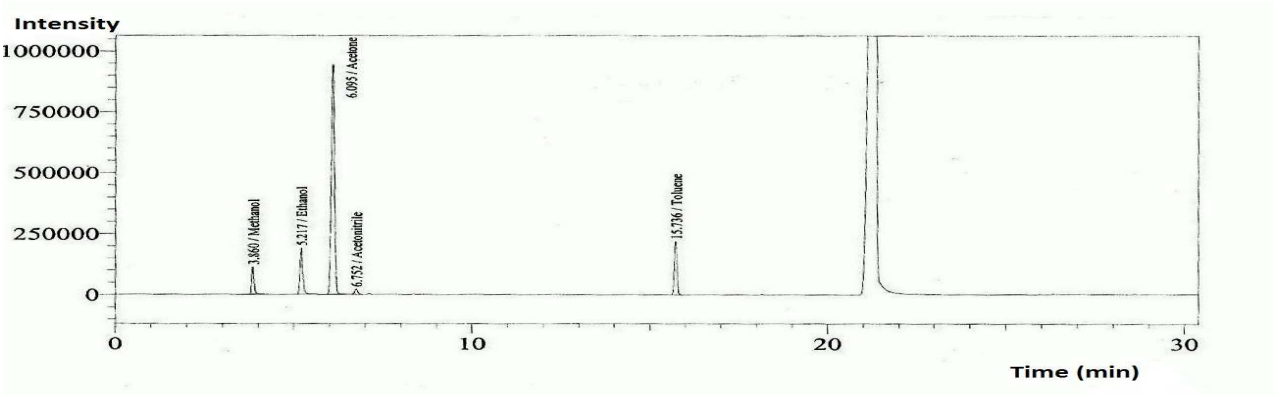

Figure 2: Chromatogram for System Suitability (Bosentan Monohydrate)

RESULTS AND DISCUSSION

In this study, a HS-GC analytical method was developed and validated for the quantitative determination of the solvents methanol, ethanol, 2-propanol, acetone, acetonitrile and toluene in Bosentan monohydrate. The proposed method uses the standard addition technique with internal standard quantitation for determination of seven solvents. The method was validated within ICH guidelines Q2A and Q2B. Selectivity, limits of detection and quantitation, linearity, range, precision (system repeatability), recovery and robustness (changes in HS and GC conditions and solution stability) were determined. Excellent results were obtained, within global validation reference values, particularly taking into account the low concentration levels investigated. The test method was validated and had good reproducibility and linearity for the solvents used in the manufacturing process. The recovery was good and justified the preparation of the standard in DMSO without the product as matrix.

The concentration of residual solvents (ppm) in the drug samples can be determined using the formula:

$$\frac{\text{Area of individual solvent in test solution}}{\text{Average area (six injections) of individual solvent in standard solution}} \times \frac{\text{Individual solvent wt. in standard solution (mg)}}{\text{Wt. of sample in (mg)}} \times \frac{10}{100} \times \frac{10}{100} \times 10^6$$

The linear range and correlation coefficients were determined between 10 ppm and 1500 ppm. The results for the 2-ml sample volume are documented in table-2.

Table 1. Linearity and accuracy of residual solvents

	Linearity			Accuracy			
Solvents	Range (%)	R^2	Slope	Recovery (%)	Average value (ppm)	Average	RSD (%)
Methanol	0.04-8.0	0.9985	32.02	97.66-101.34	3028.35	99.54	5.26
Ethanol	0.02-8.0	0.9988	46.25	98.60-100.76	5093.04	99.01	4.72
Acetone	0.05-8.0	0.999	178.79	93.09-96.65	4770.95	94.65	5.63
Acetonitrile	0.01-0.8	0.999	186.43	98.72-101.55	420.95	99.21	5.47
toluene	0.04-8.0	0.991	492.56	92.86-95.80	846.54	93.89	4.81

Table 2. Summary of validation parameters for the proposed method

Specificity	No interference was found W.R.T. excipients
Linearity (R)	0.999
Range	LOQ - 150 %
precision (RSD)	
a.system suitability	2.38-4.70
b. System precision	2.31-3.19
c. method precision	1.60-2.51
Accuracy(% recovery)	Within the limits for LOQ level to 150 %
LOD	3.17 - 4.08
LOQ	10.80 - 11.78
Ruggedness	% RSD for each solvents peak area are within the limits
Robstness	% RSD for each solvents peak area and content in ppm are within the limits
Solution stability	No interference was found W.R.T. excipients

Selectivity

The ZB-624 column, in the 30 m x 0.32 mm I.D. configuration, was chosen because this column has a standard stationary phase, which is recommended by the European and American Pharmacopeias, and has provided baseline separations of all solvents used in the validation, including the diluent (DMSO). The method showed good peak shape, and the narrow peak width resulted in excellent column efficiency. The blank chromatogram did not show any interference with the solvent peaks.

Linearity and range

To carry out this study, six concentrations were prepared of each solvent. All concentrations were prepared in triplicate, by individually weighing amounts of solvents. The experimental results were represented graphically to obtain a calibration curve and carry out the corresponding statistical study (Anova). The method is linear within a wide range for the solvents included in the validation. The correlation coefficients were all above 0.99 and linear regression showed a positive response throughout the range (Fig 3-7). The specified range is normally derived from linearity studies and depends on the intended application of the procedure [7]. The wide measurement range allows determination with adequate precision of different analyte contents in various matrices. The measurement ranges are shown in the Table 1 and 2 with the respective RSD values.

Repeatability

Repeatability was determined in accordance with ICH guidelines, i.e.: nine independent determinations were carried out during single day and on their basis the values of the standard deviations were established. The repeatability, representing the spread of the results, was expressed as RSD (Table 2).

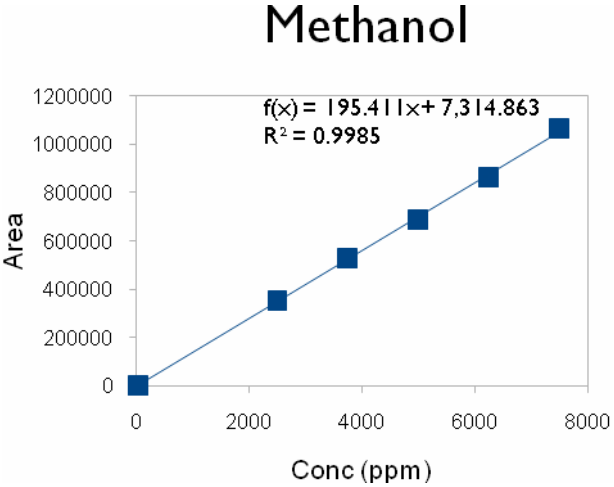

Figure 3: linearity curve of methanol

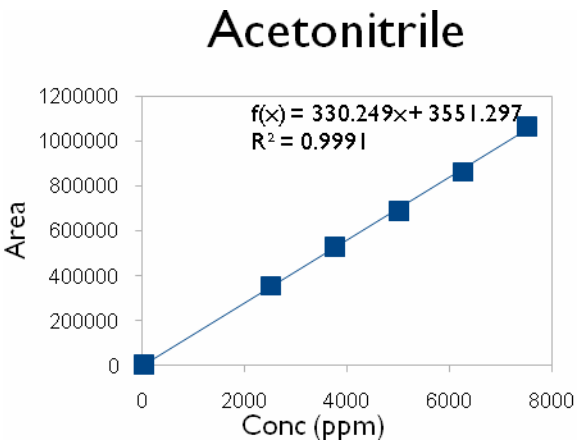

Figure 4: linearity curve of Acetonitrile

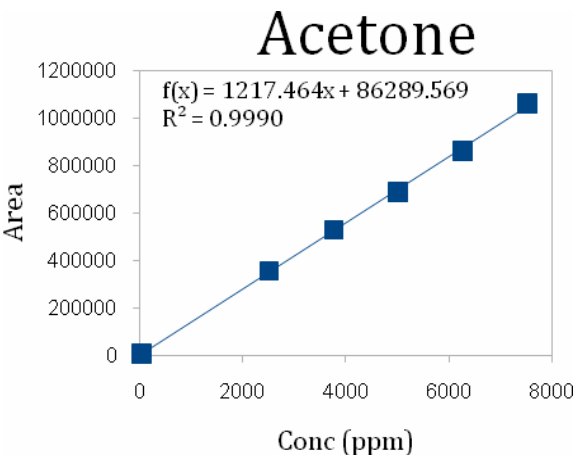

Figure 5: linearity curve of Acetone

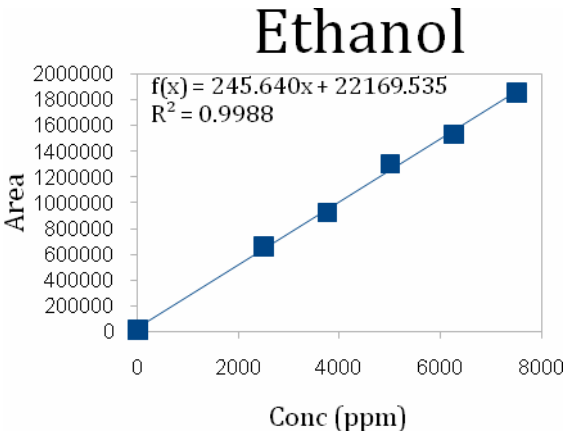

Figure 6: linearity curve of Ethanol

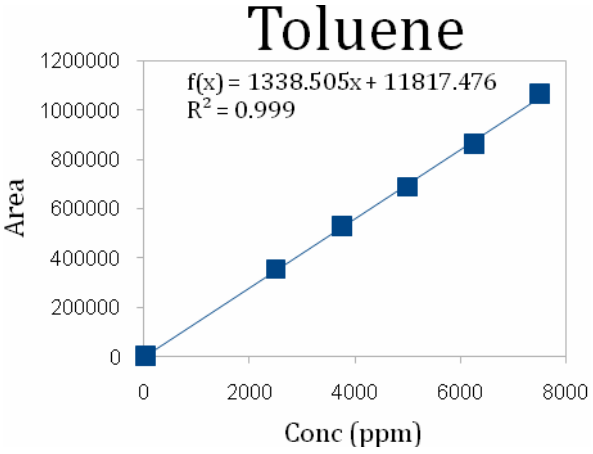

Figure 7: linearity curve of Toluene

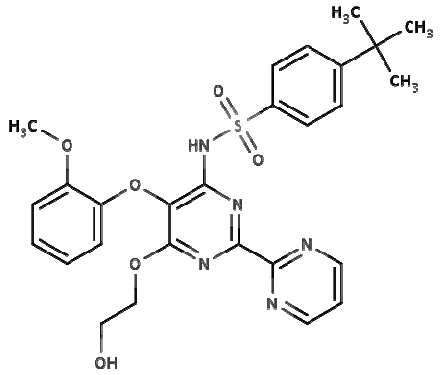

Figure 8: Chemical Structure of Bosentan.

Detection (LODs) and LOQs) quantification limits

LODs were calculated as those concentrations that gave an S/N ratio of approximately 3. LOQs were calculated as those concentrations that gave an S/N ratio≥10 and low-residual linearity values. The sensitivity of the method was demonstrated by the low-LOD values obtained for all the solvents analyzed (Table 2).

Recovery

The mean recoveries for all the solvents were between 93.09–101.55 and were lower than tabulated t for p = 0.05 (Table 2), so the recoveries and 100% values were not significantly different.

The method was found to be applicable for the routine analysis of the APIs like Bosentan monohydrate in pharma.

CONCLUSION:

The analytical method proposed for the quality control Bosentan monohydrate in relation to the residual methanol, ethanol, acetone, acetonitrile and toluene contents, met the validation requirements. Excellent results were obtained, within globally accepted validation reference values, particularly taking into account the low concentration levels investigated. The method was sensitive, linear, accurate and precise. Three randomly selected batches of each drug substance were analyzed under validated method conditions and the concentrations of residual methanol, ethanol, acetone, acetonitrile and toluene were much lower than their maximum ICH limits. Moreover, the validated method can be applied to others drug substances.

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