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DEVELOPMENT AND VALIDATION OF A HEADSPACE GAS CHROMATOGRAPHIC METHOD FOR DETERMINATION OF RESIDUAL SOLVENTS IN BOSENTAN MONOHYDRATE PURE DRUG

M. Paul Richard*[1], S. Haseena[1], G.Swarnalatha[2], C.Vanitha[2], B.Vijayakumar[2] and M.Prakash[3]

[1]OTRI, JNTUA, Ananthapuramu- 517 002, Andhra Pradesh, India.
[2]Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor-517127, Andhra Pradesh, India.
[3]FR&D, Caplin Point Laboratories Limited, Chennai, Tamilnadu, India.

ABSTRACT

There are only few published reports on determination of residual solvents in the analytical method development and there exists no detailed guidelines. Residual solvents from the process in the manufacturing of pharmaceuticals are hazardous and cause serious problems, so must be removed. This is much effort in this work is focused on the determination of analytical method for the determination of residual solvents. Bosentan monohydrate pure drug, various solvents, Shimadzu GC-2010 with head space auto injector and Perkin Elmer – 500 with head space auto injector were used. The method development based on residual solvent properties and many trails conducted on conditions like column selection, carrier gas flow, oven temperature and diluent. A simple HS-GC method for the determination of residual solvents in Bosentan monohydrate using nitrogen as the carrier gas at 3.5mL/min with DB-624 (30 meters X 0.53 mm ID) as column using FID as detector was developed. The developed method was validated and parameters were to be found within the limits of USP. The retention time for residual solvents individually and in spiked standard solution was determined. The %RSD for six injections should be NMT15%. The percentage recovery ranges from 85-115%. The correlation coefficient R2 $\geq$ 0.999. The limit of detection and limit of quantification was found to be specific. Precision, method precision and intermediate precision was found to be within the acceptance limit. Finally the sample was tested for the presence of residual solvents mainly benzene as it is a class1 solvent and should be avoided.

Key words: Bosentan monohydrate, DB-624, FID, %RSD.

INTRODUCTION

Residual solvents from the processes in the manufacturing of pharmaceuticals are hazardous and cause serious problems and must be removed. Certain methods like thermo gravimetric analysis, loss on drying are simple but lack specificity to identify the volatile analyts, spectroscopic and spectrometric methods lacked sensitivity [1-3].Gas chromatographic techniques are ideal for residual solvent analysis. They are selective for determination of residual solvents and also sensitive to accurately determine these solvents in trace amounts, when present in pharmaceutical substances. Recognizing the need to control the presence of these solvents, which are likely to cause undesirable toxic effects [4-6].

Residual solvents are the organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. These solvents are not completely removed by practical manufacturing techniques. Since there is no therapeutic benefit from residual solvents, these solvents should be removed [7-9]. As benzene is a class-1 solvent it should not be present in our sample. Gas chromatography requires only very small samples with little preparation and it is good at separating complex mixtures into components.

Corresponding Author :- **M. Paul Richard** Email:- richie2626@gmail.com

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

These impurities found was volatile in nature hence gas chromatography technique was used [10]. Residual solvent (Benzene) in Bosentan monohydrate (Fig 1) determined by gas chromatography method.

MATERIALS AND METHODS

Materials: Benzene, Propylene Glycol, DMSO, TEA, MDC, MTBE, Isopropyl Methane Sulphonate, Methyl Methane Sulphonate.

Instrument: Gas chromatography (shimadzu, GC-2010), Perkin Elmer FT-IR 600.

Method: Head space Gas chromatography

The analysis was performed on Shimadzu GC-2010 with head space auto injector and FID detector with nitrogen as the carrier gas. The chromatographic conditions are given in Table No 1.

Table 1. Chromatographic conditions

Column	DB-624
Dimension	30 meters x 0.53 mm ID (3µm)
Detector	FID Detector
Temperature	260 c
Injector Temperature	180C
Injector volume	1.0 m L
Oven Conditions	Initially kept at 40°C-hold for 5min-Raise @10°C/min to 220°C hold for 5min
Runtime	40 ml/ml
Spli t Ratio	2:1
Carrier Gas	3.5 m L/min. (Nitrogen)
Makeup Gas	25 m L /min. (Nitrogen)
Head space conditions	
Oven temperature	80° C
Transfer temperature	100° C
Needle temperature	90° C
Thermostat time	25 min
Injector time	1.0 min
GC cycle time	30 minutes

Sample preparation

Weigh approximately 200mg of sample and transfer to a 20mL headspace vial add 2 mL of diluents.

Standard preparation

Dissolve 20mg of benzene in 100mL volumetric flask, then diluted to the mark with diluent. Further dilute 0.1 mL to 100mL with diluent.

Standard stock solution

Prepared solutions are taken into 2mL head space vial, sealed with aluminium closure. These standards are run under the specified conditions and retention times are noted to calculate %RSD.

Method Validation

The parameters like specificity, accuracy, LOD and LOQ, system suitability were performed that are mentioned in the International conference on harmonization (ICH) guidelines. Specificity is performed to know the retention time further residual solvents individually and in spiked sample solution. Linearity was done to know the test results which are directly proportional to the concentration of analyte in the sample. It was performed from LOQ to 150% and results were found to be within the limits. Precision was validated to know the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. %RSD for precision was also found to be NMT 15%.

Accuracy is the amount of drug recovered from the spiked sample. It is assessed by 9 Determinations over a minimum of 3 concentration levels covering the specified range. Robustness is tested by introducing small variations in method parameters. From the results it was observed that the method remain unaffected. System suitability is performed to ensure that the complete testing system is suitable for Intended application finally the sample is checked for the presence of residual solvents especially benzene.

RESULTS

All the validated parameters were found to be within the limits. Drug recovery should be 85-105%. System suitability for 6 injections %RSD was found to be NMT 15%. LOD and LOQ values, specificity of solvents are also within the limits. The specification limit of solvents given in Table No 2.

Table 2. Specification limits of solvents

S.No	Name of the solvent	Specification limit in ppm
1	Benzene	2
2	DMSO	5000
3	Propylene glycol	1500
4	MDC	600
5	MTBE	5000
6	n-hexane	290
7	TEA	320
8	Methyl methane sulphonate	75
9	Isopropyl methane sulphonate	75

DISCUSSION AND CONCLUSION

The determination of residual solvent (Benzene) in Bosentan monohydrate performed by gas chromatography method. The specificity of Benzene given in Table No 3. No interference between peaks of interest and blank were found.

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

The system suitability parameters of Benzene figures given below.

Determination of residual solvents like Benzene, Propylene glycol, DMSO, MDC, MTBE, n-Hexane and TEA in Bosentan monohydrate. In that Propylene glycol and DMSO are not co-eluted with other volatile solvents used in the manufacturing process of Bosentan monohydrate. The details are shown in table no 3.

The system suitability parameters of various solvents given in table no 4.

The validation parameters LOD & LOQ for Propylene glycol, DMSO, benzene, Bosentan monohydrate were shown in firure 5-14.

LOD and LOQ values were established based on signal to noise ratio method. Calculated signal to noise ratio of standard solution through system software and prepared LOD and LOQ solutions. Signal to noise ratio (S/N) for LOD values are about 3:1 and signal to noise rato for LOQ 10:1. Precision at LOQ level was noted more than 10. The S/N concentration values of LOD given in table no 5 and LOQ shown in table no 6.

The recovery studies of the solvents repeated for different concentrations and the %RSD value calculated. Hence the method was accurate. The results were shown in table no 7.

Table 3. Specificity of solvents

S. No	Name of the solvent	Concentration in ppm	Retention time	
			1st injection	2nd injection
1	Benzene	1.95	10.472	10.472
2	Propylene glycol	1503.10	15.27	15.27
3	DMSO	5051	15.90	15.90
4	MDC	601.80	6.793	6.793
5	MTBE	5001	7.663	7.663
6	n-Hexane	290.30	8.631	8.631
7	TEA	322.30	13.272	13.272

Table 4. System suitability of solvents

Solvent	Concentration Ppm	Area	% RSD
BENZENE	1.95	9721 11039 11026 11152 11153 11327	5.40
PROPYLENE GLYCOL	1532	1671171.23 1757680.10 1418976.80 1615882.89 1630002.45 1431005.98	8.52
DMSO	5051	6104407.91 6345216.01 5205161.37 5923247.47 5981019.84 5329900.39	7.73
MDC	601.80	196275.17 197925.76 194232.57 195176.04 192849.78 196480.38	0.92
MTBE	5001	5728521.41 5849991.09 5698670.72 57787719.64	1.22

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

		5646285.23 5756128.06	
n-Hexane	290.30	438852.10 444936.73 430372.93 432383.59 416276.64 421901.60	2.45
TEA	322.30	442702.13 444819.49 441585.30 446291.74 454570.14 460688.69	

Table 5. Concentration and average S/N values of LOQ for solvents

S no	Solvent	Concentration in, ppm	S/N Value	Average S/N Value (n=3)
1	Benzene	1.03	10.65 11.35 11.72	11.24
2	Propylene Glycol	46.1	10.374 11.374 10.501	10.74
3	DMSO	48.05	12.076 14.896 12.895	13.29
4	MDC	48	11.82 11.46 14.22	12.51
5	MTBE	20	11.80 10.15 12.38	11.44
6	n-Hexane	13.5	11.10 11.54 12.12	11.59
7	Tea	14	12.24 10.28 11.77	11.43

Table 6. Concentration and Average S/N values of LOD for solvents

S No	Solvent	Concentration in ppm	S/N Value	Average S/N Value (n=3)
1	Benzene	0.34	3.135 3.142 3.144	3.14
2	Propylene Glycol	13.97	3.165 3.119 3.073	3.12
3	DMSO	14.56	3.828 3.634 3.515	3.66
4	MDC	14.40	3.62 4.12 4.07	3.94

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

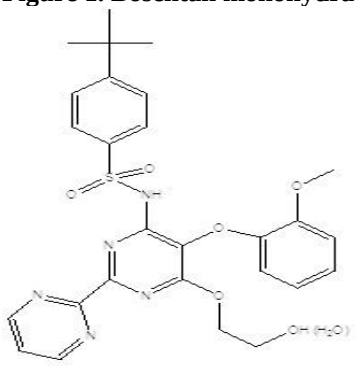
5	MTBE	6.00	3.78 3.87 3.60	3.75
6	n-Hexane	4.05	3.73 4.38 4.50	4.20
7	Tea	4.20	3.97 5.44 5.86	5.09

Table 7. Percentage of recovery studies for solvents

S no	Solvent	Area	Obtained Value ppm (n=6)	Theoretical Value ppm	% of RSD	% of Recovery Studies
1	Benzene	6242 6253 6299 6541 6258 6238	1.19 1.12 1.13 1.17 1.12 1.11	1.03	2.90	115.53 108.74 109.71 113.59 108.74 107.77
2	Propylene Glycol	52160.21 42995.95 42508.15 43896.72 48608.70 44038.67	44.08	46.10	8.4	109.10 89.96 88.94 91.87 101.67 92.16
3	DMSO	61292.02 54294.89 50605.14 56942.13 51193.41 51224.14	47.10	48.05	7.76	110.71 98.10 91.44 102.92 92.46 92.57
4	MDC	14576.69 15404.93 15448.87 15516.61 15863.23 15252.37	47.20	48	2.78	93.40 98.76 99.03 99.45 101.66 97.76
5	MTBE	25748.06 25223.38 21257.48 24547.35 22081.13 20859.06	20.27	20	9.18	112.02 109.79 92.52 106.82 96.07 90.77
6	n-Hexane	18211.78 19923.52 18951.62 20877.65 20047.49 19433.30	13.18	13.5	4.74	90.83 99.42 94.56 104.15 99.99 96.94
7	Tea	21107.62 21771.26 18565.69 21306.13 18381.29 20478.82	14.56	14.56	7.17	108.26 111.73 95.27 109.31 94.29 105.06

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

Figure 1. Bosentan monohydrate



4-tert-butyl-N-(6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl)benzenesulfonamide hydrate

Fig 2. Chromatogram of system suitability for Benzene

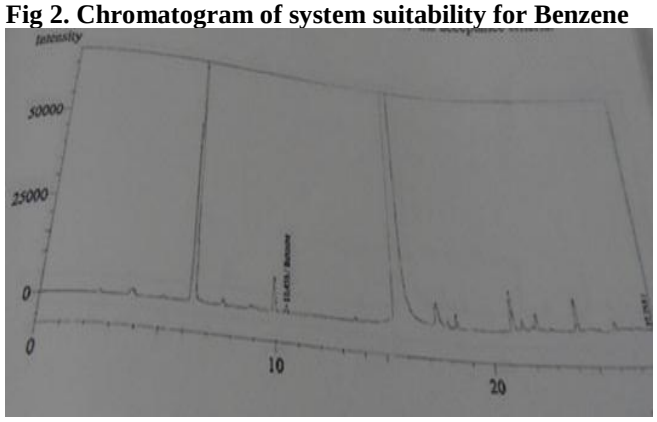


Fig 3. Chromatogram of specificity for Benzene

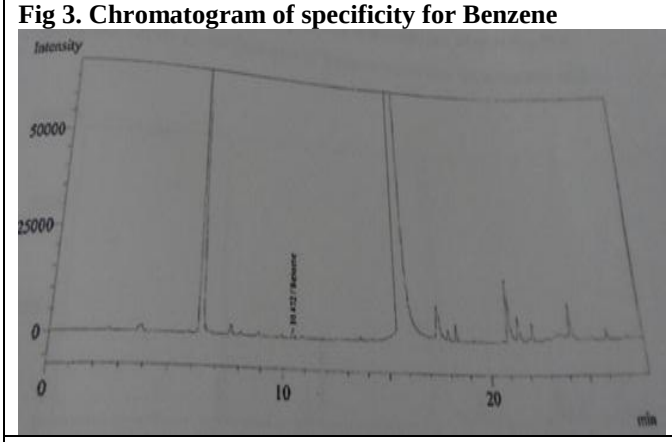


Fig 4. Chromatogram of LOD for Benzene

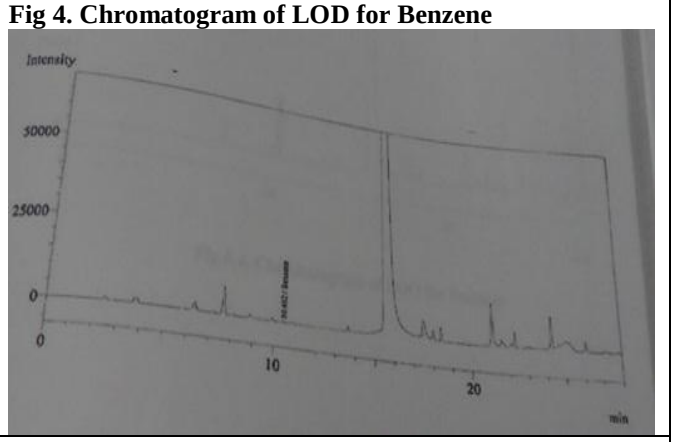


Fig 5. Chromatogram of LOD for Propylene glycol & DMSO

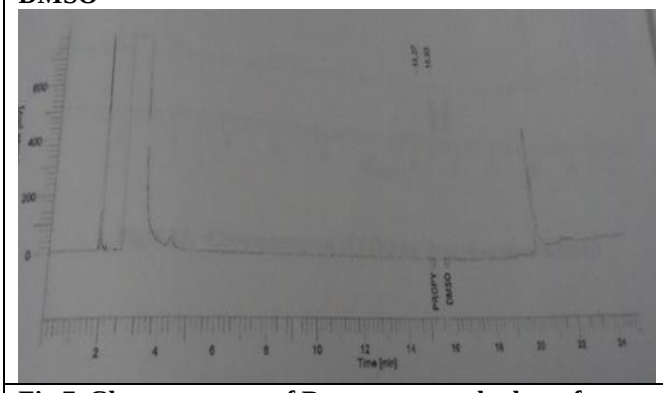


Fig 6. Chromatogram of selectivity for DMSO

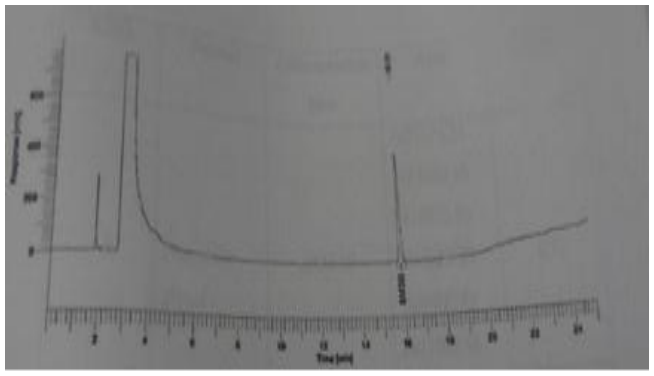


Fig 7. Chromatogram of Bosentan monohydrate for benzene

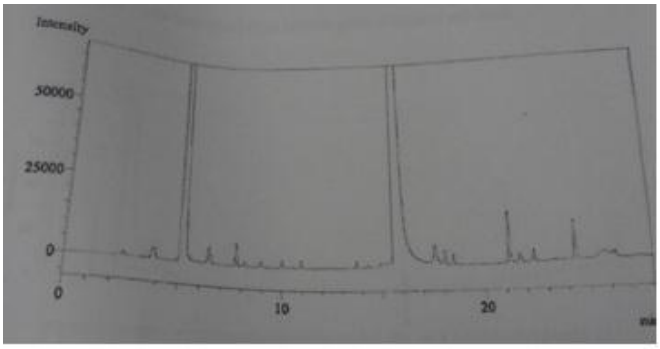
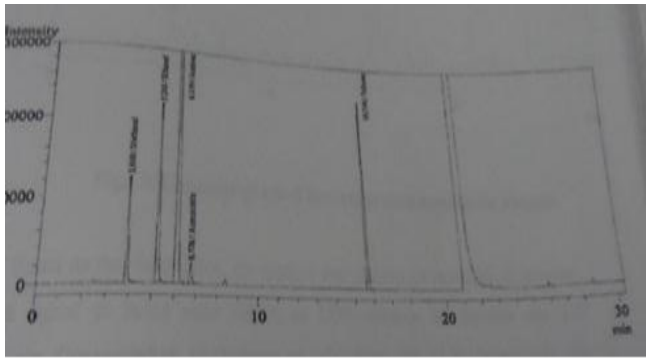


Fig 8. Chromatogram of 100% LOQ for Benzene



Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

Fig 9. Chromatogram of diluent for Benzene

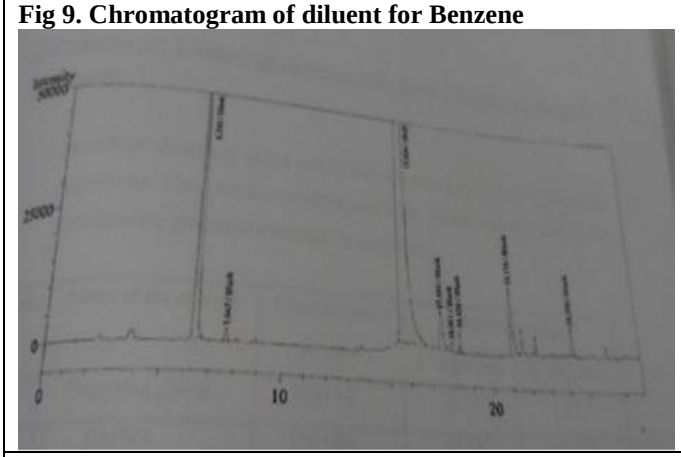


Fig 10. Chromatogram of LOQ for Benzene

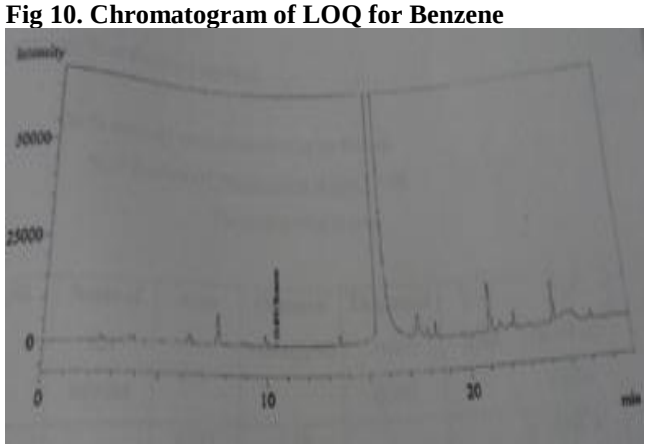


Fig 11. Chromatogram of LOD for Benzene

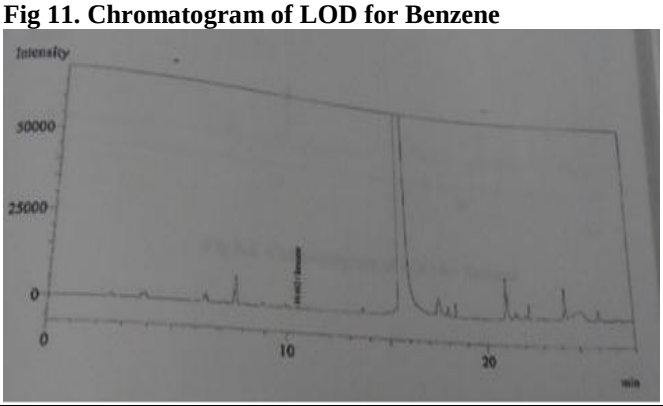


Fig 12. Chromatogram of diluent for Propylene glycol and DMSO

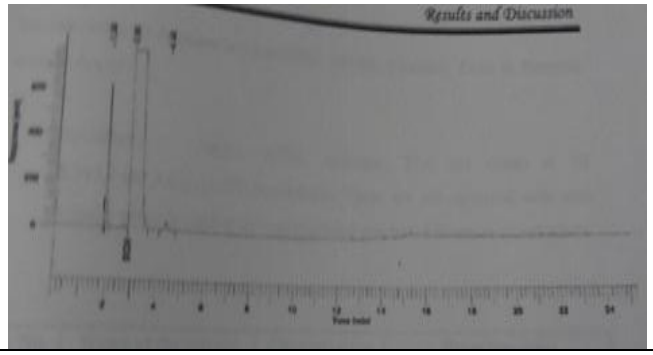


Fig 13. Chromatogram of 100% LOD for Propylene glycol & DMSO

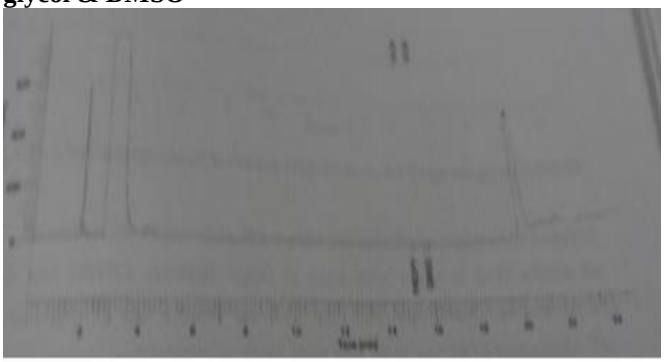
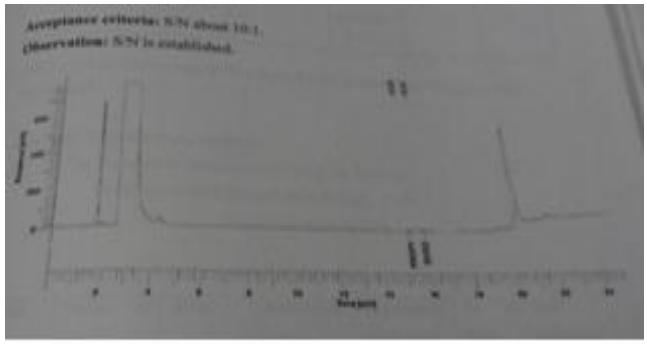


Fig 14. Chromatogram of LOQ for Propylene glycol & DMSO



CONCLUSION

A single, rapid and highly selective HS-GC method was developed and validated for the quantification of residual solvents present in Bosentan monohydrate API through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The developed gas chromatographic method has to evaluate reliable and economical result for the determination of Benzene, Propylene Glycol, DMSO, TEA, MDC, MTBE and n-Hexane as residual solvents present in Bosentan monohydrate. The results of various validation parameters confirmed that the method is specific, System Suitability, Limit of Detection, Limit of Quantification, Accurate (% of recovery studies) as per ICH guidelines.

REFERENCES

1. European medicines agency. ICH topic Q3C (R4) impurities, Guidelines for residual solvents, 2009.
2. Gurudeep RC, Sham KA. Instrumental methods of chemical analysis, Himalaya publishing house, New Delhi, 1979, 2.678-2.694.

Asian J. Pharm. Res. Vol 5, Issue 1, 29-36, 2015.

3. Willard HH, Merritt LL, Dean JA, Frank AS. Instrumental methods of analysis, CBS Publishers and distributors, New Delhi, 7th ed., 1986, 540.
4. Camarasu CC, Mezel SM, Varga GB. Residual solvents in pharmaceutical products by GC-HS & GC-MS-SPME. *Jpharm Biomed Anal*, 18, 1998, 623-638.
5. Chen TK, Moekel W and Superhant HL. Modifications to the Europeon pharmacopoeias proposal on residual solvents. *Pharmeuropa*, 4, 1992, 62-67.
6. Skett P. Low-level measurement of potent toxins in analysis of drug impurities. R.S.A.M Webb, Blackwell publishing, 2007.
7. Anton P, Lutz M, Miklos S, Judith KJ, Peter L and David T. Elevated ethyl methane sulfonate(EMS) in nelfinavir mesylate (viracept,Roche):overview. *AIDS Res Ther*, 6, 2009, 18.
8. Green N, Judson PN, Langowsky JJ, Marchnt CA. Knowledge based expert systems for toxicity and metabolism prediction: DEREK, SAR and METEOR.SAR QSAR. *Environ.Res*., 1999, 299-314.
9. Snodin DJ. Genotoxic impurities: from structural alerts to qualification. *Org. process Res.Dev*, 14(4), 2010, 960-976.
10. Bauer M, Barth ElEmy C. Handbook of solvents, wypych G.Ed., 1st ed. Chem Tec publishing, Toronto, New York, 2001, 112.