International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(4): July-August 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Medical Sciences **Original Article……!!!**

Received: 14-08-2012; Revised; Accepted: 19-08-2012

DEVELOPMENT AND VALIDATION OF HEAD SPACE GC METHOD FOR RESIDUAL SOLVENTS OF PIPERACILLIN SODIUM AND TAZOBACTAM SODIUM BLEND (8:1)

Shah A.B., Patel A.T., Umalkar D.*

Department of Quality Assurance, Parul Institute of Pharmacy, Limda, Dist. Vadodara-391760, Gujarat, India

Keywords:

Piperacillin, Tazobactam, Headspace gas chromatography, Residual solvents, Validation

For Correspondence:

Umalkar D.

Department of Quality Assurance, Parul Institute of Pharmacy, Limda, Dist. Vadodara-391760, Gujarat, India

E-mail:

deepakumalkar@gmail.com

ABSTRACT

In order to increase efficiency and sensitivity, head space-gas chromatography (HS-GC) method was successfully developed and validated for determination of residual solvents present in Piperacillin sodium and Tazobactam sodium blend (8:1). Four residual solvents i.e. Ethanol, Acetone, Dichloromethane, and Ethyl Acetate were estimated on GC column Agilent DB-624 (30m x 0.32mm x 1.8µm) using Nitrogen as carrier gas at a flow rate of 0.7ml/min. In order to increase the method sensitivity and efficiency in sample equilibration, N, N-Dimethylformamide (DMF) was selected as the sample diluent based on its high capacity of dissolving drug substance, stability and high boiling point. The HS sample equilibration temperature and equilibration times were found to be 90ºC and 20 min, respectively. Injector port temperature and detector temperatures were found to be 200ºC and 250ºC respectively. The two-stage gradient GC programme was from 40ºC to 230ºC. Retention time of Ethanol was about 8.6 min and relative retention times of Acetone, Dichloromethane, and Ethyl Acetate with respect to Ethanol were found to be 1.16, 1.37 and 1.74 respectively with total run time 25 min. The resolution between residual solvents was greater than 2.0 for all pairs of components. The linearity Range was found to be 600-1800µg/ml for Ethanol, Acetone and Ethyl Acetate while 40-120µg/ml for Dichloromethane. The performance of the method was validated for specificity, LOD, LOQ, linearity, accuracy, precision and robustness. The method has been successfully applied for the quantification of residual solvents present in Piperacillin sodium and Tazobactam sodium blend (8:1) in routine analysis.

INTRODUCTION

Residual solvents are solvents that are used during the manufacturing process and may be detected after the product is in its final dosage form. Residual solvent testing is important in the pharmaceutical industry for several reasons. For a drug substance, solvent content will affect its potency. From a safety perspective, depending on the dosage strength of the drug substance and the duration of the treatment, the amount of solvent or solvents entering a patient must be safe toxicologically. Residual solvent content must be within the limits specified in USP General Chapters <467> "Residual Solvents" to minimize their effects on potency of drug and its toxicological effects. A most common and conventional technique for measuring residual solvent content is gas chromatography (GC) because of the small size and volatile nature of solvent molecules. HS-GC methods are automated, accurate and more sensitive than any other possible non-specific methods for residual solvents. Literature survey revealed that no specific method was available for determination of residual solvents of the Piperacillin Sodium and Tazobactam Sodium blend (8:1). Therefore, it was thought of interest to develop method for determination of residual solvents present in the piperacillin and tazobactam blend (8:1).

MATERIALS AND METHODS

Materials and Reagents

Piperacillin sodium and Tazobactam sodium blend (8:1) sample was provided by Astral Pharmaceuticals Ltd, Makarpura, G.I.D.C., Vadodara, Gujarat (India). Residual solvents used were of ≥99% purity, and purchased from the following sources: Ethanol, Acetone, Dichloromethane, Ethyl Acetate from Merck specialties pvt ltd, Mumbai. N,N-Dimethylsulfoxide(DMSO), N,N-Dimethylformamide(DMF) were used as diluents and purchased from Merck specialties pvt ltd, Mumbai.

Instrumentation

GC-2010 (Shimadzu) equipped with Flame Ionization Detector (FID) and Head-Space Autosampler (Teledyne Tekmar) was used for the experiment. The HSGC system was controlled using two different softwares: for GC system GC-Solution and for autosampler HT3 Software. The GC column was an Agilent DB-624 (6% cyanopropylphenyl/94% dimethyl polysiloxane) fused silica capillary column, 30m long, 0.32mm I.D., 1.8µm film thickness. (Temperature Limits of column: -20ºC to 260ºC)

Standard Solutions

According to given Specification limits for residual solvents as per USP General Chapter <467> "Residual Solvents" Ethanol, Acetone and Ethyl Acetate being class 3 solvents were prepared at about 5000ppm individually in DMF, and Dichloromethane being class 2 solvent was prepared at about 320ppm in DMF then injected to the HSGC system.

Preparation of standard stock solution (Containing all 4 residual solvents):

About 750 mg of absolute ethanol, about 750 mg of acetone, about 50 mg of dichloromethane, 750 mg of ethyl acetate, accurately weighed, in to a 25 ml volumetric flask containing about 10 ml of DMF, diluted to volume with DMF and mixed well. This solution contains about 30,000µg/ml of Ethanol, Acetone, Ethyl Acetate and about 2000µg/ml of Dichloromethane.

Preparation of Working Standard Solution-1

5 ml of Standard Stock Solution was taken into a 25 ml volumetric flask. Diluted to volume with DMF and mixed well. This solution contains about 6000µg/ml of Ethanol, Acetone, Ethyl Acetate and about 400µg/ml of Dichloromethane.

Preparation of Working Standard Solution-2

5 ml of the Working Standard Solution-1 was taken into 25 ml volumetric flask. Diluted to volume with DMF and mixed well. This solution contains about 1200µg/ml of Ethanol, Acetone, Ethyl Acetate and about 80µg/ml of Dichloromethane.

For the HS-GC analysis, 2mL of the standard solutions was pipetted into 15-20mL headspace sample vial and immediately sealed with a Teflon-lined septum and an aluminum crimp cap.

Sample Solutions

Preparation of Sample Solution (Without Spike)

About 500 mg of sample (i.e. Piperacillin and Tazobactam blend) accurately weighed into headspace vial and diluted with 2 ml of DMF, mixed well and immediately sealed with a Teflon-lined septum and an aluminum crimp cap.

Preparation of Sample Solution (With Spike)

About 500 mg of sample (i.e. Piperacillin and Tazobactam blend) accurately weighed into headspace vial and diluted with 2 ml of Spiking Solution, mixed well and immediately sealed with a Teflon-lined septum and an aluminum crimp cap.

Procedure

During the HSGC method development, in order to select the most appropriate system parameters to obtain the best separation, sensitivity and time efficiency, solvent mixtures were injected under a variety of conditions, e.g. at different GC Columns (AB-5, HP-5, DB-624), HS temperatures; vial-room temperature(70-90ºC), needle temperature(90-100ºC), transfer line temperature(100-130ºC), detector temperatures(200-300ºC), injector temperatures(100-200ºC), equilibration time(10-20min), GC gradients (40-230ºC, at the rate of 10–40ºC /min), carrier gas flow rates (0.6-0.8ml/min), different diluents (DMSO, DMF) etc. The final HS-GC conditions used for method validation were obtained based on optimized HS and GC parameters. The HSGC system was equilibrated under the experimental conditions by injecting 3 blank DMF samples every day before sample sequence injections. Each of the solvents was injected once separately to determine method specificity and signal response sensitivity.

RESULT AND DISCUSSION

Final optimized Head-Space conditions:

Table No.1-Final optimized Head-Space conditions

Head-Space Conditions	
Vial-room Temperature	90˚C
Needle Temperature	95˚C
Transfer line Temperature	130˚C
Sample Equilibration Time	20 min
Sample Mixing Time	40 min
Pressurization Time	3 min
Inject Time	0.1 min
Withdrawal Time	0.2 min

Final optimized GC conditions:

Carrier Gas: Nitrogen

Column: DB-624 (30m x 0.32mm x 1.8µm)

Flow rate: 0.7 ml/min

Diluent Selected: DMF

GC cycle time: 25 min

Split ratio: 1:20

Table No.2- Final optimized GC conditions:

Temperature programming:	
Injector port Temperature	200˚C
Detector Temperature	250˚C
Temperature 1	40˚C
Hold time 1	12 min
Rate 1	35˚C/min
Temperature 2	180˚C
Hold time 2	5 min
Rate 2	35˚C/min
Temperature 3	230˚C
Hold Time 3	3 min

Fig.1 Chromatogram for standard solution using optimized HS-GC conditions

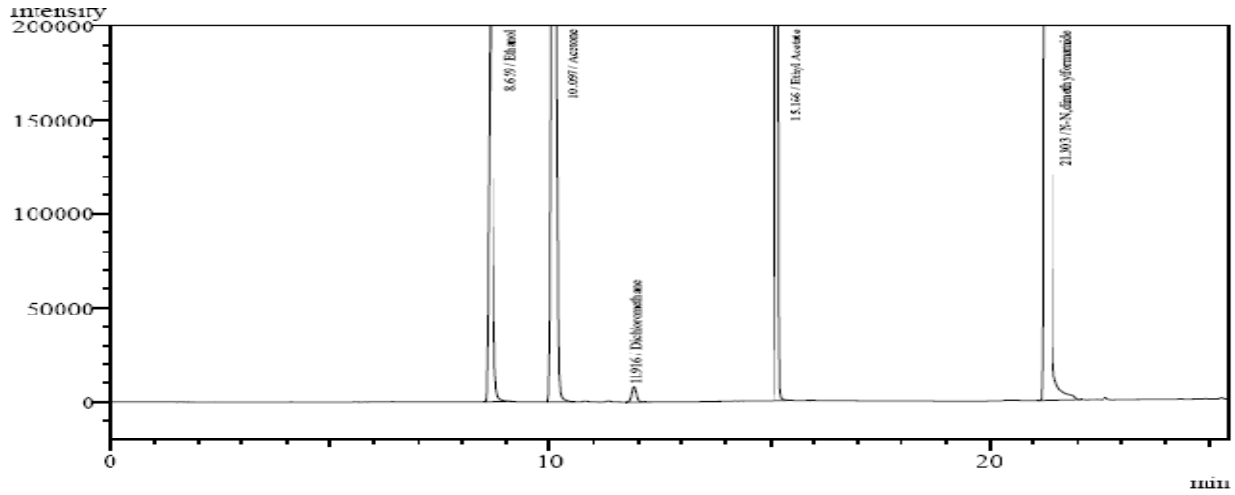
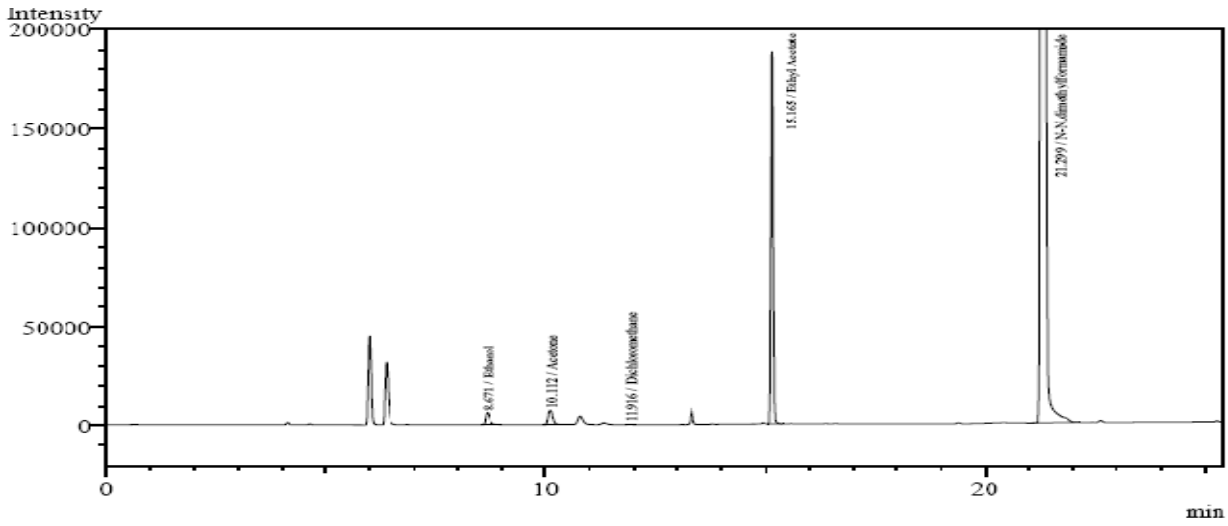


Fig.2 Chromatogram for Sample solution using optimized HS-GC conditions



SYSTEM SUITABILITY TESTING

Table No.3- System Suitability Parameters

No.	System Suitability Parameter	Observed Values				Specification
		Ethanol	Acetone	Dichloromethane	Ethyl Acetate	
1	Resolution (R_s)	--	8.48	10.223	23.671	>2.0
2	%RSD	6.80	4.85	5.34	5.22	<15.0
3	Tailing Factor (T_f)	1.092	1.049	1.030	1.060	Not greater than 2.0

METHOD VALIDATION

Specificity:

Specificity of the method was shown by injecting the blank; Sample preparation and Standard solution and showing the resolution between all peaks are in both sample solution and Standard Solution and there was no interference from the blank at the retention times of analyte peaks those were obtained from standard solution and resolution of more than 2.0 was obtained between two closely eluting peaks which meets the acceptance criteria.

Table No.4-Specificity of the method

Name of the Solvent	Retention Time (min)	Relative Retention Time	Resolution
Ethanol	8.626	1.0	0.0
Acetone	10.088	1.16	8.48
Dichloromethane	11.851	1.37	10.223
Ethyl Acetate	15.143	1.74	23.671

Linearity & Range:

Linearity Stock Solution was prepared in DMF in such a way that it contains about 6000µg/ml of Ethanol, Acetone, Ethyl Acetate and about 400µg/ml of Dichloromethane. From this stock solution five concentration levels i.e. 50%, 80%, 100%, 120% and 150% were prepared.

The linearity and range was determined at these five concentration levels for Ethanol, Acetone, Dichloromethane and Ethyl Acetate. The linearity and range were found as 600-1800µg/ml for Ethanol, Acetone, Ethyl Acetate and 40-120µg/ml for Dichloromethane. The calibration curve was constructed by plotting absorbance against concentration (µg/ml).

Fig.3 Linearity curve for Ethanol

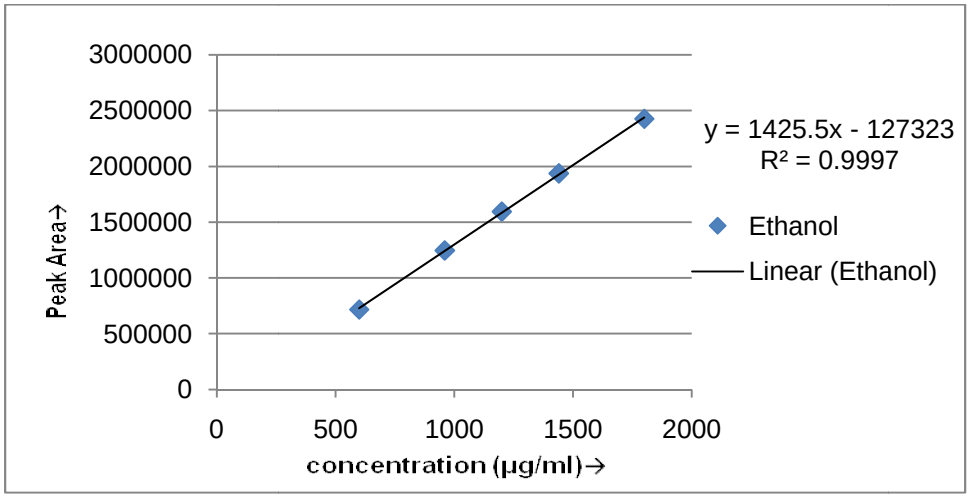


Fig.4 Linearity curve for Acetone

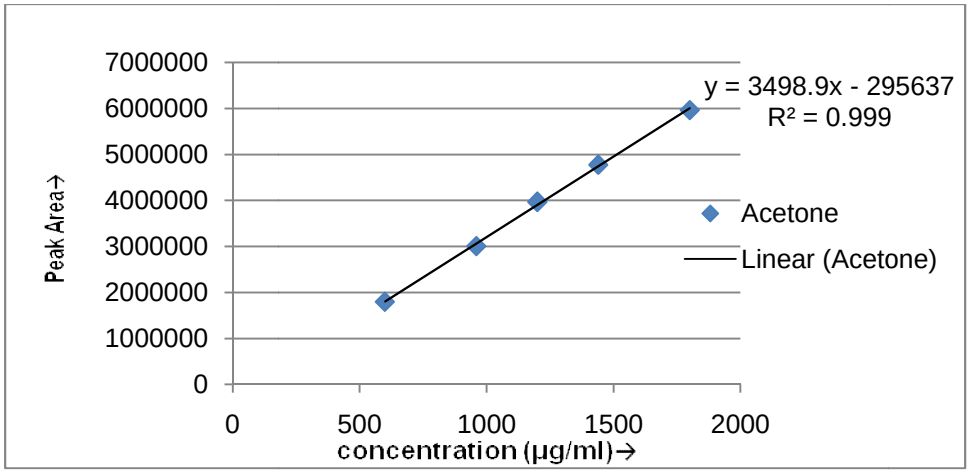


Fig.5 Linearity curve for Dichloromethane

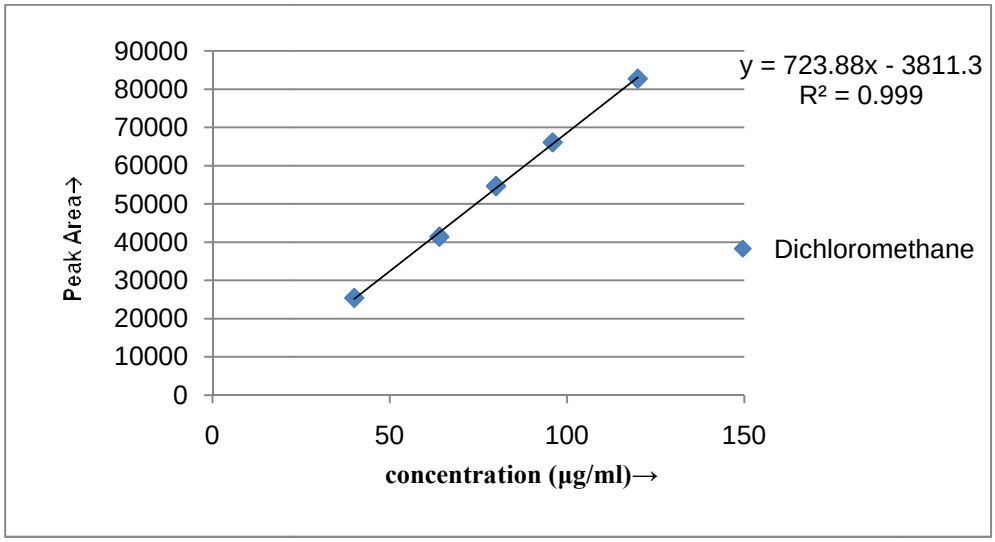
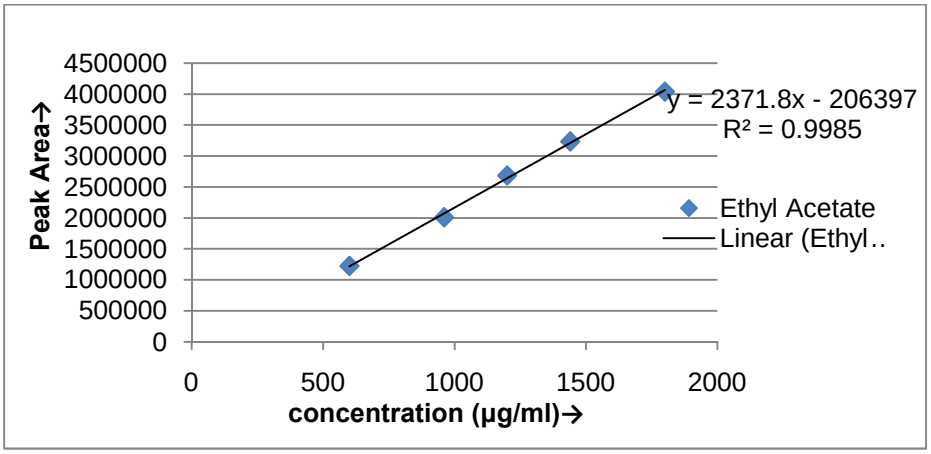


Fig.6 Linearity curve for Ethyl Acetate



Precision:

a) Repeatability: (intra-day precision)

Standard solution (100% Specification level) was injected six times, areas of peaks were measured and % RSD was calculated.

b) Intermediate Precision: (Inter-day Precision)

Stock Solution was prepared in DMF in such a way that it contains about 6000µg/ml of Ethanol, Acetone, Ethyl Acetate and about 400µg/ml of Dichloromethane. From this stock solution three concentration levels i.e. 80%, 100% and 120% were prepared.

Inter-day precision was shown by injecting the standard solution at 80% level, 100% level, 120% level (in triplicate) on three consecutive days. % RSD at each level was calculated.

c) Precision at LOQ:

LOQ Solution was prepared in such a way that it contains 1.2µg/ml of Ethanol, 0.3µg/ml of Acetone, 0.8µg/ml of Dichloromethane and 0.3µg/ml of Ethyl Acetate. LOQ Solution was injected six times, areas of peaks were measured and % RSD was calculated.

Table No.5-Precision Study

PRECISION	**%RSD (NMT 15%)**			
	Ethanol	**Acetone**	**Dichloromethane**	**Ethyl Acetate**
Intra-day Precision	8.31	6.44	6.42	6.92
Inter-day Precision				
80%	10.88	7.07	7.90	8.78
100%	7.19	6.76	6.75	7.05
120%	4.01	3.10	3.57	3.88
Precision At LOQ	10.20	11.80	9.86	10.90

Accuracy:

The accuracy of the method was determined by standard addition method at three different levels. The recovery studies were carried out by spiking standard solution equivalent to 50%, 100%, and 150% to the original amounts present in each drug formulation. The recoveries were as reported.

Acceptance Criteria: % Recovery should be within 80% to 120%.

Table No.6-Recovery Studies

RESIDUAL SOLVENT	**RECOVERY OF SAMPLE SOLUTION (WITHOUT SPIKE):**	**CONCENTRATION**	**RECOVERY OF SAMPLE SOLUTION (WITH SPIKE):**	**NET AMOUNT RECOVERED (µg/ml)**	**AMT OF STD SOLVENT ADDED (µg/ml)**	**% RECOVERY**
ETHANOL	130.86	50	5193	5062.14	4777.957	105.95
		100	5266.86	5136	4781.78	107.41
		150	4849.83	4718.97	4778.91	98.74
ACETONE	63.192	50	4824.07	4760.87	4763.59	99.94
		100	4669.42	4606.23	4767.40	96.62
		150	5135.92	5072.73	4764.55	106.47
DICHLORO METHANE	4.6518	50	339.89	335.238	326.352	102.72
		100	356.15	351.50	326.613	107.62
		150	335.45	330.80	326.417	101.34
ETHYL ACETATE	1221.758	50	6195.09	4973.34	4785.30	103.93
		100	6584.30	5362.54	4789.13	111.97
		150	6237.85	5016.09	4786.26	104.80

LOQ & LOQ:

Limit of Quantification (LOQ): LOQ level was estimated by injecting progressively lower concentration of the standard solution and measuring the S/N ratio. Lowering of the concentration was continued till the value of S/N was greater than 10.0

Acceptance Criteria: Signal to Noise ratio (S/N) is 10:1

Limit of Detection (LOD): LOD level was estimated by injecting progressively lower concentration of the standard solution and measuring the S/N ratio. Lowering of the concentration was continue till the value of S/N was greater than 3.0

Acceptance Criteria: Signal to Noise ratio (S/N) is 3:1

Table No.7-Limit of Quantification and Limit of Detection

Name of the Solvent	**Limit of Quantification-LOQ(ppm)**	**Limit of Detection-LOD (ppm)**
Ethanol	1.2	0.3
Acetone	0.3	0.12
Dichloromethane	0.8	0.4
Ethyl Acetate	0.3	0.12

Robustness:

Following method parameters were varied to check the robustness of the proposed method.

Flow Rate: 0.6 ml/min and 0.8 ml/min

Vial Temperature: 85ºC and 95ºC

Standard solution (100% Specification level) was injected six times at different above mentioned conditions, areas of peaks were measured and % RSD was calculated.

Acceptance Criteria: % RSD should not be more than 15.0%.

CONCLUSION

Head space- gas chromatographic (HS-GC) method for the determination of residual solvents in Piperacillin Sodium and Tazobactam Sodium blend (8:1) was successfully developed and validated to utilize in routine analysis.

Table No.8-Validation Summary

Validation Parameter	**Ethanol**	**Acetone**	**Dichloromethane**	**Ethyl Acetate**
Linearity: Correlation Coefficient (r) should NLT 0.999				
Regression Equation	y = 1425.5x - 127323	y = 3498.9x - 295637	y = 723.88x - 3811.3	y = 2371.8x - 206397
Correlation Coefficient(r)	0.9998	0.9997	0.9999	0.9999
Range (µg/ml)	600-1800	600-1800	40-120	600-1800
Precision: %RSD should NMT 15%				
Repeatability	8.31	6.44	6.42	6.92
Inter-day Precision				
80%	10.88	7.07	7.90	8.78
100%	7.19	6.76	6.75	7.05
120%	4.01	3.10	3.57	3.88
Precision at LOQ	10.20	11.80	9.86	10.90
LOQ-LOD				
LOQ(µg/ml)	1.2	0.3	0.8	0.3
LOD(µg/ml)	0.3	0.12	0.4	0.12
Accuracy: %Recovery should within 80-120%				
Concentration	%Recoveries			
50%	105.95	99.94	102.72	103.93
100%	107.41	96.62	107.62	111.97
150%	98.74	106.47	101.34	104.80
Robustness: %RSD should NMT 15%				
Flow Rate: 0.6 ml/min	10.18	7.55	8.96	8.01
Flow Rate: 0.8 ml/min	12.13	5.54	5.88	6.28
Vial temperature: 95ºC	11.74	5.06	6.02	6.39
Vial temperature: 85ºC	11.10	8.08	8.05	8.42

REFERENCES

1. United States Pharmacopoeia , Chapter <467> ,Residual Solvents , pp 1-10
2. ICH Guidelines: Impurities: Guideline For Residual Solvents Q3c (R5), Current Step 4 version dated 4 February 2011
3. The Merck Index, Fourteenth edition, Merck Research Laboratories, Division of Merck & Co., Inc., Whitehouse station, NJ,USA,2006
4. A WHO guide to good manufacturing practice (GMP) requirements, Part 2: Validation
5. ICH Topic Q 2 (R1) ,Validation of Analytical Procedures: Text and Methodology, June 1995
6. Rao AL, Krishna KS, Kumar CK and Raja TS. "Simultaneous determination of piperacillin and tazobactum in bulk and pharmaceutical dosage forms by RP-HPLC", International Journal of Pharmacy and Pharmaceutical Sciences, 2011; Vol. 3(2): 134-136.
7. Gupta AR, Singh YM, Semwal VP. "Development and validation of a headspace gas chromatographic method for the determination of residual solvents in arterolane (RBx11160) maleate bulk drug" Journal of pharmaceutical bioallied sciences, 2010; Vol. 2(1): 32-37.
8. Cheng C, Yan Z. "A generic static headspace gas chromatography method for determination of residual solvents in drug substance", Journal of Chromatography A, 2010; Vol. 1217(41).
9. Bilal Yilmaza, Yucel Kadioglua. "Method development and Validation for the GC-FID assay of 17 ß-Estradiol in pharmaceutical preparation", International Journal of pharmaceutical science review and research, 2010; Vol. 2(2): 44-47.
10. Zhou Weimin, "Determination of piperacillin sodium and tazobactam sodium (4:1) for injection by HPLC", CNKI Journal, Chinese Modern Medicine, 2009; Vol. 09.
11. Hari PR, Naseerali CP, Sreenivasan K. "A sensitive estimation of residual ethylene glycol in ethylene oxide sterilized medical devices by HPLC with electro spray ionization mass spectrometric detection", Journal of Chromatography B. Analytical Technologies in the Biomedicine and Life Sciences, 2009; Vol. 877(3): 328-32.
12. Perez Pavon JL, Fernandez Laespada ME, Moreno Cordero B., "Use of mass spectrometry methods as a strategy for detection and determination of residual solvents in pharmaceutical products", Analytical Chemistry, 2006; Vol. 78(14):4901-8.
13. Pai PNS, "H Prathibha. simultaneous estimation of tazobactam and piperacillin in injection formulation", Indian Journal of Pharmaceutical Sciences, 2006, 799-801.