ejpmr, 2016,3(10), 61-66

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUAL SOLVENTS IN OXACILLIN SODIUM BY HEADSPACE GAS CHROMATOGRAPHY

Habibur Rahman[1], Elaref Ratemi[2], S. K. Manirul Haque[2,]* and Prem Shanker[3]

[1]Department of General Studies, Jubail Industrial College, Jubail Industrial City, P. O. Box No – 10099, Jubail – 31961, Saudi Arabia.

[2]Department of Chemical and Process Engineering Technology, Jubail Industrial College, Jubail Industrial City, P. O. Box No – 10099, Jubail – 31961, Saudi Arabia.

[3]Department of Quality Control, Blue Circle Organics Ltd, Ambernath, Maharashtra, India.

***Corresponding Author: Dr. S. K. Manirul Haque**
Department of Chemical and Process Engineering Technology, Jubail Industrial College, Jubail Industrial City, P. O. Box No – 10099, Jubail – 31961, Saudi Arabia.

Article Received on 04/08/2016 **Article Revised on 25/08/2016** **Article Accepted on 16/09/2016**

ABSTRACT

A validated simple and sensitive headspace gas chromatographic (HSGC) method was developed for simultaneous determination of methanol, ethyl acetate and toluene in oxacillin sodium. The separation was achieved using a 30 m long Elite - 5 fused silica capillary column with 0.32 mm internal diameter. The developed gas chromatographic method offers a symmetric peak shape, good resolution and reasonable retention time for all the solvents. Beer's law was obeyed in the concentration ranges of 100 – 1500, 100 – 800 and 100 – 500 ppm for methanol, ethyl acetate, and toluene, respectively for oxacillin sodium. The method was validated according to the international conference on harmonization (ICH) guidelines. The degrees of linearity of the calibration curves, the percent recoveries, relative standard deviation for the method were also determined. The correlation coefficient for methanol, ethyl acetate and toluene were found to be 0.9997, 0.9998 and 0.9996, respectively for oxacillin sodium.

KEYWORDS: Oxacillin sodium, residual solvents, headspace gas chromatography, ICH.

1. INTRODUCTION

In human medicine and animal husbandry, penicillin is widely used for the treatment of bacterial infections. Oxacillin sodium is a penicilliinase- resistant ß- lactam and is chemically known as 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-6-[[(5-methyl-3-phenyl-4-isoxazolyl)carbonyl]-amino]-7-oxo-, monosodium salt, monohydrate, [2S-(2α,5α,6ß)]. Its molecular formula and molecular weights are $C_{19}H_{18}N_3NaO_5S.H_2O$ and 441.44 g/mol, respectively. Since it is resistant to the penicillinase enzyme, it is widely used clinically in the treatment of penicillin-resistant Staphylococcus aureus. It eliminates bacteria that cause infections including pneumonia, meningitis, urinary tract, skin, bone, joint, blood and heart valve infections. The use of the oxacillin would lead to drug residues in different matrix. It has low toxicity and may be administered orally as additives or directly by injection.

There are some solvents which are routinely applied during synthesis of drug substances, excipients, or during drug product formulations but they are not desirable in the final product, mainly because of their toxicity, influence on the quality of crystals of the drug substance, and their odor or taste, which can be unpleasant for patients. These solvents still remain in small quantities even after various manufacturing process are used to eliminate them. These small quantities of the solvents are commonly known as residual solvents. Analysis of residual solvents in drug formulations is an important issue because of the potential risk to human health due to the toxicity of such solvents. Thus, analysis of residual solvents is regarded as one of the most difficult and demanding analytical tasks in the pharmaceutical industry. The manufacturing of active pharmaceutical ingredients (API) under good manufacturing practice (GMP) conditions requires adequate control of the quality of the different ingredients involved in the synthesis. These residual solvents must therefore be controlled, and their purity determined, before any synthesis.

Headspace gas chromatography (HSGC) is a technique where the liquid or solid sample is set in a closed vessel until the volatile components reach equilibrium between the sample and the gas volume above, i.e., the so called "headspace". The advantage of the headspace sampling is that direct liquid or solid probing is avoided and complex sample matrix in a liquid or solid sample can be simplified or even eliminated in its vapor phase[1]. The basic principle of headspace gas chromatography can be

found in textbooks[2] and review articles[3 - 5]. The key in HSGC is the sampling and transfer of the samples in headspace to GC. Several techniques have been developed and some are commercialized, such as "purge and trap", vial pressurization for static headspace sample transfer and multiple headspace extraction (MHE) in static headspace. Solid phase micro extraction (SPME) attracted great attention for its capability in analyzing at the part per billion (ppb) levels[6]. With the advances in gas chromatographic technology, the application of HSGC has been broadened significantly. Recent trends in HSGC application have shifted to agricultural and food science[7] pharmaceutical and medical analysis[8], and environmental analysis[9]. Review of different sample transfer methods and their applications can be found in the article by Snow and Slack[10].

Regulatory agencies and pharmacopoeias suggest headspace gas chromatography as the most suitable and applicable technique for residual solvent testing for active substances, pharmaceutical quality control and formulations soluble in water. Residual solvent specification limits, set in accordance with the toxicity of solvents, vary from a few ppm to thousands of ppm. The amount of such solvents is therefore limited by international conference on harmonization (ICH) guidelines[11]. The ICH recommends and limits the amount of residual solvents considered safe in pharmaceutically finished goods and for human use. The ICH has published guidelines and daily exposure limit of many solvents. Since Class I solvents are highly carcinogenic or toxic, they are generally avoided in pharmaceutical manufacturing. Only ICH Class II and Class III solvents were evaluated during this method development. Few analytical methods have been reported in the scientific literature for the determination of oxacillin sodium in pure and pharmaceutical formulations. These methods are based on spectrophotometry[12 – 14], ion exchange[15], photometric extraction[16], potentiometric[17] and high performance liquid chromatography (HPLC)[18 – 22].

The objective of this study was to develop and validate a new HSGC method with a shorter sample equilibrium time by assessing a number of HSGC parameters for the simultaneous determination of methanol, ethyl acetate and toluene in oxacillin sodium. The method validation was performed to demonstrate the method specificity, accuracy, precision, linearity and sensitivity. There are a number of calibration methods for the determination of residual solvents in drug substances by HSGC including those using external standard, internal standard and standard addition, but there are no significant differences among these approaches with respect to accuracy and precision. Therefore, in our method we used an external standard approach and evaluated the drug substance matrix impacts on residual solvents recoveries using four synthetic small molecule drug substances during method validation. These solvents should be estimated and checked so that they may not exceed the amount specified by the ICH guidelines.

2. EXPERIMENTAL

2.1. Reagents and Chemicals

- Pharmaceutical grade Oxacillin Sodium was purchased from Sigma Aldrich, Germany, and was used as a working standard.
- The drug formulation Bactocill (250mg, Baxter Healthcare), Biocilina® (250mg, Biogalenic), Bristopen®(250mg, Bristol-Myers Squibb), Dicloxal Ox® (250mg, Magma) and Ocillina® (250mg, CCPC) were purchased from a local pharmacy shop. Methanol, ethyl acetate, toluene, and dimethyl sulphoxide (DMSO) were of ≥99% purity and were purchased from Ranbaxy Fine Chemicals Ltd, India.

2.2. Instrumentation and Conditions

A Perkin Elmer (Model Clarus 400, Shelton, USA, Serial No: 646N8111901) HSGC equipped with flame ionization detector was used. The Clarus 400 GC model carries a microprocessor controlled gas chromatograph with an optional built–in auto sampling system. The GC system is supported with the additional equipment Turbo Matrix 40 Sampler. All gas conduits that deliver carrier gas or any detector gas to the Clarus 400 GC must be formed from copper or stainless steel tubing that is free of grease, oil, or other organic materials. The data processing system was run with the Total Chrome Navigator software connected with a PC.

The GC column was elite-5 fused silica capillary column, 30 m long and 0.32 mm in internal diameter. The column temperature was maintained at 50°C for 2 min, then raised at a rate of 20°C per min to 220°C and maintained at 220°C for 2 min. The injection port and detector temperature was maintained at 250°C. The carrier nitrogen gas passed with a velocity of 37.3 cm per second at 10 Kpa pressure and a split ratio of 1: 1. The injections, pressurized, withdraw and thermostat times were 0.1, 2, 0.2 and 10 min., respectively. The gas chromatography cycle time was only 35 min.

2.3. Standard solutions and Sample Preparation

Standard Solution A: Accurately weighed 1000 mg of ethyl acetate were added into a 100 ml volumetric flask containing about 40 ml of dimethyl sulfoxide, the solution was made up to volume with dimethyl sulfoxide and mixed thoroughly.
Standard Solution B: This solution contained 1000 mg of methanol and was prepared as in solution A above.
Standard Solution C: This solution contained 890 mg of toluene and was prepared as in solution A above.
Reference solution A: Pipette 2 ml of standard solution A, 2 ml of solution B and 2 ml of solution C into a 200 ml volumetric flask containing approximately 50 ml of dimethyl sulfoxide. Make up to the required volume with dimethyl sulfoxide and mix thoroughly.

Reference solution B: Pipette 5 ml of standard solution A, 3 ml of solution B and 1 ml of solution C into a 100 ml volumetric flask containing approximately 40 ml of dimethyl sulfoxide. Make up to the required volume with dimethyl sulfoxide and mix thoroughly.

Sample preparation

To determine the content of residual solvents in commercial dosage forms, the contents of 500 mg tablets were weighed and finely powdered. A portion of the powder equivalent to 200 mg of active ingredient was weighed accurately, stirred well with 20 ml DMSO and let stand for 10 min. The residue was filtered on Whatmann No. 42 filter paper (Whatman International Limited, Kent, UK) and washed with DMSO. The residue was washed well with DMSO for complete recovery of product and was further diluted to give a final concentration of 2 mg/ml. An aliquot of the diluted solution was analyzed for residual solvent content following the recommended procedure.

3. RESULTS AND DISCUSSION

3.1. Method development and validation

3.1.1. System suitability

The performance of the system was evaluated by injecting the reference solution A, each and every day before starting the analysis. The number of theoretical plates and resolution between each peak was determined. The relative standard deviation of six replicate injections of reference solution A was calculated to study the system repeatability. The % RSD of methanol, ethyl acetate and toluene were found to be 1.96, 2.59, and 5.13, respectively (The ICH limit is 10%).

3.1.2. Linearity and linear range

To investigate the linearity of the proposed method for residual solvent, a range of concentrations (100-1500 ppm) were prepared and injected. The linear equation of each solvent was determined by linear regression. The results are reported in Table 1. The correlation coefficient was in the range of 0.9996 to 0.9998. The linearity range of methanol, ethyl acetate and toluene were 100-1500, 100-800 and 100-500 ppm, respectively.

3.1.3. Instrument precision

Instrument precision was determined by six replicate injections of the reference solution B, and the relative standard deviations (RSD) of peak area of the solvents were calculated to evaluate the repeatability. The % RSD of methanol, ethyl acetate and toluene were found to be 0.263, 0.368 and 0.189, respectively (Table 2).

3.1.4. Method precision

Method precision was determined by three replicate injections of the sample solution, and the relative standard deviations (RSD) of peak area of the solvents were calculated to evaluate the method repeatability. The average of three replicate injections of the sample solution showed % RSD for methanol and ethyl acetate to be 1.93, 0.596%, respectively. The toluene was not detected. The results for method precision are found to be under acceptable limit for each residual solvent (Table 3).

3.1.5. Accuracy

Accuracy was determined by using the proposed method in which known amount of each solvent corresponding to 50%, 100% and 150% was spiked with reference solution A. The accuracy was then calculated as a percentage of analyte. From the result, it is evident that the recovery of each solvent ranged from 97.92% to 101.59% (Table 4).

3.1.6.Limit of Detection (LOD) and limit of quantitation (LOQ)

The analyte of a sample at limit of quantitation can be quantified with acceptable precision and accuracy. Limit of detection is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact amount. LOD and LOQ were calculated using the signal to noise ratio (S/N) method. Three replicate solutions were injected and the % RSD was calculated. The results are shown in Table 5.

3.1.7. Robustness and ruggedness

To study the robustness of the validated method of reference solution A, some variations from the normal conditions were assessed including a change in column flow, increase or decrease in vial equilibrium time, column temperature and injector temperature. The results (not shown) indicate that the flow rate increases from 1.43 % to 5.13 % and decreases from 2.84 % to 7.04 %, increase in vial temperature from 0.23% to 2.92%, increase in vial temperature from 1.23% to 2.49%. The %RSD of the analyses ranged from 2.13% to 5.03%. The ruggedness tests were performed by using different columns and the analyst. The % RSD was in the range of 2.02% to 6.95%, 3.03% to 6.93% and 3.24% to 6.74%, respectively.

3.1.8. Solution Stability

The reference solution A was prepared and injected after 24 hours. The relative standard deviation of methanol, ethyl acetate and toluene were found to be 1.10, 0.42 and 1.07, respectively (Table 6). The %RSD values are acceptable within a limit of 15 %.

Commercial formulation products

Various pharmaceutical formulations were used including Bactocill (250 mg, Baxter Healthcare), Biocilina (250 mg, Biogalenic), Bristopen (250 mg, Bristol-Myers Squibb), Dicloxal Ox (250 mg, Magma) and Ocillina (250 mg, CCPC). The concentration of solvents present in the selected pharmaceutical producst was determined by using the calibration graph. The concentration of ethyl acetate in each sample was determined (Table 7) whereas methanol and toluene were not present in the formulation products.

Table 1. Analytical characteristics of the proposed method

Solvent	Linearity range (ppm)	Slope	Intercept	Correlation Coefficient	Regression equation
Methanol	100- 1500	1197.5	8251.2	0.9997	Y = 1197.5 X + 8251.2
Ethyl acetate	100- 800	250.89	1115.9	0.9998	Y = 250.89 X + 1115.9
Toluene	100- 500	161.54	891.2	0.9996	Y = 161.54 X + 891.2

Table 2. Instrument precision of the proposed method

Injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Standard - 1	301245	485236	252124
Standard - 2	302587	486124	252125
Standard - 3	302897	485225	251358
Standard - 4	302578	483325	252257
Standard - 5	302956	481589	252785
Standard - 6	301245	482570	252458
Mean	302251	484012	252185
% RSD[a]	0.263	0.368	0.189

[a]Relative Standard Deviation

Table 3. Method precision of the proposed method.

Sample injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Sample injection - 1	11458	1231258	Not Detected
Sample injection - 2	11587	1245896	Not Detected
Sample injection - 3	11895	1236987	Not Detected
Mean	11646	1238047	
% RSD	1.93	0.596	

Table 4. Accuracy and Recovery.

Concentration		**200 ppm**	
Solvent	Peak area	Concentration by graph	% Recovery
Methanol	251240	202.91	101.46
Ethyl Acetate	511047	199.02	99.51
Toluene	33521	201.99	101.00
Concentration		**300 ppm**	
Solvent	Peak area	Concentration by graph	% Recovery
Methanol	360014	293.75	97.92
Ethyl Acetate	77525	304.55	101.52
Toluene	50124	304.77	101.59
Concentration		**500 ppm**	
Solvent	Peak area	Concentration by graph	% Recovery
Methanol	601425	495.34	99.069
Ethyl Acetate	125012	493.83	98.76
Toluene	82138	502.95	100.59

Table 5. LOD and LOQ of the proposed method.

Injection of lowest detection (30 ppm)	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Injection -1	521254	11254	501247
Injection- 2	520257	11325	512589
Injection - 3	521541	11458	503254
Mean	521017.33	11345.67	505696.66
% RSD	0.13	0.91	1.20

Table 6. Solution stability of the proposed method.

Standard	Peak area for methanol	Peak area for ethyl acetate	Peak area for toluene
Injection -1	272580	485214	265478
Injection -2	271251	487512	261874
Injection-3	276985	423478	259933
Mean	273605	485401	262428
% RSD	1.10	0.42	1.07

Table 7. Application of proposed method on formulation products.

Bactocill (250mg, Baxter Healthcare)			
Sample injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Sample injection – 1	Not Detected	1452	Not Detected
Sample injection – 2	Not Detected	1541	Not Detected
Sample injection – 3	Not Detected	1014	Not Detected
Mean		1335.7	
Concentration (ppm)		0.88	
Biocilina (250mg, Biogalenic)			
Sample injection	**Peak area of methanol**	**Peak area of ethyl acetate**	**Peak area of toluene**
Sample injection – 1	Not Detected	1245	Not Detected
Sample injection – 2	Not Detected	1301	Not Detected
Sample injection – 3	Not Detected	1189	Not Detected
Mean		1245	
Concentration (ppm)		0.52	
Bristopen (250mg, BMS)			
Sample injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Sample injection – 1	Not Detected	1620	Not Detected
Sample injection – 2	Not Detected	1452	Not Detected
Sample injection – 3	Not Detected	1354	Not Detected
Mean		1475.3	
Concentration(ppm)		1.43	
Dicloxal Ox (250mg, Magma)			
Sample injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Sample injection – 1	Not Detected	1387	Not Detected
Sample injection – 2	Not Detected	1440	Not Detected
Sample injection – 3	Not Detected	1521	Not Detected
Mean		1449.3	
Concentration(ppm)		1.33	
Ocillina (250mg, CCPC)			
Sample injection	Peak area of methanol	Peak area of ethyl acetate	Peak area of toluene
Sample injection – 1	Not Detected	1152	Not Detected
Sample injection – 2	Not Detected	1245	Not Detected
Sample injection – 3	Not Detected	1312	Not Detected
Mean		1236.3	
Concentration(ppm)		0.48	

CONCLUSION

The above results clearly show that the current developed method is a good analytical technique for the quantitative analysis of residual solvents in Oxacillin sodium. This method is highly sensitive and accurate for the determination of residual methanol, ethyl acetate and toluene in Oxacillin. Till now there is no pharmacopoeias method for the determination of residual solvent for

oxacillin. The present method has a wider linear dynamic range with good accuracy and precision. The relative standard deviation values obtained are accurate and within the limit. Thus, the present method is an attractive method for the determination of residual solvents in Oxacillin Sodium in bulk and pharmaceutical formulations.

REFERENCES

1. J. Y. Zhu, X. S. Chai; 2005. Some recent developments in headspace gas chromatography; Current Analytical Chemistry, 1: 79 – 83.
2. Kolb, B., Ettre, L.S., Static Static Headspace Gas Chromatography : Theory and Practice, Wiley – VCH, 1997 ; New York, p. 26.
3. Hamilton, S.E., Marc, Rossington, M.D., Bertrand, A., 2016. Org. Process Res. Dev. 20: 189 – 194.
4. M., Zaazaa, H., Amer, S., Korany, M., 2015. RSC Adv. 5: 17150 – 17159.
5. Pandey, S., Pandey, P., Kumar, R., Singh, N.P., 2011. Braz. J. Pharm. Sci. 47: 379 – 384.
6. Burrows, D.L., Nicolaides, A., Stephens, G.C., Ferslew, K. E., 2004. Journal of Forensic Science 49: 394 – 397.
7. Miller, B.A., Day, S. M., Vasquez, T. E., Evans, F. M., 2004. Science Justice, 44: 73 – 76.
8. Nikolaou, A. D., Lekkas, T. D., Golfinopoulos, S. K., Kostopoulou, M. N., 2002. Talanta, 56: 717 – 726.
9. Zhu, J.Y., Chai, X.S., 2005. Current Analytical Chemistry 1: 79-83.
10. Snow, N. H., Slack, G. C., 2002. Trends in Analytical Chemistry 21: 608 – 617.
11. ICH guidelines for residual solvents Q3 Step 4, Geneva, Switzerland, 1997.
12. Gujral, R. S., Haque, S. M., Shanker, P., 2009. International Journal of Biomedical Science 5: 373 – 379.
13. Munson, J. W., Papadimitriou, D., Deluca, P. P., 1979. Journal of Pharmaceutical Sciences Pharma. Sci. 68 : 1333 – 1335.
14. Zhiping, L., Jianling, S., 2013. Analytical Instrumentation 4: 12 – 15.
15. Yanga, G., Fenga, S., Liua, H., Yinb, J., Zhanga, L., Caia, L., 2007. Journal of Chromatography B, 854: 85 – 90.
16. Bontchev, P. R., Papazova, P., 1975. Microchimica Acta 64: 503 – 513.
17. Shvedene, N. V., Borovskaya, S. V., 2003. Journal of Analytical Chemistry 58: 1085-1090.
18. Li, F., Qiao, M., Guo, X. 2003. Biomedical Chromatography 17: 53 – 57.
19. Sorensen, L. K., Rasmussen, B. M., Boison, J.O., Keng, L. J., 1997. Journal of Chromatography B: Biomedical Science and Applications 694: 383 – 391.
20. Verdon, E., Couedor, P., 1998. Journal of Chromatography B: Biomedical Science and Applications 705: 71 – 78.
21. Grover, M., Gulati, M., Singh, S. 1998. Journal of Chromatography B: Biomedical Science and Applications 708: 153 – 159.
22. Markovich, R. J., Ong, S., Rosen, J., 1997. Journal of Chromatography Science 35: 584 – 592.