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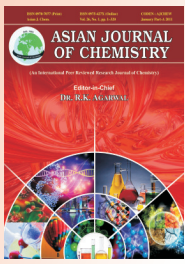

NOTE

Detection of the Related Substances of Methicillin Sodium by RP-HPLC

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To develop and validate a simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method for the determination of methicillin sodium, the HPLC separation was carried out by reverse phase chromatography on Agilent ZORBAX SB-C18 (250 × 4.6 mm i.d. 5 µm particles, Agilent Corporation, USA) with mobile phase which mobile phase A was 10 % acetic acid-0.1 M potassium dihydrogen phosphate-acetonitrile-water (1:50:50:890) and the mobile phase B was 10 % acetic acid 0.1 M potassium dihydrogen phosphate-acetonitrile-water (1:50:500:400). The detected wavelength was 254 nm. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of methicillin sodium. Limit of detection and limit of quantification for methicillin sodium were found to be 0.02 and 0.08 µg/mL, respectively. The proposed method is simple, fast, accurate and precise for quantification of methicillin sodium as well as for routine analysis in quality control.

Keywords: Methicillin sodium, RP-HPLC.

Methicillin was the first semisynthetic penicillin highly effective in penicillinase-producing *Staphylococcal* infections in which activity is conferred mainly by steric hindrance. At doses of penicillinase activity which would completely destroy benzylpenicillin, serum concentrations of methicillin appear to be minimally affected[1-6].

Jusko[7] developed the first fluorimetric determination of penicillins based on the formation of a strongly fluorescent yellow product during acid hydrolysis at elevated temperature of ampicillin and other α-aminopenicillins[8-10].

Methicillin contains a bound group in the 6-position, which can be used as a source of fluorescence emission when excited at appropriate wavelengths. Using this property, a rapid spectrofluorimetric assay method for the determination of methicillin has been developed.

All reagents and spectroscopic solvents pure grade employed for HPLC and GFC analyses were provided by Nanchang University.

Samples were analyzed on a SHIMADZU LC-20A (Shimadzu Corporation, Kyoto, Japan) equipped with diode-array detector using a Agilent ZORBAX SB-C18 (250 × 4.6 mm i.d. 5 µm particles, Agilent Corporation, USA). For chromatographic separations, mobile phase A was 10 % acetic acid-0.1 M potassium dihydrogen phosphate-acetonitrile-water (1:50:50:890). The mobile phase B was 10 % acetic acid 0.1 M potassium dihydrogen phosphate-acetonitrile-water (1:50:500:400). The HPLC gradient program was time (min) /%A(v/v): 0/85, 30/0, 45/0, 55/85 and 60/85. Flow rate was 1 mL/min. PDA detector wavelength was 254 nm. Column oven temperature was maintained at 30 °C.

The methicillin sodium sample was prepared in diluent (water) at 1 mg/mL concentration and 20 µL of sample solution was injected into HPLC system.

System suitability: As per USP 27 system suitability tests were carried out on freshly prepared. Standard solution of methicillin sodium to check the various parameters. Such as efficiency, retention time and peak tailing which was found to comply with USP requirements. The instrumental precisions as determined by six successive injections of the standard solution give RSD below 2% of retention time and area.

Linearity: 0.1 g methicillin sodium sample was weighed accurately in 10 mL volumetric flask. The sample was dissolved by water and diluted to the scale. Then 0.60, 0.9, 1, 1.125, 1.20 and 1.50 mL of the solution were measured into 10 mL volumetric flask. These prepared solutions were injected into HPLC. According to the peak area and concentration, we calculated the linear regression equation. The linear regression equation was A = 0.766C + 220.42, r = 0.9993.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the

developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for methicillin sodium was found to be 0.02 µg/mL. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of methicillin sodium was found to be 0.08 µg/mL. It was concluded that the developed method is sensitive.

Reproducibility: In 0, 1, 2, 3, 4, 5, 6, 7, 8 h, reference solution of methicillin sodium was injected continuously. Result indicated the RSD was 1.2 %. Ruggedness of the method was determined by different analysts, different columns and different instruments on different days. Relative standard deviation was found below 2 %. The results indicating that the developed HPLC method was found to be precise.

Accuracy: The accuracy of the proposed analytical method was determined by recovery experiments. The recovery studies were carried out at three different concentration levels in triplicate (80, 100 and 120 %). The analyzed samples yielded high recovery values from the developed method. The recovery results of the method were 97.05, 100.08 and 95.32 %.

Precision: The precision of the method for the determination of methicillin sodium was studied using the parameters like system precision, method precision and intermediate precision. System precision was determined by six replicate injections of standard solution injected in to the HPLC system. The relative standard deviation was less than 2 %. Method precision was determined by the six individual sample preparations injected to the HPLC system. The relative standard deviation was less than 2 %. Ruggedness of the method was determined by different analysts, different columns and different instruments on different days. Relative standard deviation was found below 2 %. The results indicating that the developed HPLC method was found to be precise.

Sample test: The samples of methicillin sodium were analyzed by the developed HPLC method. The result was shown in Table-1.

TABLE-1
RESULTS OF TEST SAMPLES

Batch number	201403001	201403002	201403003
Content (%)	0.42	0.55	0.54

Conclusion

A convenient and rapid RP- HPLC method has been developed for estimation of methicillin sodium. The assay provides a linear response across a wide range of concentrations. Low % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of methicillin sodium as well as for routine analysis in quality control.

REFERENCES

1. C. Lozano, C. Aspiroz, Y. Saenz, M. Ruiz-Garcia, G. Royo-Garcia, E. Gomez-Sanz, F. Ruiz-Larrea, M. Zarazaga and C. Torres, *J. Antimicrob. Chemother*., **67**, 2804 (2012).
2. B. Li, S. Wendlandt, J. Yao, Y. Liu, Q. Zhang, Z. Shi, J. Wei, D. Shao, S. Schwarz, S. Wang and Z. Ma, *J. Antimicrob. Chemother*., **68**, 1251 (2013).
3. S. Wendlandt, B. Li, Z. Ma and S. Schwarz, *Vet. Microbiol*., **166**, 650 (2013).
4. X.-S. Li, W.-C. Dong, X.-M. Wang, G.-Z. Hu, Y.-B. Wang, B.-Y. Cai, C.-M. Wu, Y. Wang and X.-D. Du, *J. Antimicrob. Chemother*., **69**, 1424 (2014).
5. S. Wendlandt, C. Lozano, K. Kadlec, E. Gomez-Sanz, M. Zarazaga, C. Torres and S. Schwarz, *J. Antimicrob. Chemother*., **68**, 473 (2013).
6. S. Wendlandt, B. Li, C. Lozano, Z. Ma, C. Torres and S. Schwarz, *J. Antimicrob. Chemother*., **68**, 1679 (2013).
7. W.J. Jusko, *J. Pharm. Sci*., **60**, 728 (1971).
8. E. Puigdellivo, G. Obiols and J. Moreno Dalmau, *Afinidad*, **30**, 749 (1973).
9. M. Ortega and B. del Castillo, *Afinidad*, **30**, 651 (1973).
10. F. Belal, A. El-Brashy and F. Ibrahim, *Microchem. J*., **39**, 106 (1989).