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Method development and validation of meropenem in pharmaceutical dosage form by RP-HPLC

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An analytical reversed phase HPLC (RP-HPLC) method has been developed and validated for the estimation of meropenem using RP-HPLC column [Inertsil C18 (4.6 mm × 250 mm) 5µ]. The validation parameters has been followed as per FDA and ICH specifications under the study. Water, acetonitrile and methanol have been used as mobile phases in specific composition. The *p*H of the mobile phase is adjusted with phosphoric acid. The isocratic flow rate of the mobile phase is 1.5 mL/min with UV detection of 300 nm. The method is validated for accuracy, linearity and precision with analytical samples. Moreover, the specificity of the method has determined by the comparison of the drug content in a marketed preparation of meropenem solution. The linearity has been found to be more than 0.999 as well as the precision and accuracy values are less than 2% and from 95% to 105%, respectively. The developed and validated method is found to be the most suitable for quality control programs and can be used as fast, economic, accurate and reproducible.

Keywords: Meropenem, RP-HPLC, Method validation, Pharmaceutical dosage form.

Meropenem is a carbapenem broad-spectrum third generation antibiotic, which is actively used against Gram-positive and Gram-negative organisms. This drug is used as a single agent for the treatment of skin and skin structure infections caused by *Staphylococcus pyrogens*, *Streptococcus aureus*[1-3].The bactericidal activity of meropenem affects through the inhibition of cell wall synthesis. Generally, Meropenem readily penetrates the cell wall of the most Gram-negative and Gram-positive bacteria to achieve penicillin-binding- protein (PBP) targets. Its strongest affinities are towards PBPs 2, 3 and 4 of *Pseudomonas aeruginosa* and *Escherichia coli*; and PBPs 1, 2 and 4 of *Staphylococcus aureus*[4].

The Code of Federal Regulations (CFR) 311.165c explicitly states that "the accuracy, sensitivity, reproducibility and specificity of test methods employed by the firm shall be established and documented". The parameters performed for the development of method validation can be used to determine the consistency, reliability and quality of analytical results. The demonstration of the validation of analytical procedures is directed into the four categories of analytical procedures such as identification tests, quantitative tests for impurities content and limit tests for the control of impurities and quantitative tests of the active moiety in samples of drug product or drug substances or other selected components in the drug product[5]. Meropenem is commonly used as intravenous injections. Method used in formulation of Drug Delivery System (DDS) undergoes into various processes such as solvent evaporation or solvent removal techniques, may lead to degradation of the drug content. The method validation of Meropenem have to be perform that it could achieve at most precise and accurate therapeutic efficacy by the techniques and procedure of formulation which we have done and come to know, whether our formulation is potential in resulting desired therapeutic effect. Thus, the objectives of the present study were to develop and validate the authentic RP-HPLC method for the detection of (i) pure Meropenem, (ii) the drug content of Meropenem in marketed preparation[6]. Considering the importance of RP-HPLC method, attempts have been made to develop inexpensive, fast, effective and reproducible liquid chromatographic methods for the validation of meropenem in pharmaceutical dosage form by RP-HPLC and the results are presented herein. The validation of meropenem is necessary whenever the conditions changes, therefore the method have been validated. The literature reveals that few expensive works have been done for estimation of analytical method validation of meropenem pure drug and dosage form[7-29].

Experimental Section

Chemicals and reagents

The marketed preparation of Meropenem for injection (IP) was purchased from a pharmacy containing 500 mg Meropenem and 45.10 mg sodium carbonate. Chromatographic grade acetonitrile (Merck), analytical grade 85% Orthophosphoric acid buffer salts (Merck), Tetrabutylammonium hydroxide (Himedia) and ultra-pure water (UPW, Milipore®, 0.22 μm filtered) were used for validation analysis.

Chromatographic conditions

High Performance Liquid Chromatography (HPLC) instrument (Shimadzu LC-2010HT), equipped with a ultra-violet (UV) detector, rheodyne manual injector and 20 μL sample loop was used in the study. HPLC analyses were conducted with an analytical reversed phase column (Inertsil C18, 4.6 mm × 250 mm, 5μ- Shiseido) and 50 μL glass syringe for chromatography (SGE, Australia).

The mobile phase used was consisting of water, acetonitrile and methanol in a ratio of 15:3:2. The *p*H of the mobile phase was adjusted at 7.5 with 10% v/v phosphoric acid. All samples were analysed with isocratic flow rate of 1.5 mL/min as well as with column temperature of 25°C and 300 nm UV detection. The mobile phase is used as diluent in throughout the process. Reconstitute 1 vial (1g) of test sample with 20 mL of water for injection. Quality Control (QC) concentrations were then prepared at 55, 110 and 165 ppm, as the respective low, medium and high concentration control samples. These concentrations were selected based on recommended guidelines by the International Conference on Harmonisation (ICH) and Food and Drug Administration (FDA) for analytical method validation[30-35]. Also the standard solution of meropenem was prepared.

Method validation

The linearity of the method was evaluated by standard solutions (QC sample preparations) within the concentration range of about 55 mg (50%), 88 mg (80%), 110 mg (100%), 132 mg (120%), and 165 mg (150%) of Meropenem. The samples were injected to the HPLC system and the area response was recorded. The peak area was plotted against drug concentration (in ppm) and the linearity was thus calculated by the linear regression equation $y = mx + c$.

The accuracy of the method was determined by measurement of recovery. Recovery solutions were prepared by spiking meropenem to placebo powder to obtain solutions in the range of 50 to 150% (i.e. at 55, 110 and 165 ppm) of the target concentration of Meropenem in triplicate. The accuracy of the method for assay of Meropenem was demonstrated at 110 ppm concentrations and for assay of recovery solution 55, 110 and 165 ppm concentration preparations were used. Percentage recovery was calculated for the intra-day assay experiments. The precision of the method was assessed by the study of Inter–day Assay (intermediate precision) and Intra-day Assay (Method Precision and repeatability). For estimation of system precision, the working standard solution was prepared and injected in six replicates into the HPLC system same as done in determination of accuracy. The peak area was recorded. The mean and RSD were calculated and evaluated with reference to one calibration curve on the same run.

The Intra-day assay study was carried out as described in ICH guidelines on six samples prepared from the same homogenous blend of powder of Meropenem 100 mg and the percentage assay was calculated. The mean, RSD and 95% confidence interval of the assay results obtained from six sample preparations were calculated. For Inter-day assay study, the analysis was carried out on six sample solutions prepared as described in repeatability study by different analysts, on a different day, using a different HPLC system and a different lot of column.

The specificity of the developed HPLC method was evaluated by the analysis of aqueous meropenem by spiking pure substances with 5 replicate injections of sample in different stress condition i.e (i) Treat test with 10 mL of 0.1N NaOH solution and kept for 10 min. (ii) Treat test with 10 mL of 0.1N HCl solution and kept for 10 min. (iii) Treat test with 10 mL of 3% Hydrogen Peroxide and kept for 10 min, (iv) Heat solution with 10 mL of water at 70°C for 10 min, (v) Keep the solution in UV light for 1 h. The assay result is unaffected in the presence of excipients by compression with the assay result obtained by the spiked and unspiked sample which is calculated by Peak Purity Index and Threshold.

Results and Discussion

Linearity

A linear relationship was obtained between the peak area of Meropenem and the corresponding concentration. The mean standard calibration curve was plotted. The calibration curve exhibited the linearity over the concentration range of 50 to 150% i.e. 55 to 165 ppm of Meropenem and correlation coefficient was

found to be 0.99952, which is well within the acceptance criteria of not less than 0.999. Hence, it was concluded that the method is linear and in range. The % relative standard deviation (RSD) for the five replicate injections of Meropenem standard solution is less than 2.0%.

Accuracy

The percentage recovery for Meropenem at 50, 100 and 150% of target concentration of Meropenem ranges from 99.19 to 99.70% which is well within the acceptance criteria of 95.0 to 105%.

Precision

The result for intra-day precision (Method Precision) is calculated by RSD for areas of peak due to Meropenem for 6 replicate injections of the standard solution is 0.08%. The result for intra-day precision (Repeatability) is calculated by RSD of the assay results for six individual sample preparations in the repeatability study and is 1.15%. On the other hand, the inter-day precision (Intermediate) results generated from the samples of 12 sets of Analyst-1 and Analyst-2 with the method indicated. The RSD for the assay results of six individual sample preparations in the intermediate precision is 0.26%. Therefore all the results were within the acceptance criteria of not more than 2.0%. The results are depicted in the Table 1.

Specificity

The most suitable isocratic conditions to resolve Meropenem with a C-18 column after the chromatographic conditions optimized for specificity, resolution and retention time was analysed. When the ratio of the mobile phase was altered or any polar solvent other than acetonitrile was used, the resultant chromatograms either had an increase in background noise or peaks indicated the tailing effect. Pure Meropenem was found to be eluted at a retention time of 7 min, as shown in the chromatogram Fig.1. The procedure is performed same as done in the method development in selection of mobile phase ratio and temperature for reliable and efficient validation. Besides, no interference was observed due to blank and placebo at the retention time of meropenem in standard solution and sample solution chromatograms. The Purity Index at different stress conditions was found greater than threshold. Peak purity index greater than the threshold

Table 1 — Accuracy and precision values of quality control (QC) samples of meropenem.

Qc sample % concentration	Accuracy	Precision		
		Intra-day (Method)	Intra-day (Repeatability)	Inter-day (Intermediate)
50 %	99.70	1818324	0.194	99.97
100%	99.53	1818447	0.332	99.91
150%	99.19	1817327	0.092	100.53
		RSD- 0.085%	RSD-1.15%	RSD-0.26%

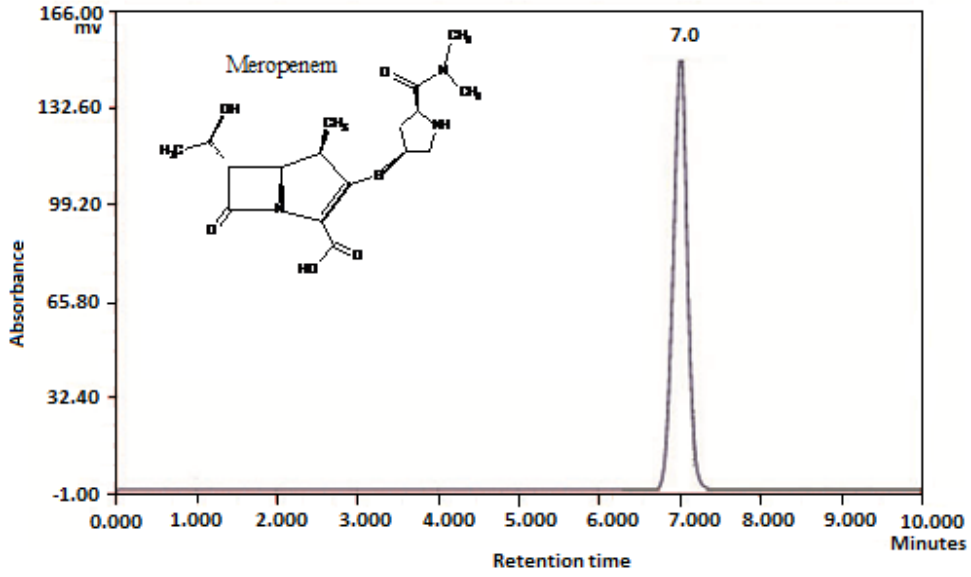

Fig. 1 — Chromatogram showing the optimum retention time

indicate that there is no evidence of spectral heterogeneity or no evidence of co-elution.

System suitability

Evaluation of system suitability is performed by injecting repetitive injection (n=6) of Meropenem (100 µg/mL) to the chromatograph. Inject the test preparation into the system, record the chromatogram and measure the peak response for Meropenem. It is evaluated before and after every experimental work performed. The system suitability parameters of RSD, tailing factor and theoretical plates for the peak under the study were calculated and presented in the Table 2. While the chromatograms in Figs 2(a) and (b) are showing the retention time of meropenem injection 1 and injection 5 on RP-HPLC.

The absolute difference between the assay results obtained in the repeatability of sample-1 and those obtained by carrying out modifications in the method are not more than 2.0%. The modifications should not affect the system suitability criteria. The absolute difference between the assay results obtained in the

Table 2 — System suitability test result for component for meropenem

S.N.	No. of Injection	Retention time (min.)	Area (mV.s)	Tailing factor	Theoretical plates
1	1	7.23	1818447	0.38	3650
2	2	7.06	1811689	0.39	3467
3	3	7.22	1818324	0.38	3644
4	4	7.17	1817327	0.39	3580
5	5	7.15	1815937	0.38	3506
6	6	7.09	1814904	0.39	3489
	Mean	7.15	1816104.7	0.38	3556
	%RSD	0.95	0.14	1.42	1.84
	Limit of % RSD	2.0 %	2.0 %	2.0 %	2.0 %
	Result	Pass	Pass	Pass	Pass

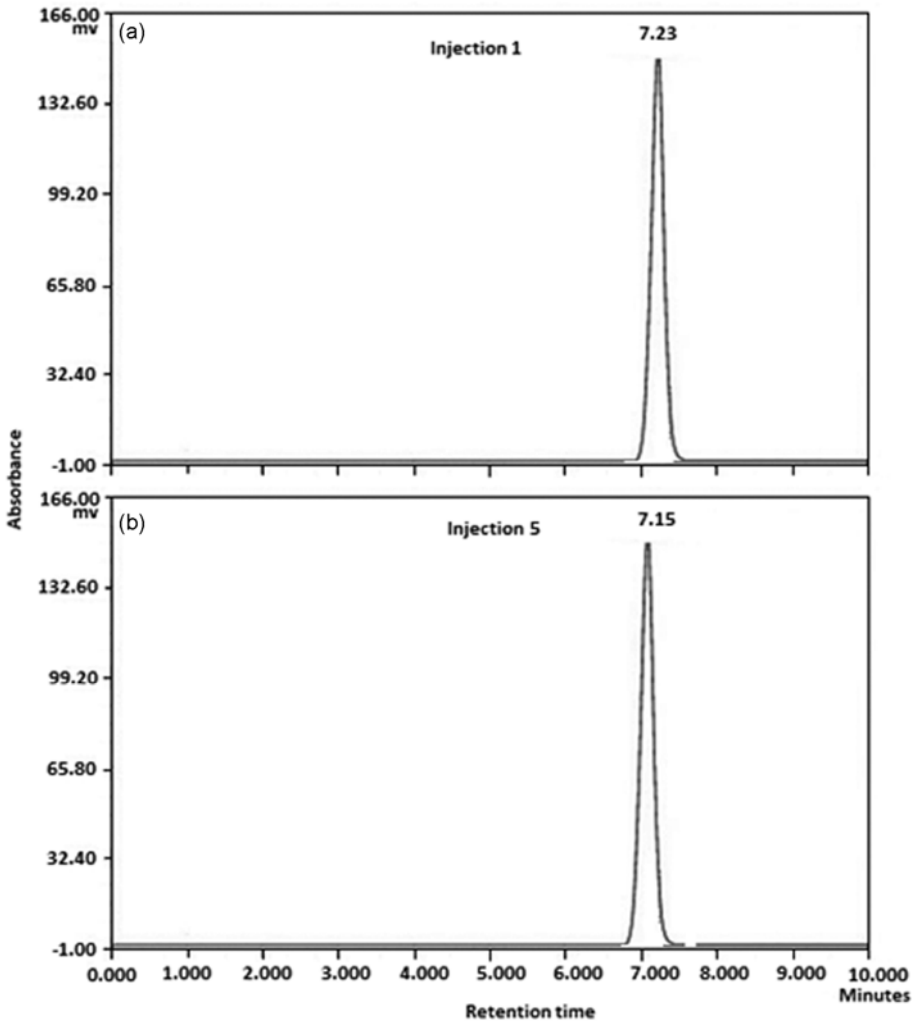

Fig. 2 — (a) Retention time of injection 1 and (b) Retention time of injection 5.

Table 3 — Recovery of solution stability

Time period	% Assay
After 0 h	101.74
After 3 h	10025
After 6 h	98.93
After 12 h	95.78
After 18 h	93.00
After 24 h	89.21
Avg.	96.49
STD	4.76
%RSD	4.93

robustness studies and those obtained in repeatability were well within the acceptance criteria of not more than 2.0%. The system suitability parameters like % RSD for six replicate injections of standard solution, tailing factor and theoretical plates were not significantly changed with altered conditions.

Solution stability

Five injections of sample solutions are given after 3, 6, 12, 18 and 24 h respectively stored at room temperature for determining a suitable time period and under prescribed storage condition where, analytical solution remain stable. The recovery of solution stability is obtained by calculating % RSD of % assay of every determinant. The obtained results for standard solution are given in Table 3. The % relative standard deviation for the five replicate injections of Meropenem standard solution is 4.93% which is found within acceptable limit i.e. not more than 5%.

Meropenem was successfully detected with reversed phase HPLC utilizing a higher percentage of buffered water in its mobile phase. The reliability and sensitivity of the validated method was ensured with good linearity, accuracy and precision within the ICH and FDA limits for method validation of analytical samples.

In the present study, simple sample preparation and good chromatographic separation with less run time for sample analysis offers high throughput quantization. Specificity study reveals that the method can quantitatively measure the compound of interest i.e. Meropenem in the sample matrix without any interference from other components. Linearity study reveals that the test results are directly proportional to the analytic concentration over a wide range. Intermediate precision study shows that the method is resistant to within laboratory variations.

Conclusion

The RP-HPLC method developed for the detection of meropenem has been successfully performed in laboratory. Moreover, the developed method is found to be reliable, sensitive, reproducible and inexpensive. Therefore, the specific detection of meropenem in a common marketed preparation can be used not only for routine quantitative analysis but for qualitative analysis. Finally, on the basis of obtained results, it is concluded that the selected method for the validation of meropenem by RP-HPLC was found to be specific and precise under the study.

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