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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF DAPTOMYCIN

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ABSTRACT: A simple, Accurate, precise method was developed for the estimation of the Daptomycin in Tablet dosage form. The detection was carried out by using PDA detector at 223nm.Phenomenex IB-SIL C_8 column was used for the study as this yield peak of good shape.Buffers of different pH were tried. 3.4g/L $NH_4H_2PO_4$ adjust P^H to 3.1 ± 0.05 with H_3PO_4 was finally used because of the good symmetrical peaks.. Injection volume used was 25µl .This has yielded peaks of good shape without any splitting. The flow rate was fixed at 0.9ml/min. This has eluted the drug around 37min.By using the method the retention time of the Daptomycin was found to be 37.74min.System suitability conditions meet the recommended criterion.Linearity of the solution was demonstrated between 0.1506 mg/ml and 0.4519 mg/ml concentration range.Accuracy was demonstrated as reported. Recovery and % of RSD are within the recommended limits.Limit of detection value is at 0.0000154mg/ml and limit of quantification is at 0.0000467mg/ml. Under forced degradation studies, the peak single point threshold is always lower than purity index for Daptomycin peak and all degradant peaks were well resolved. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test inIndustries.Hence the method can be adopted as a stability indicating method.

Keywords: Quality control, Daptomycin, Recovery, Wavelength.

INTRODUCTION:

Pharmaceutical Analysis is that centre branch of drug store training and research, which is advancing speedy. It can be arranged as union of new medications particles and pharmaceutical investigation.The liquid chromatographic techniques, the pivoted arrange systems in perspective of balanced silica offers the most imperative probability of triumphs. In any case, an extensive number of (structure) factors (parameters) impact the selectivity and the assurance. Trade legitimate methods are made for the prescription thing to diminish the cost and time[1]. Then evaluate the best division condition from trial runs. In the wake of improving the separation condition, favour the procedure for release to routine research focus.Daptomycin arepale yellow crystalline powder, both the medicine are freely soluble in methanol and practically insoluble in water[2]. Themechanismofactionofdaptomycinisdistinctfromthatofanyotherantibiotic. Daptomycin bindstobacterialmembranesandcauses arapiddepolarisationofmembranepotential inbothgrowingandstationaryphasecells.

Thislossofmembranepotentialcausesinhibitionofprotein,DNA,andRNAsynthesis. Thisresultsinbacterialcelldeathwithnegligiblecelllysis. Nausea,vomiting, constipation, diarrhoea, headache,dizziness,trouble sleeping, anxiety, or pain/redness/swelling at the injection site may occur. Cubicin may cause serious muscle damage, causing muscle pain or weakness, pseudomembranous colitis, causing diarrhoea.It may affect nerve conduction, causing tingling, numbness, burning, or weakness.

MATERIALS AND METHOD:

Instrumentation: Shimadzu Prominence HPLC system equipped with UV/PDA detector, auto injector, binary high pressure gradient pump, 100µl sample loop and a column heater, LC solutions data handling system was used for the analysis. Phenomenex IB-Sil column,having length of 250mm, 4.6mm internal diameter, 5µm film thickness having C_8 stationary phase with fully porous silica solid support of reverse phase separation mode used for method development and validation. The data was recorded using LC 2010 solutions software

Materials and Reagents: Standardized known purity of Daptomycin from the CoA was used as Daptomycin standard.HPLC grade acetonitrile was used as the solvent as well as the mobile phase. It was procured from Rankem, India. Ammonium dihydrogen phosphate, Sodium hydroxide, Hydrochloric acid, Hydrogen peroxide was purchased from Rankem, India.All the reagents and chemicals are of analytical grade except HCl which is of gradient reagent grade. Pure water for the analysis was prepared by using Millipore Milli-Q purification system.

Method:

Diluent: The nature of the drug reveals certain information about the drug such as solubility, pk_a. Based on the solubility of the drug, the diluent is selected. The solvent in which the drug has maximum solubility is selected as the diluents

Preparation of Standard stock solutions: Daptomycin drug substance (30.69mg) was accurately weighed into a 100ml volumetric flask, dissolved and made up to volume with diluent (concentration 0.3069 mg/ml).

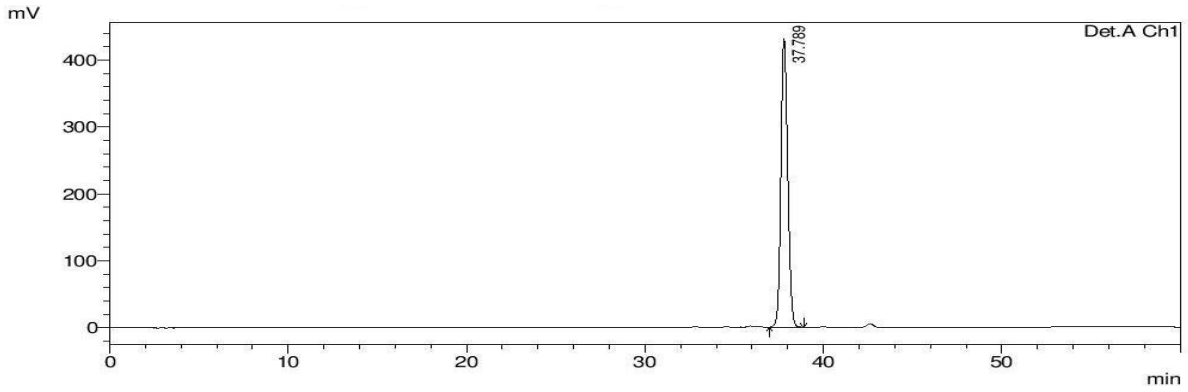
Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Selection of mobile phase: For efficient separation various mobile phases of different composition are used. Initially elution was done using different buffers such as ammonium dihydrogen phosphate adjusted P^H with ammonia buffer and phosphoric acid buffer. When ammonium dihydrogen phosphate adjusted P^H with ammonia buffer was used there was no proper resolution of peaks. So ammonium dihydrogen phosphate adjusted P^H with phosphoric acid buffer was then used for separation. Ammonium dihydrogen phosphate adjusted P^H 3.1± 0.05 with phosphoric acid along with Acetonitrile was used as the mobile phase.

RESULTS AND DISCUSSION:

25 µl of the blank, stock and sample was administered into the chromatographic system and areas for the peak were used for computation with flow rate 0.9ml/mincolumn employed Shimadzu seperations module with PDA/UV Detector connected to LC solution software wavelength 223nm, column temperature 25°C, run time 60min. diluent used were Milli Q Water in the ratio 50:50 hence both peaks have good resolution, tailing Factor, theoretical plate count and resolution. Daptomycin was eluted at 37.74min.respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.The optimized chromatogram given fig.1

Fig.1: Optimized Chromatogram



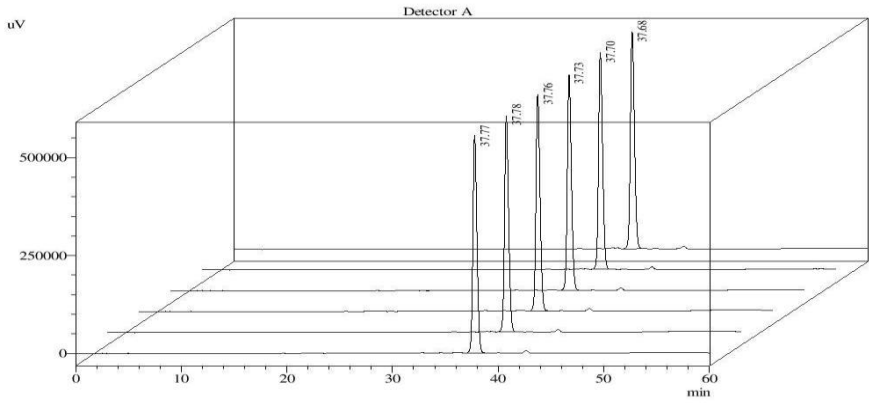
Validation Parameters:

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines[3]. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The results were given in Table 1 and Fig. 2.

Table 1: Systemsuitabilityparameters forDaptomycin

Injection Number	**Retention Time**	**Peak Area**	**Theoretical plates**	**Tailing factor**
Injection – 1	37.68	14950534	44210	1.2
Injection – 2	37.70	14958668	44271	1.2
Injection – 3	37.73	14914628	43948	1.2
Injection – 4	37.76	14914948	44788	1.2
Injection – 5	37.78	14926741	44254	1.2
Injection – 6	37.77	14931230	45220	1.2
Mean	**37.74**	**14932791**	**44448**	**1.2**
Acceptance	**< 1.0%**	**< 2.0%**	**≥ 2500**	**< 2.0**
Result	**Pass (RSD – 0.11)**	**Pass (RSD – 0.12)**	**Pass**	**Pass**

Fig. 2: SystemsuitabilityChromatogram



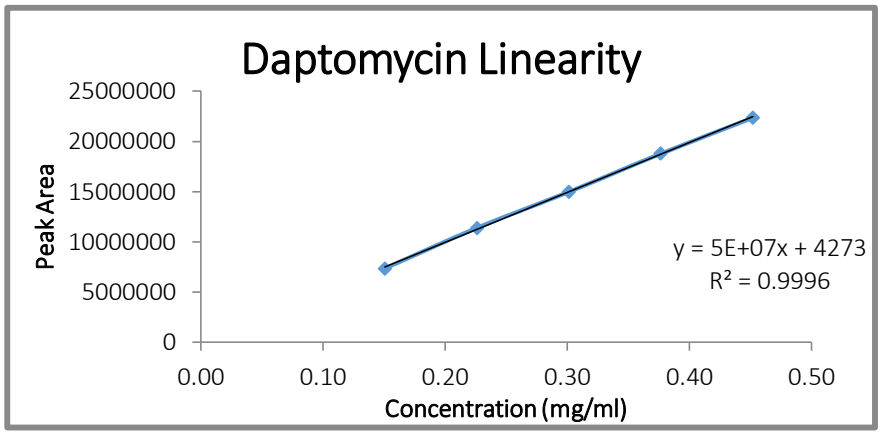
Specificity: Retention times of Daptomycinwere 37.74minrespectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific and the result were given in fig. 2.

Linearity: Six linear concentrations ofDaptomycinwere injected Correlation coefficient obtained was 0.999. The results are mentioned in table 2 and fig. 3.

Table 2: Linearity table for Daptomycin

Level Solutions	Concentration (mg/ml)	Area Response	Average Area Response
50%	0.15064	7340697	7351496
		7362294	
75%	0.22596	11405853	11386681
		11367508	
100%	0.30128	14927682	14979526
		15031370	
125%	0.37660	18786366	18788391
		18790416	
150%	0.45192	22372353	22362431
		22352509	

Fig. 3: CalibrationcurveofDaptomycin

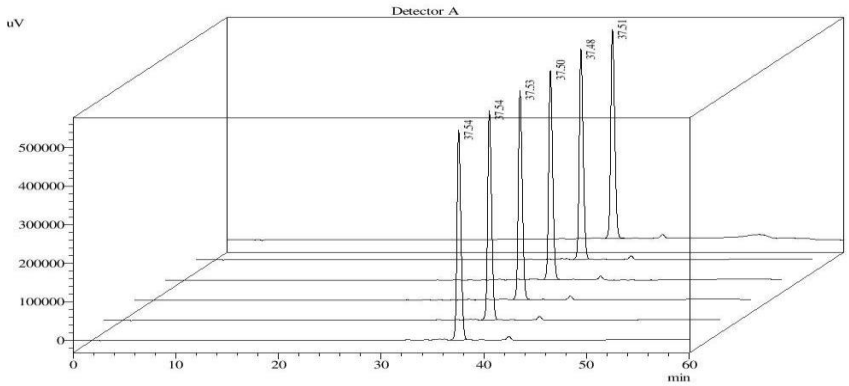


Reproducibility: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given. Injection reproducibility was demonstrated and the % of relative standard deviation for retention time and area were within the limits. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.15% and 99.65% for Dapomycin respectively.

Sensitivity: Limit of detection value is at 0.0000154mg/ml and limit of quantification is at 0.0000467mg/ml.

Fig. 4:LOD& LOQ Chromatogram ofStandard drugs Daptomycin



Conclusion:

A simple, Accurate, precise method was developed for the estimation of the Daptomycin. Retention time of Daptomycin were found to be to 37.74min. The sample recoveries of said formulations was agreed with individual label claim amount as per ICH guidelines. Hence the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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