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Validated RP-HPLCMethod for the Estimation of Amphotericin B in Bulk and Pharmaceutical Dosage Form

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Abstract:

For fast assessment of Amphotericin B in bulk, pharmaceutical dose format, a basic, choosy, linear, precise, yet accurate RP-HPLC technique was devised, validated. Under ambient temperature, isocratic elution with flow rate of 1 mL/min was performed using Luna C18 column: - 250 x 4.6 mm; 5µ.The mobile phase was composed of 60:30:10 v/v/v of acetonitrile, tetrahydrofuran, and o-phosphoric acid - pH 6.0 was amended by Triethylamine. UV detection frequency was 287 nm, and also the sample injection volume was 20µl. Amphotericin B had a retention duration of 7.722 minutes.The percent recovery ranged from 99.60 to 100.42 percent. The percent RSD for the method's accuracy and precision was determined to be lower than 2%. The technique was verified in accordance with the International Conference on Harmonization (ICH) criteria. The approach was fruitfully then used analyze Amphotericin B in bulk samples and formulations on a regular basis.

Keywords: AmphotericinB, RP-HPLC, UVdetection, Validation, Analysis

Introduction:

Amphotericin B is an antibiotic that is polyene antifungal [Figure 1]. It has been documented to be fungistatic at clinical doses. In treating for acute systemic fungal infections, amphotericin B is administered as an intravenous infusion including aspergillosis, candidiasis, blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosi, cryptococcosis, mucormycosis, and sporotrichosis, which is a common therapy for choice in fungal endocarditis, peritonitis, meningitis, or severe respiratory-tract infections. [1R-(1R*,3S*,5R*,6R*,9R*,11R*,15S*16R*,17R*,18S*,19E, 21E,23E, 25E, 27E, 29E, 31E, 33R*,35S,36S*,37S*)]-33-[(3-Amino-3,6-Dideoxy-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[13.3.1]nona-triaconta-19,21,23,25,27, 29,31-hepta-ene-36-carboxylicacidis its chemical form [1]. A review of the literature indicated that liquid chromatography [2- 19] and spectrophotometry [20-25] have been used in individual or coupled forms for the detection of Amphotericin B in pure medication, pharmaceutical dosages, and biological materials. The goal of this study is to create and test a simple, quick, and reliable method for measurement of Amphotericin B in bulk and tablet dosage forms, an isocratic RP-HPLC technique incorporating UV detection had been used. The usefulness of the proposed technique for determining Amphotericin B in bulk & tablet dosage form was confirmed per the International Conference on Harmonization - ICH [26].

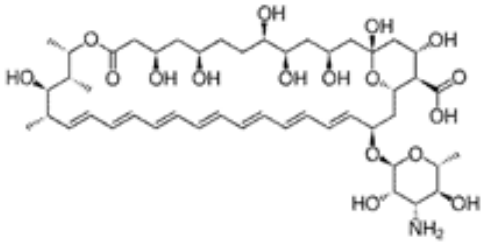

Fig. 1: Amphotericin B – Molecular Structure

Materials and Methods

Chemicals and reagents

Merck Specialties Pvt. Ltd. from Mumbai, India provided HPLC quality acetonitrile, tetrahydrofuran, and o-phosphoric acid. Dr. Reddy's Laboratories from Hyderabad, India, gave an amphotericin B standard sample.

Instrumentation and analytical conditions

A LC-20 AD pump having a Rheodyne model-7161 injection valve of a 20l loop (Rheodyne Inc., Cotati, CA, USA) as well as a UV-visible detector calibrated at 287 nm comprised the HPLC system (Shimadzu, Japan). The Luna C18 analytical column: - 250 x 4.6mm i.d., 5µ particle size was in performed room temperature. Isocratic elution by means of Acetonitrile:Tetrahydrofuran:*o*-phosphoric acid in 60:30:10 v/v/v with pH 6.0 was employed with1 mL/min rate of flow. Before usage, the mobile phase was newly produced and degassed after 5 min of sonification. A UV-Visible spectrophotometer, the Elico SL-159, was used to capture the UV spectrum of Amphotericin B.

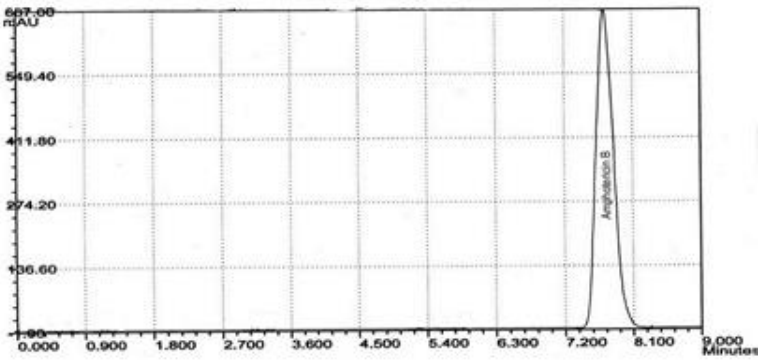

Fig 2: Typical chromatogram ofAmphotericinBthru analysis of standard solution

Preparation of stock and working standard solutions of Amphotericin B

Approximately 100 mg of Amphotericin B was carefully weighed and put to a 100 mL volumetric flask holding 50 mL of methanol. To make a 1mg/mL solution, the solution sonification was run for 5 minutes and then the volume was adjusted with a more mobile phase. This solution was diluted by mobile phase to obtain a workable standard solution containing 100 µg/mL of Amphotericin B, which was then diluted further with mobile phase to obtain a level of 20 µg/mL, and thereafter administered. All of the operational standard solutions were strained through a 13mm membrane syringe filter of pore size 0.2 µm before being analyzed. The column subsequently equilibrated for a minimum 60 minutes with the mobile phase running thru the apparatus before adding solutions. With 7 concentrations of 10-30 µg/mL operational standard solutions, the calibration curve was

produced. Each dilution's chromatogram was collected three times. Calibration solutions had been made on a regular basis and examined as soon as they were ready.

Assay of sample preparation

Amfotex injectable vials were taken in fives. The closures made of aluminium were eliminated. The powders in every vial were combined, and also the mean weight of powder in each vial was determined. In a 100mL volumetric flask, a quantity of powder corresponding to 100 mg Amphotericin B was combined with 50 mL methanol. The flask's components were sonicated for around 20 minutes to ensure full drug solubility, and the volume was increased to 100 mL by mobile phase to have a 1 mg/mL solution. The mix was then strained using a membrane filter of 0.45µ.A 2 mL aliquot of the aforementioned solution was transferred to separate volumetric flask of capacity 100 mL and brought to volume using mobile phase and thoroughly mixed to get a solution with a 20 µg/mL concentration.The column was then filled with the aforesaid solution (20 µL). In the HPLC apparatus, an aliquot of the solution was administered and the peak area of Amphotericin B was measured.

Table 1:statistical analysis of calibration curves intheHPLCdeterminationofAmphotericinB(n=6)

Parameters	Values
λmax(nm)	287
Beer'slawlimit(µg/ml)	10-30
Correlationcoefficient	0.9996
Regressionequation	Y=11004*x* × 737.2
Limitofdetection(µg/ml)	0.18
Limitofquantification(µg/ml)	0.58

Validation procedure

The goal of method validation is always to show that technique is appropriate for its specified function as indicated in the ICH standards. For specificity, linearity, precision (repeatability & intermediate precision), accuracy, short-term sustainability, and system compatibility, the technique was verified. To evaluate linearity, standard graphs were made containing 7 concentrations ranging of 10-30 µg/mL, generated in triplicates. The calibration plot was created by plotting the peak shape of Amphotericin B against concentration. The linearity was determined using the least square regression technique and linear regression analysis. The assay's accuracy was investigated in terms of repeatability as well as intermediary precision. Six replicate shots of newly created Amphotericin B test solution in the same apparatus having a concentration level of 100% (20 µg/mL) of desired sample concentration level same day were used to estimate repeatability. To evaluate intermediate precision, the test was reiterated again evaluating newly produced solution at the very same concentration for 2 successive days. Amphotericin B's peak area was evaluated, while precision was expressed as a percent RSD. By evaluating the Amphotericin B sample at three unique levels of pure solutions employing three separate preparations for every level, the method's accuracy had also been evaluated (percent recovery & percent RSD of separate readings). The Amphotericin B% rallied within samples was used to calculate the findings.

Short-term sustainability of the sample solution had been evaluated over 3 days at ambient temperature (20±1°C). Both solutions shielded against light were subsequently re-injected at 24 and 48h at ambient

temperature then matched to newly produced solutions to validate the stability both of standard solutions at 100 percent level as well as tablet sample solutions.

Results and Discussion

Selection of the detection wavelength

The Amphotericin B - UV spectra in methanol was surveyed in the 200-400 nm range, with a maximum @ 287 nm.

Optimization of the chromatographic conditions

The type of the sample, molar weight, and solubility all play a role in choosing the right stationary phase. Amphotericin B is non-polar. Reverse phase columns are preferred for analyzing non-polar chemicals. The C18 column was chosen among C8 and C18. Using reverse phase columns, non-polar compounds are particularly appealing. As a result, the polar mobile phase impacted the elution of the chemical from the column.

The mobile phase was chosen as a combination of acetonitrile, Tetrahydrofuran, and o-phosphoric acid, and the impact of such mobile phase composition on the holding time of Amphotericin B was carefully studied. Acetonitrile, Tetrahydrofuran, and o-phosphoric acid concentrations were calibrated to produce a symmetric peak having a short operation period (Fiigure 2). In the ratio of 60:30:10v/v/v of acetonitrile, tetrahydrofuran, and o-phosphoric acid, short operation duration and peak asymmetry stability were detected. It was discovered that the concentration of the mobile phase was optimal.

Validation of method

Linearity

7-point calibration plots encompassing a concentration span of 10-30 µg/mL were created (3 separate results at every concentration).The peak region indication of Amphotericin B and the matching drug concentration were shown to have linear correlations. The slope and intercept's standard deviations were both modest. The correlation coefficient (r^2) was greater than 0.9996. Table 1 provides the data analysis of the calibration.

Precision

For the analysis of commercial doses comprising Amphotericin B, the established technique was used. After extraction the medication as described through the experimental section's assay sample prep, the sample was tested six times. The outcomes agrees well with the content labeled. Outcomes of assay given by label claim% was 98.4± 0.8exhibiting injection form of AmphotericinB inveterate content needs to (95–105%) of the label claim. Small standard deviation results indicated that the measurement was highly repeatable. As a result, it demonstrated that the study's equipment was accurate and that the established analytical procedure is very repeatable. Research conducted by the very same analyst same day for 3 successive days (n=3) revealed an R.S.D of 0.0355 for moderate precision. This implies that the approach precision is effective.

Accuracy

The accuracy statistics were represented as a percentage of Amphotericin B recovered in actual samples. The average Amphotericin B recovery statistics in actual samples was between 99.60 and 100.42 percent. The mean percent R.S.D. was 0.31 percent, which met the study's admission requirements. Excipients utilized in tablet formulation were shown to have no impact on the results. As a result, the method's accuracy was validated.

Stability

The Amphotericin B's stability in standardized & sample solutions comprising it was evaluated by keeping them at room temperature (20 ± 1^0c). Following 3 consecutive days of storage, the solutions were tested in triplicate, and the results were compared to newly produced samples. In every scenario, the solutions remained constant for 24 hours, since the findings did not fall below 98 percent throughout that period. This indicates that Amphotericin B is sustainable in standardized & sample solutions @ ambient temperature for minimum a day.

System suitability

Asymmetry element, tailing component, HETP, and amount of theoretical plates were indeed computed as system suitability factors[27-29]. All of values were found to be below the limits (Table 2). The suggested method's statistical assessment demonstrated its good linearity, repeatability, and validity for various parameters[29,30], leading to the conclusion that it may be utilized for the quick and accurate measurement of Amphotericin B in injectable formulations.

Table2: Voriconazole – Systemsuitabilityandstudy

S.No.	Parameters	Voriconazole
1	Tailingfactor	1.32
2	Asymmetricfactor	1.13
3	Theoreticalplates	4515
4	HETP	0.0506

Conclusion:

For assessment of Amphotericin B in injection form, a validated isocratic RP-HPLC technique has indeed been devised. The suggested approach is basic, quick, precise, and accurate. Its 10-minute chromatographic operation time permits it to analyses a big amount of samples in a limited amount of time. As a result, it is appropriate for analyzing Amphotericin B in pharmaceutical dosage form on a regular analysis.

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