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Difference Spectrophotometric Determination of Mitomycin-C

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Difference spectrophotometric determination of mitomycin-C was studied and found that it is preferable over other methods since it is simple, convenient, reproducible and free from interference due to additives, as there was no shift in isobestic points.

MITOMYCIN-C is an antitumor agent used in the treatment of malignant lymphomas, wide range of sarcomas and in other malignant conditions such as metastatic adenocarcinoma of breast[1]. Various methods have been reported for its estimation in dosage forms and in biological fluids. The Super critical fluid chromatographic method has been reported[2] for its assay. In addition to this Voltametry[3] and H. P. L. C.[4] methods have also been described. We hereby describe a sensitive and reproducible, difference spectrophotometric method for estimation of mitomycin-C. This method is advantageous over the others, as it achieves the spectrophotometric isolation of the drug and is simple and convenient in terms of cost and feasibility. Moreover interference due to additives can be nullified if any, as can be proved by no change in isobestic points[5].

MATERIALS AND METHODS

Shimadzu-UV 1601 spectrophotometer (Japan) with 1#cm layer cell was used for measurement of absorbance and recording of UV spectra. Systronic pH meter 324 (India), was used for pH determination.

Mitomycin-C, standard solution was prepared by dissolving it in dimethylformamide and then diluting it with HCl/KCl buffer (pH = 1.2) and phosphate buffer (pH = 10.0) solution respectively. Dimethylformamide, hydrochloric acid, sodium hydroxide, potassium chloride, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate used were of analytical grade.

Different dilutions of drug representing 4-32 μg/ml were prepared by dissolving the drug in dimethylformamide. Two series of dilutions were prepared using HCl/KCl buffer (pH = 1.2) and phosphate buffer (pH = 10.0) respectively. The UV spectra in acidic and alkaline medium were recorded against respective blanks. Difference spectra was recorded by placing acidic solution of known concentration (28μg/ml) in the reference cell and alkaline solution in the sample cell (fig 1). The λ max in acidic solution was found to be at 506, 296, 243 nm and λ max in alkaline solution was found to be at 588, 363, 253 nm. The λmax was found to be at 364, 253 and λ min was found to be at 498, 310, 244 nm, when acidic and basic solution were kept in reference and sample cell respectively, the λ max was found at 364, 253 nm. and λ minimum was found at 498, 310, 244 nm. The isobestic points (points representing zero absorbance corresponding to intersecting points of acidic and alkaline spectra) were recorded at 408 nm and 326 nm which were identical irrespective of the pH of solution kept in reference cell.

A marketed injection of mitomycin-C (2 mg) was analyzed by making solutions of suitable dilution in two series in presence of dimethylformamide and volume was made up with buffer solution of pH 1.2 and pH 10.0 respectively and difference curve was recorded.

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Table 1 : DIFFERENCE STANDARD CURVE OF MITOMYCIN C

S.No.	Concentration μg/ml	Absorbance* 364 nm	300 nm	Amplitude**
1.	4	0.176	-0.026	0.202
2.	8	0.365	-0.037	0.402
3.	12	0.581	-0.035	0.616
4.	16	0.741	-0.047	0.788
5.	20	0.954	-0.065	1.019
7.	28	1.361	-0.083	1.444
8.	32	1.475	-0.072	1.547

* = Average of three determinations

** = Sum of absorbance at 364 nm and 300 nm.

Correlation coefficient = 0.998, Slope = 0.031, Intercept= 0.012

Table 2 : VALIDATION OF THE PROPOSED METHOD

S.No.	Amount added (mg)	Amount found Y1	Y2	Y3	Y4	Y mean±sd	% recovery
1.	1	0.95	1.00	0.95	1.00	0.97±0.02	
2.	2	2.00	1.95	1.95	2.00	1.97±0.04	
3.	3	2.95	2.95	3.00	2.95	2.96±0.03	98.72
4.	4	4.00	3.90	4.00	3.95	3.96±0.2	
5.	5	4.95	4.90	5.00	4.95	4.95±0.08	

% recovery was calculated by $N(\Sigma XY) - (\Sigma Y)(\Sigma X) / N(\Sigma X)^2-(\Sigma Y)^2$ where X is the amount of standard drug added and Y is the amount of drug determined by the method developed in this investigation. N represents the total number of determinations.

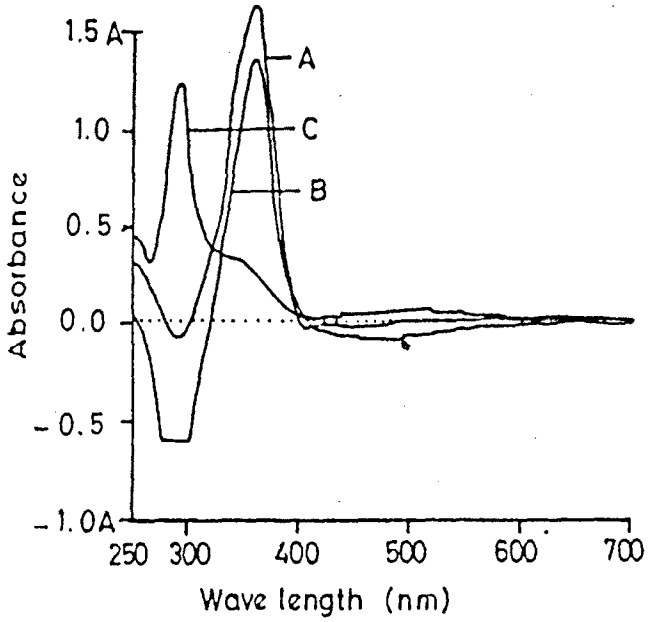
RESULTS AND DISCUSSION

The difference spectra of mitomycin C has been shown in fig.1 Despite getting two peaks and three valleys, the 364 nm peak and the 300 nm valley were selected due to high resolution and all gave linear absorbance concentration plots. The amplitude which is the sum of magnitude of absorbance at the above two wavelengths (364 and 300 nm) was selected for the preparation of standard curve. It is also found that the Beer Lambert's law is obeyed in the concentration range of 4-32 μg/ml. There was no change in the isobestic points which reveal absence of interference due to additives. The equation obtained is as follows:

Concentration = (0.050 x abs) + (0.012)

When a marketed formulation (containing labelled amount 2 mg of mitomycin C in injection) was analysed

Fig. 1: Difference spectra of mitomycin-C



Scanning spectra were recorded from 250-700 nm of the solution of mitomycin C in acidic (A) and basic (B) medium. The difference spectra is represented by (C)

by this method, 1.97 mg of the drug was estimated showing 98.50 percent recovery.

In order to confirm the reliability and suitability of the proposed method, recovery studies were conducted by adding a known concentration of standard drug to the previously analyzed formulation and then the drug contents were estimated. The study reveals that 98.72 percent recovery was obtained [Table 2].

Thus, the difference spectrophotometric method is a simple, convenient and reproducible which can be potentially applicable for the estimation of mitomycin C in injections.

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