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Spectrophotometric Determination of Irinotecan in Pharmaceutical Formulations

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A new simple, sensitive and cost effective visible spectrophotometric method was developed for the estimation of irinotecan in both bulk drug samples and injections. The method was based on the formation of ion pair complexes of the drug with two acidic dyes namely bromocresol green and bromophenol blue in acidic buffer solution followed by their extraction in organic solvent (chloroform).The absorbance of the organic layer was measured at their respective absorption maxima at 420 nm for bromocresol green and 380 nm for bromophenol blue against the corresponding reagent blank. The method obeyed Beer's law between 3.0-37.5 µg/mL for bromocresol green and 1.0-12.5 µg/mL for bromophenol blue.The results of recovery experiments indicated average recovery was above 99.86 % and using the t-statistic, hypothesis was tested for the correlation between the amount added and amount found, at a confidence level of 0.05 (5 %). It was proved that there was a strong correlation between the amount added and amount found. The interference studies also revealed the common excipients and other additives usually present in pharmaceutical dosage forms did not interfere in the proposed method.The proposed method is precise, accurate, sensitive and cost effective and can be used in routine analysis in quality control laboratories.

Key Words: Irinotecan, UV-Visible spectrophotometric, Bromocresol green, Bromo phenol blue, Formulation.

INTRODUCTION

Irinotecan is an antineoplastic agent that is primarily used in the treatment of metastatic colourectal cancer. It is chemically (S)-10-[4-(-piperidino)-piperidinocarbonyloxoyl]-4,7-diethyl-4-hydroxy-1*H*-pyrano-[3,4:6,7]indolizino-[1,2-b]diethyl-3,14[4*H*,12*H*]-dione. The drug is official in Martindale, The Extra Pharmacopoeia[1]. Chemical structure of irinotecan is shown in Fig. 1.

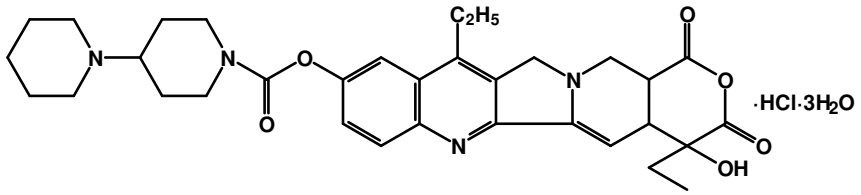

Fig. 1. Structure of irinotecan

It is a semi-synthetic, water-soluble derivative of camptothecin, which is a cytotoxic alkaloid extracted from plants such as *Camptotheca acuminata*. Irinotecan and its active metabolite, SN-38, inhibit the action of topoisomerase I, an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Irinotecan and SN-38 bind to the topoisomerase I-DNA complex and prevent religation of the DNA strand, resulting in double-strand DNA breakage and cell death. Irinotecan is cell cycle phase-specific (S-phase)[2].

Few HPLC methods for quantitative determination of irinotecan were reported in the literature. Majority of these HPLC methods were applied in the determination of irinotecan and it's metabolites in biological fluids[3-8] and are mainly useful for therapeutic monitoring of the drug. No visible spectrophotometric method for quantitative determination of irinotecan in bulk drug samples and injections was reported. The objective of this research was to develop and validate rapid, economical and sensitive visible spectrophotometric method for quantitative determination of irinotecan in bulk drug samples and injectable preparations. Irinotecan is an indolizino derivative, having an amino group in the molecular structure making it possible to form the ion-pair complexes with acidic dyes namely bromocresol green (BCG) and bromophenol blue (BPB)[9-15].

EXPERIMENTAL

Elico-SL-164 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements. Digisun model DI-707 pH meter was used for all the pH measurements.

All the chemicals used were of analytical grade. Bromocresol green (BCG) (0.1 % w/v), bromophenol blue (BPB) (0.1 % w/v), phthalate buffer of pH 2.2 and chloroform.

100 mg of bromocresol green was dissolved in 0.72 mL of 0.1 N NaOH and 20 mL of methanol. After solution was affected, sufficient distilled water was added to produce 100 mL. 100 mg of bromophenol blue was approximately weighed and taken in a 100 mL volumetric flask. To that 1.5 mL of 0.1N NaOH and 20 mL of methanol were added. The solution

was then diluted with distilled water to make up the volume to 100 mL. This solution was treated with methanol to remove methanol soluble impurities. Phthalate buffer (pH 2.2) was prepared according to Indian Pharmacopoeia.

Preparation of standard drug solution: 50 mg of irinotecan was accurately weighed and dissolved in 50 mL of distilled water in a standard volumetric flask to obtain a stock solution of 1 mg/mL. 5 mL of this solution was further diluted with distilled water to 50 mL to get 100 μg/mL of working standard solution.

Preparation of sample solution: Volume of injection equivalent to 25 mg of the drug was diluted to 50 mL and further diluted with distilled water to get the required concentration as given in the preparation of standard drug solution.

Bromocresol green (BCG) method: Aliquots of standard drug solution (0.3-3.75 mL) were added to 5 mL of phthalate buffer of pH 2.2 contained in a separating funnel followed by 0.5 mL of 0.1% (w/v) bromocresol green solution. The solution was extracted with chloroform and collected chloroform layer was dried over anhydrous sodium sulfate. Volume was made up to 10 mL. A linear graph was obtained at 420 nm after the waiting period of 15 min, against reagent blank prepared simultaneously.

Bromophenol blue (BPB) method: Aliquots of standard drug solution (0.1-1.25 mL) were added to 3 mL of phthalate buffer of pH 2.2 contained in a separating funnel followed by 1.0 mL of 0.1% (w/v) bromophenol blue solution. The solution was extracted with chloroform and collected chloroform layer was dried over anhydrous sodium sulfate. Volume was made up to 10 mL A linear graph was obtained at 380 nm against reagent blank prepared simultaneously.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen. The optimum pH required for complexation, effect of dye concentration and efficiency of the solvent to extract the ion pair were studied with respect to maximum sensitivity, colour stability, adherence to Beer's law and other optimum conditions are incorporated in the procedure (Table-1). The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-2.

The regression analysis using the method of least squares was made to evaluate the slope (m), intercept (b) and correlation coefficient (r) obtained from different concentrations and the results are presented in Table-2. The graph showed negligible intercept as described by the regression equation $y = mx + b$, where y is the absorbance and x is the concentration in μg/mL.

TABLE-1
OPTIMUM CONDITIONS AND RESULTS OF THE PROPOSED METHOD FOR THE DETERMINATION OF IRINOTECAN

Condition	Bromocresol green	Bromophenol blue
Drug solution (μg/10 mL)	30-375	10-125
Volume of buffer (mL)	5	3
λ_{max} (nm)	420	380
pH of buffer solution	2.2	2.2

TABLE-2
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE METHOD

Parameter	Bromocresol green	Bromophenol blue
λ_{max} (nm)	420	380
Beer's law range (μg/mL)	3.0-37.5	1.0-12.5
Molar extinction coefficient (1 mol^{-1} cm^{-1})	1.4×10^4	5.8×10^7
Sandell's sensitivity (μg/cm^2/0.001)	0.0660	0.0940
Regression equation (y = mx + b)*		
Slope (m)	0.0024	0.0073
Intercept (b)	0.0055	0.0272
Correlation coefficient (r)	0.9998	0.9989
Precision (% Relative standard deviation)	0.2740	0.8470
% Range of error (95 % confidence limits)	0.3260	0.5060

*y = mx + b, where y is the absorbance unit and x is the concentration in μg/mL.

Commercially available injections of irinotecan were analyzed by the proposed method and as additional check on the accuracy of the method. The recovery experiments were also conducted by spiking known amounts of pure drug in preanalysed injection and the recovery was calculated in each of the case using the regression line equation developed under the linearity experiment. The results of recovery experiments were given in the Table-3 for bromocresol green and Table-5 for bromophenol blue.The average recovery across the concentration range studied was 99.86 % with a relative standard deviation of 0.52 % for bromocresol green and 100.05 % with a relative standard deviation of 0.21 % for bromophenol blue. The correlation coefficient between the amount added to amount found was calculated as 0.9999 in both the methods, indicating a strong correlation. A regression line graph was drawn using the amount added on the x-axis and the amount found on the y-axis. The slope and intercept were calculated for the regression line (Method of Least Squares) and hypothesis was tested

for the correlation between the amount added and amount found at a confidence level of 0.05 (5 %), using the t-statistic.

TABLE-3
DETERMINATION OF IRINOTECAN IN INJECTIONS-BCG METHOD

Amount added in µg (Anhydrous basis)	Amount found (µg)	Recovery (%)
46.35	46.15	99.57
46.35	45.85	98.92
46.35	46.25	99.78
92.70	92.50	99.78
92.70	92.90	100.22
92.70	93.50	100.86
185.40	185.50	100.05
185.40	185.00	99.78
185.40	185.00	99.78
Mean		99.86
SD		0.0052
RSD (%)		0.52

Results were expressed in Table-4 for bromocresol green and Table-6 for bromophenol blue. The results revealed that there was a strong correlation between the amount added and amount found.

TABLE-4
STATISTICAL EVALUATION, t-TEST FOR
BROMOPHENOL GREEN METHOD

Parameter	Value
Alpha	0.05
t-Statistic	2.3646
t-Calculated	483.35
Degrees of freedom (df)	7
Correlation coefficient (r)	0.9999

The interference studies revealed the common excipients and other additives usually present in pharmaceutical dosage forms did not interfere in the proposed method.

The proposed visible spectrophotometric method enables quantitative determination of irinotecan in bulk drug samples and injections. Efficient visible spectrophotometric detection at the respective absorption maxima was found to be suitable without any interference from injectable solution excipients or solvents. The calibration curves were linear (r = 0.9989) over

TABLE-5
DETERMINATION OF IRINOTECAN IN INJECTIONS-
BROMOPHENOL BLUE METHOD

Amount added in µg (Anhydrous basis)	Amount found (µg)	Recovery (%)
32.445	32.43	99.94
32.445	32.35	99.71
32.445	32.45	100.02
60.255	60.45	100.32
60.255	60.35	100.16
60.255	60.32	100.11
92.700	92.50	99.78
92.700	93.00	100.32
92.700	92.80	100.11
Mean		100.05
SD		0.0022
RSD (%)		0.21

TABLE-6
STATISTICAL EVALUATION, t-TEST FOR
BROMOPHENOL BLUE METHOD

Parameter	Value
Alpha	0.05
t-Statistic	2.3646
t-Calculated	577.18
Degrees of freedom (df)	7
Correlation coefficient (r)	0.9999

a concentration range from 3.0-37.5 µg/mL for bromocresol green and 1.0-12.5 µg/mL for bromophenol blue. The relative standard deviation's (RSD) were less than 1 % and average recovery was above 99.86 %. Results were statistically evaluated using t-test. T-calculated is greater than t-statistic for 7 degrees of freedom at a confidence level of 0.05 (5 %) showing that there was strong correlation between amount added and amount found. The analytical results of samples were in accordance with those of standard solution in the same concentrations. The proposed method is fast, precise, accurate, sensitive, efficient and can be used in routine analysis in quality control laboratories.

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