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DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF VINBLASTINE SULFATE AND MOXIFLOXACIN HYDROCHLORIDE

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Keywords:

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ABSTRACT: Objective: To develop a simple, accurate and precise method for the simultaneous estimation of Moxifloxacin hydrochloride (MOX) and Vinblastine sulfate (VIN). **Methods:** The normal spectrum of VIN and MOX were converted to its second derivative spectrum and the amplitude minima of VIN and MOX were measured at 214 nm and 297 nm, respectively. MOX and VIN solution were simultaneously determined in 0.1M HCl at 297 nm and 214 nm. **Results:** The amplitude of MOX and VIN were found to be more distinct in 0.1M HCl with compared to water, methanol and 0.1M NaOH (order = 2 and $\Delta\lambda$=1). Linearity was obtained over the range 1-8 µg/ml and 3-24 µg/ml with a lower limit of quantitation of 1.7 µg/ml and 0.4 µg/ml for MOX and VIN, respectively. For each level of samples, inter- and intra-day precision (% RSD) was <2.1% and <2.3%for MOX and < 2.3 and <2.7 % for VIN, respectively. The mean recovery of MOX and VIN were in the range 99.98%-103.1%and 101.03%-104.3%, respectively. The developed method was validated as per ICH guidelines for parameters like linearity, accuracy, method precision, and ruggedness. **Conclusion:** The results obtained were well within the acceptable criteria. The method can be used for routine analysis of MOX and VIN.

INTRODUCTION: Vinblastine sulfate (VIN) is alkaloids which is one the chief compound of *Catharanthus sroseus* (Linn.) G. Don (Family Apocynaceae) [1]. It has also been used for lymphoma, testicular cancer, choriocarcinoma, breast cancer or Kaposi's sarcoma [2]. The most striking effects are produced in Hodgkin's disease. VIN is moderately active clinically against advanced breast cancer [3]. VIN works by stopping the cancer cells from separating into two new cells [3].

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Vinblastine sulphate is methyl (3a*R*, 4*R*, 5*S*, 5a*R*, 10b*R*, 13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*, 7*R*, 9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1, 4, 5, 6, 7, 8, 9, 10-octahydro-2*H*-3,7-methanoazacycloundecino [(5,4-*b*)indol-9-yl]-6-formyl-5-hydroxy-8-methoxy -3a, 4, 5, 5a, 6, 11, 12, 13a-octahydro-1*H* indolizino[8,1-*cd*]carbazole-5-carboxylate sulphate and its molecular formula is $C_{46}H_{58}O_9N4.H_2SO_4$.[6]

Moxifloxacin hydrochloride (MOX) on the other hand is a synthetic fluoroquinolone antibiotic agent, chemically 1-Cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7 -((4as, 7as) -octahydro -6H-pyrrolo (3, 4-b) pyridin-6-yl)-4-oxo-3-quinoline-carboxylic acid with the empirical formula $C_{21}H_{24}FN_3O_4$. [6] Its antibacterial spectrum includes enteric gram-(−) rods (*Escherichia coli*, *Proteus sp*, *Klebsiella species*), *Haemophilus influenzae*, atypical bacteria (*Mycoplasma*, *Chlamydia*,

Legionella), and *Streptococcus pneumonia*, and anaerobic bacteria. Moxifloxacin binds DNA and forms DNA gyrase (topoisomerase II) complex and blocks further DNA replication; it also blocks topoisomerase IV interferes with the separation of interlocked replicated DNA molecules [7].

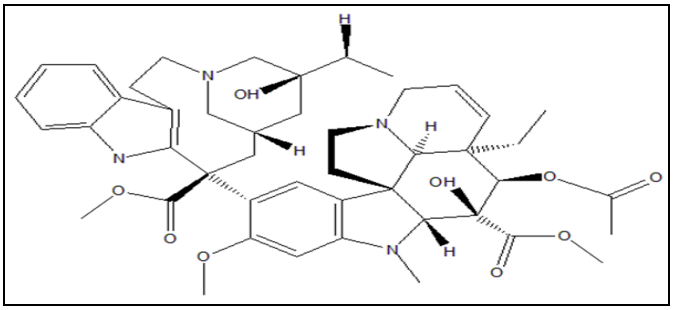

FIG. 1: STRUCTURE OF VINBLASTIN SULFATE

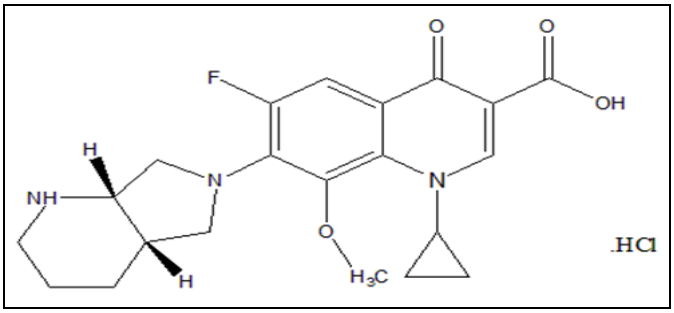

FIG. 2: STRUCTURE OF MOXIFLOXACIN HYDRO-CHLORIDE

Chemotherapy regimen used in clinical practice are empiric drug combinations [9], the dose prescribed for VIN is 3.7 mg/m^2 [10-11] and for MXR is 400 mg [12]. There are evidence of use of adjunct antibiotics like fluoroquinolones with anticancer drugs enhancing the cytotoxic effects while, at the same time, decreasing chemotherapy-induced pro-inflammatory cytokine secretion from cells, which may be harmful during chemotherapeutic treatment [13, 14, 15]. Few spectrophotometric methods [16-21] and High- Performance Liquid Chromatography (HPLC) [22-29] have been reported for the determination of MOX and VIN in single or combined pharmaceutical dosage forms or biological fluids. But none of these methods demonstrate the simultaneous estimation of these two drugs in combination by a derivative method in the pharmaceutical dosage form. A drug which has narrow therapeutic range need regular monitoring of drug plasma concentration [4, 5]. In continuation of our previous simultaneous estimation of Moxifloxacin with Doxorubicin hydrochloride, Imatinib mesylate and Vinblastine sulfate by vierodt's method[3, 20-31] in the present paper, second-order derivative UV spectrophotometry was used for the simultaneous estimation of VIN and MOX.

The derivative spectrophotometric method has been used for the estimation of two drugs in combination. This method involves the conversion of the normal spectrum to its first, second and higher derivative spectrum. In these method substances with narrow bandwidth display larger derivative amplitude than those of broad bandwidth substances. Derivative spectrophotometry discriminates in favor of substances with narrow spectral bandwidth against broad spectral bandwidth substances. This is because the derivative amplitude, *i.e.* the distance from a maximum to a minimum is inversely proportional to the fundamental spectral bandwidth (W) raised to the power (n) of the derivative order. With derivative spectrophotometry absorbance of excipients and other probable components, noise can be eliminated in case of a product with a high amount of excipients which is not possible in normal UV spectrophotometry [33].

EXPERIMENTAL:

Chemicals and Reagents: MOX was obtained as a gift sample from Alkem Laboratories (Sikkim plant). All reagents employed were of analytical grade ordered from S.D Fine Chem. Ltd. (Mumbai, India). Stock solutions of MOX and VIN (1 mg/ml) were prepared in 0.1M HCl and stored at 2-8 °C. Water used was from Direct-Q3water purification system (Millipore, India).

Instrumentation: Analytical balance model CP225D (Sartorius, Germany) was used. UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan; System Ver.1.12) was used for the method.

Selection of Solvent: Individual sample of the pure VIN and MOX was checked for their solubility in a different solvent, *i.e.*, methanol, water, 0.1HCl, and 0.1M NaOH. The molar absorptivity of the respective drug in each of the four solvents was calculated taking the concentration of 5 µg/ml, and corresponding derivative spectrum of the drugs was processed. The solvent showing a distinct and higher amplitude of second derivative spectrum were selected as the choice of solvent for the rest of the experiment.

Preparation of Analytical Solution: A stock solution of VIN and MOX were prepared by dissolving 10 mg of the drug in 10 ml of 0.1M HCl and was stored in the temperature ranging from 2-8 °C. For each analytical solution of desired concentration, suitable dilution was carried out.

Method Validation: [32]

Linearity: The linearity of the method was established by preparing a different concentration of the drug ranging from 1-8 µg/ml and 3-24 µg/ml of VIN and MOX, respectively. The amplitude of absorbance against the corresponding analyst concentration was plotted and slope, intercept and correlation coefficient were determined using linear regression analysis.

Precision: Intra-day precision was reported as % RSD for three replicate samples at three different concentrations (different ratio of drugs) levels against a qualified standard drug.

Inter-day precision was also carried out similar, but in two different days and the %, RSD was calculated.

Accuracy: The accuracy was evaluated in triplicate by adding a pure drug of MOX and VIN in already analyzed sample solution consisting of 3 µg/ml of VIN and 9 µg/ml of MOX. A known amount of VIN (20%, 40%, and 60%) and MOX (20%, 40% and 60%) standard solutions were added to the already analyzed sample solution and the analysis was carried out. The total amount of drug present was determined by the proposed method, and the % recovery of the pure drug was calculated.

Limit of Detection: Limit of detection was carried out as per ICH guideline. It was determined by using a formula:

LOD= SD of amplitude × 3.3 / slop

Where SD of amplitude is obtained from 6 replicates of amplitude obtained from the sample solution and the slope is obtained from the linearity curve.

Limit of Quantification: Limit of quantification was carried out as per ICH guideline. It was determined by using a formula:

LOQ= SD of amplitude × 10 / slop

Where SD of amplitude is obtained from 6 replicates of amplitude obtained from the sample solution and the slope is obtained from the linearity curve.

RESULTS:

Method Development: A distinct minima of the second order derivative spectrum (Δλ=1) of VIN and MOX was found at 214 nm and 297 nm in acidic (0.1M HCl) solution than in water, methanol, and 0.1 M NaOH. The ratio of the two drugs selected was 1:3 (VIN: MOX). The derivative spectrum of VIN and MOX solution showed enhanced resolution and the bandwidth discrimination as shown in **Fig. 3**, **4**.

The amplitude of the respective drug concentration was measured, and the linearity curve prepared using concentration against amplitude. Concentration of the drug was calculated using the calibration equation for respective VIN and MOX.

Linearity: The calibration curve for VIN was linear over the concentration range of 1-8 µg/ml. The correlation coefficient value obtained was 0.998 with the regression equation y = 01.175x + 0.026.

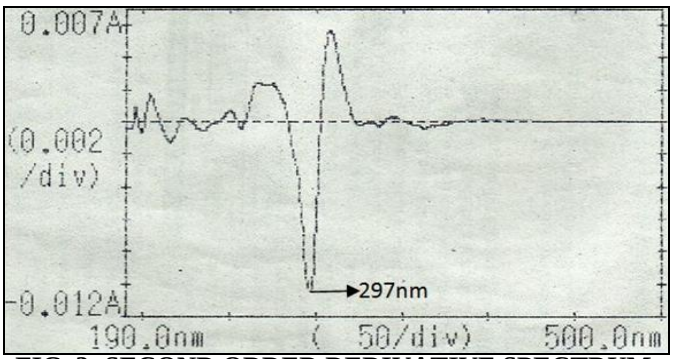

FIG. 3: SECOND ORDER DERIVATIVE SPECTRUM OF MOX (MINIMA AT 297 nm) IN 0.1M HCl

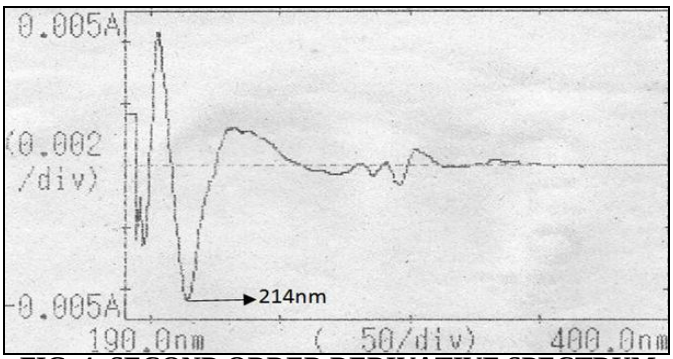

FIG. 4: SECOND ORDER DERIVATIVE SPECTRUM OF VIN (MINIMA AT 214 nm) IN 0.1M HCl

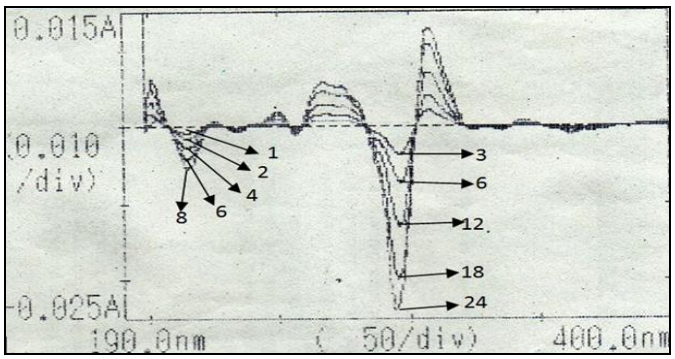

FIG. 5: SECOND ORDER DERIVATIVE SPECTRUM OF VIN (1, 2, 4, 6 & 8 µg/ml) AT 214 nm AND MOX (3, 6, 12, 18 & 24 µg/ml) AT 297nm in 0.1M HCl

Similarly, the calibration curve for MOX was linear over the concentration range of 3-24 µg/ml. The correlation coefficient value obtained was 0.999 with the regression equation y = 0.868x - 0.050. The high value of the correlation coefficient indicates the method is linear over the concentration range.

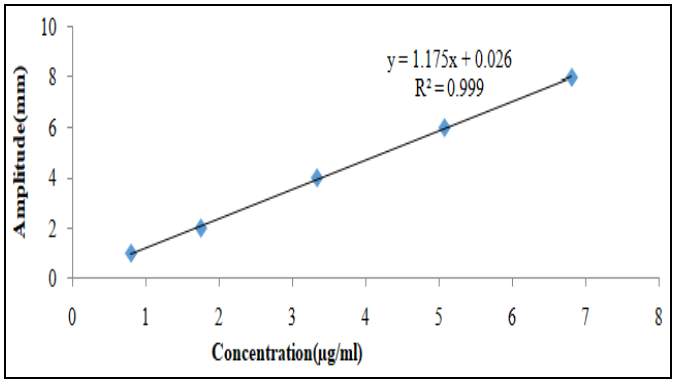

FIG. 6: STANDARD CALIBRATION CURVE FOR VIN

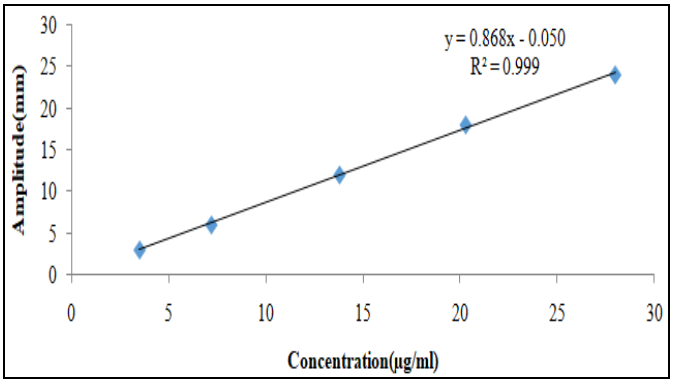

FIG. 7: STANDARD CALIBRATION CURVE FOR MOX

Precision: The precision of the method was determined by intra-day and inter-day precision studies by taking three different concentrations of the sample. Values of % RSD for intra-day were 2.7 and 2.3 for 5 µg/ml and 15 µg/ml concentration of VIN and MOX respectively; for inter-day 2.3 and 2.1 for 5 µg/ml and 15 µg/ml concentration of VIN and MOX respectively, as shown in **Table 1**.

TABLE 1: INTRA-DAY AND INTER-DAY PRECISION DATA OF VIN AND MOX

Parameters	Intra-day		Inter-day	
Drug concentration	VIN (5 µg/ml)	MOX (15 µg/ml)	VIN (5 µg/ml)	MOX(15 µg/ml)
%Assay	93.05	95.78	94.5	95.21
	96.60	99.15	97.13	95.63
	93.05	95.15	99.17	94.51
	91.52	99.15	94.58	95.92
	96.62	101.9	100.19	92.9
	98.15	101.9	97.13	99.15
% Mean	94.84	99.53	97.13	95.56
% RSD	2.70	2.30	2.30	2.10

Accuracy: Recovery studies were performed by the standard addition method. It was performed with a view to justifying the accuracy of the proposed method. Previously analyzed sample was spiked with known amounts of standard VIN and MOX to get three different levels (20%, 40%, and 60%) and the mixture was analyzed by the proposed method. The experiment was performed in triplicate % recovery, mean% recovery and RSD (%) was calculated for each concentration. The method has shown good and consistent recoveries ranging within 99%-103.2% and 101%-104.2% for VIN and MOX respectively, confirming the accuracy of the method, as shown in **Table 2**.

TABLE 2: ACCURACY DATA FOR THE DETERMINATION

Drug	Conc. of sample (µg/mL)	Conc. of standard added (µg/mL)	Amt. added %	Total concentration found (µg/mL)	Recovery % (n=3)	Mean Recovery % (n=9)	% RSD
VIN	3	0.6	20%	3.5	98.38	99.98	2.70
				3.5	98.38		
				3.6	103.16		
	3	1.2	40%	4.23	103.79	103.11	2.00
				4.24	104.81		
				4.2	100.72		
	3	1.8	60%	4.8	100.67		
				4.84	103.72		
				4.87	104.44		
MOX	9	1.8	20%	10.8	104.73		
				10.78	99.37		
				10.85	103.99		
	9	3.6	40%	12.6	100.24		
				12.63	101.03		
				12.63	101.82		
	9	5.4	60%	14.67	105.7		
				14.67	105.7		
				14.45	101.5		

Limit of Detection and Limit of Quantification: The limits of detection and quantification were determined from the calibration curve. The LOD and LOQ were 0.82 µg/ml and 2.51 µg/ml, respectively, for VIN and 0.082 µg/ml and 0.25 µg/ml, respectively for MOX.

DISCUSSION AND CONCLUSION: MOX and VIN have been simultaneously estimated by the derivative spectroscopic method which showed a relatively superior spectrophotometric method for estimation of these two drugs. The developed method was validated in compliance with ICH guidelines for parameters. The developed method was validated in compliance with ICH guidelines for parameters like linearity, accuracy, method precision, robustness, and ruggedness. The results obtained were well within the acceptable criteria. The method can be used for routine quality control analysis of MOX and VIN simultaneously.

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CONFLICT OF INTEREST: Declared none.

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