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RESEARCH ARTICLE

Simultaneous Spectophotometric Estimation of Moxifloxacin Hydrochloride and Vinblastin Sulphate

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ABSTRACT

Moxifloxacin is used as prophylactic antibiotics for the cancer patients undergoing therapy with anticancer drug like vinblastin sulfate. There is a need for development of a simple, accurate, and precise method for the simultaneous estimation of moxifloxacin hydrochloride (MOX) and vinblastin sulfate (VIN). MOX and VIN solution were simultaneously determined in 0.1M HCl at their respective λ_{max}. The absorbance λ_{max} of MOX and VIN were 295nm and 214nm, respectively. The developed method was validated according to ICH guidelines for parameters like linearity, accuracy, precision, ruggedness and robustness. Molar absorptivities of MOX and VIN were found to be more in 0.1M HCl with compared to water, methanol and 0.1M NaOH. Linearity was obtained over the range 0.5 – 20.0 µg/ml and 1-32 µg/ml with a lower limit of quantitation of 0.25 µg/ml and 1.5 µg/ml for MOX and VIN, respectively. For each level of samples, inter- and intra-day precision (% RSD) was <1% and <2%for MOX and < 3.2 and <4 % for VIN, respectively. The mean recovery of MOX and VIN were in the range 95%-100%and 95%-101%, respectively. The method developed was validated as per 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 analysis of MOX and VIN.

Key words: Moxifloxacin hydrochloride, Doxorubicin hydrochloride, Simultaneous estimation, Method development, Validation.

INTRODUCTION

Moxifloxacin hydrochloride (MOX) 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-quinolinecarboxylic acid with the empirical formula $C_{21}H_{24}FN_3O_4$ [1]. Its antibacterial spectrum includes enteric Gram-(−) rods (*Escherichia coli*, *Proteus*species, *Klebsiella* species), *Haemophilus influenzae*, atypical bacteria (*Mycoplasma*, *Chlamydia*, *Legionella*), and *Streptococcus pneumoniae*, 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 [2].

Vinblastin sulfate (VIN) in the other hand is a chemotherapy drug used most often for bladder cancer.VIN has the molecular formula $C_{46}H_{58}O_9N4.H_2SO_4$. VIN It may also be used for lymphoma, testicular cancer, choriocarcinoma, breast cancer or Kaposi's sarcoma. These drugs are sometimes called microtubule inhibitors. VIN works by stopping the cancer cells from separating into two new cells [3]. The most striking effects are produced in Hodgkin's disease, in the treatment of which it may be the present drug of choice, but regression of other tumors can be produced, VIN is moderately active clinically against advanced breast cancer [4, 5].

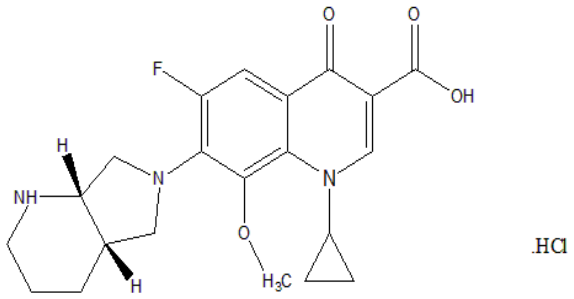

Fig 1: Structure of moxifloxacin hydrochloride

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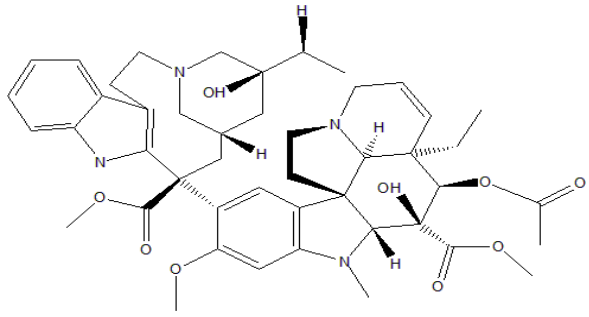

Fig 2: Structure of vinblastin sulphate

Few spectrophotometric methods [6-11] and high-performance liquid chromatography (HPLC) [12-19] 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 in pharmaceutical dosage form. There are evidences of use of adjunct antibiotics like flouroquinolones 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 [20-22]. Chemotherapy regimen used in clinical practice are empiric drug combinations [23, 24], maximum dose prescribed for VIN is 3.7 mg/m^2 [25] and for MXR is 400 mg [26].

Fluoroquinolones including MOX is most commonly used as the prophylaxis, along with chemotherapeutic agents like VIN in cancer patients. Simple and sensitive method developments have become necessary in recent years the importance of quality control of drugs and drug products. Sensitive, precise and accurate method for the simultaneous estimation of VIN and MOX has not been carried out till date. There is a need of developing method for the simultaneous determination of these drugs body fluids. Thus the authors have developed this novel method for simultaneous estimation of DXR and MOX, which could also aid in determining those drugs in plasma or body fluids.

Simultaneous method by UV-spectrophotometry has been used for estimation of two drugs in combination. In this method, a sample contains two absorbing drugs each of which absorbs at λ_{max} of the other . It may be possible to determine both drugs by the technique of simultaneous equations (vierodt's method) provided that certain criteria apply [21].

EXPERIMENTAL

Chemicals and reagents:

MOX was obtained as a gift sample from Alkem Laboratories (Sikkim plant). Methanol used where spectrophotometric grade and all other 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℃. These solutions were diluted appropriately with 0.1M HCl to the working solution. Water used was from Direct-Q3water purification system (Millipore, India)

Instrumentation

Analytical balance model CP225D (Sartorius, Germany) was used. Simultaneous estimation was performed using a UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan).

Selection of solvent

Individual sample of the pure VIN and MOX was checked for their solubility in different solvent, i.e. methanol, water, 0.1HCl and 0.1M NaOH. Then molar absorptivity of the respective drug in each four solvent was calculated taking an approximate concentration of 5µg/ml. The solvent having highest molar absorptivity was selected as the choice of solvent for the rest of the procedure.

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℃. For each analytical solution of desired concentration, suitable dilution was carried out.

Method Validation

Method development

Fluoroquinolones including MOX is most commonly used as the prophylaxis, along with chemotherapeutic agent in cancer patients. Simple and sensitive method development have become necessary in recent years the importance of quality control of drugs and drug products. Sensitive, precise and accurate method for the simultaneous estimation of VIN and MOX has not been carried out till date.

Linearity

The linearity of the method was established by preparing different concentration of the drug ranging from 0.5 to 20 µg/ml and 1 to 32 µg/ml of MOX and VIN, respectively. Absorbance against the corresponding analyst concentration was plotted and slope, intercept and correlation co-efficient 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 1.5 µg/ml of VIN and 4.5 µg/ml of MOX. Known amount of VIN (0.37 µg/ml, 0.75 µg/ml and 1.125 µg/ml) and MOX (1.1 µg/ml, 2.25 µg/ml and 3.37 µg/ml) standard solutions was 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 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 absorbance × 3. 3 ÷ slope

Where, SD of observance is obtained from 6 replicates of absorbance 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 absorbance × 10 ÷ slope

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

Robustness

Robustness were carried out by changing the wavelength by ± 2 nm, the strength of the solvent, i.e. 0.1M HCl±0. 05, and room temperature 23℃ and 30℃.

Ruggedness

Ruggedness was performed by carrying out analytical procedure with different analyst.

RESULTS

Method Validation

The molar absorptivity of the VIN was found to be maximum, i.e. 4.53×10^4 at 214nm in acidic (0.1M HCl) solution than in water, and 0.1 M NaOH. Similarly molar absorptivity of MOX HCl was found to be maximum i.e 4×10^4 at 295nm in basic (0.1M NaOH) solution than in water, methanol and 0.1M HCl. Comparison of molar absorptivity is shown in (**Table 1 & 2**).

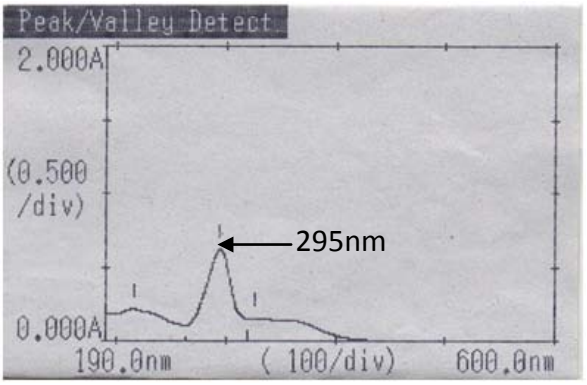

Fig 3: Absorbance spectrum of MOX in 0.1 M HCl

Though MOX HCl shows maximum absorptivity in a basic solution, its molar absorptivity in acidic solution is still same as VIN and the λ_{max} is also far from one another making it possible to carry out simultaneous estimation. VIN solution showed absorption λ_{max} at 214nm and MOX hydrochloride solution showed absorption λ_{max} at 295nm in 0.1M HCl as shown in (**Fig 3 & 4**).

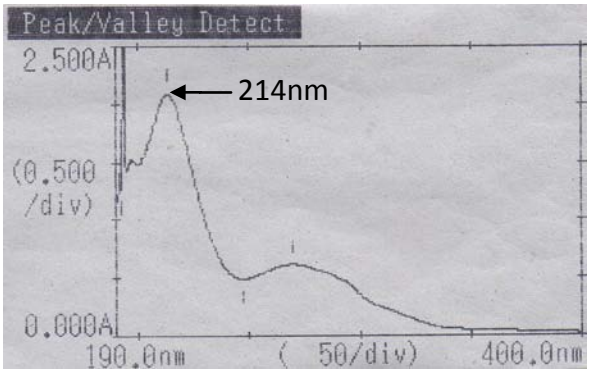

Fig 4: Absorbance spectrum of VIN in 0.1 M HCl

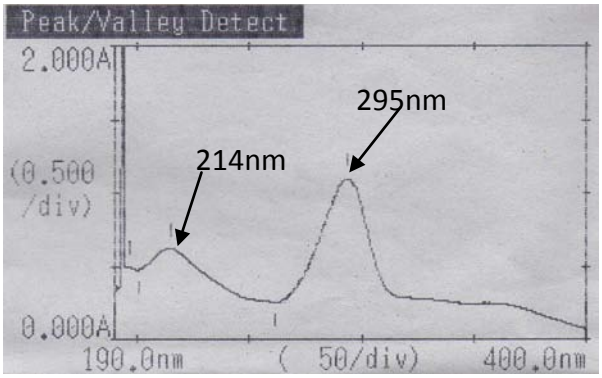

Fig 5: Absorbance spectrum of MOX (λmax at 295nm) and VIN (λmax at 214nm) in 0.1 M HCl

Table 1: Absortivity of VIN in different solvent

	NaOH	HCl	Water	Methanol
λmax(nm)	270	214	214	214
Molar conc.	4.2×10^{-3}	4.23×10^{-4}	4.4×10^{-4}	4.21×10^{-4}
Absorbance	0.577	2.089	2.1124	2.012
Molar absorptivity	12.4×10^3	4.9×10^3	4.8×10^3	4.78×10^3

Table 2: Absortivity of MOX in different solvent

	NaOH	HCl	Water	Methanol
λmax(nm)	290	295	289	292
Molar conc.	1.25×10^{-5}	1.25×10^{-5}	1.25×10^{-5}	1.25×10^{-5}
Absorbance	0.512	0.498	0.390	0.405
Molar absorptivity	4.096×10^4	3.984×10^4	3.12×10^4	3.24×10^4

Linearity

The calibration curve for VIN was linear over the concentration range of 1-32 µg/ml. The correlation coefficient value obtained was 0.9994 with the regression equation y = 0.019x + 0.024. Similarly the calibration curve for MOX was linear over the concentration range of 0.2-20 µg/ml. The correlation coefficient value obtained was 0.998 with the regression equation y = 0.114x + 0.031. The high value of correlation coefficient indicates the method is linear over the concentration range.

Precision

The precision of the method was determined by intra-day and inter-day precision studies by taking three different concentrations of sample. Values of %RSD for intra-day were 3.8, 2.26, and 3.2 for concentration of 3, 2, 1µg/ml for VIN and 1.809, 0.327 and 1.44 for concentration of 8, 9, 8µg/ml for MOX. Similarly, for inter-day %RSD were 3.14, 1.78, and 1.8, for concentration of 3, 2, 1 µg/ml of VIN and 0.99, 0.84, and 0.93 for 8, 9, 8 µg/ml concentration of MOX, as shown in (**Table 3 & 4**).

Table 3: Intra-day precision data of VIN and MOX by simultaneous equation method

Parameters	VIN			MOX		
Drug concentration (µg/ml)	A	B	C	A	B	C
	3	2	1	8	9	8
%Assay	93.81	99.13	107.19	103.36	103.27	104.52
	101.93	99.4	110.57	106.49	104.0	104.5
	96.44	103.69	115.17	107.45	103.82	107.47
% Mean	97.40	104.22	110.9	105.77	103.70	105.50
% RSD	3.8	2.26	3.23	1.809	0.327	1.44

Table 4: Inter-day precision data of VIN and MOX by simultaneous equation method

Parameters	VIN			MOX		
Drug concentration (µg/ml)	**A**	**B**	**C**	**A**	**B**	**C**
	3	**2**	**1**	**8**	**9**	**8**
%Assay	94.81	99.15	108.57	104.36	102.27	103.52
	100.93	100.40	109.57	105.49	104.0	104.52
	98.44	102.69	112.17	106.45	102.82	105.47
% Mean	98.06	100.75	109.97	105.43	103.7	105.50
% RSD	3.14	1.78	1.8	0.99	0.841	0.9

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed sample were spiked with known amount of standard VIN and MOX so as to get three different levels (25%, 50% & 75%) and the mixture were analyzed by the proposed method.

The experiment was performed in triplicate % recovery, mean% recovery, RSD (%) were calculated for each concentration. The method has shown good and consistent recoveries ranging from 95%-99% and 95%-98% for VIN and MOX respectively, confirming the accuracy of the method, as shown in (**Table 5**).

Table 5: 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
MOX	4	1	25%	4.95	99.16	98.9	0.62
				4.96	99.38		
				4.91	98.22		
	4	2	50%	5.80	96.79	96.6	0.7
				5.81	96.9		
				5.74	96		
	4	3	75%	6.52	96.2	95.98	0.43
				6.49	96.20		
				6.50	95.49		
VIN	2	0.5	25%	2.25	98.04	99.59	1.4
				2.29	99.86		
				2.32	100.88		
	2	1	50%	2.72	96.45	97.4	0.9
				2.73	97.57		
				2.75	98.18		
	2	1.5	75%	3.1	95.45	95.88	0.3
				3.23	96.12		
				3.11	96.09		

Robustness

Robustness were performed by small variations in spectrophotometric conditions like solvent strength, wavelength and room temperature. It can be observed from the table 6 that the method is

Table 6: Robustness of simultaneous equation method for VIN and MOX

Parameters	%RSD (n=3)	
	VIN	MOX
Temperature (23℃ and 30℃)	0.32	0.14
Solvent strength (0.1M±0.05)	0.02	0.01
Wavelength (±2nm)	2.6	3.1

unaffected by small variation in the spectrophotometric conditions.

Ruggedness

The results were well within acceptable limits of 99%-102% for both the drugs as shown in (**Table 7**).

Table 7: Ruggedness data of simultaneous equation method for VIN and MOX

DRUG	Analyst I amount found%±SD (n=3)	% RSD	Analyst II amount found %±SD (n=3)	% RSD
VIN	99.2	0.3	97.4	0.3
MOX	102.4	0.9	97.42	1

Limit of detection and limit of quantification

Typically the concentration level that generates a signal to noise (S/N) of 10 is regarded as the LOQ and the concentration level that generates S/N=3 is regarded as the LOD. 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

Some studies [20-22] show that, moxifloxacin significantly enhanced the anti-proliferative effect of chemotherapeutic agents, adding its significance to be used along with the chemotherapeutic agents. There is a need for the development of method for clinical monitoring of these two drugs simultaneously, so that complications (eg. cardio toxicity) and relative effectiveness can be efficiently monitored when used in combination. Development of in vitro simultaneous method for the estimation of these two drugs aids for the development of method for clinical monitoring.

For the first time, MOX and VIN have been simultaneously estimated by the spectroscopic method. 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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