Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF MOXIFLOXACIN HYDROCHLORIDE AND DOXORUBICIN HYDROCHLORIDE

NAYAN PRADHAN*, HIYASHREE RAJKHOWA, HEMANT GIRI, BHUPENDRA SHRESTHA

Department of Pharmaceutical Analysis and Quality Assurance, Himalayan Pharmacy Institute, Majhitar 737132, East Sikkim, India
Email: nayanpradhan7@gmail.com

Received: 12 Dec 2014 Revised and Accepted: 10 Sep 2015

ABSTRACT

Objective: To develop a simple, accurate, and precise spectrophotometric method for the simultaneous estimation of moxifloxacin hydrochloride (MOX) and doxorubicin hydrochloride (DXR).

Methods: MOX and DXR solution were simultaneously determined in 0.1M HCl at their respective λ_{max}. The absorbance λ_{max} of MOX and DXR was 295 nm and 480 nm, respectively. The developed method was validated according to ICH guidelines for parameters like linearity, accuracy, precision, ruggedness and robustness.

Results: Molar absorptivities of MOX and DXR 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 1.5-50 µg/ml with a lower limit of quantitation of 0.25 µg/ml and 1.5 µg/ml for MOX and DXR, respectively. For each level of samples, inter-and intra-day precision (% RSD) was<1.3 % and<1.4 % for MOX and<2.5 and 2.4 % for DXR, respectively. The mean recovery of MOX and DXR was in the range 96.21 %-98.77 % and 97.13 %-99.64 %, respectively.

Conclusion: 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 DXR.

Keywords: 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]. MOX is a fourth-generation synthetic fluoroquinolone antibacterial agent. 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. It differs from earlier antibacterials of the fluoroquinolone class such as levofloxacin and ciprofloxacin in having greater activity against Gram-(+) bacteria and anaerobes. Because of its potent activity against the common respiratory pathogen *Streptococcus pneumoniae*, it is considered a respiratory quinolone. 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].

Doxorubicin hydrochloride (DXR) is a cytotoxic anthracycline antibiotic isolated from cultures of the fungus *Streptomyces peucetius*var. *caesius*. The most common uses of DXR in cancer therapy is for various types of testicular cancer, leukemia, Ewing's sarcoma, Hodgkin's disease, and Kaposi's sarcoma [3]. DXR is chemically 5, 12-Naphthacenedione, 10-[(3-amino-2, 3, 6-trideoxy-a-Llyxo-hexopyranosyl) oxy]-7, 8, 9, 10-tetrahydro-6, 8, 11-trihydroxy -8-(hydroxyacetyl)-1-methoxy, hydrochloride. Its empirical formula is $C_{27}H_{29}NO_{11}$ [4].

DXR is a cytotoxic anthracycline antibiotic [5, 6], closely related to the natural product Daunomycin [7, 8] and like all anthracyclines, it works by inter calating DNA [9-10] with the most serious adverse effect being life-threatening heart damage. It is prescribed in the treatment of a wide variety of malignant neoplastic diseases, including leukemias, lymphomas, sarcomas, germ cell tumors, and carcinomas [12].

Literature showed anticancer and flouroquinolones are frequently prescribed in clinical practice and interaction may result without recognition. 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

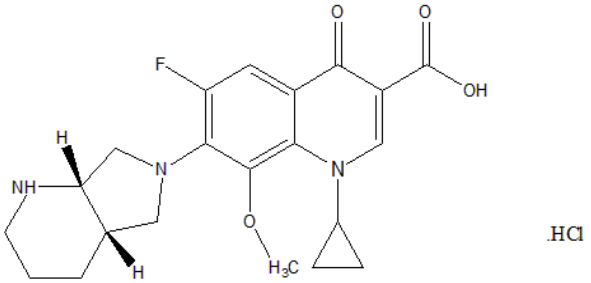

Fig. 1: Structure of moxifloxacin hydrochloride

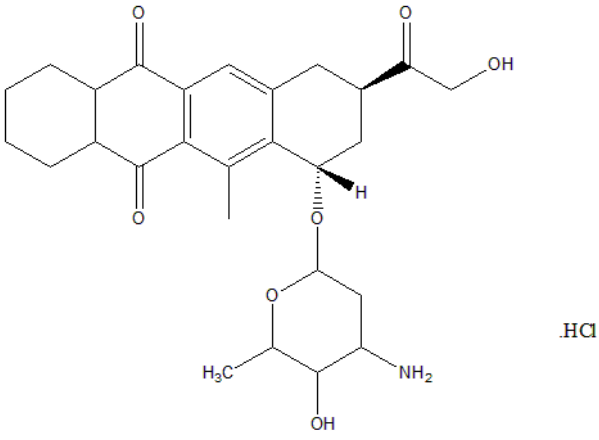

Fig. 2: Structure of doxorubicin hydrochloride

May be harmful during chemotherapeutic treatment. [13-15] Chemotherapy regimen used in clinical practice are empiric drug combinations [16-17], maximum doses prescribed for DXR is 75 mg/m^2 [18] and for MXR is 400 mg [19]. Few spectrophotometric methods [20-29] and high-performance liquid chromatography methods [29-42] have been reported for the determination of moxifloxacin and DXR in single or combined pharmaceutical dosage forms or biological fluids.

But till date there is no report of simultaneous estimation of these two drugs in pharmaceutical dosage form or in biological fluid. The combined drug related toxicities or change in therapeutic efficacy should be monitored. 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 aid in determining those drugs in plasma or body fluids.

MATERIALS AND METHODS

Chemicals and reagents

MOX hydrochloride and DXR hydrochloride were obtained as a gift sample from Alkem Laboratories (Sikkim) and Yarrow Chem. Ltd (Mumbai, India). Methanol used was spectrophotometric grade and all other reagents employed were of analytical grade (S. D Fine Chem. Ltd., Mumbai, India). Distilled water was used throughout the experiment.

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 and ratio of drugs to be analyzed

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

Preparation of analytical solution

A stock solution of DXR and MOX was prepared by dissolving 50 mg of the drug in 50 mL of 0.1M HCl. The final concentration of 3 µg/ml of DOX and 5 µg/ml of MOX was prepared by suitably diluting the stock solution with 0.1M HCl.

Method validation

Linearity

The linearity of the method was established by preparing different concentration of drug ranging from 0.5 to 30 µg/ml and 0.5 to 40 µg/ml of MOX and DXR, respectively. Absorbance against the corresponding analyte 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 similarly 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 DXR in already analyzed sample solution consisting 3 µg/ml of DXR and 6 µg/ml of MOX. Known amount of DXR (0.6 µg/ml, 1.8 µg/ml and 2.4 µg/ml) and MOX (1.2 µg/ml, 3.6 µg/ml and 4.8 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

LOD 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

LOQ 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 was carried out by changing the wavelength by±2 nm, the strength of the solvent, i.e. 0.1M HCl±*0.05*, and room temperature 25±2 ℃.

Ruggedness

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

RESULTS

Method development

The molar absorptivity of the DXR was found to be maximum i. e 2×10^3 at 480 nm in acidic (0.1M HCl) solution than in water, methanol and 0.1 M NaOH. Similarly molar absorptivity of MOX was found to be maximum i. e 4×10^4 at 290 nm in basic (0.1M NaOH) solution than in water, methanol and 0.1M HCl. Comparison of molar absorptivity is shown in table 1 and 2.

Though MOX shows maximum absorptivity in a basic solution, its molar absorptivity in acidic solution is still 20 times higher than DXR and the λ_{max} is also very far from one another making it possible to carry out simultaneous estimation. DXR hydrochloride solution showed absorption λ_{max} at 480 nm and the MOX hydrochloride solution showed absorption λ_{max} at 295 nm in 0.1M HCl as shown in fig. 3 and 4.

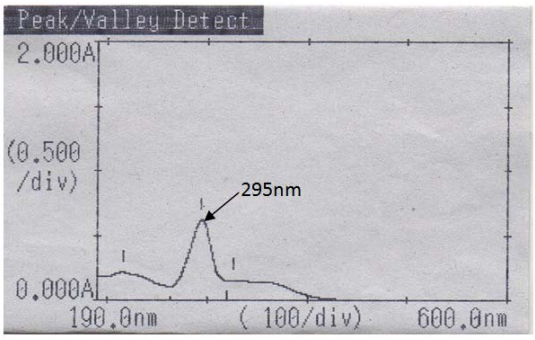

Fig. 3: Absorbance spectrum of MOX in 0.1M HCl

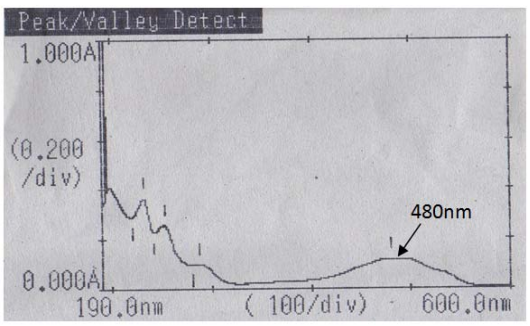

Fig. 4: Absorbance spectrum of DXR in 0.1M HCl

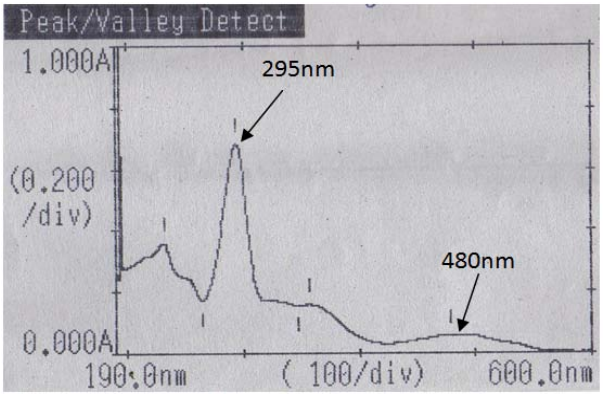

Fig. 5: Absorbance spectrum of MOX (λ_{max} at 295 nm) and DXR (λ_{max} at 480 nm) in 0.1M HCl

Method validation

Linearity

The calibration curve for DXR was linear over the concentration range of 1.5-50 µg/ml. The correlation coefficient value obtained was 0.998 with the regression equation y = 0.019x+0.024.

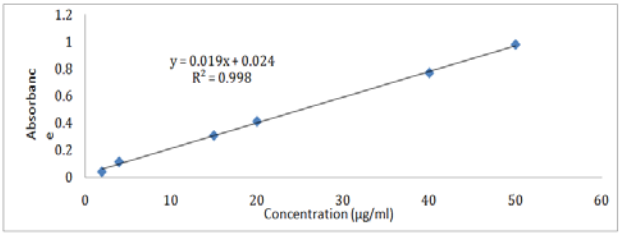

Fig. 6: Standard calibration curve for DXR

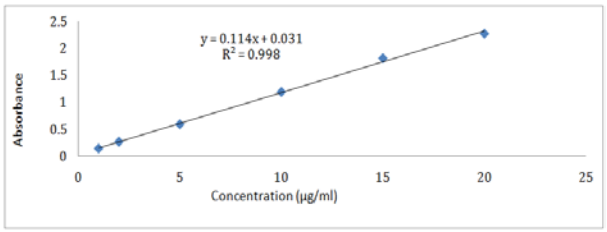

Fig. 7: Standard calibration curve of MOX

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 the sample. Values of % RSD for intra-day were 2.4, 1.3, and 1.7 for 1.5, 3 and 4.5 µg/ml concentration of DXR and 0.95, 1.0 and 1.3 for 2.5, 5 and 7.5 µg/ml concentration of MOX; for inter-day 2.5, 0.7 and 2.1 for 1.5, 3 and 4.5 µg/ml concentration of DXR and 0.7, 1 and 1.3 for 2.5, 5 and 7.5 µg/ml concentrations of MOX respectively, as shown in table 3 and 4.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed sample was spiked with known amounts of standard DXR and MOX so as to get three different levels (20 %, 60 % and 80 %) 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 from 96.21 %-98.77 % and 97.13 %-99.64 % for DXR and MOX respectively confirming the accuracy of the method, as shown in table 5.

Table 1: Absortivity of DXR in different solvent

	NaOH	HCl	Water	Methanol
λmax (nm)	489	480	496	495
Molar conc.	11.5×10^{-5}	2.87×10^{-5}	2.87×10^{-5}	2.87×10^{-5}
Absorbance	0.045	0.498	0.390	0.405
Molar absorptivity	392	2.021×10^{3}	1.463×10^{3}	1.847×10^{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}

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

Parameters	DXR			MOX		
Drug concentration (µg/ml)	A	B	C	A	B	C
	1.5	3	4.5	2.5	5	7.5
%Assay	103.1	101.5	102.2	99.1	99.4	99.2
	106.4	99.9	100.1	97.8	98.6	101.2
	103.1	99.8	98.5	98.0	100.3	99.5
% Mean	104.2	100.4	100.1	98.3	98.7	99.9
% RSD	2.4	1.3	1.7	0.95	1.0	1.3

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

Parameters	DXR			MOX		
Drug concentration (µg/ml)	A	B	C	A	B	C
	1.5	3	4.5	2.5	5	7.5
	106.20	98.21	102.00	97.04	98.32	99.01
%Assay	104.10	97.23	98.00	96.89	97.30	102.21
	100.10	99.03	103.01	98.32	96.03	100.34
% Mean	104.20	98.16	101.00	97.42	97.22	100.51
% RSD	2.5	0.75	2.1	0.66	0.96	1.31

Robustness

Robustness was performed by small variations in spectro photometric conditions like solvent strength, wavelength and room temperature. It can be observed from the table 6 that the method is unaffected by the small variation in the spectro photometric conditions.

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 0.46 µg/ml and 1.4 µg/ml, respectively, for DXR and 0.082 µg/ml and 0.25 µg/ml, respectively for MOX.

Ruggedness

The results of ruggedness were well within acceptable limits of 98 % -102 % for both the drugs as shown in table 7.

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	5	1	20 %	5.76	96.02	97.13	1.05
				5.88	98.04		
				5.84	97.32		
	5	3	60 %	7.86	98.30	98.45	0.56
				7.84	97.98		
				7.92	99.06		
	5	4	80 %	8.92	99.09	99.64	1.27
				8.91	99.02		
				8.89	98.80		
DOX	1.5	0.3	20 %	1.71	95.02	96.21	1.09
				1.74	96.6		
				1.75	97.00		
	1.5	0.6	60 %	2.06	98.30	97.70	0.72
				2.06	97.88		
				2.04	96.93		
	1.5	1.2	80 %	2.65	98.30	98.77	1.07
				2.64	98.02		
				2.70	99.98		

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

Parameters	% RSD (n=3)	
	DOX	MOX
Temperature (23 ℃ and 30 ℃)	0.34	0.56
Solvent strength (0.1M±*0.05*)	0.03	0.05
Wavelength (±*2* nm)	2.63	3.84

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

Drug	Analyst I amount found %±SD (n=3)	% RSD	Analyst II amount found %±SD (n=3)	% RSD
DOX	98.03±1.20	1.22	98.32±1.89	1.92
MOX	101.10±0.76	0.75	99.05±1.55	1.56

DISCUSSION

DXR a prototype drug from the active class of anticancer agents used to treat solid cancers and hematological malignancies [43], while, MOX belongs to wide spectrum antibiotics fluoroquinolones group and is successfully used in clinical practice to treat a number of infections and as an antibacterial prophylactic in cancer patients [44]. Several clinical studies have adopted the different dose schedules of DXR alone or in combination with other drugs to improve the anticancer activity. [45] Some studies [13-15] 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 the method for clinical monitoring of these two drugs simultaneously, so that complication like 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 DXR have been simultaneously estimated by the spectroscopic method. Vierodt's method was used for the estimation of the two drugs in solution which showed a relatively good degree of accuracy and precision. 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 DXR simultaneously.

ACKNOWLEDGEMENT

The authors are grateful to the management of the Himalayan Pharmacy Institute (HPI) for providing excellent laboratory facilities. We are grateful to Alkem Laboratories Ltd Kumrek, East Sikkim for a gift sample of MOX. We are thankful to Dr. Ankur Gupta, Ex. HOD of Department of Pharmaceutical Analysis and Quality Assurance, HPI for his encouragement and Mr. Jeewan Pradhan, Laboratory technician, HPI, for laboratory assistance.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. http://www.drugbank.ca/drugs/DB00218. [Last accessed on 24 Sep 2014]
2. Hauser AR. Antibiotic basics for clinicians: choosing the right antibacterial agent: Lippincott Williams and Wilkins; 2007.
3. Stewart J, Warren F, King D, Venkateshwaran T, Ponder G, Fox J. Stability of ondansetron hydrochloride, doxorubicin hydrochloride, and dacarbazine or vincristine sulfate in elastomeric portable infusion devices and polyvinyl chloride bags. Am J Health Syst Pharm 1997;54:915-20.
4. The Indian Pharmacopoeia Commission Ghaziabad. Indian Pharmacopeia; 2007. p. 441.
5. Trevor A, Katzung B, Masters S. Pharmacology: Examination and Board Review. Chapter 54, Anthracycilne Antibiotics; 2012. Available from: URL: www.accesspharmacy.com. [Last accessed on 25 Jan 2013]
6. Fujiwara A, Hoshino T, Westley JW. Anthracycline antibiotics. Crit Rev Biotech 1985;3:133.
7. Cutler SJ. Biologically active natural products: pharmaceuticals. CRC Press; 2000. p. 5.
8. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 y. J Nat Prod 2007;70:461-77.
9. Richards AD, Rodgers A. Synthetic metallomolecules as agents for the control of DNA structure. Chem Soc Rev 2007;36:471–83.
10. Johansson S, Goldenberg D, Griffiths G. Elimination of HIV-1 infection by treatment with a doxorubicin-conjugated anti-envelope antibody. AIDS 2006;20:1911–5.
11. Pigram WJ, Fuller W, Hamilton LD. Stereochemistry of intercalation: interaction of daunomycin with DNA. Nature New Biol 1972;235:17–9.
12. Facts and Statistics. The Leukemia and Lymphoma Society; 2009.
13. Reuveni D, Halperin D, Shalit I, Priel E, Fabian I. Quinolones as enhancers of camptothecin-induced cytotoxic and anti-topoisomerase I effects. Biochem Pharmacol 2008;75:1272–81.
14. Reuveni D, Halperin D, Fabian I, Tsarfaty G, Askenasy N, Shalit I. Moxifloxacin increases anti-tumor and anti-angiogenic activity of irinotecan in human xenograft tumors. Biochem Pharmacol 2010;79:1100–7.
15. Barot M, Gokulgandhi MR, Pal D, Mitra AK. *In vitro* moxifloxacin drug interaction with chemotherapy: Implications for retinoblastoma management. Exp Eye Res 2014;118:61-71.
16. Lai ECS, Lo CM, Fan ST, Liu CL, Wong J. Postoperative adjuvant chemotherapy after curative resection of hepatocellular carcinoma: a randomized controlled trial. Arch Surg 1998:133:183-8.
17. Zoli W, Ricotti L, Tesei A, Barzanti F, Amadori D. *In vitro* preclinical models for a rational design of chemotherapy combinations in human tumors. Crit Rev Oncol Hematol 2001;37:69-82.
18. http://www.drugs.com/dosage/doxorubicin.html. [Last accessed on 24 Sep 2014]
19. http://www.drugs.com/dosage/moxifloxacin.html. Last accessed on 24 Sep 2014]
20. Motwani SK, Chopra S, Ahmad FJ, Khar RK. Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations. Spectrochim Acta Part A 2007;68:250-6.
21. Kaur K, Kumar A, Malik AK, Singh B, Rao A. Spectrophotometric methods for the determination of fluoroquinolones: a review. Crit Rev Anal Chem 2008;38:2-18.
22. Sturgeon RJ, Schulman SG. Electronic absorption spectra and protolytic equilibria of doxorubicin: direct spectrophotometric determination of microconstants. J Pharm Sci 1977;66:958-61.
23. Patel RK, Parmar RR, Patel VM, Shah DA. Method development and validation of cefixime and moxifloxacin in pharmaceutical dosage form by UV spectrophotometric method. Int J Pharm Res Bio-Sci 2012;1:81-93.
24. Vyas P, Dave J, Patel C. Simultaneous estimation of Moxifloxacin Hcl and Bromfenac sodium in eye drops by spectrophotometric methods. Int J Pharmal Sci Res 2012;3:2137-42.
25. Abdellaziz LM, Hosny MM. Development and validation of spectrophotometric, atomic absorption and kinetic methods for determination of moxifloxacin hydrochloride. Anal Chem Insights 2011;6:67.
26. Sahu S, Azam MA, Sahu D, Banarjee M. Spectrophotometric estimation of moxifloxacin in bulk and its pharmaceutical formulations. Pharmacologyonline 2011;2:491-502.
27. Mishra M. Simple and validated UV-spectroscopic method for estimation of moxifloxacin. HCL in bulk and formulation. J Global Pharm Technol 2010;2:21-7.
28. Chintawar PP, Pawar PN, Harde MT, Joshi SV, Chaudhari PD. Spectrophotometric methods for simultaneous estimation of moxifloxacin HCl and ketorolac tromethamine. Asian J Res Chem 2010;3:767-71.
29. Srinivas N, Narasu L, Shankar BP, Mullangi R. Development and validation of a HPLC method for simultaneous quantitation of gatifloxacin, sparfloxacin and moxifloxacin using levofloxacin as internal standard in human plasma: application to a clinical pharmacokinetic study. Biomed Chromatogr 2008;22:1288-95.
30. Baietto L, D'Avolio A, De Rosa FG, Garazzino S, Patanella S, Siccardi M, *et al.* Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. Ther Drug Monit 2009;31:104-9.
31. Kamruzzaman M, Alam A-M, Lee SH, Suh YS, Kim YH, Kim GM, *et al.* Method for determination of fluoroquinolones based on the plasmonic interaction between their fluorescent terbium complexes and silver nanoparticles. Microchim Acta 2011;174:353-60.
32. Sultana N, Akhtar M, Shamim S, Gul S, Arayne MS. Simultaneous determination of moxifloxacin and H_2 receptor antagonist in pharmaceutical dosage formulations by RP-HPLC: application to *in vitro* drug interactions. Quim Nova 2011;34:683-8.
33. Dewani A, Barik B, Kanungo S, Wattyani B, Chandewar A. Development and validation of RP-HPLC method for the determination of moxifloxacin in presence of its degradation products. Am Eurasian J Sci Res 2011;6:192-200.
34. Devika G, Sudhakar M, Rao JV. Simultaneous estimation of cefixime and moxifloxacin in bulk and its pharmaceutical dosage form by RP-HPLC. Orient J Chem 2012;28:1743-50.
35. Sousa J, Alves G, Fortuna A, Falcão A. Analytical methods for determination of new fluoroquinolones in biological matrices and pharmaceutical formulations by liquid chromatography: a review. Anal Bioanal Chem 2012;403:93-129.
36. Barth HG, Conner AZ. Determination of doxorubicin hydrochloride in pharmaceutical preparations using high-pressure liquid chromatography. J Chromatogr A 1977;131:375-81.
37. Sastry CS, Lingeswara Rao JS. Determination of doxorubicin hydrochloride by visible spectrophotometry. Talanta 1996;43:1827-35.
38. Shinozawa S, Oda T. Determination of adriamycin (doxorubicin) and related fluorescent compounds in rat lymph and gall by high-performance liquid chromatography. J Chromatogr A 1981;212:323-30.

39. Dubois J, Hanocq M, Atassi G. Determination of doxorubicin, Daunorubicin and some of their metabolites in mouse plasma by high-performance reversed-phase liquid chromatography with amperometric detection. Anal Lett 1987;20:1611-34.
40. King D, Stewart J. HPLC determination of dacarbazine, doxorubicin, and ondansetron mixture in 5 % dextrose injection on underivatized silica with an aqueous-organic mobile phase. J Liq Chromatogr Relat Technol 1993;16:2309-23.
41. King D, Venkateshwaran T, Stewart JT. HPLC determination of a vincristine, doxorubicin, and ondansetron mixture in 0.9 % sodium chloride injection. J Liq Chromatogr Relat Technol 1994;17:1399-411.
42. Fogli S, Danesi R, Innocenti F, Di Paolo A, Bocci G, Barbara C, *et al.* An improved HPLC method for therapeutic drug monitoring of daunorubicin, idarubicin, doxorubicin, epirubicin, and their 13-dihydro metabolites in human plasma. Ther Drug Monit 1999;21:367.
43. Gianni L, Munzone E, Capri G, Fulfaro F, Tarenzi E, Villani F, *et al*. Paclitaxel by 3h infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. J Cereals Oilseeds 1995;13:2688-99.
44. Cullen M. Antibacterial prophylaxis after chemotherapy for solid tumors and lymphomas. N Engl J Med 2005;353:988-98.
45. Lebrecht D, Walker UA. Role of mtDNA lesions in anthracycline cardiotoxicity. Cardiovasc Toxicol 2007;7:108-13.