www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Simultaneous estimation of Cefixime and Ofloxacin in tablet dosage form by RP- HPLC

Subramanian Natesan*, Rameshkumar Loganathan, Venkateshwaran Krishnaswami and Abimanyu Sugumaran

Laboratory for Lipid Based Systems, Department of Pharmaceutical Technology,
Anna University of Technology Tiruchirappalli, Tiruchirappalli, Tamil Nadu, India

ABSTRACT

A simple, precise, accurate RP-HPLC method has been developed for the simultaneous estimation of cefixime and ofloxacin in combined tablet dosage form. The chromatographic separation was achieved using Phenomenex Luna C18 (250 X 4.60 mm, 5 µm) analytical column and the mobile phase consisting of methanol and 25 mM phosphate buffer 40: 60% (v/v) pH adjusted to 5.5 using ortho phosphoric acid at a flow rate of 1.2 mL/min. The UV detection was carried out at 290 nm using photodiode array detector. The retention time of cefixime and ofloxacin was found to be 2.5 min and 7.8 min respectively. The proposed method was validated for specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection and limit of quantitation. Linear calibration curves were obtained in the concentration of 5-25 µg/mL for cefixime and ofloxacin. The mean percentage recovery obtained for cefixime and ofloxacin were respectively between 98.0% and 105.7%. Limit of detection and quantification for cefixime were 13.3 ng/mL and 40.1 ng/mL and for ofloxacin 12.3 ng/mL and 37.1 ng/mL, respectively. The developed method can be used for routine analysis of titled drugs combination in tablet formulation.

Keywords: cefixime; ofloxacin; RP-HPLC; simultaneous analysis; validation.

INTRODUCTION

Cefixime [(6R,7R)-7[(Z)-2-(2-amino- 4-thiazolyl) -2-(carboxymethoxyimino]-acetamido)-8-oxo-3-vinyl-5-thia-1-azo bicyclo-(4,2,)-oct-2-ene-2 carboxylic acid] (Fig. 1) is an orally absorbed third generation cephalosporin antibiotic possessing antibacterial spectrum against various gram positive bacteria and gram negative bacteria, including *Haemophilus influenza*, *Neisseria gonorrhoea* (Nanda 2009), *Escherichia coli*, and *Klebsiella pneumonia.*

It was not hydrolyzed by the common plasmid or by chromosomal β-lactamases which inactivate the oral penicillins and cephalosporins and thus cefixime is useful to treat some of the most difficult respiratory infections, gonorrhoea, otitis media, pharyngitis and urinary tract infections. It has been reported that the aminothiazole ring is responsible for both excellent activity and oral absorption and in particular amino group in the thiazole ring is essential for the potential antibacterial activity (Meng 2005, Kathiresan 2009).

Ofloxacin [9-fluoro-2, 3-dihydro-3-methyl-10(4-methyl-1- piperazinyl) 7- oxo-7H-pyrido [1, 2, 3de]-1, 4-benzoxazine- 6-carboxylic acid] (Fig. 2) is a synthetic fluoroquinolone derivative, which has demonstrated broad spectrum activity against many pathogenic gram-negative and gram-positive bacteria. The bactericidal action of ofloxacin results from interference with enzyme DNA gyrase which is needed for the synthesis of bacterial DNA (Mouton 1999, Kasabe 2009).

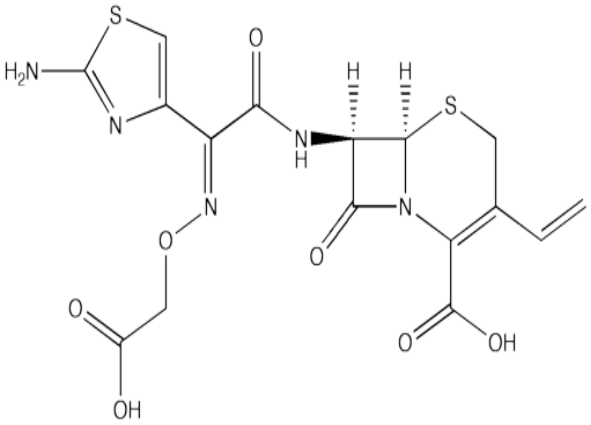

Figure 1: Chemical structure of Cefixime

Ofloxacin is used to treat pneumonia and bronchitis caused by influenza, *Streptococcus pneumonia*, skin infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, sexually transmitted diseases such as gonorrhoea and chlamydia, urinary tract and prostate infections caused by *Escherchiae coli* and used as an alternative treatment to ciprofloxacin for anthrax (Sultana 2007).

* Corresponding Author
Email: natesansubbu@gmail.com
Contact: +91-431-2407978
Received on: 10-02-2011
Revised on: 13-03-2011
Accepted on: 15-03-2011

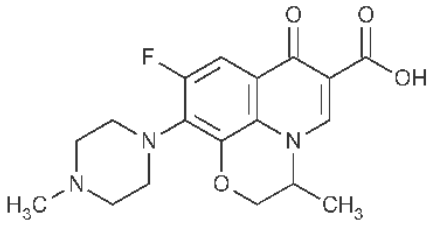

Figure 2: Chemical structure of Ofloxacin

New tablet formulation containing cefixime and ofloxacin each 200 mg is commercially available in Indian market to treat nephrotoxicity, since no single drug is considered to be bactericidal for enterococci. The different methods for the estimation of cefixime such as HPLC with UV detection (Nemutlu 2009, Dhoka 2009, Rathinavel 2008, Zendelovska 2003), HPTLC (Jovanovic, 1998), electrophoretic method (Raj 2010, Walily 2002), flow injection spectrophotometry (Momani 2001) and simple UV spectrophotometry **(**Nanda 2009**)** have been described. HPLC method with UV detection (Mignot 1998, Ohkubom 1991, Kumar 2009, Zeng 1999, Shervington 2005), fluorescence detection (Chan 2006), spectrofluorimetry method (Ballesteros 2002) and spectrophotometric methods (Wankhede 2002) have been described for the determination of ofloxacin. However, no references have been found for simultaneous determination of cefixime and ofloxacin in pharmaceutical preparation up to best of our knowledge. The present manuscript describes a simple, rapid, precise and accurate isocratic reverse-phase HPLC method for the simultaneous determination of cefixime and ofloxacin in combined tablet dosage form.

EXPERIMENTAL

Chemicals

Cefixime and ofloxacin were obtained from Orchid Pharmaceuticals Ltd, Chennai and Saimira Pharmaceuticals, Chennai respectively. Methanol (HPLC Grade) was purchased from SD Fine chemicals Ltd, Mumbai, potassium ortho phosphate (AR Grade) and ortho phosphoric acid (AR Grade) were purchased from Qualigens fine chemicals, Mumbai. Milli Q water was used throughout the experiment. Tablets were purchased from Indian market, containing cefixime 200 mg and ofloxacin 200 mg per tablet.

Equipments

Analysis was performed on a chromatographic system of Shimadzu Prominence consisting of LC 20 AD liquid pump equipped with manual 20 µl sample injector. Chromatographic separation was achieved on Phenomenex Luna C18 (250 X 4.60 mm, 5 µm) analytical column. Data acquisition was made with LC solution v.1.24 Spinchrome-1 soft ware. The peak purity was checked with the photodiode array detector.

Standard preparation

Cefixime (25 µg/mL) and ofloxacin (25 µg/mL) standard stock solution was prepared by transferring 100.0 mg of cefixime and 100.0 mg of ofloxacin to a 100 mL volumetric flask containing 10 mL of methanol. It was then sonicated for 15 min. The solution was diluted up to volume using milli Q water; appropriate dilutions were made to prepare 25 µg/mL solution using milli Q water.

Sample preparation

Twenty tablets were accurately weighed, crushed in a mortar and finely powdered. The average weight of tablets was determined with the help of weight of 20 tablets. A portion of tablet equivalent to 93.0 mg was accurately weighed in to 100 mL volumetric flask, 10 mL of methanol was added, sonicated with intermittent shaking for 20 min and after sonication filtered through 0.22 µm membrane filter. Aliquot portion of the filtrate was further diluted to get final concentration of 25 µg/mL of cefixime and ofloxacin. 20 µL of the test solution was injected, the chromatogram was recorded, and the amount of the drugs present was calculated.

Method validation

The optimized chromatographic conditions were validated by evaluating linearity, recovery, method and system precision, limit of detection (LOD), limit of quantification (LOQ), robustness, intra- day, inter- day variability and solution stability in accordance with ICH guideline Q2 (R1). The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with

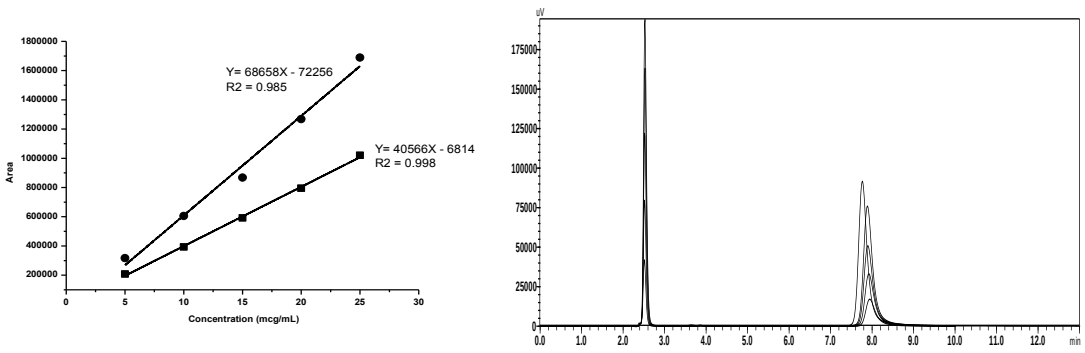

Figure 3: Linearity of Cefixime and Ofloxacin

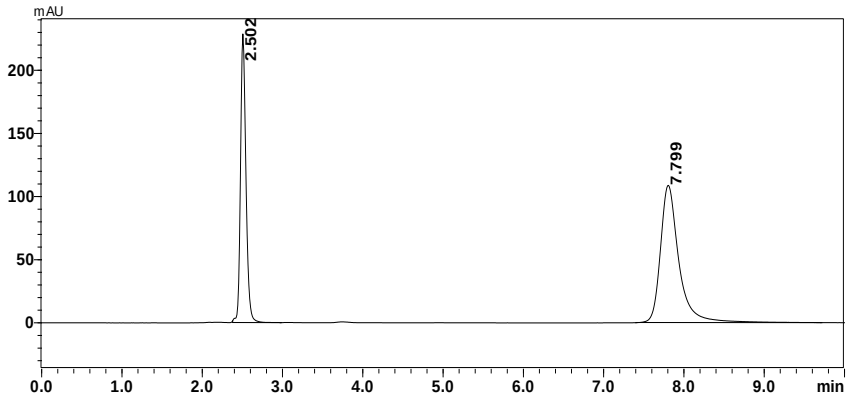

Figure 4: System precision Chromatogram of Cefixime and Ofloxacin

Table 1: Method validation and System suitability parameters of Cefixime and Ofloxacin

Parameters	Cefixime		Ofloxacin	
Recovery	**RSD (%) of peak area**	**Recovered (%)**	**RSD (%) of peak area**	**Recovered (%)**
80	1.64	105.2	1.12	102.3
100	1.41	100.4	1.45	105.7
120	1.92	99.2	1.41	98.0
Precision				
System precision	4.18	-	2.01	-
Method precision	2.57		1.90	
Variations				
Analyst-1 variation	1.19		1.75	
Analyst-2 variation	1.75	-	0.31	-
Inter day variation	0.75		1.79	
Intra day variation	1.35		1.31	
System suitability				
Retention time (minutes)	2.5		7.8	
Tailing factor	1.2	-	1.5	-
Theoretical plates	6499		7989	
Resolution	-		19.59	
Area	1024746		1457645	

Table 2: Robustness datas for Cefixime and Ofloxacin

% RSD of peak area							
S.No	**Drug**	**altered flow rate (mL/min)**			**altered pH**		
		1.1	1.2	1.3	pH5.4	pH5.5	pH5.6
1	Cefixime	0.22	1.07	1.60	0.27	1.07	1.90
2	Ofloxacin	1.92	0.09	1.49	0.76	0.09	1.32

Table 3: Solution stability datas for Cefixime and Ofloxacin

S. No	Drugs	Conditions	12 Hours	24 Hours
			Peak area	**Peak area**
1	Cefixime	RT	1122063	1126373
		REF	1114321	1094527
2	Ofloxacin	RT	1653240	1629908
		REF	1541480	1521006

RT – Room temperature, REF – Refrigerated condition

known concentration. Robustness of the method was determined by purposely altering the final experimental conditions like flow rate and pH of mobile phase. The nominal concentrations of standard and test solutions for ofloxacin and cefixime were 5 and 25 µg/mL. Response function was determined by preparing standard solution at five different concentration levels using different analyst on two different days. The accuracy of the assay method was evaluated with recovery of the standards from excipients.

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

During optimizing the method two organic solvents (methanol, acetonitrile) were tested. The chromatographic conditions were also optimized by using different buffers like phosphate, acetate, citrate for mobile phase preparation. After a series of screening experiments, it was concluded that phosphate buffer gave better peak shapes than their acetate, citrate counterparts. The resolution of chromatogram obtained with methanol is better than acetonitrile. The cost of acetonitrile also favoured to choose methanol as solvent for further studies. The chromatographic separation was achieved on a Shimadzu Prominence consisting of LC 20 AD liquid pump, Phenomenex C18 column (250 X 4.6 mm, 5 µm), and using methanol- phosphate buffer (25 mM) 40:60% (v/v) as mobile phase. The pH of buffer was adjusted with orthophosporic acid to pH 5.5; pH of buffer was selected according to pka value of cefixime 3.73 and ofloxacin 6.97. The detection wavelength of cefixime is 288 nm and ofloxacin is 292 nm, and for the simultaneous estimation of drugs the detection wavelength was fixed at 290 nm at which the peak response of both drugs were maxima. The flow rate of 1.2 mL/min was kept to achieve adequate retention time of two peaks as 2.5 min and 7.8 min of cefixime and ofloxacin respectively.

Method Validation

Linearity

Linearity was determined for cefixime and ofloxacin in the range of 5-25 µg/ml. The correlation coefficient ('r^{2}') values for both the drugs were >0.999. Typically, the regression equation for the calibration curve was found to be y = 40567x - 6814.5 for ofloxacin and y = 68112x - 72256 for cefixime. Linearity of cefixime and ofloxacin were shown in Fig. 3.

Recovery

Recovery of the method was calculated at three levels like low, middle and high concentrations (80%, 100% and 120%) by standard addition method. These mixtures were determined by the proposed method in triplicates the mean percentage recoveries obtained for cefixime and ofloxacin were between 98.0% and 103.7% respectively (Nemutlu 2009). Results of recovery (%) and R.S.D (%) were shown in Table 1.

Method and system precision

Precision of this method was determined by injecting the standard solution of the analytes five times. The system precision (injection repeatability) is a measure of the method variability that can be expected for a given analyst performing the analysis for five repeated analysis of the same sample working solution (Zendelovska 2003). The RSD% of peak area for five replicates were found to be less than 2.6% for method precision and 4.2% for system precision. Typical chromatogram for system precision was shown in fig. 4.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of cefixime and ofloxacin were determined by calibration curve method which includes signal- to- noise ratio, use of standard deviation of the response and the slope of the calibration curve, solutions of both cefixime and ofloxacin were prepared in the range of 2.5-12.5 µg/mL and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were 13.3 ng/mL and 40.1 ng/mL for cefixime and 12.3 ng/mL and 37.1 ng/mL for ofloxacin calculated by using the following equations.

$$LOD = \frac{3.3\,\sigma}{S} \qquad LOQ = \frac{10\,\sigma}{S}$$

where σ is the residual standard deviation of response and S is the slope of the calibration curve.

System suitability and specificity

System suitability tests are an integral part of a liquid chromatographic method, and they were used to verify whether the proposed method was able to produce good resolution between the peaks of interest with high reproducibility, determined by making five replicate injections (Kumar 2009) from freshly prepared standard solutions and analyzing the peaks for theoretical plates (*N*) and tailing factors (*T*). The USP theoretical plate was found to be greater than 6000 for both peaks and tailing factor was found to be 1.2 for cefixime and 1.5 for ofloxacin, the resolution between two peaks was found to be 19.59. In peak purity analysis with photodiode array detector, purity angle was less than purity threshold for both cefixime and ofloxacin indicates the peak of analytes was pure and excipients used in the formulation did not interfere the analytes.

Intra- inter day and analyst variability

The intra-day (repeatability), inter-day (intermediate precision) variability and analyst to analyst variations were determined in standard solutions. These experiments were repeated on second day to evaluate day to day variability (Ballesteros 2002). The %RSD of peak area for intra- day, inter- day variability and analyst to analyst variations as shown in Table 1 was found to be less than 2.0%. The result shows that there is no statistical difference between analysts, hence the method is found to be rugged.

Robustness

In order to demonstrate the robustness of the method, deliberate change in chromatographic conditions, i.e. change in flow rate by ±0.1 mL/min, change in pH of the buffer by ±0.1 unit was carried out (Mignot 1998). The standard deviation of peak areas was calculated for each parameter and % R.S.D was found to be less

than 2.0% for both ofloxacin and cefixime. The lowest values of %R.S.D. as shown in Table 2 indicates the method to be robust over an acceptable working range by its HPLC operational condition.

Stability of standard and sample solution

Duplicate standard and sample solutions (stored at room temperature around 25˚C and another set stored at refrigerator condition) were separately injected at 0, 12, 24 hours (Wankhede 2008, Joshia 2010). The results remained almost unchanged as shown in Table 3 and no significant degradation was observed within the given period, indicating that the standard and sample solutions were stable for at least 24 hours.

CONCLUSION

A simple, reproducible, linear, precise, affordable and accurate RP- HPLC method has been developed and validated for quantitative simultaneous determination of cefixime and ofloxacin in tablet formulation. In the case of ofloxacin the polarity around the tryptophan residues decreased hence the hydrophobicity increased (Nia 2010). The method is very specific as both peaks were well separated from its excipient peaks with a short chromatographic time course of 10 min. This analytical method enables quantification of the cefixime and ofloxacin in a range from 2.5 to 25 µg/mL, with acceptable precision, recovery, linearity, inter day and intraday precision validated as per ICH guidelines which considers to be a promising technique that has obvious advantage compared with conventional analytical techniques. Thus the proposed method can be applied for routine quality control analysis work and pharmacokinetic studies.

REFERENCES

Ballesteros, O., Vilchez, J.L., Navaion, A., 'Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry', J. Pharm. Biomed. Anal. 30, 2002 pp. 1103-1110.

Chan, K.P., Chu, K.O., Lai, W.W.K., Choy, K.W., Wang, C.C., Lam, D.S.C., Pang, C.P., 'Determination of ofloxacin and moxifloxacin and their penetration, In human aqueous and vitreous humor by using high-performance liquid chromatography fuorescence detection', Anal. Biochem. 353, 2006 pp. 30-36.

Dhoka, M.V., Gawande, V.T., Joshi, P.P., 'Simultaneous estimation of cefixime trihydrate and erdosteine in pharmaceutical dosage form by using reverse phase - high performance liquid chromatography', Int. J. Chem. Tech. Resear. 2, 2009 pp. 79-87.

Horie, M., Saito, K., Nose, N., Nakazawab, H., 'Simultaneous determination of benofloxacin, danofloxacin, enrofloxacin and ofloxacin in chicken tissues by high-performance liquid chromatography', J. Chromatogr. B. 653, 1994 pp. 69-76.

Joshia, S.J., Karbharia, P.A., Bhoira, S.I., Bindub, K.S., Dasc, C., 'RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation', J. Pharm. Biomed. Anal. 52, 2010 pp. 362-371.

Jovanovic, S.E., Agbaba, D., Stakic, D.Z., Vladimirov, S., 'HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms', J. Pharm. Biomed. Anal. 18, 1998 pp. 893-898.

Kasabe, J., Shitole, V.V., Waghmare, V.V., Mohite, V., 'Simultaneous estimation of metronidazole and ofloxacin in combined dosage form by reverse phase high performance liquid chromatography method', Int. J. Chem. Tech. Resear. 1, 2009 pp. 1244-1250.

Kathiresan, K., Murugan, R., Shahul Hameed, M., Gokula Inimai, K., Kanyadhara, T., 'Analytical method development and validation of cefixime and dicloxacillin tablets by RP-HPLC', Rasayan. J. Chem. 2, 2009 pp. 588-592.

Kumar, R.S., Nallasivan, P.K., Saravanakumar, S., Kandasamy, C.S, Venkatnarayanan, R., 'Simultaneous RP-HPLC estimation of nitazoxanide and ofloxacin in tablet dosage forms', Asian. J. Resear. Chem. 2, 2009 pp. 43-45.

Meng, F., Chen, X., Zeng, Y., Zhong, D., 'Sensitive liquid chromatography tandem mass spectrometry method for the determination of cefixime in human plasma application to a pharmacokinetic study', J. Chromatogr. B. 819, 2005 pp. 277-282.

Mignot, A., Lefebvre, M.A., 'High-performance liquid chromatographic determination of ofloxacin in plasma and urine', J. Chromatogr. 430, 1998 pp. 192-197.

Momani, I.F.A., 'Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis' J. Pharm. Biomed. Anal. 25, 2001, pp. 751-757.

Mouton, Y., Leroy, O., 'Ofloxacin', Int. J. Antimicrob. Agent. 1, 1991, pp. 57-74.

Nanda, R.K., Gaikwad, J., Prakash, A., 'Simultaneous spectrophotometric estimation of cefixime and ornidazole in tablet dosage form', Int. J. Pharm. Tech. Res. 3, 2009 pp. 488-491.

Nemutlu, E., Kir, S., Katlan, D., Beksac, M.S., 'Simultaneous multi response optimization of an HPLC method to separate seven cephalosporins in plasma and amniotic fluid application to validation and quantification of cefepime, cefixime and cefoperazone', Talanta 80, 2009 pp. 117-126.

Nia, Y., Sub, S., Kokot, S., 'Spectrometric studies on the interaction of fluoroquinolones and bovine serum albumin', Spectrochimica. Acta. Part. A. 75, 2010 pp. 54

Ohkubom, T., Kudo, M., Sugawara, K., 'High performance liquid chromatography determination of ofloxacin in serum', Anal. Sci. 7, 1991 pp. 741-743.

Okoye, N.N., Nwokedi, G.I.C., Ukwueze, N.N, Okoye, F.B.C., 'Spectrophotometric determination of some cephalosporin antibiotics using Prussian blue reaction', Sci. Resear. Essay. 2, 2007 pp. 342-347.

Raj, K.A., 'Determination of cefixime trihydrate and cefuroxime axetil in bulk drug and pharmaceutical dosage forms by electrophoretic method', Int. J. Chem. Tech Resear. 2, 2010 pp. 337-340.

Rathinavel, G., Mukherjee, P.K., Valarmathy, J., Joshua, L.S., Ganesh, M., Sivakumar, T., 'A validated RP-HPLC method for simultaneous estimation of cefixime and cloxacillin in tablets' E. J. 5, 2008 pp. 648-651.

Shervington, L.A., Abba, M., Hussain, B., Donnely, J., 'The simultaneous separation and determination of five quinolone antibotics using isocratic reversed-phase HPLC application to stability studies on an ofloxacin tablet formulation', J. Pharm. Biomed. Anal. 39, 2005 pp. 769-775.

Smet, J.D., Bousserya, K., Colpaertb, K., Suttera, P.D., Paepec, P.D., Decruvenaereb, J., Bocxlaer, J., 'Pharmacokinetics of fluoroquinolones in critical care patients a bio-analytical HPLC method for the simultaneous quantification of ofloxacin, ciprofloxacin and moxifloxacin in human plasma', J. Chromatogr. B. 877, 2009 pp. 961-967.

Sultana, N., Arayne, M.S., Yasmeen, N., 'In vitro availability of ofloxacin in presence of metals essential to human body', Pak. J. Pharm. Sci. 20, 2007 pp. 36-42.

Walily, A.M.E., Gazy, A.A., Belal, S.F., Khamis, E.F., 'Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride', J. Pharm. Biomed. Anal. 22, 2002 pp. 385-392.

Wankhede, S.B., Praksh, A., Chitlange, S.S. 'Simultaneous spectrophotometric estimation of ofloxacin and satranidazole in tablet dosage form', Asian. J. Resear. Chem. 1, 2008 pp. 9-11.

Zendelovska, D., Stafilov, T., Milosevski, P., 'High-performance liquid chromatographic method for determination of cefixime and cefotaxime in human plasma', Bulletin. Chem. Tech. Macedonia. 22, 2003 pp. 39-45.

Zeng, S., Zhong, J., Pan, L., Li, Y., 'High-performance liquid chromatography separation and quantitation of ofloxacin enantiomers in rat microsomes', J. Chromatogr. B. 728, 1999 pp. 151-155.