International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277-7873
RESEARCH ARTICLE **V-1, I-3, 2012**

Development and Validation of First Derivative Spectrophotometric Method for Simultaneous Estimation of Cefixime and Moxifloxacin in Synthetic Mixture

Chaudhari BG*[1], Patel B[1]
[1]*Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar-384012, Mehsana, Gujarat, India.*
Manuscript No: IJPRS/V1/I3/00166, Received On: 05/09/2012, Accepted On: 22/09/2012

ABSTRACT

A simple and economical first derivative spectrophotometric method has been developed for the simultaneous estimation of cefixime and moxifloxacin in their synthetic mixture. The method involved determination of Zero Crossing points (ZCP) in their respective derivative spectra. By scanning first derivative spectra of cefixime and moxifloxacin, ZCP for cefixime was found to be at 289 nm and for moxifloxacin at 316.4 nm. For cefixime 316.4 nm and for moxifloxacin 289 nm was chosen as an analytical wavelength. The method involved solving of an equation based on measurement of absorbances at wavelengths 289 and 316.4 nm. The proposed method was found to be simple, economical, accurate, and reproducible for routine analysis of both drugs in tablet dosage form.

KEYWORDS

Cefixime, Moxifloxacin, Spectrophotometric, First Derivative.

INTRODUCTION

Cefixime, an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Chemically, it is [6R, 7R] – 7- [[(2Z)- 2- (2-amino thiazole- 4-yl)- [(carboxy methoxy) imino] aetyl] amino]-3-ethenyl -8-oxo 5-thia 1-aza bicyclo [4.2.0] oct-2- ene-2 carboxylic trihydrate1. Moxifloxacin is a synthetic fluoroquinolone antibiotic agent. Chemically, it is 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo- quinoline-3-carboxylic acid[2]. Cefixime is official in IP' 20103, BP' 20104, USP' 20095, JP' 20066 while Moxifloxacin is official in BP' 20107.Literature survey reveals that number of methods such as spectrophotometric[8,9], HPLC[10-17], HPTLC[17,18], LC-MS[19], HPCPE[20], Voltametric[21] are reported for the estimation of cefixime from its formulation or biological fluids. Similarly number of methods such as spectrophotometric[22], HPLC[23,24], HPTLC[25], TLC[26], Fluorescence spectroscopy[27], Voltametric[28] are reported for the estimation of diltiazem from its formulation or biological fluids. There was no any method reported for the simultaneous estimation of cefixime and moxifloxacin from their combined dosage form. So, present study was aimed to develop and validate[29] spectrophotometric method for simultaneous estimation of rosuvastatin and diltiazem from their synthetic mixture which would be simple, cost effective and easily adopted by small laboratories.

***Address for Correspondence:**
Chaudhari Bharat G.
Department of Quality Assurance,
S. K. Patel College of Pharmaceutical Education and Research,
Ganpat University, Ganpat Vidyanagar-384012,
Gujarat, India.
E-Mail Id: bharat_pharmacy@yahoo.co.in

MATERIALS AND METHOD

Instruments and Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System (Millipore Bioscience Division Pvt.Ltd, India) was used during study.

Chemicals and Reagents

Kindly gifted reference standards of Cefixime (Acme Pharmaceuticals, Gujarat, India), and Moxifloxacin (Astron Research Centre, Ahmedabad) was used without further purification. Synthetic mixture containing 40 mg cefixime and 40 mg moxifloxacin was prepared in the laboratory. AR grade methanol (S.D. Fine Chemical Ltd., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

Preparation of Reagents and Solutions

Preparation of standard solutions

To Prepare standard solution of cefixime (100 µg/ml) and moxifloxacin (100 µg/ml), 10 mg of each drugs were transferred in two different 100 ml volumetric flask. Dissolve and diluted up to mark with methanol, from these stock solution, 0.8 ml aliquots were transferred in two different 10 ml volumetric flask and were diluted up to mark with methanol to get working standard solution having concentration of cefixime (CEF) and moxifloxacin (MOXI) of 8µg/ml each

Preparation of synthetic mixture

CEF (40 mg) and MOXI (40 mg) were taken and then mixed with starch, Lactose, Magnesium stearate, S.S.G. and Talc. Total 400 mg of mixture was prepared and it was used in further study.

Preparation of sample solution

From synthetic mixture, powder equivalent to 100 mg of drugs was taken and diluted up to 100 ml with methanol. The content was mixed with methanol (50ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a whatman filter paper no. 41.

Determination of the Zero Crossing Points

The standard solutions of CEF (8 µg/ml) and MOXI (8 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. It appeared that CEF showed zero crossing at 289 and 260.3 nm while MOXI showed zero crossing at 316.4 and 226 nm. At 289nm CEF showed zero absorbance and MOXI showed reasonable absorbance, while at 316.4nm MOXI showed zero absorbance so these two wavelength were selected for further measurement.

Calibration Curve for CEF and MOXI

To check linearity of the method, working standard solution having concentration in range of 4-24 µg/ml were prepared from the standard stock solutions of both drugs. For this prepare aliquots of 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml of standard stock solutions of both drug were transferred separately to a series of 10 ml volumetric flasks and diluted to mark with methanol, and first-derivative absorbances (D1) were measured at 316.4 nm for CEF and 289 nm for MOXI. The calibration curves were constructed by plotting absorbance verses concentrations.

Analysis of CEF and MOXI in synthetic mixture

The response of the sample solution was measured at 316.4 nm and 289 nm for quantitation of CEF and MOXI, respectively. The amounts of the CEF and MOXI present in the sample solution were calculated by fitting the responses into the regression equation for CEF and MOXI in the proposed method.

RESULTS AND DISCUSSION

Method development

In the derivative spectrum at 289 nm (zero crossing point of CEF), MOXI gives some abs. whereas at 316.4 nm (zero crossing point of MOXI), CEF gives some abs. Hence the wavelengths 316.4 nm and 289 nm were selected as analytical wavelengths for determination of CEF and MOXI, respectively. These two wavelengths can be employed for the estimation of CEF and MOXI without any interference from the other drug in their combined formulation. The overlain spectra for standard CEF and MOXI are shown in Figure: 1. The overlain first derivative spectrum is shown in Figure: 2. First-derivative spectra give good quantitative determination of both drugs at their respective ZCPs without any interference[30-32].

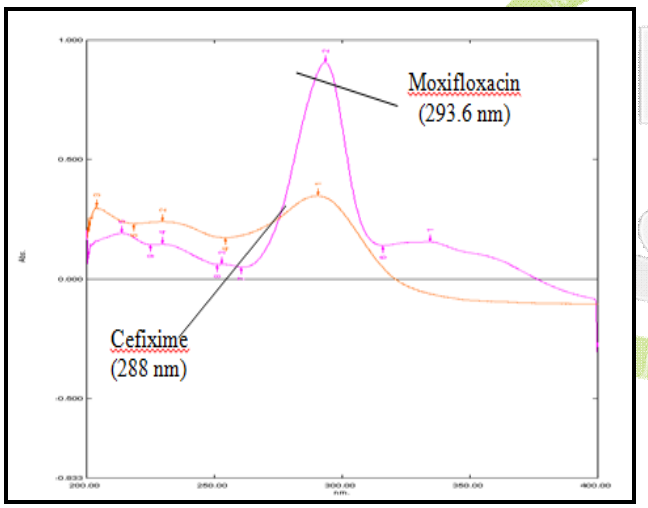

Figure 1: Overlain Zero Order Absorption Spectra of Standard Solution of CEF and MOXI In Methanol (12 µg/ml)

Validation of the proposed method:-

3.2.1 Linearity

Linear correlation was obtained between first derivative absorbance versus concentrations of CEF and MOXI in the range of 4-14 µg/ml. Regression parameters are mentioned in Table 1 and 2. The calibration curves of these two drugs at 316.4 nm and 289 nm are shown in Figure-3, and Figure-4.

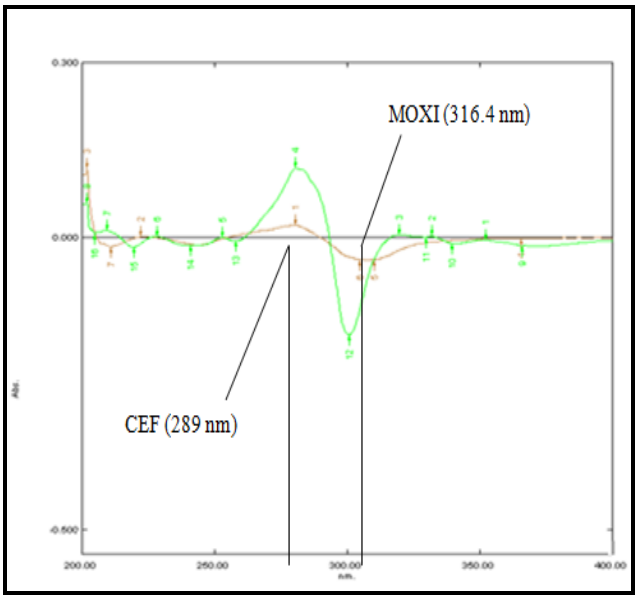

Figure 2: Overlain First Derivative Absorption Spectra of Standard Solution of CEF and MOXI in Methanol (12 µg/ml)

Table 1: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

PARAMETERS	CEF at 316.4nm	MOXI at 289nm
Linearity (µg/ml)	4-14	4-14
Slope	0.003	0.007
Intercept	0.001	0.005
Correlation coefficient	0.995	0.993
LOD (µg/ml)	0.55	0.42
LOQ (µg/ml)	1.66	1.28
Repeatability (RSD, n = 6), %	1.92	1.63
Precision (RSD), %		
Interday (n = 6), % Intraday (n = 6), %	0.74 – 1.85 0.62 – 1.95	0.65 – 1.74 0.42 – 1.63

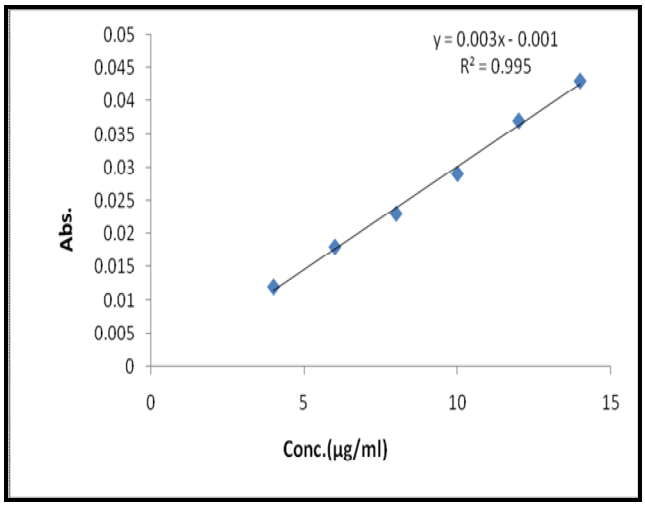

Figure 3: Calibration Curve of CEF at 316.4 nm

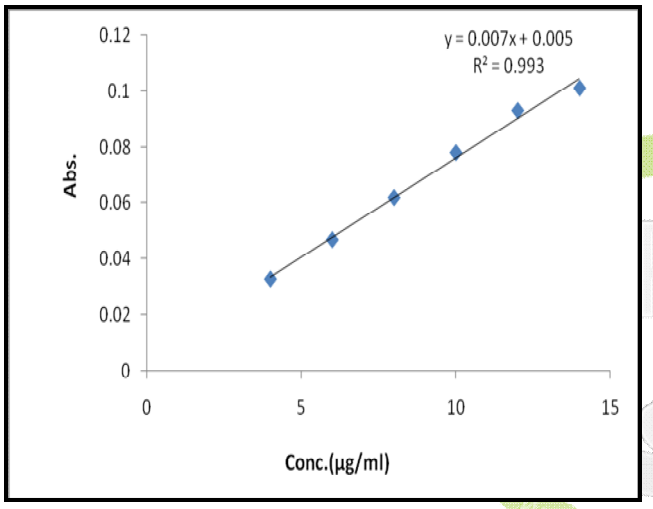

Figure 4: Calibration Curve of MOXI at 289 nm

Accuracy

The accuracy of the method was determined by calculating recovery of CEF and MOXI by the standard addition method. Known amounts of standard solutions of CEF and MOXI (4, 8, 12 µg/ml for both drugs) were added to prequantified separate sample solutions of CEF 8 µg/ml and MOXI 8 µg/ml. The amounts of CEF and MOXI were estimated by applying obtained values to the regression equation of the calibration curve. The mean recoveries were 100.44 ± 1.32 and 100.6 ± 1.24 % for CEF and MOXI, respectively (Table 1). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

Table 2: Calibration Curve Data of CEF and MOXI by First Derivative Spectrophotometric Method

Sr no.	Concentration (µg/ml)	derivative value of CEF at λmax 316.4 nm	derivative value of MOXI at λmax 289 nm
1	4	0.012	0.033
2	6	0.018	0.047
3	8	0.023	0.062
4	10	0.029	0.078
5	12	0.037	0.093
6	14	0.043	0.101

Table 3: Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± SD*
CEF	I	8	50 %	101.2 ± 0.72
	II	8	100 %	101.2 ± 1.45
	III	8	150 %	98.9 ± 1.80
MOXI	I	8	50 %	101.2 ± 0.60
	II	8	100 %	100.7 ± 1.42
	III	8	150 %	99.9 ± 1.72

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution of (n = 6) of CEF and MOXI (8 µg/ml) without changing the parameter of proposed method. The RSD values for CEF and MOXI were found to be 1.92 and 1.63 %, respectively (Table- 1 & 4). Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable.

Table 4: Precision Data for CEF and MOXI

CEF and MOXI (8 µg/ml)	Absorbance at 316.4nm (ZCP of CEF)	Absorbance at 289nm (ZCP of MOXI)
1	0.026	0.057
2	0.025	0.055
3	0.024	0.053
4	0.026	0.057
5	0.029	0.056
6	0.027	0.054
Mean	0.026	0.055
S.D.	0.0005	0.0009
%CV	1.92	1.63

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 6 different concentrations of standard solutions of CEF and MOXI (4, 6, 8, 10, 12, and 14 µg/ml). The result was reported in terms of relative standard deviation (RSD). The low RSD values of interday (0.74 – 1.85 and 0.62 – 1.95%) and intraday (0.65 – 1.74 and 0.42 –1.63%) variations for CEF and MOXI, respectively, reveal that the proposed method was precise (Table 5).

LOD and LOQ

LOD and the LOQ of the drug were calculated using the following equations as per ICH guidelines. LOD values for CEF and MOXI were found to be 0.55 and 0.42 µg/ml, respectively and LOQ values CEF and MOXI were found to be 1.66 and 1.28 µg/ml, respectively (Table- 1). These data show that proposed method is sensitive for the determination of CEF and MOXI.

Assay of the Synthetic Mixture

The proposed validated method was successfully applied to determine CEF and MOXI in their synthetic mixture. The results obtained for CEF and MOXI were comparable with the corresponding taken amounts (Table 5.6). The first order derivative spectrum of sample was recorded.

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 4-14 µg/ml with co-efficient of correlation, r^2 = 0.995 for CEF at 316.4 nm and r^2 = 0.993 for MOXI at 289 nm, proposed method is highly reproducible and reliable.

The result of the analysis of synthetic mixture by the proposed method was highly reproducible and reliable and it was in good agreement with the taken amount of the drug. The additives usually present in the synthetic mixture did not interfere with determination of CEF and MOXI. The method can be used for the routine analysis of the CEF and MOXI in synthetic mixture.

ACKNOWLEDGMENTS

Authors are greatly thankful to S K Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar-384012 for providing facilities to carry out the work.

Table 5: Intermediate Precision Data for CEF and MOXI

CEF (µg/ml)	MOXI (µg/ml)	Intraday (RSD), %		Interday (RSD), %	
		CEF	MOXI	CEF	MOXI
4	**4**	0.66	0.45	0.75	0.64
8	**8**	0.92	0.89	0.98	0.99
12	**12**	1.73	1.61	1.80	1.90

Table 6: Analysis of Sample by Proposed Method (n = 6)

Sample No.	Amount taken		Amount found		% Assay	
	CEF (mg)	MOXI (mg)	CEF (mg)	MOXI (mg)	CEF (mg)	MOXI (mg)
1	400	400	400.6	400.5	100.2	100.5
2	400	400	402.7	398.6	101.8	98.6
3	400	400	402.4	399.2	100.9	99.2
4	400	400	398.3	400.5	99.3	100.5
5	400	400	403.1	399.8	101.4	99.8
6	400	400	399.7	401.2	99.8	101.2
Mean			401.1	399.9	100.5	99.9
S.D.			1.91	0.95	0.96	0.95

REFERENCES

1. The Merck Index "An encyclopedia of chemicals, drugs and biologicals" Merck and Co. Inc., whitehouse station, NJ, USA, 14th edition, 2006, 1924.
2. The Merck Index "An encyclopedia of chemicals, drugs and biologicals" Merck and Co. Inc., whitehouse station, NJ, USA, 14th edition, 2006, 6291.
3. Indian pharmacopoeia, Government of India, the Controller Publication, New Delhi 2010 vol.2; 1012.
4. British pharmacopeia, 6th edition, 2010; Vol I, Vol III, London: Her Majesty's Stationary Office 410.
5. The United State Pharmacopeia Drug Information, Maryland, the United States Pharmacopeial Convention Inc. 2009 vol.2, 1835.
6. Japanese Pharmacopoeia, Society of Japanese Pharmacopoeia, Tokyo, 15th edition 2006;. 443, 938-39.
7. British pharmacopeia, 6th edition, 2010; Vol II, Vol III, London: Her Majesty's Stationary Office 1460.
8. Virypaxappa BS, Shivaprasad KH, Latha MS, "A Simple Method for The Spectophotometric Determination of Cefixime In Pharmaceuticals", International Journal of Chem. Eng. Res, 2010, 2, 23-30.
9. Sharma R, Pathodiya G, "Simultaneous Estimation and Validation of Cefixime Trihydrate and Ornidazole in Bulk and Tablets", J. Pharm. Res, 2010, 3(12), 2953-2955
10. Falkowski JA, Look MZ, B. Hideyo ND, Silber M, "Determination of Cefixime in Biological Samples by RP-HPLC", J. Chromatography, 1987, 422, 145-152.

11. Eric-Jovanovic S, Agbaba D, Zivanov-Stakic D, Vladimirov S, "HPTLC Determination of Cephalosporins in Dosage Forms', J. Pharm. Biomed. Anal, 1998, 18, 893-898.
12. Arshad HM, Gauhar S, Bano R, "Development of HPLC-UV Method for Analysis of Cefixime In Raw Materials and In Capsule", Jordan J. Pharm. Sci, 2009, 2, 53-65.
13. Rathinavel G, Mukherjee PB, "A Validated RP – HPLC Method for Simultaneous Estimation of Cefixime and Cloxacillin in Tablets", E-J Chem. 2008, 5, 648-651.
14. Saikrishna K, Akula G, Pandey VP, Sreedevi K, "Validation of RP-HPLC for the estimation of cefixime in cefixime oral suspension", Int. J. Pharm. Technol, 2010, 2, 385-395.
15. Zendelovska D, "HPLC method for determination of cefixime and cefotaxime in human plasma", Bull. Chem. Technol. of Macedonia, 2003, 22, 39–45.
16. Khan IU, Sharif S, Ashfaq M, Asghar MN, "Simultaneous determination of potassium clavulanate and cefixime in synthetic mixtures by high-performance liquid chromatography", J AOAC Int. 2008, 91, 744-749.
17. Deshpande MM, Kasture VS, Gosavi SA, "Application of HPLC and HPTLC for the simultaneous determination of cefixime trihydrate and ambroxol hydrochloride in pharmaceutical dosage form", Eurasian J. Anal. Chem. 2010, 5, 227-238.
18. Dhoka MV, "Validated HPTLC method for determination of cefixime trihydrate and erdosteine in bulk and combined pharmaceutical dosage", Eurasian J. Anal. Chem, 2011, 6, 1-4.
19. Mang F, Chen X, Zeng Y, Zong D, "Cefixime trihydrate in human plasma: application to a pharmacokinetic study", J. Chromatography B, 2005, 819, 277-282.
20. Honda S, Taga A, Kakehi K, "Determination of cefixime & its metabolites by high performance capillary electrophoresis", J. Chromatography, 1992, 590, 364-368.
21. Golcu A, Dogan B, Ozkan SA, "Voltametric determination of cefixime in dosage form & biological fluid", Talanta, 2005, 67, 703-712.
22. Misra M, Misra AK, Zope P, Panpalia GM, Dorle AK, "Simple and validated UV–spectroscopic method for estimation of moxifloxacin in bulk and formulation", J. Global Pharm. Technol. 2009, 2, 6-9.
23. Guerra FL, Paim CS, Steppe M, Schapoval EE, "Simple and rapid liquid chromatography method for determination of moxifloxacin in tablet dosage form", J. AOAC Int, 2005, 88, 1086-1092.
24. Djurdjevic P, Ciric A, Djurdjevic A, Stankov MJ, "Optimization of separation and determination of moxifloxacin and its related substances by RP-HPLC", J. Pharm. Biomed. Anal, 2009, 50, 117-126.
25. Shah SA, Rathod IS, Suhagia BN, Baldaniya MV, "High performance thin layer chromatographic method for estimation of moxifloxacin in tablet dosage form", Indian J. Pharm. Sci, 2006, 67, 112-115.
26. Motwani_SK, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar S, "Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin" J. Analytica chemica acta, 2007, 582, 75-82.
27. Hemanthkumar AK, Ramachandran G, **"**Simple and rapid liquid chromatography method for determination of moxifloxacin in plasma", J. Chromatography B, 2009, 877, 1205-1208
28. Aparecido M, Trindade G, Silva GM , Ferreira VS, "Determination of moxifloxacin in tablets and human urine by square-wave adsorptive voltammetry", J. Microchem, 2005, 81, 209-216.
29. International Conference on Harmonisation, Topic Q2B, Validation of Analytical Methods: Methodology. The Third

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), (1996) Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland.

30. Beckett AH., Stenlake JB., Practical Pharmaceutical Chemistry, 14th Edition, CBS Publishers and distributors, 297-299, 1997.
31. Karajgi SR, Simultaneous Estimation of Atorvastatin and Ramipril by First Derivative Spectrophotometric method, J. Pharm. Res, 2009, 2, 874-877.
32. Mahaparale S, Telekone RS, Raut RP, Damle SS., Kasture PV, "Simultaneous Spectrophotometric Determination of Drotaverine Hydrochloride and Paracetamol in Tablet", Indian J Pharm Sci, 2010, 72, 133–136.

