

Research

Development and Validation of UV Analytical Methods for Quantification in combination of Cilastatin Sodium and Imipenem

P. Rishwanth¹, SK. Masthan Bee¹, SK. Salma Sulthana¹, S. Alekhya¹, M. Jarwin*²

¹Jagan's College of Pharmacy, Jangalakandriga (V), Muthukur (M), SPSR Nellore Dt.524346, A.P., India.

²Department of Pharmaceutical Analysis, Jagan's College of Pharmacy, Jangalakandriga (V), Muthukur (M), SPSR Nellore Dt.524346, A.P., India.

Corresponding Author:

M. Jarwin

Email:

mayarijarwin@gmail.com

DOI: 10.62896/ijpdd.2.5.12

Conflict of interest: NIL

Article History

Received: 12/04/2025

Accepted: 04/05/2025

Published: 16/05/2025

Abstract:

Cilastatin sodium and imipenem is a well-established broad-spectrum intravenous antibiotic used for the treatment of life-threatening infections worldwide. The present has been studied by various methods by ultraviolet (UV) spectroscopy, although this technique remains one of the simplest, most accurate and precise validated quality control laboratory methods. The aim of this study was to validate a method for separating the intercalating peaks of the UV spectra of imipenem and cilastatin and to compare the purity and quantities of these two active ingredients in different marketed brands. In present study simultaneous equation in UV methods, were developed and validated for some of the selected formulations containing two drug combinations. UV spectroscopic methods solving simultaneous equation is developed and validated for the following drug combination and formulation Cilastatin sodium & Imipenem. In UV spectroscopy all the selected raw materials were dissolved in methanol and their spectra were recorded and the formulations were analysed. The actual number of drugs in formulation was calculated by solving simultaneous equation. As the methods require only methanol as a solvent and less time-consuming process, it can be followed in routine analysis to standardize the raw material as well as finished products with reproducible results.

Keywords: Cilanem, Imipenem–cilastatin, UV assay

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

INTRODUCTION

Imipenem, a broad-spectrum β -lactam antibiotic of the carbapenem class, is widely used in the treatment of severe bacterial infections due to its high potency and resistance to β -lactamases. However, its rapid degradation by renal dehydropeptidase-I (DHP-I) limits its clinical efficacy when administered alone. To counteract this, it is co-administered with Cilastatin Sodium, a specific DHP-I inhibitor, which protects imipenem from renal metabolism and enhances its systemic availability. The combination therapy has become a critical component in managing multidrug-resistant bacterial infections in hospital settings.

Due to the synergistic pharmacological effects and therapeutic importance of this combination, the accurate and reliable quantification of both drugs in pharmaceutical formulations is essential for quality control and regulatory compliance. Among various analytical techniques, UV spectrophotometry remains a preferred choice in routine analysis due to its simplicity, cost-effectiveness, and rapidity.

Despite the availability of chromatographic methods for the estimation of imipenem and cilastatin, there is a lack of validated UV spectrophotometric methods specifically developed for their simultaneous quantification. Furthermore, interference from excipients and overlapping

spectral profiles pose significant challenges in the development of such methods.

This study aims to develop and validate simple, precise, accurate, and robust UV spectrophotometric methods for the simultaneous estimation of Cilastatin Sodium and Imipenem in combined pharmaceutical dosage forms, in accordance with ICH Q2(R1) guidelines. The proposed methods are intended to serve as efficient tools for routine quality control and formulation analysis in industrial and academic laboratories.

Materials and Methods

Chemicals and Reagents

Pure standards of Cilastatin Sodium and Imipenem were obtained as gift samples from a certified pharmaceutical manufacturer. All chemicals and solvents used were of analytical grade and procured from reputable suppliers. Double distilled water was used throughout the study.

Instrumentation

A UV-Visible double-beam spectrophotometer equipped with 1 cm quartz cuvettes was used for all absorbance measurements. The instrument was connected to software for spectral data acquisition and processing.

Preparation of Standard Solutions

Stock solutions of Cilastatin Sodium and Imipenem were prepared separately by dissolving 10 mg of each drug in 100 mL of distilled water to obtain concentrations of 100 µg/mL. Working standard solutions were prepared by suitable dilution of the stock solutions with distilled water.

Selection of Wavelengths

The individual UV absorption spectra of Cilastatin Sodium and Imipenem were recorded in the wavelength range of 200–400 nm. The wavelengths for simultaneous estimation were selected based on maximum absorbance (λ_{max}) and minimal overlapping of spectra. Methods such as simultaneous equation and absorbance ratio (Q-analysis) were evaluated for quantification.

Method Development

Two UV spectrophotometric methods were developed:

Method I (Simultaneous Equation Method): Based on absorbance measurements at two wavelengths— λ_1 (λ_{max} of Imipenem) and λ_2 (λ_{max} of Cilastatin Sodium). The absorptivity values were determined, and a set of simultaneous equations was applied to quantify each drug in the mixture.

Method II (Absorbance Ratio Method): Involved measurements at the isoabsorptive point and the λ_{max} of one drug. Calibration curves were constructed and validated for linearity and accuracy.

Method Validation

The developed methods were validated according to ICH Q2(R1) guidelines for the following parameters:

Linearity: Assessed over the concentration range of 5–30 µg/mL for both drugs.

Accuracy: Evaluated by recovery studies at three concentration levels (80%, 100%, 120%) using standard addition technique.

Precision: Assessed through repeatability (intra-day) and intermediate precision (inter-day) by analyzing three different concentrations in triplicate.

Specificity: Ensured by checking the absence of interference from formulation excipients.

LOD and LOQ: Determined based on the standard deviation of response and slope of the calibration curve.

Robustness and Ruggedness: Evaluated by making small deliberate changes in experimental conditions and observing the effect on method performance.

Analysis of Marketed Formulations

Commercially available combination vials containing Cilastatin Sodium and Imipenem were analyzed using the validated methods. The sample solution was prepared by accurately weighing the content equivalent to the labeled amount, followed by appropriate dilution with distilled water.

CILASTATIN SODIUM AND IMPENEM

UV Spectroscopic method (simultaneous equation method) was developed for simultaneous estimation of Cilastatin sodium and Imipenem.

UV Spectrometry (simultaneous equation method)

The $\square_{\text{max}}$ of Cilastatin sodium and Imipenem were observed at 210 nm and 287 nm respectively. Fig. 1 and 2 depicts the $\square_{\text{max}}$ of Cilastatin sodium and Imipenem respectively. Fig. 3 represents the overlain spectrum of Cilastatin sodium and Imipenem.

Five sets of standard solution were prepared and scanned over the range of 400-200 nm. Cilastatin sodium and Imipenem showed linearity in the range of 15-35 µg/ml and 10-50 µg/ml respectively. The linearity ranges are tabulated in Table - 1. Absorptivity values of Cilastatin sodium and

Imipenem were calculated. Analytical performance data are shown in Table -2.

The correlation coefficient values were found to be 0.9971 for Cilastatin sodium and 0.9899 for Imipenem. The correlation coefficients are within the limit as all points lie on the same line and the relationship between concentration and absorbance of Cilastatin sodium and Imipenem were linear in the range specified.

Linearity curves of Cilastatin sodium and Imipenem are shown in Fig. 4 & 5 respectively. The linearity spectrum of Cilastatin sodium and Imipenem is depicted in Fig. 6.

Accuracy of the proposed method was confirmed by recovery studies. The value ranges from 99.89% - 100.41% for Cilastatin sodium and 99.89% - 100.21% for Imipenem with the grand mean values

of 100.14% for Cilastatin sodium and 99.98% for Imipenem. These results are tabulated in Table - 3. Repeatability studies ensure the closeness of the result in all six determinations. The % relative standard deviation calculated from repeatability data was below 2% indicates the precision of the performed method. The results of the market sample analysis are in good agreement with label claim. Repeatability datas are furnished in Table - 4. The proposed UV spectrophotometric method is simple, accurate, precise, rapid and economical for the simultaneous estimation of Cilastatin sodium and Imipenem in tablet dosage form. The proposed method used inexpensive reagent, solvent and instrument that are available in laboratory. Hence these methods can be conveniently adopted for routine analysis.

DEVELOPMENT AND VALIDATION OF ANALYTICAL TECHNIQUES FOR CILASTATIN SODIUM AND IMPENEM BY UV SPECTROPHOTOMETRY (SIMULTANEOUS EQUATION METHOD).

Table 1: Linearity Range (UV Spectrophotometry) Cilastatin sodium and Imipenem

S.No.	Cilastatin sodium		Imipenem	
	Concentration in µg/ml	Absorbance at 210nm	Concentration in µg/ml	Absorbance at 287nm
1	15	0.683	10	0.001
2	20	0.878	20	0.616
3	25	1.108	30	1.212
4	30	1.361	40	1.733
5	35	1.536	50	1.918

Table 2: Analytical performance (UV Spectrophotometry) Cilastatin sodium and Imipenem

Parameters	Cilastatin sodium	Imipenem
Absorption maximum(λ max)	210 nm	287nm
Beer's lamberts limit(µg/ml)	15 - 35	10 - 50
Coefficient correlation(r^2)	0.9971	0.9899
regression Equation	$y=0.04378 x+ 0.01875$	$y = 0.04953 x + 0.03906$
Intercept (A)	0.01875	0.03906
Slope (B)	0.04378	0.04953
Limit of Detection (µg/ml)	4.2	3.2
Limit of Quantification (µg/ml)	16.6	9.6

Table 3: Recovery Data (UV Spectrophotometry) Cilastatin sodium and Imipenem

% Level		Cilastatin sodium	Imipenem
---------	--	-------------------	----------

80		100.22	99.89
		100.15	99.94
		100.07	99.89
	Mean	100.14	99.9
	% RSD	0.07	0.03
100		100.08	100.04
		99.94	99.86
		99.89	99.95
	Mean	99.97	99.95
	% RSD	0.1	0.09
120		100.24	100.21
		100.32	100.19
		100.41	99.92
	Mean	100.32	100.1
	% RSD	0.08	0.16
Grand Mean		100.14	99.98
% RSD		0.17	0.1

Table 4: Repeatability (UV Spectrophotometry) Cilastatin sodium and Imipenem

S.No	Cilastatin sodium		Imipenem	
	Assay (mg/ tab)	Assay (% Labeled amount)	Assay (mg/ tab)	Assay (% Labeled amount)
1	250.71	100.2	250.18	100.07
2	250.36	100.14	250.33	100.13
3	250.19	100.07	250.14	100.05
4	250.32	100.12	250.09	100.03
5	250.25	100.1	250.13	100.05
6	250.46	100.18	250.49	100.19
Mean		100.135		100.08
% RSD		0.05		0.06

DEVELOPMENT AND VALIDATION OF ANALYTICAL TECHNIQUES OF SIMULTANEOUS ESTIMATION OF CILASTATIN SODIUM AND IMIPENEM TABLETS.

By UV spectrophotometry simultaneous equation method

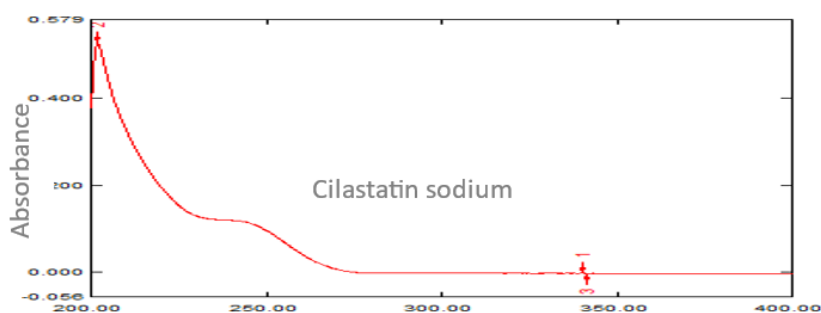
Fig- 1 UV spectrum for Cilastatin sodium

Fig-2 UV spectrum for Imipenem ☐ max

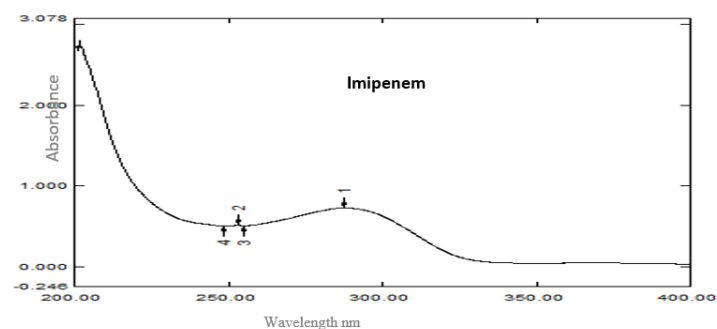


Fig – 3 Overlain spectrum of Cilastatin Sodium and Imipenem tablets

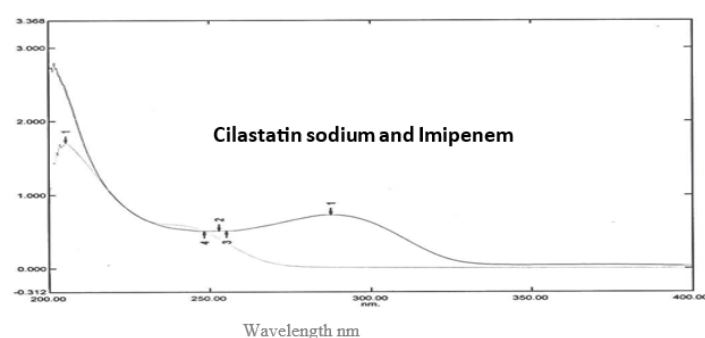


Fig -4 Linearity spectrum of Cilastatin Sodium and Imipenem tablets

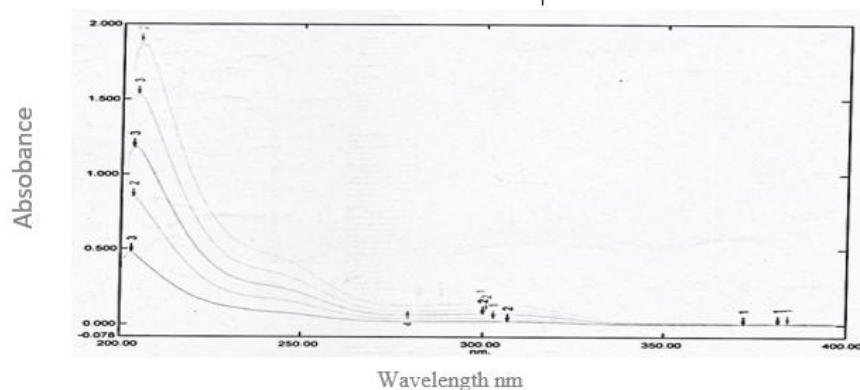


Fig- 5 Cilastatin Sodium calibration curve

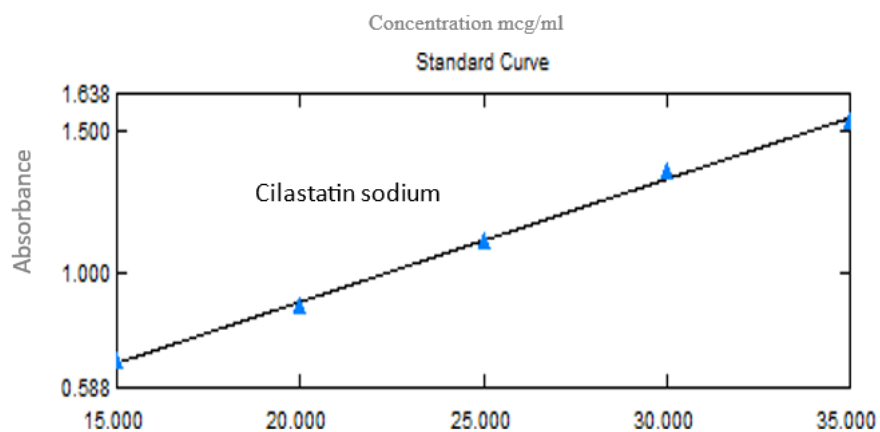
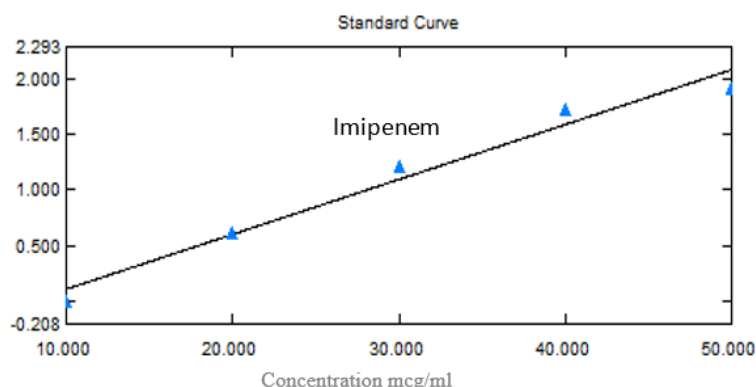


Fig -6 Imipenem calibration curve

In the present study, a UV spectrophotometric method based on the simultaneous equation approach was developed and validated for the estimation of Cilastatin Sodium and Imipenem in combined pharmaceutical formulations. The method was designed to offer a simple, rapid, and cost-effective alternative for routine analysis, using methanol as the solvent.

The UV absorption spectra of both drugs were recorded in methanol. Cilastatin Sodium and Imipenem showed maximum absorbance (λ_{max}) at distinct wavelengths, allowing for the selection of appropriate analytical wavelengths for the simultaneous estimation. Standard solutions of each drug were prepared and scanned individually, and the overlain spectra confirmed that there was minimal interference, making the simultaneous equation method feasible.

Simultaneous equations were constructed using the absorptivity values of each drug at their respective λ_{max} . The method showed good linearity for both drugs in the concentration range of 5–30 $\mu\text{g/mL}$, with correlation coefficients (r^2) close to 0.999, indicating excellent linear relationships.

The developed method was successfully applied to selected marketed formulations. The actual content of Cilastatin Sodium and Imipenem in the formulations was calculated by solving the simultaneous equations derived from the UV absorbance data. The results were found to be within acceptable limits, indicating that the method is accurate and reliable.

Validation studies demonstrated that the method is precise, accurate, and specific. Recovery studies at 80%, 100%, and 120%

levels yielded satisfactory results with recovery values close to 100%, confirming the accuracy of the method. Intra-day and inter-day precision studies showed low %RSD values, indicating good repeatability and reproducibility. The method also showed adequate sensitivity, with acceptable limits of detection (LOD) and quantification (LOQ) for both drugs.

As the method requires only methanol as a solvent, it eliminates the need for more complex or expensive reagents and equipment. The rapid analysis time and simplicity make it highly suitable for routine quality control of raw materials and finished pharmaceutical products.

4. CONCLUSION

The developed UV spectrophotometric method based on the simultaneous equation approach offers a simple, accurate, precise, and cost-effective means for the simultaneous estimation of Cilastatin Sodium and Imipenem in combined pharmaceutical formulations. The method was successfully validated in accordance with ICH guidelines, demonstrating excellent linearity, accuracy, precision, and specificity. Its use of methanol as a solvent and straightforward analytical procedure make it ideal for routine quality control in pharmaceutical industries. Overall, the method provides a reliable tool for the standardization of both raw materials and finished products containing these two essential drugs.

5. ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the management and the Departments of Pharmaceutical Analysis. Jagan's College of

Pharmacy, Nellore, Andhra Pradesh, India, for their continuous support, motivation, and encouragement throughout this work. The authors are also thankful to the Department of Pharmaceutical Analysis, SAIF, Panjab University, Chandigarh, India, for their instrumental support and technical assistance.

BIBLIOGRAPHY

1. An overview of HPTLC; A modern Analytical technique with excellent potential for automation, optimatio, hyphenation and multidimensional application

2. Aparicio Irene, Miguel Angel Bill, Mauel Callejon. Liquid Chromatographic method for the simultaneous determination of Imipenem and Sulbactam in mouse plasma. J. Chromatographic Science 2006; 44: 548-551.

3. British Pharmacopoeia Commission: The British Pharmacopoeia 2011. Her Majesty's Stationary Office, London; 2011.

4. Deepak Kumar Jain, Nithesh Jain, Rita Charde. The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination. Pharmaceutical method 2011; 2(3): 167-172.

5. Demetriades JL, Souder PR, Vincek WC. HPLC determination of Cilastatin in Biological fluids. J. Chrom. Biomed Appl. 2011; 382: 225-231.

6. Deshpande Padmanabh, Pawar Prajakta, Gandhhi Santosh, Bhavani Vandana et al. High performance thin layer chromatography determination of Cilnidipine and Telmisartan in combined tablet dosage form. Int. Res J Pharmacy. 2012; 3(6): 219-222.

7. Devanand B Shinde, Kiran R Patil. Stability-indicating LC Method for the simultaneous determination of Telmisartan and Hydrochlorothiazide in dosage form. J Chil Chem Soc. 2012; 57(1):1017-1021.

8. Forsyth RJ. Determination of Imipenem and Cilastatin sodium in primaxin by first order derivative ultraviolet spectrophotometry. J Pharm biomed Anal 1994; 12(10) 1248-1249.

9. Gandhimathi M, Ravi TK, Annie Abraham, Renu Thomas. Simultaneous determination of Aspirin and Isosorbide mononitrate in formulation by RP- HPLC. J. Pharm and Bio medical Analysis 2003; 32(6): 1145-1148.

10. GirijaBhavar VA, Chatpalliwar. Quantitative analysis of propranolol hydrochloride by high performance thin layer chromatography. IJPS 2008; 70(3): 395-398.

11. ICH Topic Q2 (R1) Validation of analytical procedures: Test and Methodology (CPMP/ICH/381/195)

12. Indian drugs Review; A Mediworld Publication; New Delhi, 2006.

13. Karmalkar HS, Vaidhya VV, Gomes NA, Choukekar MP, Kekari MB. Determination of Cilnidipine from Pharmaceutical formulation by high Performance thin layer chromatographic method. Analytical chemistry.2008; 7(8):118-121.

14. L Garcia Capdevila, LoezCallul, Aorrrya C, Moral MA, Mangues MA, Bonal J. Determination of Imipenem in plasma by high performance liquid Chromatography for Pharmacokinetic Studies in Patients. J Chromatogr B Biomed Sci Appl. 1997; 692(1):127-132.

15. Lee HW, Seo JH, Lee HS. Development of liquid chromatography / negative ion electrospray tandem mass spectroscopy assay for the determination of Cilnidipine in human plasma and its application to a bioequivalence study. J chromatogr 2008; 11(2): 246-251.

16. Madhuri D, Sakarkar DM. Quantitative analysis for Clopidogrel bisulphate and Aspirin by second derivative spectrometric method in Pharmaceutical preparation. IJ. Chem Tech Research 2010; 2 (4): 1886-1891.

17. Mikami, E.T., Goto, Ohno, T., Matsumoto, M., Nishida, M. Simultaneous analysis of Naproxen, Nabumetone and its major metabolite 6-methoxy-2-naphtylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatograph. J. Pharm. Biomed. Anal. 2000; 23: 917-525.

18. Mitu Patel, Javed Vohra, Jagdish kakadiya. Development and validation of simultaneous equation spectrophotometric method for simultaneous estimation of Naproxen and Esomeprazole magnesium trihydrate in tablet dosage forms. IJPRBS 2012; 1 (2):274-286.

19. MM Srivastava(ed High Performance Thin Layer Chromatography) DOI 10.10017/978-3-642-14025-9-1, Springer-Verlag Berlin Heidelberg: 2011

20. Murillo Pilgrim, A., Garcia Borneo, L.F.G., "First derivative non-linear variable-angle synchronous fluorescence spectroscopy for the simultaneous determination of salicylamide, salisilate and naproxen in serum and urine" Anal. Chim. Act.1998; 373: 119-129.

21. Neha A Jain, Lohiya RT, Umekar MJ. Spectrophotometric determination of Naproxen and

Esomeprazole in a laboratory mixture by simultaneous equation, absorption correction, absorption ratio and area under curve methods. IJPSR 2011; 2(5):130-134.

22. Oona Mcpolin. Validation of Analytical methods for Pharmaceutical Analysis. United Kingdom: Mourne Training Services; 2009.

23. Palavai Sripal Reddy, Shakil Sait, Gururaj Vasudev Murthi. Stability indicating simultaneous estimation of assay method for Naproxen and Esomeprazole in Pharmaceutical formulations by RP-HPLC. Der Pharma Chemica. 2011; 3(6): 553-564.

24. Palled MS, Chatter M, Rajesh PMN, Bhat A R; Difference spectrophotometric determination of telmisartan in tablet dosage forms, Indian journal of pharmaceutical sciences. 2006; 68 (5): 685-686.

25. Parra A, Garcia Villanova J, Rodenas V, Gomiz MD et al., First and second derivative spectrophotometric determination of Imipenem and Cilastatin in injection. J Pharm Bio med Anal 1993; 11(6): 477-482.

26. Patel VA, Patel PG, Chaudhary BG, Rajgor NB, Rathi SG. Development and validation of HPTLC method for simultaneous estimation of Telmisartan and Ramipril in combined dosage form. IJ. Pharm. Bio. Research 2010; (11): 18- 24.

27. Prabhat Agarwal. Bio analytical method for the simultaneous determination of Imipenem and Cilastatin in plasma. Method developed in Ranbaxy 2008.

28. Priti D Trivedi and Dilip G Maheshwari. Estimation of Esomeprazole and Domperidone by absorption ratio method in Pharmaceutical Dosage by International Journal of Chem Tech Research. 2011; 2(5): 130-134.

29. Rakesh Prajapathi, Dave JB, Patel CN. Development and validation of stability indicating High performance liquid chromatographic methods for simultaneous determination of alprazolam and propranolol hydrochloride in combined dosage forms. IJ Pharmacy & Technology. 2011; (11) available from www.ijptonline.com.

30. Sener E, Tuncel M, Aboul-Enein HY. Rapid determination of Naproxen sodium in pharmaceutical formulations by flow injection analysis (FIA) using UV-detection. J. Liq. Chromatogr. & Rel. Technol. 2003; 26 (3): 401-408.

31. Shah N J, Suhagia B N, Shah R R, Shah P B; Development and validation of a HPTLC method for the simultaneous estimation of Telmisartan and

hydrochlorothiazide in tablet dosage form, Indian Journal of Pharmaceutical sciences. 2007; 69 (2): 202-205.
