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A Simple Rp- HPLC Method for Simultaneous Estimation of Six Cardiovascular Drugs in Bulk and Dosage Form

Celina Nazareth[1]*, B. Shivakumar[2], P. Reddy[3], S. Acharya[4] and S. Verekar[4]
[1]*PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Goa, India,*
[2]*BLDEA College of Pharmacy, Bijapur, India,*
[3]*Samskruti College of Pharmacy, Kondapur, Hyderabad, India,*
[4]*VerGo Pharma Research Laboratories Pvt. Ltd., Verna, Goa, India*.

Abstract: *A simple, convenient Rp-HPLC method has been developed and validated for the simultaneous estimation of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone. The column used was an Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size of adsorbent. Separation was achieved using isocratic elution in a buffer-acetonitrile-methanol mobile phase at a flow rate of 1.2 ml/min. The detection was performed at wavelength of 225 nm using a UV detector. The column temperature was 45^{0}C and injection volume was 20µl. The method was validated for precision, linearity and accuracy. The % RSD for all the drugs was found to be less than 2 %. The correlation coefficient (r^2) was not less than 0.999 for all drugs. The mean percent recovery of the drugs from tablet placebo at 50%, 100% and 150% were within limits. The marketed formulations of the drugs were analyzed and the mean assay results were found to be within limits. The developed method can thus be employed for routine simultaneous analysis of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone in bulk and in their marketed formulations.*

Keywords: *Rp-HPLC method, Indapamide, Metolazone, Spironolactone, Olmesartan, Nebivolol, Rosuvastatin.*

I. Introduction

As per World Health Organization, Cardiovascular Diseases (CVDs) are the number one cause of death globally. Although many CVDs can be treated or prevented, an estimated 17.1 million people die of CVDs each year[1]. CVDs are caused by disorders of the heart and blood vessels, and include coronary artery disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease and congenital heart disease. The major causes of CVDs are tobacco use, stress, inadequate or lack of sleep, physical inactivity, an unhealthy diet and harmful use of alcohol.

Congestive Cardiac Failure (CCF) is one of the complications of Coronary Artery Disease. It is a chronic and usually progressive illness which occurs when, cardiac output is insufficient to meet the demands of tissue perfusion, resulting in a clinical syndrome of decreased exercise tolerance with pulmonary and systemic venous congestion[2]. Combination of drugs are needed to control the risk factors associated with CCF and these include diuretics, angiotensin II receptor antagonists, β_1 receptor blockers and statins.

Metolazone [3-7] is an oral diuretic agent commonly classified with the thiazide diuretics. It is useful to treat Congestive Heart Failure and Hypertension. Indapamide [3-7] is a non-thiazide, sulphonamide diuretic drug which reduces blood pressure at doses causing little diuresis. It is generally used in the treatment of hypertension, as well as decompensated cardiac failure. Nebivolol hydrochloride [5-8] is a β1 -blocker (anti-hypertensive) which reduces peripheral vascular resistance and significantly increases stroke volume, with preservation of cardiac output. Olmesartan medoxomil [5-7, 9], a recent member of angiotensin receptor blocker (ARB) class of drugs, is indicated in the treatment of hypertension and prevention of diabetic nephropathy and congestive cardiac failure. Rosuvastatin [5, 7, 10] reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood, while increasing levels of high-density lipoprotein in the management of hyperlipidaemia. Spironolactone [3-7, 11] is a potassium sparing diuretic agent.

Literature survey revealed that different analytical methods like UV spectroscopy, Rp-HPLC, High Performance Thin Layer Chromatography (HPTLC) [12-33] have been reported for the analysis of the above drugs individually and in combination with other drugs. However, there has been no study involving simultaneous estimation by HPLC of above six drugs. Hence, in the present study a simple Rp-HPLC method, for the simultaneous analysis of above mentioned cardiovascular drugs in bulk and tablet dosage form has been developed and validated.

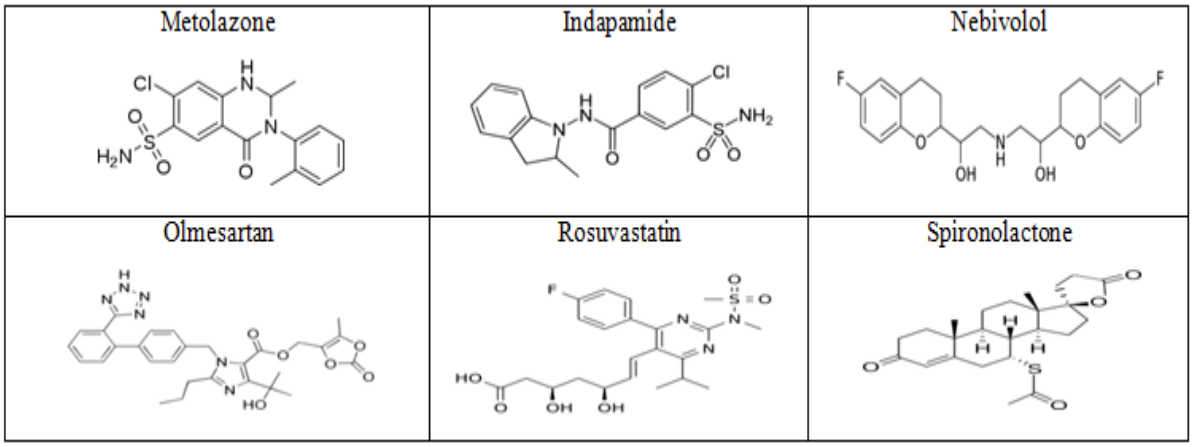


Fig. 1 Chemical structure of drugs

II. Materials And Methods

2.1 Chemicals and reagents

All chemicals and reagents used were of analytical grade. Olmesartan medoxomil was obtained as a gift sample from Unichem laboratories Ltd., Pilerne, Goa; Rosuvastatin from VerGo Pharma Research Laboratories Pvt. Ltd., Verna, Goa; Nebivolol HCl from Glenmark Generics, Goa; Metolazone and Spironolactone from Centaur pharmaceuticals Pvt. Ltd., Tivim, Goa and Indapamide from Adcock Ingram Ltd., Bangalore. Tablet formulations were procured from the local market.

2.2 HPLC Instrument and Chromatographic conditions

The instrument used for analysis was of Agilent Technologies "1120 Compact LC" with UV detector and Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size of stationary phase. The mobile phase used was buffer-methanol-acetonitrile in the proportion of 45:33:22, v/v/v. The column temperature was 45^0C, the flow rate was 1.2 ml/min and injection volume was 20µL.

2.3 Preparation of standard and sample solutions

A mixed standard solution of the drugs was prepared by accurately weighing the quantity of drugs as in Table 1, into a 100 ml volumetric flask. About 75ml of diluent (ACN & MilliQ water, 1:1, v/v) was added and the solution was sonicated for 10 minutes. The volume was made to 100ml with diluent and mixed. The solution was centrifuged at 4000 rpm for 10 mins. A volume of 4 ml diluted to100ml using diluent was injected 5 times and peak areas were determined.

Table 1 Weight of drugs in standard solution

Drug	Metolazone	Indapamide	Nebivolol HCl	Olmesartan medoxomil	Rosuvastatin	Spironolactone
Weight in mg	10	30	60	30	30	30

Ten tablets of each drug sample were accurately weighed and average weight determined. Tablet powder equivalent to 5 mg of drug was weighed and transferred to 50ml volumetric flask. About 35ml of diluent was added and the mixture was sonicated for 10 minutes. The volume was adjusted with diluent and mixed. Aliquot solution was then centrifuged at 4000 rpm for 10 mins. The centrifugate was appropriately diluted and the solution was injected and peak area was determined. The percentage purity of the tablets was calculated.

2.4 Method validation

The developed method was validated for linear range, accuracy, precision and specificity [33].

2.4.1 Linear range

The linearity of the method for each drug was studied by preparing 5 different concentrations of the drugs as in Table 2. The solutions were injected in the HPLC system and peak areas were recorded. Calibration curves were constructed by plotting peak areas versus concentration of each drug and the linear range was determined. The linear regression equation and correlation coefficient for each drug was determined.

Table 2 Concentration of drugs in working standard solutions

Vol (ml) of std stock in 100ml	Metolazone in ppm	Indapamide in ppm	Nebivolol HCl in ppm	Olmesartan medoxomil in ppm	Rosuvastatin in ppm	Spironolactone in ppm
2	2	6	12	6	6	6
3	3	9	18	9	9	9
4	4	12	24	12	12	12
5	5	15	30	15	15	15
6	6	18	36	18	18	18

2.4.2. Precision

Precision of the method for each drug was determined by performing repeatability studies by the successive analysis of six injections of above solution, corresponding to 100% of drug concentration. The percentage RSD was determined.

2.4.3. Accuracy

Accuracy of the developed HPLC method was determined by carrying out recovery studies for each drug at spike level 50% (L1), spike level 100% (L2) and spike level 150% (L3) concentration by replicate analysis (n=3). A volume of 2ml, 4ml and 6ml of standard drug solution (corresponding to 50%, 100% and 150% concentration of each drug) was added to 50 mg of placebo powder taken in 3 different 50ml volumetric flask. Around 35ml of diluent was added and solution was sonicated for 10 mins. The volume was made up with diluent and mixed. Aliquot of the solution was centrifuged at 4000 rpm for 10 mins. The clear centrifugate was diluted appropriately and injected. The percentage of total drug content recovered was calculated.

2.4.4.. Specificity

The specificity of the method was determined by injecting the diluent and placebo solution in the chromatographic system and observing the chromatograms.

2.5 System suitability testing

System suitability of the system was determined by six replicate injections. The acceptance criteria adopted was less than 2 % RSD for peak areas, greater than 2000 (USP) theoretical plates and asymmetry factor between 0.85 and 2.0.

III. Results And Discussion

3.1 Method development

The solubility of the drugs was tested in acetonitrile (ACN), methanol, ACN & MilliQ water mixture (1:1, v/v), methanol & MilliQ water mixture (1:1, v/v), 0.1N HCl and phosphate buffer pH 6.8. Based upon the free solubility of the drugs, ACN & MilliQ water (1:1, v/v) mixture was selected as diluent for method development and validation. The drug concentrations were optimized so as to obtain absorbance values in the range of 0.3 to 0.9. UV Spectra of the drugs as studied from Fig. 2 revealed that a wavelength of 225 nm could be used as a common wavelength for analysis.

Chromatographic separation of the drugs was tried on YMC Pack Pro C_{18} RS column having a length of 250mm with 4.6 mm ID and particle size of stationary phase being 5 micron. The mobile phase used was buffer-methanol-acetonitrile in the proportion of 45:33:22, v/v/v. The column temperature was maintained at 25^0C and flow rate of mobile phase chosen was 1 ml/min. However, results were not satisfactory as Nebivolol and Rosuvastatin were not resolved and Retention time (Rt) of Spironolactone was more than 20 mins. Several parameters were verified including, alteration of column temperature (30^0C, 40^0C, 45^0C) and flow rate of mobile phase (1.2 ml/min), with no improvement in the resolution. Hence, change of column, so as to increase the surface area of adsorbent and increased carbon loading, was tried. An Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size was used. Optimum separation of the drugs was finally achieved on the column as seen in Fig. 3, with column temperature of 45^0C and 1.2 ml/min flow rate of mobile phase. The injection volume was 20µL. There were no interferences from the diluent and placebo, as seen in Fig. 4.

The calibration curve of the drugs as in Fig. 5 gave linear lines. The results of accuracy and precision studies as depicted in Table 3 proved that the results were satisfactory. The proposed Rp-HPLC method was applied to marketed formulations of the drugs. The results, as tabulated in Table 4, were within acceptable limits.

The results of system suitability testing as depicted in Table 5 proved that the parameters were within the acceptable limits.

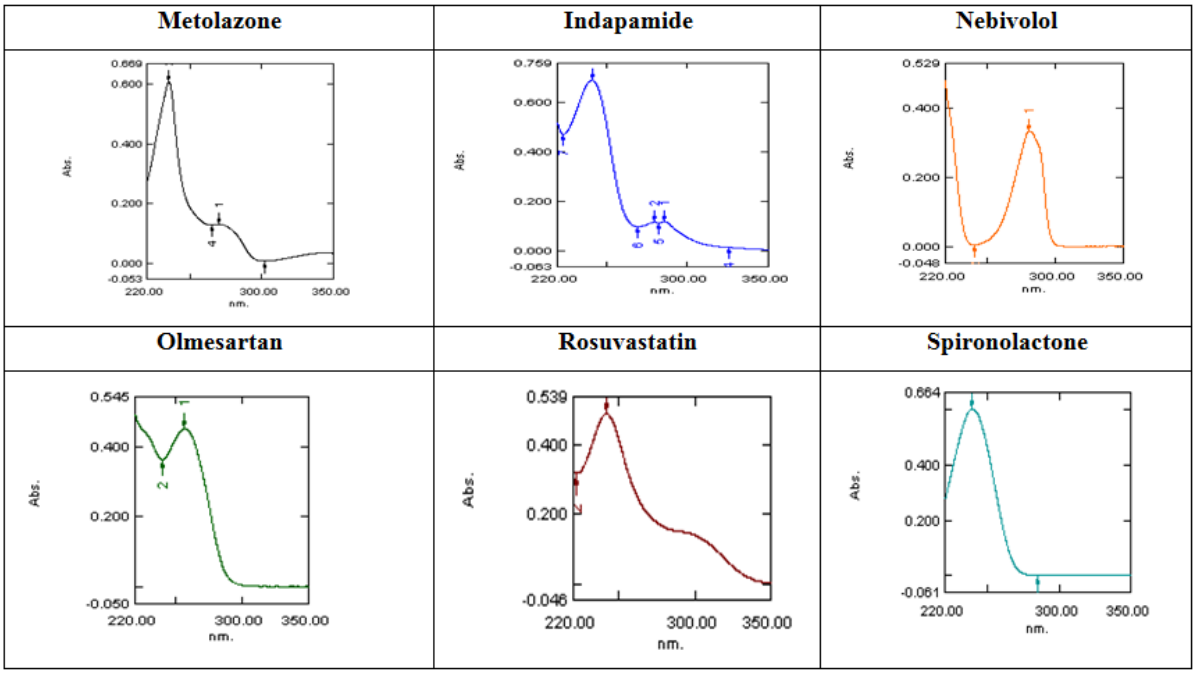


Fig. 2 UV Spectra for standard drugs

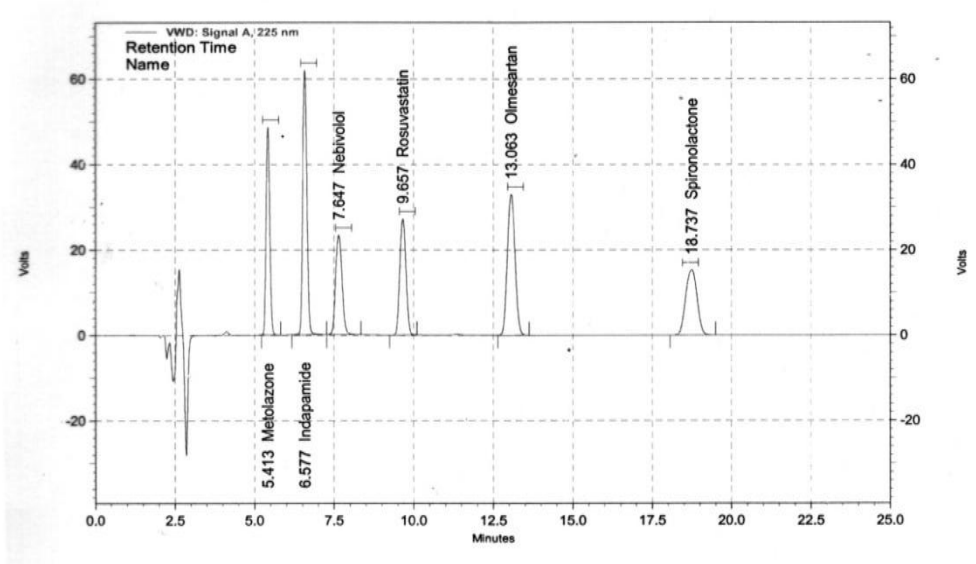
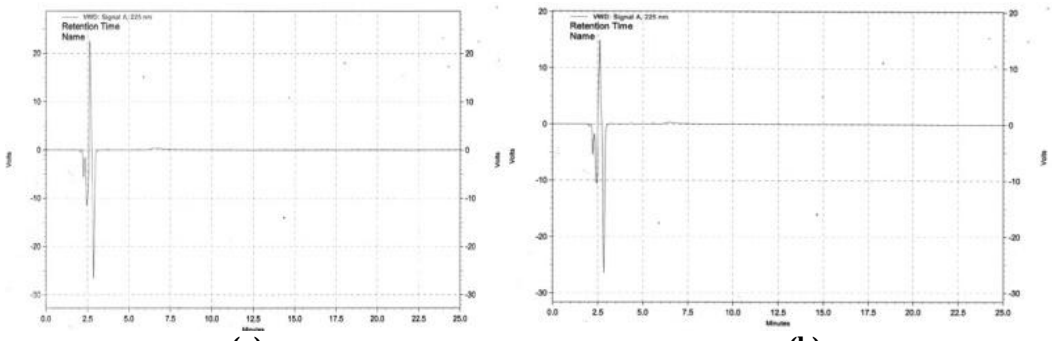


Fig. 3 Chromatogram showing separation of drugs



(a) **(b)**

Fig. 4 Chromatogram for (a) diluent and (b) placebo

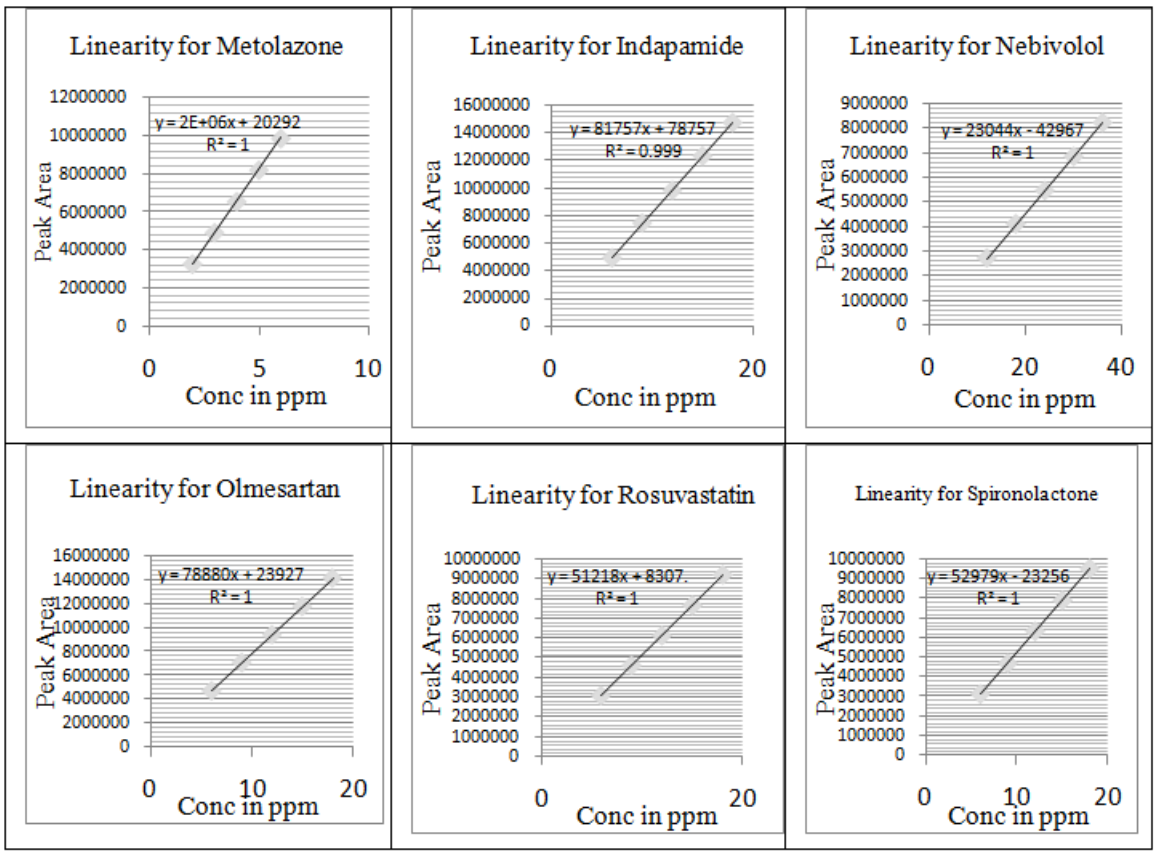


Fig. 5 Calibration curves for drugs

Table 3 Results of Accuracy and Precision analysis

	Met	Ind	Neb	Ros	Olm	Spi
% Recovery L1 (n=3)	100.95	100.97	100.75	100.71	99.83	100.47
% Recovery L2 (n=3)	100.45	100.11	100.57	100.39	99.70	100.46
% Recovery L3 (n=3)	99.73	99.19	99.98	99.79	99.24	99.93
Precision % RSD (n=6)	0.07750	0.06111	0.18673	0.13247	0.20606	0.16094

Table 4 Results for assay of marketed formulations

Formulation	Metoz 2.5	Natrilix	Nebi 5	Roseday	Olmetrack 20	Aldactone
Mfg. By	Centaur Pharma	Serdia	Otsira Genetica	USV	USV	RPG Life Sciences
Drug	Metolazone	Indapamide	Nebivolol	Rosuvastatin	Olmesartan	Spironolactone
% Purity	98.38	104.33	97.51	104.72	102.15	98.38

Table 5 Results for system suitability testing

Drug	Metolazone	Indapamide	Nebivolol	Rosuvastatin	Olmesartan	Spironolactone
Peak Area (%RSD)	0.3	0.269	0.5	0.293	0.170	0.225
Theoretical plates	8580	9291	5947	9466	10780	10690
Asymmetry factor	1.112	1.076	1.064	1.040	1.046	0.955

IV. Conclusion

A simple, isocratic LC method has been developed, optimized and validated for the simultaneous estimation of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone. The method is simple, rapid, accurate, precise and specific. It can be used for the routine analysis of the six cardiovascular drugs without the need for separation, in bulk and in their dosage form.

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References

[1]. World Health Organization, http://www.who.int/cardiovascular_diseases/prevention_control/en/ (July 2013)
[2]. L. Brunton, B. Chabner, B. Knollman, Goodman and Gilman's the pharmacological basis of therapeutics (twelfth ed., The McGraw – Hill Companies, Inc., 2011), 789-813.
[3]. United States Pharmacopoeia (Vol III, thirty fourth ed., Rockville, MD: The United States Pharmacopeial Convention, 2011), 3115-3116, 3506, 4267-4268.
[4]. British Pharmacopoeia (Vol II, The Stationary Office on behalf of MHRA, 151 Buckingham Palace Road, London, 2012), 1124-1126, 1458-1459, 2030-2032.
[5]. M.J. O'Neil, The Merck Index (fifteenth ed., RSC publishing, 2013), 4977, 6222, 6520, 6932, 8401, 8885.
[6]. K.D. Tripathi , Essentials of Medical Pharmacology (sixth ed., Jaypee Brothers Medical Publishers (P) ltd. 2008), 561-578.
[7]. Drug Index, (Passi publications, 3, 2014), 70, 79,113-114, 585.
[8]. J.G. Hardman, L.E. Limbird, A.G. Gilman, The pharmacological basis of therapeutics (tenth ed., The McGraw-Hills Company, Inc., 2001), 257, 853.
[9]. http://www.pubchem.ncbi.nlm.nih.gov (November 2013)
[10]. Martindale-The Complete Drug Reference (thirty-fourth ed., Pharmaceutical Press. London, 2005), 15-19, 966.
[11]. Indian Pharmacopoeia (Vol III, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India Ministry of Health and Family Welfare, 2014), 2783.
[12]. S. Sandeepkumar, S. Manjunath , A. Raju , V.S. Chouhan, Ultra violet and derivative spectrophotometric methods for estimation of Metolazone in pharmaceuticals, Inter J Pharma Sci, 2(3,) 2011, 204-209.
[13]. S. Sandeepkumar, S. Manjunath , A. Raju , V.S. Chouhan, Development and Validation of Visible Spectrophotometric methods for the Estimation of Metolazone in Pharmaceutical Dosage Forms, Der Pharma Chemica, 3(2) ,2011, 512-516.
[14]. A. Chaudhary, K.R. Vadalia, P. Thummer, Development and validation of ratio derivative spectrophotometric for simultaneous estimation of metolazone and spironolactone in pharmaceutical dosage form, IJPSR, 3(10), 2012, 3999-4003.
[15]. L.R. Khanchandani, K.K. Bhatt, D.A. Shah, U.K. Chhalotiya, Development and validation of liquid chromatographic method for simultaneous estimation of Spironolactone and Hydroflumethiazide in pharmaceutical dosage form, Novus International Journal of Chemistry, 2(1), 2013, 13-19.
[16]. M.I. Walash, N. El-Enany, M.I. Eid, M.E. Fathy, Simultaneous determination of metolazone and spironolactone in raw materials, combined tablets and human urine by high performance liquid chromatography, Anal. Methods, 5, 2013, 5644-5656.
[17]. A. Sheth, K.N. Patel, R. Badmanaban, N. Doshim, N. Shah, Simultaneous estimation of Olmesartan medoxomil and Indapamide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique, Chronicles of young scientists, 4(1),2013, 69-73.
[18]. K. Patel, U. Patel, H. Shah, P. Patel, M. Patel, Development and validation of spectrophotometric methods for simultaneous estimation of Olmesartan medoxomil and Indapamide in their combined tablet dosage form, International Journal of Pharmaceutical Research and Bio-science, 5, 2012, 485-501.
[19]. P. Jain, A. Jain, D. Maliwal, V. Jain V, Development and validation of spectrophotometric and RP-HPLC method for estimation of Olmesartan medoxomil in tablet dosage form, International Journal of Pharma and Bio Sciences, 1(2), 2010, 1-7.
[20]. B. Moussa, N. Mohamed, M. Youssef, Validated spectrophotometric method for estimation of Olmesartan medoxomil in pharmaceutical formulation, International Journal of Pharmaceutical and Life Sciences, 1(3), 2012, 1-7.
[21]. D.D. Patel, M.M. Patel, Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Dosage Form by UV Spectroscopy, International Journal of Research in Pharmaceutical and Biomedical Sciences, 3(2), 2012, 935-939.
[22]. S.M. Lakshmi, S.G. Kumara, Development and validation of RP-HPLC method for the estimation of Olmesartan medoxomil in bulk and tablet dosage form, Contemporary investigations and observations in pharmacy, 2(1), 2013, 3-6.
[23]. V. Amudhavalli, K.S. Lakshmi, M. Karthick, Determination of Olmesartan and Hydrochlorthiazide in pharmaceutical formulations by RP-HPLC, Int. J. Chem. Sci., 9(2), 2011, 470-476.
[24]. C. Nazareth, B. Shivakumar, P. Reddy, B.M. Gurupadayya, Development And Validation of HPTLC method for Simultaneous Analysis of Olmesartan and Indapamide in bulk drug and combined tablet formulation, Indo American Journal of Pharmaceutical Research, 3(12), 2013, 1709-1715.
[25]. C. Nazareth, B. Shivakumar, P. Reddy, B.M. Gurupadayya, Development And Validation of HPTLC method for Simultaneous Analysis of Metolazone and Spironolactone in bulk drug and pharmaceutical dosage form, IOSR Journal of Pharmacy, 4(1), 2014, 20-25.
[26]. B.D. Prasad, B.C. Kanth, R. Vasanthi, M.R. Mohan, Prabhakar, a validated UV spectroscopic method of Metolazone in bulk and its tablet dosage forms, International Journal of Biological & Pharmaceutical Research, 3(1), 2012, 154-157.
[27]. B. Umadevi, T. Vetrichelvan, Simultaneous Estimation of Metolazone and Spironolactone in Combined Tablet Dosage Form by UV Spectroscopy, International Journal Pharm Tech Res., 3(4) ,2011, 2068-2074.
[28]. T. Sharma, R. Patra, D.G. Sankar, C.S. Sudam, development and validation of UV spectrophotometric method for determination of Nebivolol Hydrochloride following ICH guidelines and study of its degradation profile, Asian Journal of Pharmaceutical and Clinical Research, 5(4), 2012, 69-72.
[29]. B. Dhandapani, N. Anjaneyulu, Y. Venkateshwarlu, R.H. Shaik, HPTLC Method Development and Validation for the Simultaneous Estimation of Amlodipine Besylate and Nebivolol Hydrochloride in tablet dosage form, Journal of Pharmacy Research, 3(2), 2010, 332.
[30]. B.B. Patel, B.B. Shah, N.G. Kirtan, P.M. Patel, Development and Validation of Spectrophotometric Method For Simultaneous Estimation of Rosuvastatin Calcium and Aspirin In Bulk and Pharmaceutical Dosage Form, International Journal of Research in Pharmacy and Science, 2(2), 2012, 115-122.
[31]. P.S.Jain, N.K. Kale, S.J. Surana, Quantitative estimation of Rosuvastatin in bulk and tablet dosage form by using area under curve method, Journal of Pharmaceutical and BioSciences, 4, 2013, 128-133.
[32]. A.K. Gajjar, V.D. Shah, Simultaneous UV-spectrophotometric estimation of Rosuvastatin and Ezetimibe in their combined dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences, 2(1), 2010, 131-138.
[33]. Q2B Validation of Analytical Procedures, http://www.ich.org/fileadmin/ Public_Web_Site/ICH_Products/Guidelines/Quality/ Q2_R1/Step4/Q2_R1__ Guideline.pdf (15 October 2013).