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Absorbance correction method for the simultaneous estimation of hydrochlorothiazide, telmisartan and amlodipine besylate in API and combined tablet dosage formulation by UV spectrophotometry

Deepali M. Gangrade* and Priyanka Y. Kulkarni

Vivekanand Education Society's College of Pharmacy, Mumbai University, Chembur (E), Mumbai

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ABSTRACT

A new, simple, sensitive and accurate UV - spectrophotometric absorption correction method has been developed for simultaneous determination of amlodipine besylate, telmisartan and hydrochlorothiazide in combined tablet dosage form. The wavelengths selected for the analysis were 365 nm, 250 nm and 335 nm for amlodipine besylate, telmisartan and hydrochlorothiazide respectively. Beer's law obeyed the concentration range of 10 - 60 µg/ ml, 4 – 20 µg/ ml and 20 - 100 µg/ ml for amlodipine besylate, telmisartan and hydrochlorothiazide, respectively. Methanol and distilled water were used as solvents. The percentage recovery was found in the range of 98.9% to 101.6% for AMB, 99.6% to101.3% for TEL and 99.8 % to 101.2% for HCT. The developed method was validated statistically. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid, accurate and can be successfully applied for simultaneous determination of amlodipine besylate, telmisartan and hydrochlorothiazide in combined tablet dosage form.

Keywords: Amlodipine Besylate, Telmisartan, HCT, Absorption correction method, ICH guidelines.

INTRODUCTION

The chemical name for Amlodipine besylate is 3-ethyl 5-methyl 4RS-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.[1]Amlodipine besylate is the Calcium channel blocker[2]. It is used as an anti-hypertensive and in the treatment of angina.

Amlodipine besylate is a white crystalline powder. It is slightly soluble in water and sparingly soluble in ethanol. It is official in BP, EP, IP, USP[1, 3, 4, 5].

Methods available for the determinations of Amlodipine besylate include UV spectroscopy [6,7,8] high performance liquid chromatography[9, 10, 11, 12], high performance thin layer chromatography[13, 14], LC - MS[15], LC - MS/ MS[16]and stability indicating assay method [17].The present work describes a validated Absorbance correction method for simultaneous determination of these drugs in tablet dosage form.

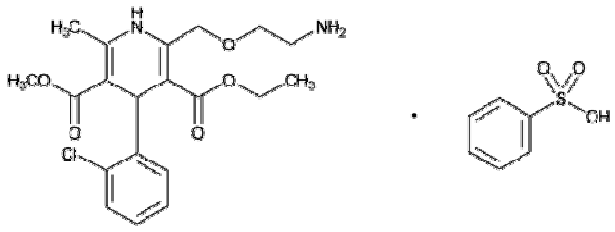

Fig :1Structure of Amlodipine Besylate

The chemical name for HCT(HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.It is a thiazide diuretic[18]. It is also used in the treatment of hyper-tension.[19]

HCT is White to off-white crystalline powder.[20] Soluble in dilute ammonia, or sodium hydroxide; also soluble in methanol, ethanol, acetone.[21]

It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia.[22,18,23]

Methods available for determination of HCT includes spectrophotometry[24], liquid chromatography[25,26], stability indicating assay[27] method and thin-layer chromatography[28], as alone or in combination with some other drugs.

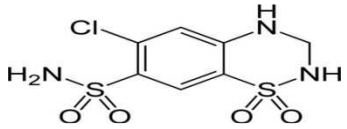

Fig :2 Structure of HCT

Telmisartan is is chemically 4′-[(1,4′-dimethyl-2-propyl [2,6′-bi-1*H*-benzimidazol]-1′-yl) methyl] [1,1′-biphenyl]-2-carboxylic acid.[29]It is an Antihypertensiv drug.[30]This is used for treatment of hypertension and diabetic nephropathy.[31,32]

It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia(BP) and U.S. Pharmacopoeia(USP).[33,34,35] Methods for determination of Telmisartan includes UV,[36,37,38] visible spectrophotometric,[39] Colorimetry [40,41], liquid chromatography-tandem mass spectrometry [42,43,44,45] and HPTLC [46,47,48].

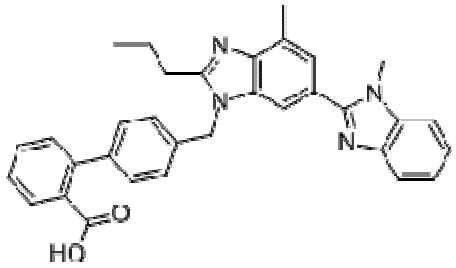

Fig : 3Structure of Telmisartan

Literature survey revealed that there are several methods were reported for the estimation of AMB, TEL and HCT individually as well as in combination with some other drugs[49]. As no method is reported for AMB, TEL and HCT in combination, the aim of the present study was to develop accurate, precise and sensitive method for the simultaneous UV spectrophotometric estimation of AMB, TEL and HCT in bulk and in combined tablet dosage form by absorption correction method. For this purpose marketed tablets TELVAS-3D containing 05 mg of AMB, 40 mg of Tel and 12.5 mg of HCT was used.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu - 1800 double beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents & Chemicals

Pharmaceutically pure sample of AMB, TEL and HCT were obtained as a gift samples from Smruti Organics, Solapur ; Glenmark Pharmaceuticals ; IPCA Laboratories respectively. All solvents were of AR grade. A combination of AMB (5 mg), TEL (40 mg) and HCT (12.5 mg) in tablet formulation was purchased from local market. (TELVAS-3D; Aristo Pharmaceuticals).

Experimental condition

According to the solubility characteristics, the common solvent for the three drugs was found to be methanol. Hence the stock solution was prepared in methanol and further dilutions were made up with distilled water.

Preparation Of standard stock solution
10 mg of AMB, TEL & HCT were accurately weighed and transferred in to 10 ml volumetric flasks separately. Dissolved in methanol and made up to the volume to 10 ml with the same. These solutions were observed to contain 1000 µg/ ml of AMB, VAL and HCT, respectively.

Study Of spectral and linearity characteristics
The standard stock solutions of AMB, TEL and HCT were further diluted with distilled water to get the concentration of 10 µg/ml of each and the solutions were scanned between the range 200 - 400 nm in 1cm cell against distilled water as blank and the overlain spectra was recorded.

From the overlain spectrum of AMB, TEL and HCT in methanol followed by distilled water, it was observed that TEL and HCT have zero absorbance at 365 nm, whereas AMB has substantial absorbance. Thus AMB was estimated directly at 365 nm without interference of TEL and HCT. At 335 nm, TEL has zero absorbance.

For estimation of HCT, the absorbance of AMB was measured at 335 nm using standard solution of AMB (10 µg/ ml). The contribution of AMB was deducted from the total absorbance of sample mixture at 335 nm. The calculated absorbance was called as corrected absorbance for HCT. At 250 nm, these three drugs were showed the absorbance. To estimate the amount of TEL, the absorbance of AMB and HCT were corrected for interference at 250 nm by using absorptivity values. A set of three equations were framed using absorptivity coefficients at selected wavelengths.

$c_x = A_1 / a_{x1}$
$c_y = A_2 - a_{x2}\, c_x / a_{y2}$
$c_z = A_3 - (a_{x2}\, c_x + a_{y3} c_y) / a_{z3}$

Where,
A_1, A_2 and A_3 are absorbance of sample solution at 365 nm, 315 nm and 250 nm, respectively.

a_{x1}, a_{x2} and a_{x3}, absorptivity coefficients of AMB at 365 nm, 315 nm and 250 nm, respectively.

a_{y2} and a_{y3},absorptivity coefficients of HCT at 315 nm and 250 nm, respectively. a_{z3}, absorptivity coefficient of TEL at 250 nm.

c_x, c_Y and c_z are concentrations of AMB, TEL and HCT, respectively in mixture.

The aliquot portions of standard stock solution of AMB, TEL and HCT were transferred into 100 ml volumetric flasks individually and made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 365 nm, 335nm and 250 nm for AMB, 335 nm and 250 nm for HCT and 250 nm for TEL. The calibration curves for AMB, TEL and HCT were prepared in the concentration range of 10 - 60 µg/ ml, 4 - 20 µg/ ml and 20 - 100 µg/ ml, respectively at their respective wavelengths by diluting aliquot portions of standard stock solution of each drug.

Analysis of Synthetic mixture of AMB, TEL And HCT
Different mixtures of the three drugs were prepared by transferring different volumes of AMB, TEL and HCT from standard stock solutions into 100 ml volumetric flasks and diluting to volume with distilled water. The concentrations of all the three drugs AMB, TEL and HCT were determined by measuring the absorbance of the prepared mixtures at 365 nm, 335 nm and 250 nm. From these absorbance values, the concentrations of AMB, TEL and HCT were determined using absorbance correction method.

Analysis of tablet formulation
Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 40 mg of TEL was transferred in to 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 4.0 ml into 100 ml with distilled water to obtain 32 µg/ ml solution of TEL which is also contains 4 µg/ ml of AMB and 10 µg/ ml of HCT theoretically. The absorbance of sample solution was measured at all selected wavelengths. The content of AMB, TEL and HCT in sample solution of tablet was calculated. This procedure was repeated for six times.

Validation of methods
The method was validated with respect to linearity, precision, accuracy and ruggedness.[50]

According to ICH guidelines for the qualitative test or assay procedure LOD, LOQ parameters are not required [51].

Linearity
Linearity was checked by diluting standard stock solution at six different concentrations. AMB was linear with the concentration range of 10-60µg/ ml at 365 nm, 335 nm and 250 nm. TEL showed the linearity in the range of 4 – 20 µg/ ml at 250 nm. HCT was linear in the concentration range of 20 - 100 µg/ ml at 335 nm and 250 nm and Calibration curves (n =6) were plotted between concentration and absorbance of drugs.

Sensitivity
The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations; LOD = 3.3σ/ s and LOQ = 10σ/ s, where σ is standard deviation of y intercept of calibration curve (n = 6) and s is slope of regression equation.

Precision
For checking precision of the method, repeatability and intermediate precision were performed. Six replicates of the same concentration were used for determining the repeatability. Inter-day and intra-day analysis was performed in triplicates for the same concentration, on the same day and for the successive three days for the determination of intermediate precision. Percentage relative standard deviation was calculated.

Accuracy
To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Ruggedness
The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

In present study, determination of AMB, TEL and HCT were carried out by using different instruments and different analysts.

RESULTS AND DISCUSSION

A rapid, sensitive, economic, precise and accurate analytical method for simultaneous estimation of AMB, TEL and HCT in pure API and in combined tablet dosage form was developed and validated.

The proposed method is based on spectrophotometric absorption correction method for the simultaneous estimation of AMB, TEL and HCT in UV region using methanol and distilled water as solvents. The overlain spectra of AMB, TEL and HCT are shown in Fig. 4

The method is based upon direct estimation of AMB at 365 nm, as at this wavelength HCT and TEL have zero absorbance and shows no interference. For estimation of HCT, corrected absorbance was calculated at 335 nm due to the interference of AMB and TEL has zero absorbance at this wavelength. At 250 nm, these three drugs were showed absorbance. To estimate the amount of TEL, the absorbance of AMB and HCT were corrected for interference at 250 nm by using their absorptivity values. The stability was performed by measuring the absorbance of same solution at different time intervals.

Beer's law obeyed in the concentration range of 10 - 60 µg/ ml at 365 nm, 335 nm and 250 nm, 4 - 20 µg/ ml at 250 nm and 20 - 100 µg/ ml at 335 nm and 250 nm for AMB, TEL and HCT, respectively. The correlation coefficient values were found above 0.999, which shows that absorbance of all the drugs was linear with concentration.The optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept were calculated andare summarized in Table 1.

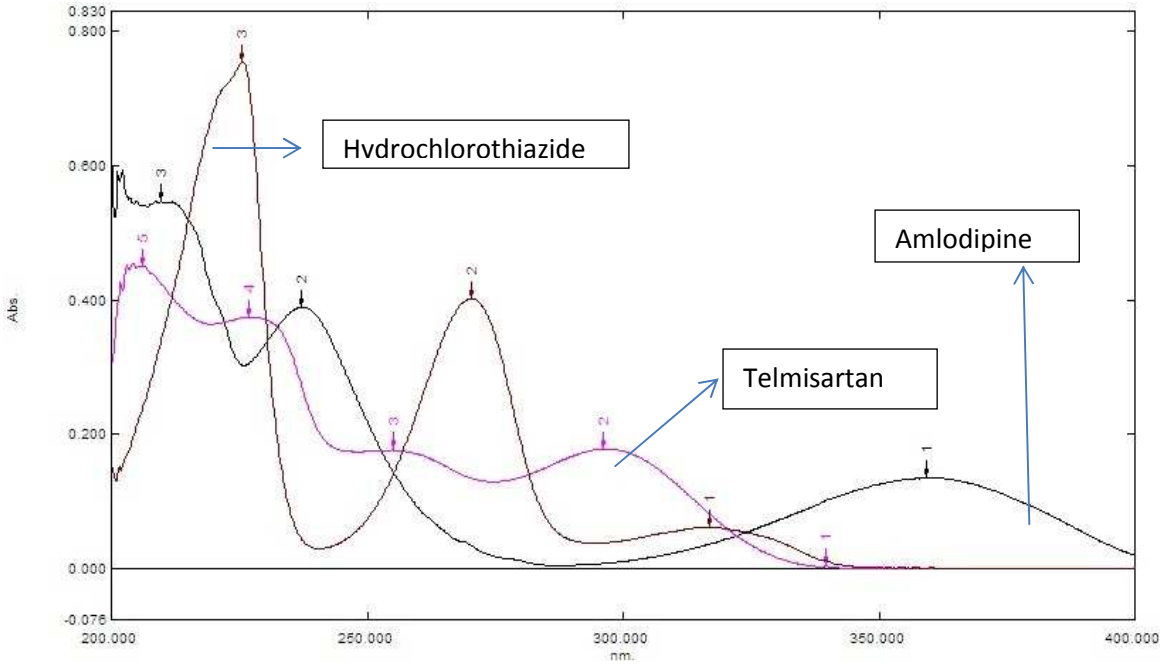

Fig.4: Overlain UV spectra of AMB, TEL and HCT(10µg/ml)

Table1: Spectral and linearity characteristics data

Parameters	HCT*	TEL*	AMB*
λmax(nm)	335	250	365
Linearity range (µg/ ml)	20-100	4 - 20	10 – 60
Correlation coefficient (r^2)	0.9997	0.9991	0.9992
Slope (m)	0.0067	0.0542	0.0012
Intercept (c)	0.0073	0.0086	0.0073
Regression equation (y = mx +c)	0.0067x -0.0073	0.0542x +0.0086	0.0012x -0.0073
LOD (µg/ ml)	0.2350	0.2489	0.1923
LOQ (µg/ ml)	0.7121	0.7542	0.5827

**Mean of six observations*

The LOD and LOQ were found to be 0.1923and0.5827 , 0.2489 and 0.7542, 0.2350 and 0.7121 for AMB, TEL and HCT, respectively. The low values indicated that the sensitivity of the method.

To study the mutual interference, if any, in the simultaneous estimation of AMB, VAL and HCT in synthetic mixture containing various proportions of AMB, VAL and HCT were prepared and the contents were estimated by proposed method. The % recovery varied from98.9 to 101.6 for AMB,99.6 to 101.3 for TEL and 99.8 to101.2 for HCT indicating that there is no mutual interference between these three drugs. The result of analysis of synthetic mixture is shown in Table 2

Table2: Results of analysis of synthetic mixtures

Conc. Of HCT (µg/ml)			**Conc. Of TEL (µg/ml)**			**Conc. Of AMB (µg/ml)**		
Theoretical	Practical	% Recovery	Theoretical	Practical	% Recovery	Theoretical	Practical	% Recovery
2.5	2.495	99.8	8	8.105	101.3	1	1.016	101.6
5	5.005	100.1	12	11.956	99.6	2	2.014	100.7
7.5	7.562	100.8	24	24.025	100.1	3	3.024	100.8
10	10.098	100.9	32	32.024	100.1	4	4.050	101.3
12.5	12.654	101.2	40	40.086	100.2	5	4.945	98.9

The percentage label claim present in tablet formulation was found to be 99.98 ± 0.574, 100.9 ± 1.002,101.05 ± 1.521 for AMB, TEL and HCT, respectively. Precision of the method was confirmed by the repeated analysis of formulation for six times. The % RSD values were found to be 0.574, 1.002 and 1.521 for AMB, TEL and HCT , respectively. The low % RSD values indicated that all the three drugs showed good agreement with the label claim ensures the precision of the method. (Table 3)

Table3:Results of analysis of tablet formulation

Parameters	HCT	TEL	AMB
Label claim (mg)	12.5	40	05
% Assay*	101.05	100.9	99.98
Standard Deviation	1.511	0.998	0.556
%RSD	1.521	1.002	0.574

**Mean of six determinations*

Further, the precision of the method was confirmed by Intraday and Inter day analysis. The % RSD values for intraday and inter day analysis was found to be 101.6 ± 0.587 and 101.3 ± 0.748for AMB, 100.5 ± 0.754and 99.8 ± 0.857 for TEL and 101.2 ± 0.956 and 101.5 ±1.254 for HCT, respectively. Hence the precision of the method was further confirmed.

The developed method was validated for Ruggedness. The analysis of formulation was done by using different instruments and different analysts. The % RSD values were found to be less than 2 indicating that the method was more rugged. The results of analysis of intermediate precision and ruggedness are shown in Table 4

Table4: Intermediate Precision and Ruggedness of the method

Parameters	% Label claim estimated (Mean ±%R.S.D.)		
	AMB	**TEL**	**HCT**
Intraday Precision (n=3)	101.6 ± 0.587	100.5 ± 0.754	101.2 ± 0.956
Inter day Precision (n=3)	101.3 ± 0.748	99.8 ± 0.857	101.5 ± 1.254
Different instruments (n=6)			
Instrument I	101.1 ± 0.698	101.8 ± 0.658	100.8 ± 0.546
Instrument II	100.9 ± 0.845	101.2 ± 0.562	101.2 ± 0.854
Different analysts (n=6)			
Analyst I	99.8 ± 0.527	100.6 ± 0.687	100.8 ± 0.684
Analyst II	100.2 ± 0.854	99.8 ± 0.526	101.6 ± 0.546

In order to check the accuracy of the developed method, known quantities of standard drugs of AMB, TEL and HCT in three different concentrations were added to its preanalysed sample and analysed by the developed method. The percentage recovery was found to be in the range of 99 – 101.25for AMB, 99.87 – 100.23forTEL and 99.66 – 101.25for HCT. The results of recovery studies are shown in Table 5

Table 5: Recovery studies

Drug	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery*	S.D	%RSD
AMB	2	1.6	3.6	1.62	101.25		
	2	2.0	4.0	1.98	99.00	1.14	1.137
	2	2.4	4.4	4.42	100.45		
TEL	16	12.8	28.8	12.83	100.23		
	16	16.0	32.0	15.98	99.87	0.182	0.181
	16	19.2	35.2	19.22	100.10		
HCT	5	4.0	9.0	4.05	101.25		
	5	5.0	10.0	5.02	100.40	0.795	0.791
	5	6.0	11.0	5.98	99.66		

**Mean of three observations*

The % RSD values for AMB, TEL and HCT were found to be 1.137, 0.181 and 0.791, respectively. The low % RSD values confirm that there is no interference due to the excipients used in formulation. This ensures the accuracy of the method.

CONCLUSION

From validation, the developed method was found to be simple, rapid, economical, precise, accurate and rugged. Hence the proposed method could be effectively applied for the routine analysis of AMB, TEL and HCT in bulk and in combined tablet dosage form.

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REFERENCES

[1] British Pharmacopoeia, International ed. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act Vol. 1, **2005**, 138, 723.
[2] Burges RA. Et al., *Cardiovascular Drug Reviews* **1990**, 8, 25-44.
[3] The European Pharmacopoeia, Counsile of Europe,Codex,France,4th Edn, **2002**, 639- 640.
[4] Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, Vol. 2, Delhi: Publication by Controller ofPublication, **2007**, 714-16, 1078.
[5] The United States Pharmacopoeia Convention, Inc., Rockville, MD, **2007**, 3496-97,1532 , 586.
[6] Khopde SA. Et al., *Indian Drugs*, **2000**, 37, 351-353.
[7] Wankhede SB. Et al., *Indian Journal of Pharmaceutical Sciences*, **2010**, 72, 1, 136-140.
[8] Gupta KR., *International Journal of ChemTech Research*, **2010**, 2,1, 551-556.
[9] Dinda SC. et al., *Research Journal Of Pharmacy and Tecnology*, **2013**, 6, 2, 204-207
[10] .Halker U P. et al., *Indian Drugs*,**1988**, 35, 168.
[11] .Shimooka K. et al., *Journal Of Pharmaceutical and Biomedical Analysis*, **1989**, 7, 1267
[12] .Sharma Ak. Et al., *International Journal Of Pharmaceutical Science and Research*,**2012**, 3, 4, 1202–1207.
[13] .Chandrashekhar TG. et al., *Journal Of Planar Chromatography - Modern* TLC, **1994**, 7, 458.
[14] .Ilango K. et al., *Indian Journal Of Pharmaceutical Sciences*, **1997**, 59, 336.
[15] .Bohumila S., *European Journal Of Pharmaceutical Science*, **2008**, 33, 91-99.
[16] .Ramani AV. Et al., *Biomedical Chromatography*, **2009**, 23, 6, 615-622.
[17] Alsarra I.A., *Journal of Chromatographic Science*, **2009**, 47, 10, 863-867.
[18] Department of Health and Social Services for Northen Ireland, Vol. 1, London: Stationery Publication, **1993**. British Pharmacopoeia, 330.
[19] Raja B. et al., International *Journal Of Research in Pharmacy & Chemistry*, **2011**, 03, 714-717.
[20] Physicians' Desk Reference. Montvale, NJ: Medical Economics Co., Inc.,**2002**, 57th ed, 2523.
[21] O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co. Inc., **2001**., 13th Edition, 854
[22] Government of India, Ministry of wealth and family welfare, Vol. 2, New Delhi: The Controller of Publication; **2007**., Indian Pharmacopoeia, 1194–1196.
[23] United State Pharmacopoeial Convention Inc; **2009** : Asian ed. Vol. 2. Rockville MD. The United State Pharmacopoeia 32 and National Formulary 27, 2566–2568.
[24] Sridharan D. et al., *International Journal Of Chem Tech Research*, **2010**, 2, 2, 876-879.
[25] Tian D. et al., *Indian Journal Of Pharmaceutical Science* **2008**, 70, 3, 372 - 374.
[26] Lara F.T., et al., *Journal Of Pharmaceutical &Biomedical Analysis*, **2010**, 51, 4, 985-990.
[27] Deanne L Hertzoget al., Pharmacopoeial Forum, **2005**, 31, 5, 1453-1463
[28] Kumbhar S.T. et al., *International journal Of Pharmaceutical Research*, **2011**, 3, 1, 62-66.
[29] Budavari S, editor. The Merck Index. 13th ed. White House Station, NJ: Merck and Co. Inc; **2001**, 1628.
[30] Maryadele J. O' Neil. The Merck Index. 14th ed. New Jersey:Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station, **2006**, 2193,9131.
[31] Ean CS, Martindale . The Complete Drug Reference. 36th ed. London: The Pharmaceutical Press; **2009**, 1409.
[32] Marydele J. O. et al., The Merck Index- An Encyclopedia of Chemicals, Drugs and Biologicals, 13thEdition, Merck Research Laboratories-a division of Merck & Co.Inc., Whitehouse Station, NJ, 491, 1628
[33] Indian Pharmacopoeia, Ministry of Health & Family Welfare, Vol-II & III 6th edn. Indian Pharmacopoeial commission, Ghaziabad, India, **2010**, 1076-77, 2186-88
[34] British pharmacopoeia-Vol-I & II. 6th Edn. The stationary office, London, **2010**, 484-85, 2042-44
[35] United States Pharmacopoeia - 34 and National Formulary-29 Vol. II & III. The United States Pharmacopoeial Convention, Rockville, MD, USA, **2011**, 2321-22, 4357-58.
[36] Tatane S., *Journal Of Advanced Pharmaceutical & Healthcare Research*, **2011**, 1, 23-26.
[37] Palled MS et al., *Indian Journal of Pharmaceutical Science*, **2006**, 68, 685-686.
[38] Gangola R. et al., *International Journal of Comprehensive Pharmacy*, 2011, 2, 1-3
[39] Kumbhar ST et al., *International Journal Of Pharmaceutical Sciences & Research*, **2011**, 2, 1254-1258
[40] Zonghui Q et al., *Journal of Analytical Chemistry*, **2009**, 69, 5, 449-454.
[41] Kalyankar TM et al., *International Journal of Pharma World Research*, **2010**, 1, 2, 1-9.
[42] Chen BM et al, *Analytica Chimica Acta*, **2005**, 540, 367-373.
[43] Hempen C et al, *Analytica Chimica Acta*, **2006**, 560, 41-49.
[44] Nozomu KN et al, *Journal Pharmaceutical Biomedical Analysis*, **2007**, 1, 1769-1774.
[45] Pengfie L et al, *Journal of Chromatography* B, **2005**, 828, 126-129
[46] Prabhu C et al., *Journal of Planar Chromatography*, **2007**, 20, 6, 77-81.
[47] Potale LV et al., *International Journal Comprehensive Pharmacy*, **2010**, 2, 2, 15-19
[48] Shah NJ, et al., *Indian Journal of Pharmaceutical Sciences*, **2007**, 69, 2, 202-205

[49] Anandakumar K. et al., *International Journal of Pharmacy and Pharmaceutical Sciences*, **2011**, 3, 1, 23-27.
[50] ICH: Proceeding of the International Conference on Hormonisation of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guidelines). Validation of Analytical Procedures: Methadology, Q2B, Geneva, Switerland: **1996**.
[51] ICH guidelines. Validation of analytical procedures: text and methodology. Q2A (R1) Nov; **2005**.