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An ecofriendly simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide in pharmaceutical dosage form by UV-spectrophotometric method

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ABSTRACT

Hydrotropy is one of the solubility enhancement techniques, which enhance solubility of poorly water-soluble drugs to many folds with use of hydrotropes. Three simple, accurate and economic methods; simultaneous equation, Q absorbance and first order derivative method have been described for the simultaneous spectrophotometric estimation of poorly water soluble drugs Olmesartan medoxomil and Hydrochlorothiazide in tablet dosage form. A mixed hydrotropic blend of 25% w/v urea and 25% w/v sodium acetate was used for the quantitative determination. Method A involved simultaneous equation method, the two wavelengths 256.8 nm (λmax of Olmesartan medoxomil) and 271.6 nm (λmax of Hydrochlorothiazide) were selected for the formation of Simultaneous equations. Whereas method B involved formation of Q absorbance equation at isobestic point (263.9 nm). Method C is First order Derivative Spectrophotometric method in which derivative amplitudes were measured at selected wavelengths (257.2 nm for Hydrochlorothiazide and 271.6 nm for Olmesartan medoxomil). Linearity was observed in the concentration range of 3-21, 3-21, 4-28 µg/ml for Olmesartan medoxomil and 2-14, 3-21, 5-35 µg/ml for Hydrochlorothiazide by method A, B and C respectively. The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Keywords: first order derivative method; mixed hydrotropic blend; Q absorbance method; simultaneous equation method; validation

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INTRODUCTION

The term hydrotropy has been used to designate the increase in solubility of poorly water soluble drugs in concentrated solutions of hydrotropic agents. A huge number of poorly water soluble drugs have been solubilized by use of various hydrotropic solutions. [1-8]. Olmesartan medoxomil (OLME) is a selective AT1 subtype angiotensin II receptor antagonist and used as antihypertensive [9]. Chemically it is 2, 3-dihydroxy-2-butenyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1-Htetrazol-5ylphenyl) benzyl] imidazole-5- carboxylate,cyclic-2, 3-carbonate [10]. Hydrochlorothiazide (HCT) is one of the oldest and widely used thiazide diuretics. Chemically it is 6-chloro-3, 4-dihydro- 2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1,1-dioxide [11,12]. OLME and HCT are available in tablet dosage form in the ratio 20:12.5. Olmesartan medoxomil is official in Martindale, The Extra Pharmacopoeia

[9] and The Merck Index [10], whereas Hydrochlorothiazide is official in I. P. [11], B. P. [12], U. S. P. [13] and Martindale, The Extra Pharmacopoeia [9]. Literature survey reveals that many analytical methods such as spectrophotometric [14,15] and RP-HPLC [15,16] methods are reported for determination of olmesartan medoxomil individually from pharmaceutical dosage form and RPHPLC [17-20] and HPTLC [20,21] methods are reported for determination of OLME and HCT in combined dosage form using different organic solvents. Some RP-HPLC methods [22-25] are reported for determination of OLME or HCT combined with other drugs. This paper represents three simple, rapid, accurate, precise, reproducible and economic UV spectrophotometric methods for simultaneous estimation of OLME and HCT in bulk and tablet dosage form using hydrotropic solubilisation technique precluding the use of organic solvents. In the present investigation, mixed hydrotropic solublizing blend of urea and sodium acetate (25% w/v each) was employed to solubilize Olmesartan medoxomil and Hydrochlorothiazide fine powder and its tablet dosage form to carryout spectrophotometric analysis.

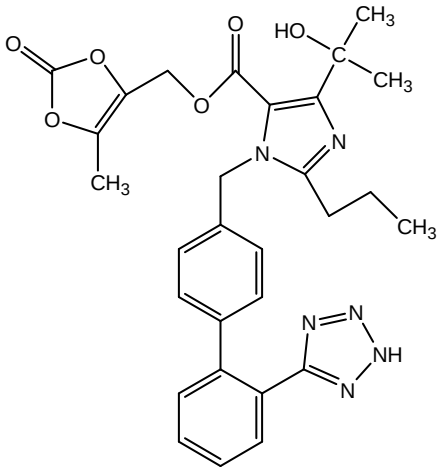

Fig. 1 Olmesartan medoxomil

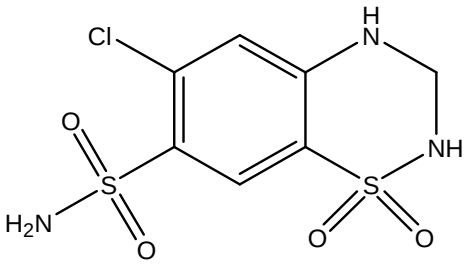

Fig. 2 Hydrochlorothiazide

MATERIALS AND METHODS

Instrument A

UV/ VIS double beam spectrophotometer, (Shimadzu-1800) with matched quartz cells corresponding to 1 cm pathlength and spectral bandwidth of 2 nm connected to a computer loaded with Shimadzu UV Probe 2.42 software was used for all the spectrophotometric measurements in all proposed spectrophotometric methods.

Materials

Standard gift samples of Olmesartan medoxomil (OLME) and Hydrochlorothiazide (HCT) were procured from Lupin Pharmaceuticals, Pune. Combined Olmesartan medoxomil and Hydrochlorothiazide tablets were purchased from local market. All other chemicals used were of analytical grade.

Solvent used

A blend of urea and sodium acetate (25:25) in distilled water was used as a solvent in the study.

Methods

Simultaneous equation method, Absorbance ratio method and first order derivative spectrophotometric method.

Preliminary solubility studies of OLME and HCT:

Solubility of OLME and HCT were determined at 28 ± 1 °C in a blend of urea and sodium acetate (25:25) solution, distilled water and buffer of pH 8 (pH of hydrotropic blend). Sufficient excess amount of each drug was added individually to screw capped glass vials of 30 ml capacity, containing distilled water, buffer of pH 8 and blend solution. The vials were shaken mechanically for 12 hours

at 28 ± 1 °C in mechanical shaker (Lab Hosp). The solutions were allowed to equilibrate for next 24 hours and then centrifuge for 5 min at 2000 rpm (Remi Instruments Limited, Mumbai, India). The supernatant of each vial was filtered through whatman filter paper #41. Filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blanks.

Stock solutions

50 mg each of Hydrochlorothiazide and Olmesartan medoxomil were accurately weighed and transferred in 50 ml volumetric flasks separately, dissolved in 30 ml of urea and Sodium acetate blend solution (25:25) and volume was adjusted to 50 ml with distilled water to obtain solution (1000 µg/ml) of each drug. Aliquot portions of the stock solutions were diluted individually with distilled water to get final concentration of 10 µg/ml for HCT and OLME respectively. These working standard solutions were scanned in the range of 400-200 nm in 1.0 cm cell against solvent blank. The absorption maximas of HCT was found at 271.6 nm while for OLME at 256.8 nm. The overlain spectra of HCT and OLME is shown in **Fig. 3.**

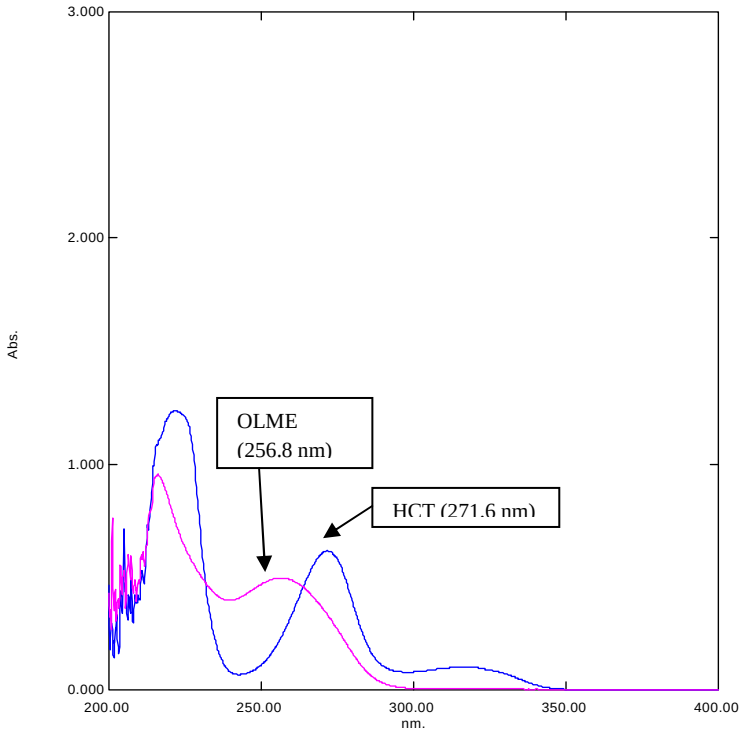

Fig. 3 Overlain zero order spectra of OLME and HCT

Determination of absorptivity value

The solutions of each drug in tripilicate were read against solvent blank at the selected wavelengths and A (1% 1 cm) value were calculated using below formula:

$$\text{Absorptivity, A (1\% 1 cm)} = \frac{\text{Absorbance at selected wavelengths}}{\text{Concentration in g / 100 ml}}$$

Preparation of calibration curves

Stock solutions each of HCT and OLME having concentration of 100 µg/ml were prepared. Aliquots of each solution were appropriately diluted and the final dilutions were read at the selected wavelengths. The linearity of HCT and OLME was found to be in the concentration ranges of 2-14 and 3-21 µg/ml for HCT and 3-21 and 3-21 µg/ml for OLME by simultaneous equation (method A) and absorbance ratio (method B). The coefficients of correlation were found to be 0.9992 for HCT and 0.9995 for OLME, respectively by method A and 0.9993 and 0.9995 for HCT and OLME respectively by method B. The methods were first applied to standard laboratory mixture which yielded encouraging results and then were applied to marketed formulation.

Application of proposed method for physical laboratory mixture:
Mixture of OLME and HCT was prepared by dissolving 10 mg, diluted with 60 ml of urea and sodium acetate blend (25:25), sonicating it for 15 min and then make up the volume up to 100 ml to afford the concentration of 100 µg/ml. From the stock solution of OLME, 1 ml of OLME solution was transferred to 10 ml of volumetric flask and diluted up to the mark to get concentration of 10 µg/ml of OLME; and from the stock solution of HCT, 0.625 ml of HCT solution was transferred to 10 ml of volumetric flask and diluted up to the mark to get final concentration of 6.25 µg/ml of HCT. The solution was scanned in the range of 200 – 400 nm, absorbance of the sample solutions were recorded, against blank. The concentrations (C_{OLME} and C_{HCT}) in sample solution were determined by using formulae given below, results are given in **Table 1**

Table 1: Results of analysis of laboratory mixture

Method	Amount present (µg/ml)		Concentration found (µg/ml)		Percentage found (%)	
	OLME	HCT	OLME	HCT	OLME	HCT
A.	10	6.25	9.919	6.157	99.19	98.51
B.	10	6.25	9.964	6.147	99.64	98.35
C.	10	6.25	9.97	6.19	99.70	99.07

Application of proposed method for analysis of tablets:
Twenty tablets were weighed and average weight was calculated. The tablets were triturated thoroughly and mixed. Tablet powder equivalent to 10 mg of OLME (~6.25 mg of HCT, on the basis of label claim) was transferred to 100 ml volumetric flask. 60 ml of urea:sodium acetate solution was added to the flask and stirred for 15 min to dissolve the drug . The content was filtered through Whatman filter paper (no.41) and volume was made upto 100 ml with distilled water. Filtrate was divided in 2 parts, A & B part. A was kept at room temperature for 48 hours to check the effect on stability of drugs in presence of urea and sodium acetate and also to note precipitation, if any during this period. Part B filtrate was appropriately diluted with distilled water to get a mixed standard containing 10 µg/ml OLME and 6.25 µg/ml HCT. The amount of each drug was estimated by proposed methods using the following formulae and the results of analysis are given in **Table 2**. After 48 hour , filtrate of part A was appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours.

Table 2: Results of analysis of tablet formulation

Method	Brand	Present amount (µg)		Concentration found (µg)		Percentage (%) found	
		OLME	HCT	OLME	HCT	OLME	HCT
A.	Olmesar	10	6.25	9.557	5.735	95.57	91.68
B.	Olmesar	10	6.25	9.848	6.246	98.48	99.93
C.	Olmesar	10	6.25	10.147	6.23	101.47	99.69

Method A: Simultaneous equation method : This method of analysis is based on the absorption of drug X (olmesartan medoxomil) and Y (Hydrochlorothiazide) at the wavelength maxima of the other. The quantification analysis was performed by using the following equations;

$$C_X = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \qquad C_Y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

Where Cx and Cy were the concentrations of OLME and HCT respectively in the diluted sample, ax_1 and ax_2 were absorptivities of OLME at λ_1 and λ_2, ay_1 and ay_2 were absorptivities of HCT at λ_1 and λ_2 respectively and A_1 and A_2 absorbances of mixed standard at 256.8 (λ_1) and 271.6 nm (λ_2) respectively.

Method B: Absorbance ratio
In absorption ratio method, absorbance of both the drugs were calculated at two selected wavelengths among which λ_1 is the wavelength of isobestic point (where both drugs show same absorbance) and λ_2

is the λ max of either drug among the drugs to be analyzed. From the overlain spectra **(Fig. 4.)** wavelength 263.9 nm (λ_1- isobestic point) and 271.6 nm (λ_2- λmax of HCT) was selected for analysis. The concentration of individual drug components was calculated by using the following equation,

$$Cx = \frac{Qm - Qy}{Qx - Qy} * \frac{A_1}{ax_1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} * \frac{A_1}{ax_1}$$

$$\text{Where } Qm = \frac{A_2}{A_1}$$

A_1 is absorbance of mixed standard at λ_1 (isobestic point), A_2 is absorbance of mixed standard at λ_2 (λmax of HCT)

$$Qx = \frac{ax_2}{ax_1}, \qquad Qy = \frac{ay_2}{ay_1}$$

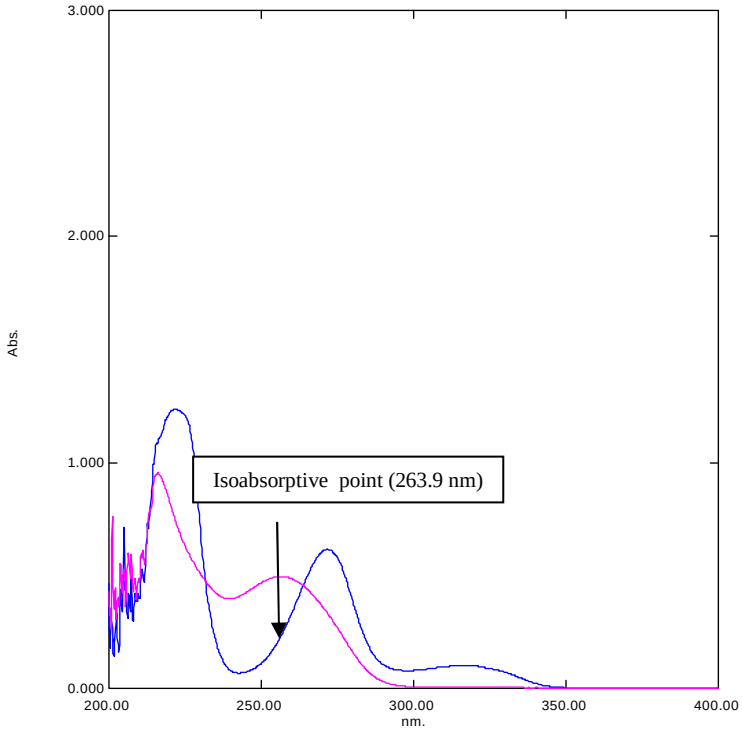

Fig. 4 Overlain Zero Order Spectra of OLME and HCT showing isoabsorptive point

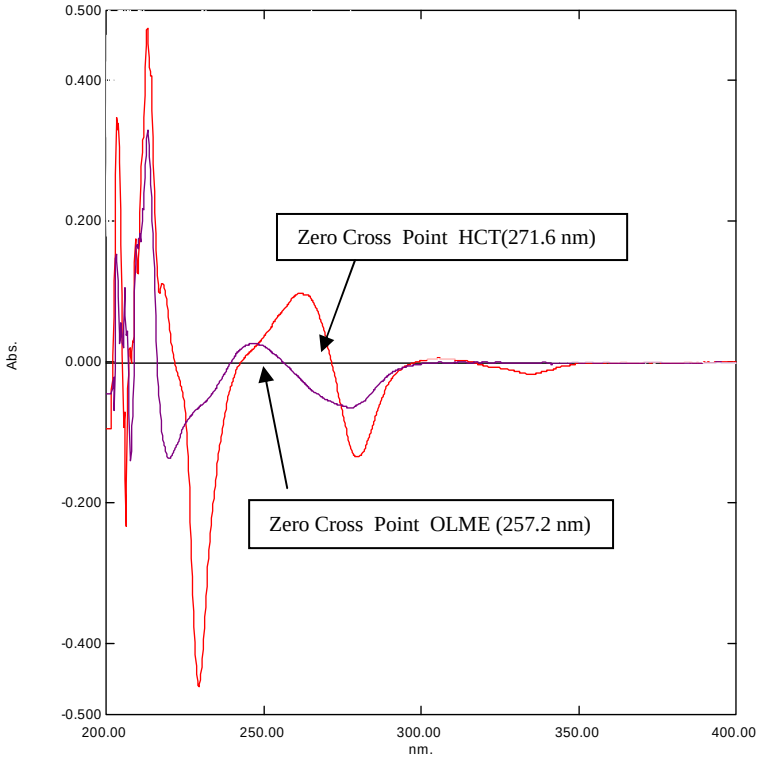

Fig. 5 Overlain First Order Derivative Spectra of OLME and HCT

Method C: First order derivative method

Solutions of 10 µg/ml of OLME and HCT were prepared separately. Both the solutions were scanned in the spectrum mode from 400.0 nm to 200.0 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative (n=1) was selected for analysis of both the drugs. The derivatized wavelength 257.2 nm for HCT which is the zero crossing of OLME and 271.6 nm for OLME which is zero crossing of HCT were selected **(Fig. 5)**

Preparation of calibration curves

The standard dilutions of 4, 8, 12, 16, 20, 24 and 28 µg/ml of OLME and 5, 10, 15, 20, 25, 30 and 35 µg/ml of HCT were prepared separately from stock solution and scanned in the spectrum mode from 400.0 nm to 200.0 nm. The absorption spectra obtained were derivatized to obtain first order derivative spectra. The absorbances of standard solutions of OLME and HCT were measured at zero crossing point of HCT (271.6 nm) and zero crossing point of OLME (257.2 nm) respectively. The working calibration curves of both the drugs were plotted separately. The mixed standard solution of 10 µg/ml for OLME and 6.25 µg/ml for HCT, respectively were prepared. The concentration of individual drug present in the mixture was determined against calibration curve of each drug in quantitation mode.

Validation of proposed methods:

The proposed methods were validated as per the ICH guidelines for various parameters like Linearity, Accuracy, Precision, Ruggedness, Limit of Detection and Limit of Quantitation.

Linearity and Range: To establish the linearity of the proposed method, three separate series of solutions of OLME and HCT were prepared from stock solution and analyzed. Least square regression analysis was done for the obtained data and shown in the **table 3.**

Table 3: Optical and Regression characteristics for analysis of OLME and HCT

Parameters	Method A		Method B		Method C	
	OLME	HCT	OLME	HCT	OLME	HCT
Beer's law limits (µg/ml)	3-21	2-14	3-21	3-21	4-28	5-35
Regression equation y = mx + c						
Slope	0.0415	0.0664	0.0413	0.0419	0.034	0.026
Intercept	0.0601	0.0331	0.0518	0.0552	0.007	0.010
Correlation coefficient [r^2]	0.9995	0.9992	0.9995	0.9993	0.9997	0.9999

Accuracy: It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of standard OLME and HCT was added to pre–analyzed sample (8 µg/mL of OLME and 5 µg/mL of HCT) and subjected them to the proposed methods. Results of Recovery studies were shown in

Table 4: Data of Recovery studies

Method	Level of % Recovery	Initial concentration (µg/mL)		Concentration found (µg/mL)		% Recovery (Mean)*		% RSD	
		OLME	HCT	OLME	HCT	OLME	HCT	OLME	HCT
A	80	8	5	14.39	4.03	99.99	100.75	0.081	0.25
	100	8	5	15.97	9.99	99.81	99.9	0.319	0.31
	120	8	5	17.61	11.01	100.05	100.16	0.3617	0.21
B	80	8	5	14.389	3.99	99.92	99.91	0.23	0.72
	100	8	5	15.99	9.97	99.955	99.76	0.26	0.56
	120	8	5	17.56	10.96	99.77	99.63	0.35	0.57
C	80	8	5	14.405	4.006	100.03	100.16	0.10	0.38
	100	8	5	15.99	9.96	99.97	99.68	0.37	0.42
	120	8	5	17.628	10.99	100.08	99.91	0.39	0.50

** mean of 3 determinations*

Precision:

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Variation of results within the same day (intraday), variation of results between consecutive days (inter day) were analyzed and results are given in **Table 5.**

Table 5: Data of Precision studies

Method	Drug	Concentration taken (µg/ml)	Interday *		Intraday *	
			Concentration found (µg/ml)	% RSD	Concentration found (µg/ml)	% RSD
A	OLME	6	5.97	0.57	0.31	0.46
		12	12.06	0.56	0.55	0.71
		18	18.02	0.65	0.80	0.51
	HCT	4	4.01	0.31	0.22	0.53
		8	7.97	0.65	0.50	0.40
		12	11.93	0.57	0.76	0.44
B	OLME	6	5.98	0.49	6.009	0.53
		12	12.01	0.13	11.99	0.50
		18	17.97	0.15	17.99	0.35
	HCT	6	6.009	0.1893	5.98	0.42
		12	12.00	0.35	11.97	0.41
		18	17.96	0.12	17.95	0.32
C	OLME	8	8.02	0.38	7.99	0.41
		16	15.94	0.38	15.97	0.28
		24	24.02	0.28	24.00	0.34
	HCT	10	9.92	0.42	10.01	0.37
		20	19.95	0.38	19.92	0.36
		30	29.96	0.45	29.92	0.37

** mean of 3 determinations*

Ruggedness: Ruggedness was determined by two different analyst by preparing sample solution of OLME (10 µg/ml) and HCT (6.25 µg/ml) from stock solution using similar operational and environmental conditions and results are given in **Table 6.**

Table 6: Data of Ruggedness

Method	Drug	Concentration Found (%) ± RSD	
		Analyst-I	Analyst-II
A	OLME	100.13 ± 0.25	99.79 ± 0.21
	HCT	99.77 ± 0.12	99.87 ± 0.13
B	OLME	99.04 ± 0.19	99.63 ± 0.07
	HCT	99.96 ± 0.25	99.99 ± 0.27
C	OLME	99.38 ± 0.14	99.66 ± 0.08
	HCT	99.42 ± 0.36	99.48 ± 0.47

Limit of Detection and Quantification (LOD & LOQ): The LOD and LOQ were estimated from the standard calibration curve. It is calculated using the formula LOD = 3.3 X σ/S and LOQ = 10 X σ/S where, σ is the standard deviation of the response and S is the slope of the calibration curve. Results are given in **Table 7.**

Table 7: LOD and LOQ

Method	Drug	LOD (µg/ml)	LOQ (µg/ml)
A	OLME	0.036	0.09
	HCT	0.100	0.30
B	OLME	0.15	0.47
	HCT	0.09	0.29
C	OLME	0.36	0.58
	HCT	0.09	0.27

RESULTS AND DISCUSSION

The solubility of HCT and OLME in urea and sodium acetate blend solution was found to be more than 40 fold and 10 fold as compared to its solubility in distilled water respectively. The pH of blend solution was 8. To check the effect of pH on solubility of drugs, their solubility was also determined in buffer of pH 8. Solubilities of both the drugs in distilled water and buffer of pH 8 were almost same thus it is concluded that enhancement in solubility of HCT and OLME in blend solution was due to hydrotropic solubilization only. Fresh filtrate and 48 hours aged filtrate (kept at room temperature) of drugs were found to have same drug contents. Also there was no precipitation within 48 hours this indicates that analysis can be accurately performed within 48 hour of extraction of the drug from tablet powder.

In simultaneous equation method, OLME showed absorbance maxima at 256.8 nm and HCT at 271.6 nm. Linearity was observed in the concentration range of 3-21 µg/ml for OLME and 2-14 µg/ml for HCT. Correlation coefficient was found to be 0.9995 and 0.9992 at 256.8 nm and 271.6 nm respectively. The proposed method was applied for the determination of OLME and HCT in the marketed dosage and estimated as 95.57% and 91.68% respectively.

In absorbance ratio method, from overlain spectra of OLME and HCT, two wavelengths were selected at 263.9 nm (isoabsorptive point) and 271.6 nm (λmax of HCT). OLME and HCT follow linearity in the concentration range 3-21 µg/ml and 3-21 µg/ml respectively. Correlation coefficient was found to be 0.9995 and 0.9993 at 256.8 nm and 271.6 nm respectively. The proposed method was applied for the determination of OLME and HCT in the marketed dosage and estimated as 98.48 % and 99.93 % respectively.

In first order derivative spectrophotometric method the derivatized wavelength 257.2 nm for HCT which is the zero crossing of OLME and 271.6 nm for OLME which is zero crossing for HCT were selected. Linearity was observed in the concentration range of 4-28 µg/ml for OLME and 5-35 µg/ml for HCT. Correlation coefficient was found to be 0.9997 and 0.9999 for OLME and HCT respectively. The proposed method was applied for the determination of OLME and HCT in the marketed dosage and estimated as 101.47 and 99.69 %.

The recovery of drugs was determined at 80, 100 and 120 % levels for all the three methods. The percentage recovery was from 99.8 to 99.9 % for OLME and 99.7 to 100.3 % for HCT. Precision, Ruggedness was performed as per ICH guidelines, results shows that % RSD < 2 % which is within

the limit for all the methods. LOD and LOQ were found to be 0.0306, 0.0927 by simultaneous equation, 0.156, 0.474 by absorbance ratio and 0.365, 0.589 by first derivative spectrophotometry for OLME and 0.1, 0.304 by simultaneous equation, 0.0986, 0.299 by absorbance ratio and 0.0901, 0.273 by first derivative spectrophotometry for HCT.

CONCLUSION

A blend of urea and sodium acetate (25:25%) was successfully used for simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide. The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise, reproducible, ecofriendly, safe, cost-effective as they preclude the use of toxic organic solvents and can be employed successfully for the estimation of Olmesartan medoxomil and Hydrochlorothiazide in bulk and formulation.

REFERENCES

[1] C. Neuberg. Hydrotropy. Biochem. Z. **1916;** 76, 107.
[2] K. Park, W. Lafayette, G. Acharya, J. Lee, S.C. Lee. Pharmaceutical applications of hydrotropic agents, polymers, thereof, and hydrogels thereof, US Patent 20030031715**. 2003.**
[3] R.K. Maheshwari. Indian Pharmacist., 2007 6: 66-67.
[4]. R.K. Maheshwari, Asian J.Chem., 2006, 18, 3194-3197.
[5] R.K. Maheshwari, S.C. Chaturvedi, N.K. Jain, *Indian Drugs***, 2005**, 42, 541-544.
[6] R.K. Maheshwari, S.C. Chaturvedi, N.K. Jain, *Indian J. Pharm. Sci.*, **2006**, 68:195-198.
[7] R.K. Maheshwari, S.C. Chaturvedi, N.K. Jain, *Indian J. Pharm. Sci.*, (In Press).
[8] R.K. Maheshwari, S.C. Chaturvedi, N.K. Jain, *Indian Drugs***., 2006**, 43, 516-518.
[9] S.C. Sweetmann, Martindale, The extra pharmacopoeia, The complete drug reference. 36th Ed., The Pharmaceutical Press, London, **2009**, 1361,1307.
[10] S. Budhwari, The Merck Index, 13thEd., Merck Research Laboratories, Whitehouse Station, New Jersey, **2001**, 6909, 1223, 4802, 854.
[11] Indian Pharmacopoeia, Vol. I., The Controller of Publication, New Delhi, **2007**, 151, 318, 1194-1195.
[12] British Pharmacopoeia, 4th Ed., Vol. I, Her Majesty's Stationary Office, London, **2004**, 979.
[13] The United State Pharmacopoeia, 26th Ed., Vol. I, United States Pharmacopoeial Convention Inc. Washington DC, **2004**, 2334.
[14] M. Celebier and S. Altinoz. Determination of olmesartan medoxomil in tablets by UV-Visible spectrophotometry, *Pharmazie*. **2007**, 62(6), 419-422.
[15] P. Jain, A. Jain, D. Maliwal and V. Jain. *International J Pharm Bio Sci.* **2010,** 1(2), 158-160.
[16] T. Murakami, H. Konno, N. Fukutsu, M. Onodera, T. Kawasaki and F. Kusu. *J Pharm Biomed Anal*. **2008,** 47(3), 553-559.
[17] O. Sagirli, A.O. Nall, S.E. Toker, D. Sensoy. *J Chromatographia*. **2007,** 66:213–218.
[18] J. Ashok Kumar, A. Sathya, K. Senthil Kumar, S.N. Patil, S.B. Prathap and V. Gopal. *International J Research in Pharm Sci.* **2010,** 1, 24-27.
[19] K.S. Chouhan, M.V. Bhure, A.T. Hemke, K.R. Gupta and S. Wadodkar S. *Research J Pharm Bio Chem Sci.* **2010**, 1(4), 2010, 78.
[20] P.D. Bari and A.R. Rote. *J Chromatographia.* June **2009**, 69(11-12), 1469-1472.
[21] N.J. Shah, B.N. Suhagia, R.R. Shah and N.M. Patel. *Indian J Pharm Sci.* **2007**, 69, 834–836.
[22] N. Sultana, M.S. Arayne, A.S. Shahid and S. Sajid. *Chin J Chromatogr.* **2008,** 26, 544-549.
[23] C.V. Patel, A.P. Khandhar, A.D. Captain and K.T. Patel. *Eurasian J Analytical Chemistry.* **2007,** 2, 159-171.
[24] G. Carlucci, G. Palumbo, P. Mazzeo, M.G. Quaglia. *J. Pharm Biomed Anal.* **2008,** 23, 185-189.

[25] F. Belal., I.A. Al-Zaagi, E.A. Gadkariem and M.A. Abounassif. *J Pharm Biomed Anal*. **2001,** 24, 335-342.