ISSN : 0974-7419 Volume 16 Issue 4

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 16(4) 2016 [156-162]

Simultaneous spectrophotometric estimation of norethindrone acetate and ethinyl estradiol in formulation

Koneru Ajitha, Ritu Kimbahune*, .G.Mubeen, Yajnesh Pai

Department of Quality Assurance, Al-Ameen College of Pharmacy, Opposite to lalbagh Main gate, Hosur Road, 560027, (BANGALORE)

E-mail: rituvivekk@gmail.com

ABSTRACT

A combination of second order derivative spectrophotometric method and absorbance correction method has been proposed for simultaneous spectrophotometric estimation of Norethindrone Acetate and Ethinyl Estradiol. Amplitude of second order derivative at 266.8 nm was used for quantitation of Norethindrone Acetate and Ethinyl Estradiol was estimated by absorbance correction method after correcting the absorbance for Norethindrone Acetate at 280 nm. The content of Ethinyl Estradiol and Norethindrone Acetate were found about 105.77% and 101.56% in tablet mixture respectively. The method has been validated in accordance with ICH guidelines and the results for all parameters were found to be within the acceptance criteria.

KEYWORDS

Norethindrone Acetate;
Ethinyl Estradiol;
Derivative Spectroscopy;
Absorbance Correction Method.

INTRODUCTION

About 100 million women worldwide use the combined oral contraceptive pills (COCPs) as the birth-control pills that include a combination of an estrogen(estradiol) and a progestogen (progestin). Progestogen inhibits sperm penetration through the cervix into the upper genital tract (uterus and fallopian tubes)by decreasing the water content and increasing the viscosity of the cervical mucus. Estrogen negative feedback on the anterior pituitary greatlydecreases the secretion of FSH, which inhibits follicular development and alsostabilizes the endometrium and thereby reduces the incidence of breakthrough bleeding. The combined effect prevents a mid-cycle LH surge and inhibitsfollicular development and thereby prevents ovulation. Norethindrone Acetate (NEA) is a synthetic derivative of progestogen whereas EthinylEstardiol (EE) is a synthetic derivative of estrogen[1, 2, 3, 4].

NEA chemically is 17-Hydroxyl-19-nor-17α-pregn-4-en-20-yn-3-one acetate and works by decreasing the pulse frequency of gonadotropin-releasing hormone (GnRH).Chemically EE is 19-Nor-17α-pregna-1, 3, 5(10)-trien-20-yne-3, 17-diol which decreases the secretion of FSH. Both analytes are white colour powder and freely soluble in methanol[5, 6].

Tablet formulations like Femhrt (2.5 µg:0.5 mg), Jintel (5 µg:1 mg), Activella (1 mg: 0.5 mg), Estrostep (20 µg:1 mg) and Junel (30 µg:1.5 mg) containing EE and NEA are available in US market and prescribed as COCPs[7].

Literature review revealed that there are many

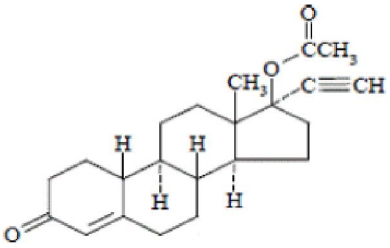

Figure 1 : Structure of norethindrone acetate (NEA)

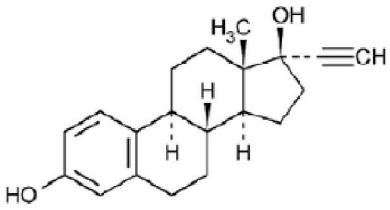

Figure 2 : Structure of ethinyl estradiol (EE)

spectrophotometric and chromatographic methods available for estimation of NEA and EE individually and in combination with other drugs[8-15]. So far there are no chromatographic methods have been reported for simultaneous estimation of NEA and EE in combination. Hence the objective of the present analytical research work is to propose a faster analytical method for simultaneous estimation of NEA and EE in fixed dose combination.

MATERIALS AND METHODS

Instrument

A Shimadzu UV-Visible double beam spectrophotometer model 1700 (Japan) with 1 cm matched quartz cells connected to a PC computer running UV-Probe processor software for absorbance measurements and treatment of data was used along with Acculab digital balance for weighing. Mark ultra sonicator was used for dissolving standard as well as sample in solvent.

Chemicals and reagents

Norethindrone Acetate and Ethinyl Estradiol were obtained in the form of gift samples from Cipla Ltd Mumbai. Norethindrone Acetate Tablets (Regestrone, 5 mg, Sandoz a Novarits company) and Ethinyl Estradiol Tablets (Lynoral, 0.1 mg, Organon (India) private Ltd) were procured from local pharmacy. Methanol (UV grade, Finar) was used as a solvent throughout the work.

Preparation of standard norethindrone acetate solution

Accurately weighed 10 mg of NEA was transferred into a clean and dry 10 mL volumetric flask and dissolved in few mL of methanol. The volume was made up to 10 mL with methanol to get concentration 1000 µg/mL. The stock solution was further diluted to get a concentration 500, 400, 300, 200 and 100 µg/mL.

Preparation of standard ethinyl estradiol solution

Accurately weighed 10 mg of EE was transferred into a clean and dry 10 mL volumetric flask and dissolved in few mL of methanol. The volume was made up to 10 mL with methanol to get concentration 1000 µg/mL. The stock solution was further diluted to get a concentration 10, 20, 30, 40, 50, 60, 70, 80 and 90 µg/mL.

Analysis of marketed formulations

Four tablets of NEA (Regestrone, 5 mg) and EE (Lynarol 0.1mg) were transferred into 100 mL volumetric flask; about 50 mL of methanol was added and sonicated for 20 minutes. The volume was made up to the mark with methanol and filtered through whatmann filter paper (no 41). First few mL of the filtrate was discarded and rest of the filtrate was used for the analysis. The concentration of the obtained filtrate was 200:4 µg/mL of NEA and EE respectively. The filtrate was scanned in the range of 200-400 nm at fast speed against methanol as a blank. The absorbance (A) was measured at 280 nm. The spectrum was converted to second order derivative with $\Delta\lambda$ 2 nm and the amplitude (D^2) was measured at 266.8 nm. The content of NEA and EE were cal-

TABLE 1 : Results of marketed formulation

Analyte	% Content						% Mean Assay	% RSD
	I	II	III	IV	V	VI		
EE	107.83	106.81	102.48	107.66	107.11	102.72	105.77	2.347
NEA	105.12	105.68	100.97	100.48	103.65	103.45	103.22	2.053

Full Paper

TABLE 2 : Results of recovery studies

Analyte	NEA (n=3)			EE (n=3)		
	CONC * (gm/100 mL)	%Assay	%Mean Recovery	CONC * (gm/100 mL)	% Assay	%Mean Recovery
Assay	0.01027	101.42	-	0.000212	106.09	-
50% Add^n	0.01458	153.92	105.05	0.000324	161.03	109.88
100% Add^n	0.02027	202.71	101.31	0.000443	221.14	115.05
150% Add^n	0.02434	243.36	99.614	0.000557	278.68	115.06
Mean		101.99%			113.33 %	

***Mean results of three observations.**

TABLE 3 : Results for precision

Parameters	Norethindrone Acetate		Ethinyl Estradiol	
Intra-day (%RSD)	1.724		1.1913	
Inter-day (%RSD)	2.886		1.831	
Reproducibility	t-test	F-test	t-test	F-test
	1.37	2.100	0.106	1.1

Tabulated values for t-1.533 and F-9.00 at degree of freedom 2, 2 (P=0.10)

culated using following equations and the results obtained are reported in TABLE 1.

$$\%\ \mathbf{NEA} = \frac{(\mathbf{D^2 at\ 266.8\ nm - Intercept}) \times \mathbf{DF} \times \mathbf{100}}{\mathbf{Slope of regression equation} \times \mathbf{label claim}}$$

$$\%\ \mathbf{EE} = \frac{[\mathbf{A\ at\ 280\ nm} - (A^{1\%}_{1cm}\ \mathbf{of\ NEA\ at\ 280\ nm} \times \mathbf{conc\ of\ NEA})] \times \mathbf{DF} \times \mathbf{100}}{A^{1\%}_{1cm}\ \mathbf{of\ EE\ at\ 280\ nm} \times \mathbf{label claim}}$$

Method validation

The proposed methods of analysis for NEA and EE in combination were validated as per the recommendations of ICH guidelines (Q2R1)for the parameters like accuracy, linearity, precision, detection limit and quantitation limit. [16]

Accuracy

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). A known amount of NEA and EE were added separately to pre-analyzed tablet solution (100:2 µg/mL), analyzed and percent recoveries were calculated. The results are presented in TABLE 2.

Precision

Precision of the proposed method was determined by measuring the absorbance of sample solution prepared as mentioned under assay of marketed formulation containing 250:5µg/ml of NEA and EE respectively at three different time intervals on same day (intra-day) and on three consecutive days (inter-day). Reproducibility was determined by applying statistical analysis (t-test and F-test) to the results obtained by different analysts to verify significant variation in precision and accuracy. The results for precision studies are presented in TABLE 3.

Linearity

The linearity of developed method was evaluated by analyzing series of the standard solutions of concentrations 100-500 µg/ml for NEA and 10-90 µg/ml for EE at selected wavelengths (n=6). The calibration curves were obtained by plotting mean amplitude (D^2) at 266.8 nm vs concentration and mean absorbance at 280 nm vs concentration and are shown in Figure 3, 4 and 5. The concentration range for the linearity has been determined on the basis of correlation of coefficient (R^2e"0.997).

Limit of detection and limit of quantification

The limits of detection (LOD) and quantification (LOQ) were established by evaluating the minimum level at which an analyte could be readily detected and quantified with accuracy, respectively. LOD and LOQ were calculated using standard deviation of the intercept from the cali-bration curve

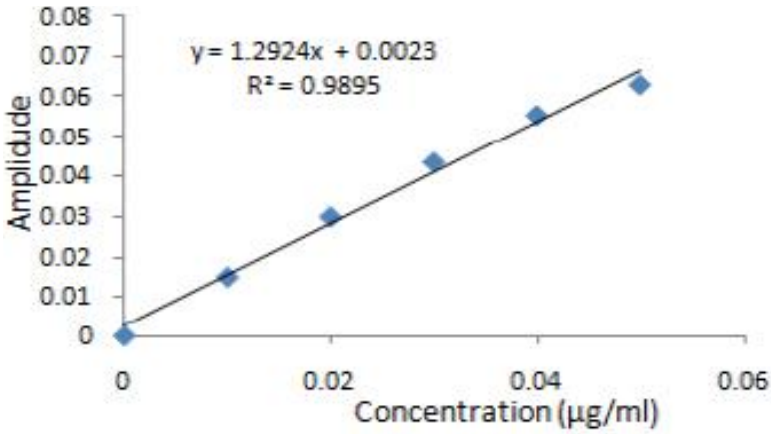

Figure 3 : The calibration curve of second order derivative of NEA at 266.8 nm *vs* concentration

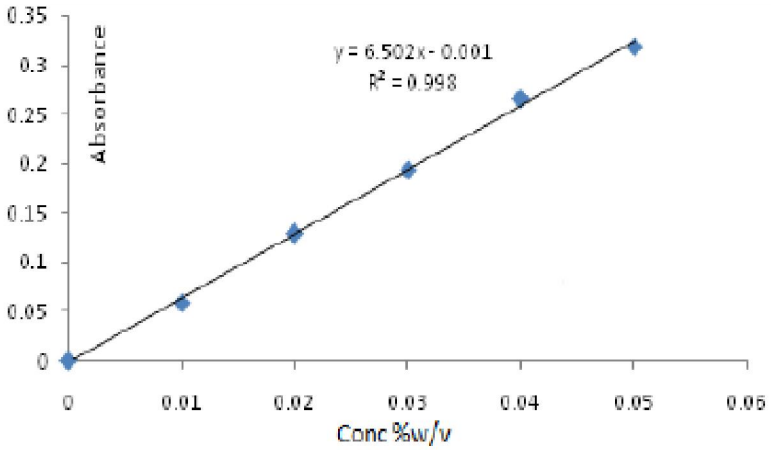

Figure 4 : The calibration curve of absorbance of NEA at 280 nm *vs* concentration

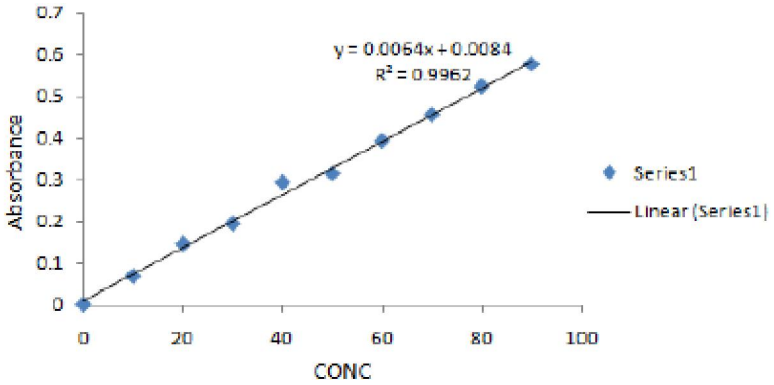

Figure 5 : The calibration curve of absorbance of EE at 280 nm *vs* concentration

(σ) and the mean slope of curve (S). The equations used were LOD = 3.3 × σ/S and LOQ = 10 × σ/S.

RESULTS AND DISCUSSION

The objective of the analytical research work is to develop and validate spectrophotometric method for simultaneous estimation of Ethinyl Estradiol (EE) and Norethindrone Acetate (NEA) in tablet formulation.

Considering the common solubility of EE and NEA, methanol was selected as a solvent for spectrophotometric analysis. The standard solutions of EE and NEA were scanned in the region of 200-400 nm at fast speed against methanol as a blank. The overlain spectra of standard EE and NEA as shown in figure 6 indicate that EE absorbs in the region of 250-300 nm with absorbance maxima at 280 nm and

Full Paper

NEA absorbs in the region of 200-280 nm with absorbance maxima at 240 nm.

The minor dose of EE with respect to NEA in COCPs (1:200, 1:500, 1:1000 etc) and equivalent molar absorptivity of Ethinyl Estradiol (ε =1902.5 at 280 nm) and Norethindrone Acetate (ε =2213.3 at 280 nm) could be the reasons for failure of simultaneous estimation of Ethinyl Estradiol and Norethindrone Acetate by simultaneous estimation method, absorption ratio method and derivative spectrophotometry. Hence a combination of second order derivative spectrophotometry and absorbance correction method has been attempted.

The zero order spectra of Ethinyl Estradiol and Norethindrone Acetate were converted to first and second order derivative with Δλ 2 nm and scaling factor 1. As shown in figure 7 NEA was exhibiting prominent amplitude whereas EE showed zero amplitude in second order derivative spectra at 266.8 nm. Hence NEA was estimated using regression equation obtained from calibration curve of amplitude (D^2) at 266.8 nm vs concentration. The determination of EE using first or second order derivative was found difficult because of its minute dose with respect to NEA in COCPs. Hence EE was estimated using standard absorptivity value and corrected absorbance at its absorbance maxima (280 nm). The specific absorbance ($A^{1\%}_{1cm}$) was calculated for EE and NEA at 280 nm and was found to be 64.188 and 6.501respectively.

As the tablet formulations containing EE and NEA are not available in India, the method was applied for the standard mixture, standard mixture in commonly used excipient solution and sample prepared by mixing marketed tablets of EE and NEA in similar proportion. The content of EE and NEA in tablet mixture were determined using the equations mentioned under Analysis of Marketed formulations and found in the range of 107.83-102.72 % and 105.12- 103.65 % respectively. The results for assay were found complying with pharmacopeial specification of individual tablets.

The developed method was validated in accordance with ICH guidelines for accuracy, precision, linearity, range, LOD and LOQ.

Accuracy was performed by standard addition

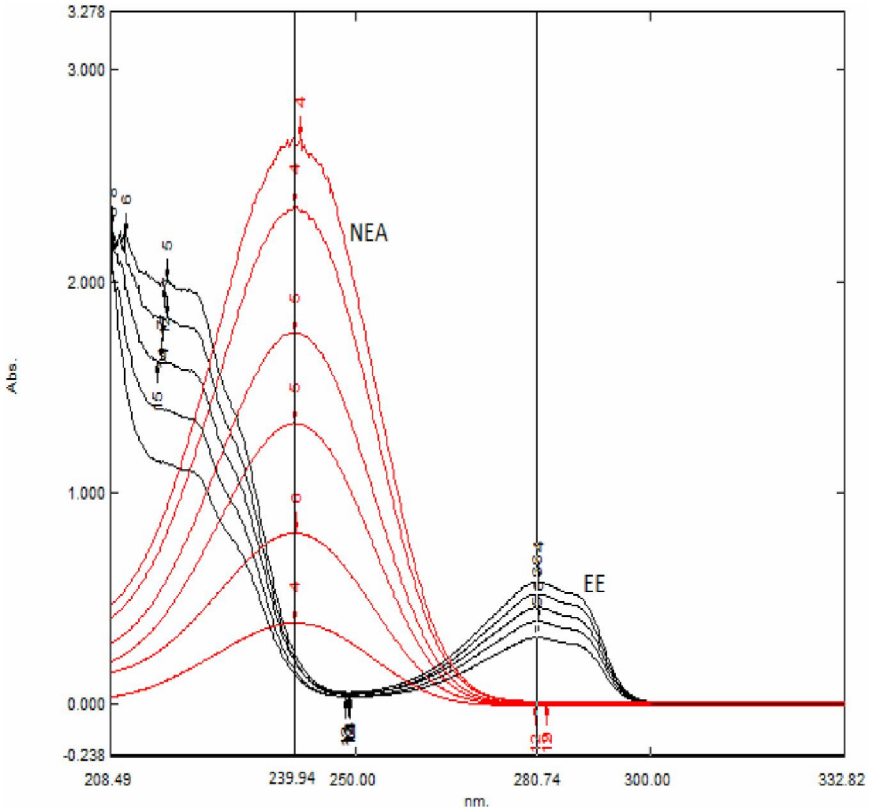

Figure 6 : The zero order overlain UV spectraof EE (50-90 µg/mL) and NEA (10-60 µg/mL)

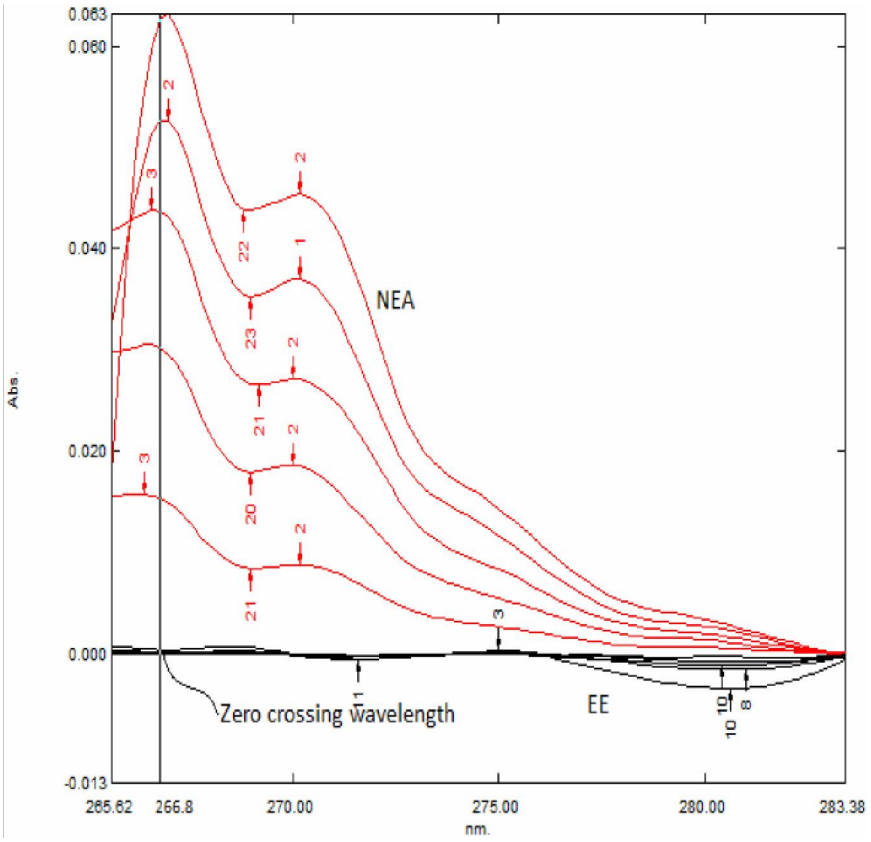

Figure 7 : The second order overlain UV spectra of EE (4-40 µg/mL) and NEA (100-500 µg/mL)

method and mean recovery was found 101.99% for NEA and 113.33% for EE. Precision of the method was studied by performing interday, intraday and reproducibility studies. The % RSD of EE was found less than 2 for inter and intra-day studies while % RSD for Inter-day studies of NEA was found more than 2. This indicates that NEA solution in methanol is not stable for more than 24 hrs. Reproducibility was determined by applying t-test and F-test for the results obtained from two different analysts. No significant difference observed between precision and results of the assay of two different analyst as calculated t and F values were found less than tabulated values at degree of freedom 2, 2 (P=0.10).

Linearity and range were obtained by plotting amplitude (D^2) at 266.8 nm *vs* concentration for NEA and by plotting absorbance at 280 nm *vs* concentration for NEA and EE. NEA was found to be linear in the concentration range of 100-500 µg/mL with coefficient of correlation 0.9895 and 0.998 at 266.8 nm and 280 nm respectively. Linearity for EE was found to be in the concentration range of 10-90 µg/mL with coefficient of correlation 0.9962 at 280 nm. LOD and LOQ were found to be 0.7013 and 3.830, 2.133 and 11.606 µg/mL for EE and NEA respectively.

A combination of second order derivative UV-Visible spectrophotometric method and absorbance correction method is rapid analytical technique for simultaneous estimation of Ethinyl Estradiol and Norethindrone Acetate and can be used for in process quality control tests. However the estimation of EE by absorption correction method is dependent on estimation of NEA. Hence extra precautions should be taken for extraction and analysis of NEA.

CONCLUSION

A combination of second order derivative UV-Visible spectrophotometric method and absorbance correction method was developed and validated for simultaneous estimation of Ethinyl Estradiol and Norethindrone Acetate and can be used for routine analysis of the standard mixture and formulation.

Full Paper

ACKNOWLEDGEMENTS

The authors shall remain grateful to Cipla limited, Mumbai, India for providing Norethindrone Acetate and Ethinyl Estradiol in the form of 'Gift samples' and Al-Ameen College of Pharmacy, Bangalore for providing lab and research facilities to complete this project.

REFERENCES

[1] W.D.Mosher, G.M.Martinez, A.Chandra, J.C.Abma, S.J.Willson; Use of contraception and use of family planning services in the United States: 1982-2002, Advanced Data. **350**, 1-36 **(2004)**.

[2] Levin, R.Ellis, Hammes, R.Stephen; Goodman & gilman's the pharmacological basis of therapeutics 12thed. In Brunton, Chabner, Bruce A, Knollmann, Bjorn C: 1163-94 **(2011)**.

[3] V.N.Sharma, Harmone, Harmone Antagonists; In sharma VN, Essential of pharmacology 3rd Edition, CBS Publishers and Distributors, 399 **(2007)**.

[4] S.R.Saif, Harmones; In Saif SR,Dr M. Haider, Dr. Zafar Niyaz, Dr. Abar; Pharmacology Review 1st Edition, CBS Publications and Distributors, 254-55 **(2005)**.

[5] The united state pharmacopoeia /the national formulary, USP XXII / NF XVII, United state pharmacopeial convention, Inc; **546**, **960**, 1710-12 **(1990)**.

[6] IP, Indian Pharmacopiea, **1**, 301-302 **(1996)**.

[7] Norethindrone Acetateand Ethinyl Estradiol(www.drugs.com/ppa/contraceptives-oral-combination-products.html)

[8] R.C.Patel, D.K.Rathod, K.S.Rajesh, V.S.Patel; Method development and validation for estimation of drospirone and ethinyl estradiol in bulk and combine dosage form by using RP-HPLC, Pharmagene, **1(1)**, 15-20 **2013**.

[9] G.M.Patel, G.VidyaSagar; Development and validation of analytical method for simultaneous estimation of Ethinyl Estradiol and Cyproterone Acetate in combine solid dosage form by using RP-HPLC, Int J.Uni.Pharm.Life.Sci., **2(3)**, 611-22 **(2012)**.

[10] M.P.Krishna, B.Thirupathirao, Kishore R.Kumar, P.Venkateswarulu; Development and validation method for the determination of related substances of Norethindrone in Norethindrone tablet and degradation studies by using RP-LC, Int.J.Chem.Tech.Res., **3(1)**, 143-8 **(2011)**.

[11] K.Bansal, P.Pant, T.R.P.Rama, Venkateswar M.Reddy; Spectrophotometric method for the estimation of Norethindrone in immediate release tablet by using UV spectrophotometer, Int.J.Pharm. Sci.Drug.Res., **3(2)**, 127-9 **(2011)**.

[12] Wenkui Li, Ying-He.Li, Z.Shaolian, W.Naidong; Simultaneous determination of Norethinedrone and Ethinyl Estradiol in human plasma by high performance liquid chromatography with tandem Mass spectrometry experience on developing a highly selective method using derivatization reagent for enhancing sensitivity, J Chromat B., **825(2)**, 223-32 **(2005)**.

[13] Berzas J.J.Nevada, F.J.Rodriguez, P.G.Castaiieda; Simultaneous spectrophotometric determination of ethinyl estradiol and levonorgestrol by partial least squares and principal component regression multivariate calibration,by using UV, Anal Chemice Acta, **340**, 257-65 **(1997)**.

[14] Kabeer S.Ahmed, Ashish Tanaji Patil, Ssdashiv Katare; A validated LC method for the simultaneous determination of drospirenone and ethinylestradiol in tablet dosage form by using combine fluorescence and UV detectors, J.Trace Anal Food Drugs, 30-37 **(2013)**.

[15] C.Praveen, M.K.Rangnath, P.Divakar; Method development and validation for simultaneous estimation of ethinyl estradiol and drospirenone and forced degradation behaviour by HPLC in combined dosage form, Pharmaceutica Anal Acta, **4(5)**, 1-5 **(2013)**.

[16] International conference on harmonization, Q2R1, Harmonised tripartite guidelines, Validation of analytical procedures: Text and methodology, Geneva, November, **(2005)**.