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A Comparative Study of Dissolution Profile and Its Validation for Levonorgestrel and Ethinylestradiol Combined Oral Doses Form Tablet

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Abstract

Dissolution is one of the most important characteristics of a drug directly effect on the drug absorption and bioavailability. The dissolution of Levonorgestrel and Ethinylestradiol from oral dosage forms at different dissolution media: Acetate, HCl, Water and Phosphate have been studied by using an RP-HPLC method. Dissolution of Levonorgestrel after 60 min in acetate, HCl, water and phosphate were 98, 95, 97 and 98% respectively. For Ethinylestradiol dissolution after 60 min at indicated media were 91, 82, 92 and 93% respectively. From that point HCl media has been rejected. Difference (f_1) and similarity (f_2) factor of test sample has been calculated comparing with Microgynon 30 mg Tablet (reference dose) and dissimilarity was found in water and phosphate media and can reject both of them. Only acetate media has been found the best option among four dissolution media for simultaneous determination of Levonorgestrel and Ethinylestradiol COCs. By using acetate buffer a complete analytical dissolution method has been validated accurding to FDA, ICH and USP category 1 requirement. It was found that this method was permeated all validation parameter. This acetate buffer has successfully been used for determination of both active from Levonorgestrel/Ethinylestradiol tablet that was manufactured by five different companies.

Keyword: Bioavailability; Buffer; Dissimilarity; Validation

Introduction

Combined oral contraceptives (COCs) have been the most popular methods of reversible birth control around the world especially in most developed countries [1,2]. A survey states that 25% of all sexually active contraception women in the US and 10% in china trust on COCs [3]. The main components of COCs are estrogen and progestogen. Of them Estrogen are responsible for preventing producing follicle stimulating hormone (FSH) and luteinizing hormone (LH) from pituitary gland in the brain and prevent development of the egg and supporting the uterine lining to prevent mid-cycle breakthrough bleeding. On the other side progestin are responsible for the Stopping LH production from occurring in the pituitary gland so no egg is released, causing changes to the uterine lining which make it harder for an egg to implant, limit the ability of an egg to be fertilized by sperm and causing cervical mucus to thicken, hindering the ability of the sperm to travel, so that no fertilization occur [4]. Combination of this two component improve contraceptive safety and tolerability [5]. There are several types of combinations of estrogen and progestin is now available and has been used in different countries. Some of the common estrogens are Estradiol, Estriol, Estrone, Ethinylestradiol, Mestranol and Diethylestradiol etc. Some of the most common progestogen is Norgestimate, Norethindrone, Norethisterone acetate, Norgestrel, Levonorgestrel, Drospirenone and Desogestrel etc. One of each group acts as combined oral contraceptives (COC). Ethinylestradiol/ Levonorgestrel oral contraceptive is one of the most renowned COC combination used.

There are various analytical methods cited in the literature used for the quantitative determination for simultaneous determination of both active from COCs dose. Examples include isotope dilution tandem mass spectroscopy [4], capillary electrophoresis [6,7], high performance liquid chromatography [8-10], ultra-performance liquid chromatographic (UPLC) [11], solid-phase extraction coupled with high-performance liquid chromatography–tandem mass spectrometry [12,13], spectrophotometry [14], ultra-high performance liquid chromatography coupled with tandem mass spectrometry [15,16], gas chromatography coupled with mass spectroscopy [13]. However some of those methods suffer from disadvantages such as a complicated and time consuming sample preparation procedures and quantification procedure. In this study a high performance liquid chromatography has been used for both Levonorgestrel and Ethinylestradiol estimation. However none of the method discussed about dissolution profile of Levonorgestrel/ethinylestradiol combined drug. In this study dissolution profile has been studied and also establish the best dissolution profile and method for simultaneous determination of Levonorgestrel and Ethinylestradiol according to USP, FDA guideline [17,18].

Materials and Method

Materials

Levonorgestrel and Ethinylestradiol chemical reference standard was purchased from the Excella GmbH & Co (Germany). Chromafil® Xtra PTFE 0.45 µm syringe filters were purchased from the Pall Corporation (Ann Arbor, MI, USA). Microgynon 30 Tablet was purchased from Bayer Pharma Germany as reference tablet for the determination of f_1 and f_2 value of test tablet. HPLC grade acetonitrile,

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Sodium acetate trihydrate and glacial acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA), Potassium Phosphate Monobasic and hydrochloric acid was purchased from Merck. HPLC ready deionized 18Milli-Q water was obtained, in-house, from a Milli-Q Gradient A-10 water purification system, Millipore, (Bedford, MA, USA).

Instrumentation and chromatographic conditions

For the analysis of dissolution sample HPLC (Shimadzu Corporation, Japan) with system controller consisted of a quaternary pump, an automatic injector, variable wavelength detector with UV detector, and a column oven was used. Active pharmaceutical ingredients were separated by Kromasil C_8 column (4.6 mm × 150 mm, 5 µm) and ProntoSIL C_8 column (4.6 mm × 150 mm, 5 µm) was used for intermediate precision analysis.

Filtered and degassed acetonitrile and deionized water in the ratio of 60:40 was used as mobile phase. The flow rate was set 1.0 ml/min for 10 min. The column temperature was controlled at 30°C and the injection volume was 100 µL. The detection wavelength was 247 nm for Levonorgestrel analysis and a spectrofluorometric detector with an excitation wavelength of 285 nm and emission wavelength of 310 nm for Ethinylestradiol.

Dissolution

Dissolution studies were carried out in a calibrated dissolution apparatus, a USP type II instrument at 50 rpm and 37 ± 0.5°C [17,18]. One single Levonorgestrel/Ethinylestradiol combined oral contraceptive placed in per vessel for dissolution test. Dissolution were performed by using four different dissolution media and sample solution were collected from each vessel using Chromafil® Xtra PTFE 0.45 µm syringe filters after 5, 10, 15, 20, 30 45 and 60 min replacing the dissolution media with fresh buffer. The amount of Levonorgestrel and Ethinylestradiol in test samples was calculated quantitatively and percent dissolution, from the measured peak area response for the test samples (A_U) and compared to the peak area response (A_S) for the standard Levonorgestrel and Ethinylestradiol by using following formula

$$\text{Quantity} = \frac{\text{Au}}{\text{As}} \times \text{C} \quad (1)$$

$$\%\ \text{Dissolution} = \frac{\text{Amount Recovery}}{\text{Amount Decleared}} \times 100 \quad (2)$$

Here, C in the concentration in ppm.

Dissolution media

Acetate buffer (pH 4.5): Acetate buffer was prepared by dissolving 2.99 gm of sodium acetate trihydrate in 700 ml Milli-Q water, add 14 ml 2M glacial acetic acid then volume 1 liter with Milli-Q water. Adjust pH to 4.5 with dilute acetic acid.

HCl (pH 1.2): 5.18 ml of concentrated HCl (37%) in 950 ml of water and mix the acidic solution properly. Adjust pH to the 1.2 by using dilute HCl and volume 1000 ml with water. This freshly prepared solution has been used as buffer media solution.

Phosphate buffer (pH 6.8): Phosphate buffer was prepared by dissolving 27.22 gm of Potassium Phosphate Monobasic in 1000 ml water. 50 ml of above solution and 22.4 ml of 0.2 M sodium hydroxide solution were taken in to a 200 ml volumetric flask. Adjust pH 6.8 with additional amount of dilute sodium hydroxide and volume to the mark with water.

Water: Fresh Milli-Q water has been used as dissolution media.

Data analysis

The dissolution profiles were constructed by plotting % dissolved Levonorgestrel and Ethinylestradiol VS time and was compared using a model independent approach which is described by the USP and FDA. It uses a difference factor (f_1) and a similarity factor (f_2) to compare dissolution profiles [19]. Difference factor calculates the percent difference between the two profiles (reference and test product) at each time point and is a measure of the relative error between the two curves (Eq-3) [20]. On the other hand similarity factor is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves (Eq-4) [20]. Generally, f_1 value up to 15 (0-15) and f_2 value greater than 50 (50-100) ensure the sameness or equivalence of the two curves of the performance and of the reference products.

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100 \quad (3)$$

$$f_2 = 50 \times \log\left[\frac{1}{\sqrt{1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2}} \times 100\right] \quad (4)$$

Where, R_t, T_t represents % dissolution value of reference product (Microgynon 30 tablet) and test product (Levonorgestrel/ethinylestradiol tablet) at time respectively, n represents number of sampling time point.

Results and Discussion

Buffer selection

According to FDA guideline for low solubility/high permeability drug, multiple media is recommended. Levonorgestrel/Ethinylestradiol COC drugs are in this category and have to select one of the suitable media that is done in this present study [21]. The dissolution profiles of Levonorgestrel/Ethinylestradiol COC tablet in different media are shown in Figure 1 and all dissolution data at different time point are recorded in Table 1. Both of them shows that for Levonorgestrel although initial dissolution rate was high comparatively than other media in HCl but after 60 min dissolution rate were close to other media and percentile dissolution of Levonorgestrel after 60 min in acetate, HCl, water and phosphate media were 98, 95, 97 and 98 respectively. On the other hand for Ethinyestradiol although initial dissolution rate was high in HCl media but after 60 min dissolution rate was found very low compared to the other media only 82% of Ethinylestradiol was dissoluble in HCl media that is comparatively lower than other, which is due to Ethinylestradiol is very degradable active and may be degrade in HCl. FDA guideline for low soluble/high permeable drugs after complete dissolution time period dissolution of each active must exceed 85% for suitability a dissolution method for those active [21]. Due to lower dissolution rate of Ethinylestradiol in HCl media, this media should be rejected as media for Levonorgestrel/Ethinylestradiol COC drug. On the other side percentile dissolution of both active exceed 85% that is the main requirement for the selection of media and may conclude all three can be used as dissolution media for this COC drug.

For the selection of best media f_1 and f_2 values should be calculated. According to FDA guideline f_1 and f_2 values should be within 0-15 and

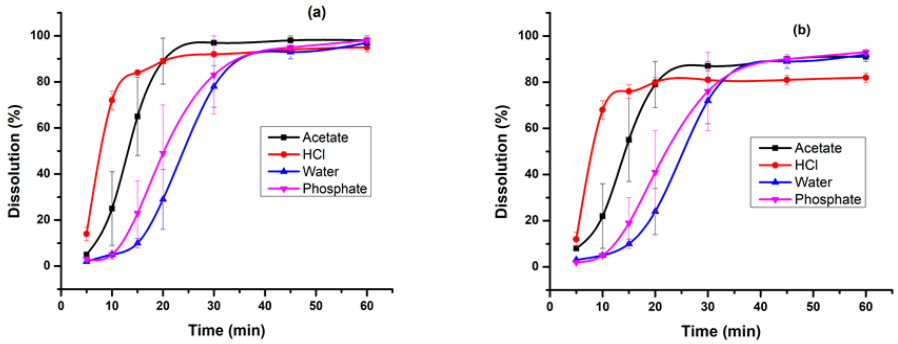

Figure 1: Disssolution profile for a) Levonorgestrel b) Ethinylestradiol.

Time point	% Dissolution of Levonorgestrel				% Dissolution of Ethinylestradiol			
	Acetate*	HCl*	Water*	Phosphate*	Acetate*	HCl*	Water*	Phosphate*
5	5 ± 0	14 ± 3	2 ± 0	3 ± 1	8 ± 0	12 ± 3	3 ± 0	2 ± 0
10	25 ± 16	72 ± 4	5 ± 1	5 ± 2	22 ± 14	68 ± 4	5 ± 1	5 ± 1
15	65 ± 17	84 ± 1	10 ± 2	23 ± 14	55 ± 18	76 ± 3	10 ± 2	19 ± 11
20	89 ± 10	89 ± 1	29 ± 13	49 ± 21	79 ± 10	80 ± 2	24 ± 10	41 ± 18
30	97 ± 1	92 ± 1	78 ± 9	83 ± 17	87 ± 2	81 ± 1	72 ± 10	76 ± 17
45	98 ± 2	94 ± 1	93 ± 3	95 ± 1	90 ± 2	81 ± 2	89 ± 3	90 ± 2
60	98 ± 2	95 ± 2	97 ± 2	98 ± 1	91 ± 2	82 ± 2	92 ± 2	93 ± 1

*12 replicate sample set of each point has been analyzed

Table 1: Dissolution of Levonorgestrel and Ethinylestradiol at various time point at four media.

50-100 respectively for selected media [17]. Table 2 shows f_1 and f_2 values of those four different media compared to Microgynon 30 mg Tablet (reference). It is clear that there are very little similarity between Microgynon and test sample in Water (f_1 =34.5, 37.7; f_2 = 28.0, 27.5) and Phosphate (f_1 =31.7, 32.7; f_2 = 32.0, 32.0) media. But on the other side in Acetate and HCl media difference (f_1) and similarity (f_2) factor are within permeable limit. But according to percentile dissolution HCl media should be rejected. For that reason the only optimum suitable media for simultaneously determine those active from COC tablet drug is acetate buffer.

Media	Levonorgestrel		Ethinylestradiol	
	Difference factor f1	Similarity Factor f2	Difference factor f1	Similarity Factor f2
Acetate	9	56.8	10.1	58.8
HCl	8.4	59.1	5.3	66.2
Water	34.3	28	37.7	27.5
Phosphate	31.7	32	32.7	32

*12 replicate sample set of each point has been analyzed

Table 2: Difference factor (f1) and Similarity factor (f2) for test sample using Microgynon 30 tablet as a reference product in different media.

Method validation of acetate buffer

Method was validated according to the International Conference on Harmonization (ICH), US Food and Drug Administration Bioanalytical method validation guidance and United States Pharmacopeia Category I requirements [22-24]. The following validation characteristics were addressed: specificity, accuracy, precision, linearity and range.

System suitability: System suitability solution was prepared daily basis from stock solution same concentration as both active present in dissolution sample. System suitability was determined from five replicate injections of the system suitability solution before sample analysis. The acceptance criteria were less than 2% relative standard deviation (RSD) for peak area, greater than 2000 theoretical plates, USP tailing factor less than 2. All critical parameters were tested before sample run and it was found that all parameter met the acceptable criteria throughout all days which are shown in the Table 3.

Specificity: For the development of chromatographic method, it should have the ability to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in the sample mixture contain analyte itself and all potential sample components (placebo, degradation products, process impurities and dissolution media response) and compare with the response of analyte. The acceptance criteria were peaks of active should be pure that means diluent and placebo does not show any interfere at the retention time of active components. It was found from the chromatogram that there were no interference at 4.9 min retention time of Levonorgestrel at 247 nm whereas diluent peak were found at 3.4 and placebo peak was at 3.1 min. Etinylestradiol peak was detected at 3.8 min at 310 nm which is completely segregated from diluent peaks 4.4, 7.3 and 7.8 min and placebo peak at 5.6 min.

Precision and intermediate precision

Precision is the measure of the degree of repeatability of an analytical method under normal condition and intermediate precision is the same as precision only the variation of some parameter such as column brand, different day, different HPLC, different person and so on. Both of them are normally expressed as the percent relative standard deviation (RSD). System precision and intermediate precision were determined for Levonorgestrel and Ethinylestradiol by analyzing the stock solution at concentration of Levonorgestre l 0.3 ppm and Ethinylestradiol at 0.06 ppm. The method precision and intermediate precision were established according to ICH/USP by six injections of the standard drug samples containing 0.3 ppm of Levonorgestrel and

0.06 ppm Ethinylestradiol. Precision and intermediate precision were expressed as a relative standard deviation (RSD %) of the analyte peak and absolute difference of average result of precision and intermediate precision. Results for precision and intermediate precision were summarized in Table 4.

Accuracy: Accuracy expresses the closeness of agreement between the measured value and the value that is accepted as either a true value or a reference value [25,26]. Accuracy of the method was determined by analyzing three different concentrations of Levonorgestrel (0.15, 0.30 and 0.45 ppm) and Ethinylestradiol (0.03, 0.06 and 0.09 ppm) that were prepared from stock solutions. According to USP guideline accuracy of dissolution samples should be within 95.0 to 105.0% [27]. Recovery from 98.5 to 99.6 % of Levonorgestrel and 98.9 to 99.9 % of Ethinylestradiol were obtained for the three concentration level which is summarized in the Table 5.

Linearity and range: Standard calibration curves were prepared with five calibrators over a concentration range from 0.15 to 0.45 ppm for Levonorgestrel and 0.03 to 0.09 ppm for Ethinylestradiol. Correlation between analyte peak area and concentration (ppm and percentage) of the samples was observed with $r^2 \geq 0.999$ for all days throughout the analysis which is summarized in the Table 6. Range was set from 50 to 150% of the active component present in the drug product which was 0.15 to 0.45 for Levonorgestrel and 0.03 to 0.09 for Ethinylestradiol.

Application of Acetate buffer in drug product

Acetate buffer was successfully applied for the evaluation of five different marketed Levonorgestrel / Ethinylestradiol 0.15 mg/0.03 mg drug products from different manufacturers in oral dosage form. Dissolution profiles of each products were performed from 5 to 60 min time period and total 6 batch from each manufacturer products were analyzed and summery of the results are shown in the Figures 2 and 3. All products maintained a dissolution rate of > 80% at the 60 min. However, Product B showed the maximum dissolution performances for Levonorgestrel and Product D for Ethinylestradiol. On the other side, Product E showed minimum dissolution for both Levonorgestrel and Ethinylestradiol. An independent t-test with equal variances for maximum and minimum release at 45 min were analyzed and it is found that for Levonorgestrel and Ethinylestradiol at a 95% confidence level p values are 0.00000015 and 0.000000071, respectively which means to fail to reject H_0 and there is no significant differences between maximum and minimum values. A one way ANOVA test also conducted for 5 drug products. It was also found that p values were 1.5×10^{-9} and 6.5×10^{-12} for Levonorgestrel and Ethinylestradiol,

Parameter	Specifications	Day 01		Day 02		Day 03	
		Lev	EE	Lev	EE	Lev	EE
Retention Time (% RSD)	≤ 2.0	0.04	0.04	0.13	0.08	0.07	0.03
Area (% RSD)	≤ 2.0	0.24	0.42	0.53	0.76	0.15	0.49
Tailing Factor	≤ 2.0	1.17	1.2	0.96	0.98	1.15	1.18
Theoretical plates	≥ 2000	6374 ± 64	5694 ± 44	6853 ± 76	6287 ± 34	6084 ± 55	5538 ± 23

n: number of replicates per concentration levels and per series

Table 3: System Suitability Test Results (n=5).

Parameter	Specifications	Observed results			
		Precision		Intermediate precision	
		Lev	EE	Lev	EE
Area of Sample	-	82016	2582988	83716	2589381
Amount Recovered	95-100%	99.67	101	100.2	99.9
Recovery (% RSD)	≤ 5.0	1.73	2.69	1.68	2.21
Area (% RSD)	≤ 5.0	1.82	2.82	1.77	2.21

n: number of replicates per concentration levels and per series

Table 4: Precision and intermediate precision Results (n=6).

Parameter	Specifications	Levonorgestrel			Ethinylestrediol		
		0.15 ppm	0.3 ppm	0.45 ppm	0.03 ppm	0.06 ppm	0.09 ppm
Recovery (%)	95-105	98.5	99.6	98.8	98.9	99.9	99.4
Retention Time (% RSD)	≤ 2.0	0.11	0.11	0.07	0.1	0.05	0.07
Area (% RSD)	≤ 5.0	0.8	0.86	0.42	0.76	0.65	0.81

n: number of replicates per concentration levels and per series

Table 5: Accuracy Results (n=6)

Standard Curve	Levonorgestrel				Ethinylestradiol			
	Analytical Range (ppm)	Slope	y-intercept	r^2 value	Analytical Range (ppm)	Slope	y-intercept	r^2 value
Validation day 1	0.15-0.45	277705	336	0.9997	0.03-0.9	4.26×10^7	10875	0.9997
Validation day 2	0.15-0.45	277065	1557	0.9996	0.03-0.9	4.61×10^7	-92093	0.9998
Validation day 3	0.15-0.45	277705	4436	0.9997	0.03-0.9	4.26×10^7	112053	0.9997

m: number of concentration levels or calibrator;
n: number of replicates per concentration levels and per series

Table 6: Linearity results (m=5; n=3).

respectively at 95% confidence interval which also confirmed that there is no significant different between all 5 drug product after 60 min (Figure 4 and 5) However, all the drug products meet the FDA specification at 60 min time, which Q >80% [17].

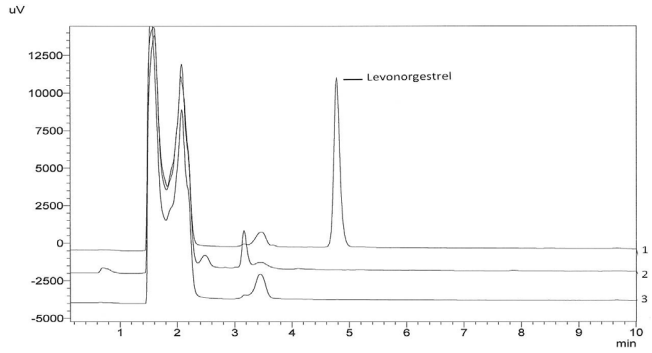

Figure 2: Peak purity of Levonorgestrel: 1) Standard Sample, 2) Placebo and 3) Diluent.

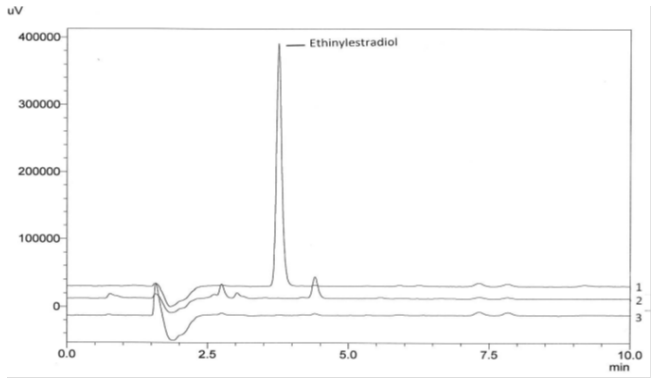

Figure 3: Peak purity of Ethinylestradiol: 1) Standard Sample, 2) Placebo and 3) Diluent.

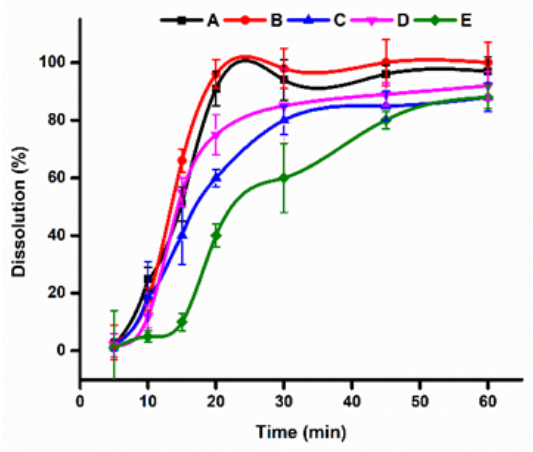

Figure 4: Dissolution profile of Levonorgestrel from 5 to 60 min.

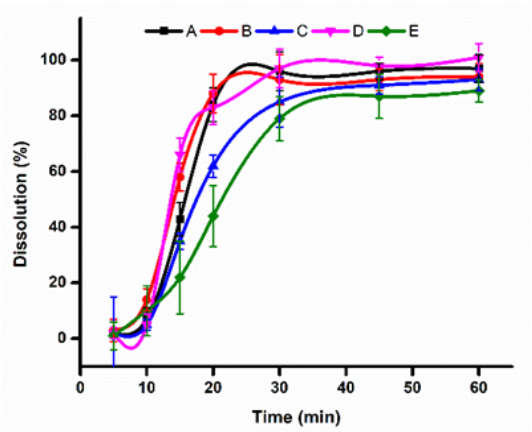

Figure 5: Dissolution profile of Ethinylestradiol from 5 to 60 min.

Conclusion

Dissolution is one of the most important characteristics of a drug directly effect on the drug absorption and bioavailability. It is very difficult to quantify Levonorgestrel and Ethinylestradiol simultaneously by using a single media. In this study a HPLC method has been used for the quantification of drug API and four different media has been used to determine best one. Dissolution profile, Difference and similarity factor suggested that acetate buffer media is the best option in this case. An analytical method for acetate media is validated according to FDA, ICH and USP requirement. The usefulness of this media (acetate) is also successfully demonstrated for the determination of both active from Levonorgestrel/Ethinylestradiol oral tablet that was collected from five different manufacturing companies.

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