SIMULTANEOUS DETERMINATION OF KETOROLAC TROMETHAMINE AND OLOPATADINE-HCl IN PURE FORM, MARKETED TABLETS AND NEW FORMULATED FAST DISSOLVING FILMS

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ARTICLE INFO	ABSTRACT
	New formulated fast dissolving films were prepared from anti-inflammatory ketorolac tromethamine and sparingly water-soluble antihistamine olopatadine-HCl. Olopatadine-HCl solubility was increased by complexation with Kleptose. Two film forming polymers used were HPMC E5 and kollicoate IR with glycerol as a plasticizer and kollidone as a superdisintegrants. D-optimal design was applied to study the effect of independent variables on the chosen responses. Consequently, the two drugs in their pure forms, pharmaceutical formulations and the new formulated fast dissolving films were analytically evaluated using three techniques. The first was UPLC in which separation was achieved on a C18 column using 0.1% O-phosphoric acid - acetonitrile (60:40 v/v) as mobile phase. The second was HPTLC where separation was performed on silica gel 60 F254 plates, with chloroform- methanol-2.5% ammonia (1:9:0.05 by volume) as a developing system and UV detection at 260nm. The third was UV - spectrophotometry for determination of both drugs without separation. It includes direct determination of ketorolac tromethamine at 325nm and measuring absorbance of olopatadine-HCl at 266 nm by isoabsorptive point. Both drugs were also measured by dual wavelength between 309 - 286.4 and 241 - 255 nm, respectively. While olopatadine-HCl was assayed by derivative ratio method at 271 nm and ratio difference between 262 and 280 nm.

INTRODUCTION

Ketorolac-tromethamine (KTR-T); (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid with 2-amino-2-(hydroxymethyl)-1,3-propanediolis a NSAID while Olopatadine hydrochloride (OPD-HCl); Z)-11-[3-(Dimethylamino) propylidene]-6, 11-dihydrodibenz [b, e] oxepin-2-acetic acid Hydrochloride} is anti-histaminic drug[1]. A number of HPLC[2-10], HPTLC[11-14], LC/MS[15,16], UV-Vis spectrophotometric[17-21], fluorometric[22] and voltametric[23-26] methods were reported for the quantification of Ketorolac tromethamine and Olopatadine HCl alone and in combination with other drugs. Meanwhile, few HPLC and UV-spectrophotometric methods [27-29] were reported for the simultaneous determination of both drugs in combination. OPD-HCl is sparingly water-soluble in complexation with cyclodextrins as Kleptose was adapted to

improve its solubility and hence its bioavailability. KTR-T administration rate is frequent as it has short plasma half-life 3-6 h. Combination of both drugs were suggested to have more potent effect for treating of allergy and inflammation compared to single drug. Fast dissolving films dissolve in the salivary fluids of the oral cavity within a minute, releasing the active drug provides a direct entry of such agents into the systemic circulation, thereby avoiding the first-pass hepatic metabolism [30]. Thus, The aim of the present work is to prepare combined fast dissolving films of Ketorolac Tromethamine (KTR-T) and Olopatadine hydrochloride (OPD-HCl) to be used in condition such as sudden episodes of allergic attacks and to analyze the two drugs simultaneously by UPLC, HPTLC and UV-spectrophotometric methods.

2.1 MATERIALS AND METHOD:

Ketorolac, was kindly supplied by Amriya Pharmaceutical Co (Egypt), its purity was 99.77% as stated by the supplier. Olopatadine HCl, was kindly supplied by Egyptian International Pharmaceutical Co. (EIPICO, Egypt), its purity was 99.84% as stated by the supplier. Ketolac® tablets, B.N. (4053015), labelled to contain 10 mg Ketorolac-tromethamine per tablet (products of Amriya Pharmaceutical Co, Egypt) and Patadine® tablets, B.N. (P064532), labelled to contain 5 mg Olopatadine HCl (products of Ajanta Pharmaceutical Company, India). Hydroxypropylmethyl cellulose (HPMC E5), Kollidon and glycerol were kindly provided from Egyptian International Pharmaceutical Co. (EIPICO,Egypt). Kleptose was provided from Roquette Pharmaceuticals Inc., (France) and Kollicoat IR was from BASF (Germany). Methanol, chloroform and O-Phosphoric acid were obtained from Sigma -Aldrich (Germany) and acetonitrile HPLC grade was obtained from Fisher (UK).

2.2. Instrumentation

- Shimadzu UV/Vis spectrophotometer (PC – 1601, Tokyo, Japan). Digital dissolution test apparatus, six tests USP standards, (Model DA-6D, Bombay-India). Differential scanning calorimeter,(model Mettler DSC60, Switzerland). Pye Unicam SP 1000 IR Spectrophotometer (type pw3710, Holland). Electric ovens (Heraeus, Ut 5060 E, Germany). Jenco pH meter (608) with jenway double junction glass electrode (England). Magnetic stirrer, Thermolyne Corporation, Dubuque Lowa, (U.S.A). The UPLC system used was an Agilent 1100 UPLC with binary pump and UV detector, analysis was performed on a Kinetex C 18 column (100 mm, 4.6 mm i.d., 2.6 μm) ; USA. - TLC plates used were 20 cm x 20 cm precoated with silicagel 60 F 254 (Flukachemie, Switzerland), a camag Linomate 5 sample applicator equipped with a 100 μL syringe (Hamilton,Germany) 20cm x 10 cm twin through glass chamber (Camag). The plates were scanned with a camag TLC scanner 3 with WINCATS computer software (Switzerland) using UV lamp with short wavelength (254 nm) (Desega-Germany).

2.3. Methods

2.3.1. Preparation of Olopatadine - Kleptose inclusion complex and its dissolution study: Inclusion complex with kleptose was prepared by co-grinding method. Where OPD-HCl and Klebtose were mixed in three geometric ratios (1:1, 1:2 and 1:3) and triturated in glass mortar pestle for 20 minutes and passed through 80 mesh screen. The dissolution 5 mg of each inclusion complex was studied using a dissolution apparatus with paddles rotating at 75 rpm. The dissolution was performed in 300 mL of phosphate buffer (pH 6.8) at 37+ 0.5ºC. At fixed time intervals, samples were withdrawn, filtered, and spectrophotometrically assayed for drug content by measuring absorbance at 298 nm.

2.3.2. Experimental design: A 12 run three factor, D-optimal design was

employed to construct polynomial models for the optimization process using Design Expert software (Version 9, State-Ease Inc., Minneapolis, MN, USA.). D-optimal design was employed to study the effect of independent variables (X_1) concentration of film forming agent (FFA), (X_2) concentration of disintegrant and (X_3) type of FFA over the dependent variables like disintegration time (sec), and *in vitro* drug release (%) as shown in design layout **Tables (1)**. In this design, three factors were evaluated each at three levels (−1, 0, +1) and all possible fourteen experimental batches were formulated. Composition of all fourteen possible combinations of orally dissolving film of KTR-T and OPD-HCl using D-optimal designs is shown in **Table (2)**.

2.3.3. Preparation of Orally Dissolving Films of ketorolac tromethamine and olopatadine-HCl Using D-optimal design:

Fast dissolving oral films were prepared by solvent casting technique. The formulations were prepared as per **Table (2)**. The hydrophilic polymers namely Hydroxy propyl methyl cellulose (HPMC E5) and Kollicoat IR were accurately weighed and dissolved in 10 mL distilled water and 0.1 mL glycerol was added as a plasticizer. Kollidone was added as superdisintegrant. Then OPD-HCl - Kleptose complex in ratio (1:1) and KTR-T were added to the polymeric dispersion under constant stirring with a magnetic stirrer for 15 minutes. The resultant homogeneous solution was poured into a petridish. Then the films were dried in an oven at 50ºC for 24 h. The films were then carefully removed and cut into strips of dimension 3×3 cm, and the dried films were wrapped in a butter paper, covered with an aluminum foil and stored in an air tight glass container for further investigations [31].

2.3.4. Evaluation of fast dissolving films

- Surface pH - The film was placed in a Petri dish and was moistened with 0.5 ml of distilled water and kept for 30 sec. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min. The average of three determinations for each formulation was done.

- Folding endurance - A small strip of the film was repeatedly folded and the number of folds till breaking the film was recorded. The experiment was performed in triplicate and the mean was calculated.

- Thickness - The film was measured by using screw gauge with a least count of 0.01 mm at different places on the film. The thickness of the film was measured at three different places and the average was measured.

- Content uniformity - The absorbance values were measured at a wavelength of 325nm for KTR-T content and 266nm for OPD-HCl using by UV spectrophotometric isoabsorptive method.

- *In vitro* disintegration time - Film was placed in a glass Petri dish (6.5 cm in diameter) containing 25 ml of distilled water at 37ºC, with swirling every 10 seconds. The time at which the film starts to break or disintegrates was recorded.

- Mouth dissolving time - Film was placed into a beaker containing 50 mL of phosphate buffer pH 6.8. Time required by the film to dissolve was recorded.

2.3.5. *In vitro* dissolution study:

The dissolution was performed by using a dissolution apparatus with paddles rotating at 75 rpm. Film was placed in a 300 ml of buffer solution (pH 6.8) at 37 ± 0.5 °C. At fixed time intervals, samples were withdrawn, filtered, and

spectrophotometrically assayed for KTR-T content at 325 nm and for OPD-HCl using is absorptive method at 266nm. Percent drug released after 15 minutes was determined.

2.3.6. Analytical evaluation of studied drugs

Preparation of stock solutions

100 mg of each of KTR-T and OPD-HCl were transferred into separate 100- ml volumetric flasks to which about 70 ml of methanol was added. The flasks were sonicated for 5 minutes then made up to the mark with the same solvent to obtain stock solutions of one mg/mL of each drug. Working solutions were freshly prepared by suitable dilution of stock solution with methanol to obtain a concentration of 0.1 mg/mL from each drug.

2.3.6.1. Chromatographic conditions

UPLC method-Separation was performed on a Kinetex C 18 column (100 mm, 4.6 mm i.d., 2.6 μm) ; USA, using a mobile phase of 0.1% O-phosphoric acid; acetonitrile 60:40 % (v/v) at a flow rate of 1 mL/ min. Wavelength of 266 nm was selected for detection.

HPTLC method-Analysis was performed on TLC plates. Before use, plates were washed with the mobile phase consisting of cholofrom-methanol-2.5% ammonia (1:9:0.05 by volume) were applied to pre-washed activated plates, as 6-mm bands, 6 mm apart, by means of a Camag Linomat IV automated spray-on band applicator equipped with a 100-μL syringe. The plates were developed with 50 mL of the mobile phase, in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were removed and air dried. Densitometry was performed at 266 nm in reflectance mode. The slit dimensions were 6.00 mm × 0.3 mm and the scanning speed was 20 mm/s.

2.3.6.2. Linearity

UPLC method- Aliquots of working standard drug solutions (0.1 mg /ml) containing 0.01-0.1mg of either drugs were introduced into two separate series of 10- ml volumetric flasks and adjusted to the volume with methanol. Triplicate 10μL injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The Peak area of each concentration was then plotted against the corresponding drug concentration.

HPTLC method**-**Different volumes of stock standard solution (1 mg/ml) containing 0.5-8.0 mg KTR-T or 1.0-8.5 mg OPD-HCl were introduced into a series of 10- mL volumetric flasks, then volume was completed with methanol. 10μLof each flask was applied to the TLC plates, in which separation was performed as previously stated. Peak area of each concentration was then plotted against its corresponding drug concentration.

Spectrophotometric method

Spectral characteristics: Aliquots equivalent to 0.05– 0.40 mg/mL KTR-T and 0.1-1.1 mg/ml OPD-HCl or 0.1-0.7 mg/ml OPD-HCl were accurately transferred from its working standard solution (0.1 mg/ml) into a series of 10 - mL volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer.

i. **Zero-Order and Isoabsorptive methods -** The zero order absorption spectra were recorded for both drugs using methanol as blank. The absorbance was measured at 325 nm for KTR-T and 266 nm (A_{iso}) for KTR-T and OPD-HCl. Two calibration graphs were constructed for each drug relating the absorbance at the selected wavelength to the corresponding drug concentrations and the regression equations were computed.

ii. **Dual Wavelength method –**From the stored data, the difference in

absorbance between 286.4 and 309) for KTR-T and between 241 and 255) for OPD-HCl were recorded. Calibration curves were constructed by plotting the obtained ΔA for each drug against its concentration.

iii. **Derivative ratio (1DR) method -** The stored spectra of OPD-HCl was divided by the spectrum of 10μg/mL KTR-T**.** Then the first derivative of the ratio spectra (1DR) at 271 nm was measured with scaling factor 1 is obtained and smoothed with Δλ = 8 nm. Then plotted against its corresponding concentration from which regression equation was computed.

iv. **Ratio difference (RD) method -** The above procedure detailed under (1DR) method was followed and after the division of the stored spectra of OPD-HCl by the spectrum of KTR-T (10 μg/ml), the amplitude difference between 262 nm and 280 nm for each spectra was plotted against its corresponding drug concentration and the regression equation was evaluated**.**

2.3.6.4. Application of the proposed methods on formulated fast dissolving films and market tablets
Ten films of the new fast dissolving film containing 10 mg KTR-T and 5 mg OPD-HCl each were cut to small pieces and introduced into a 100- ml volumetric flask, extracted with methanol, sonicated for 15 min, filtered and diluted to the volume with methanol to obtain solution labeled to contain 1 mg mL^{-1} of KTR-T and 0.5 mg mL^{-1} of OPD-HCl.
Ten tablets of each the Ketolac® and Patadine® tablet were weighed accurately and finely powdered. Powder equivalent to KTR-T or 50 mg OPD-HCL were dissolved in 30 mL methanol in two separate 100-mL volumetric flasks. Both

solutions were sonicated for 20 min and then diluted to 100 mL with the same solvent to obtain a solution containing 1 mg mL^{-1} of KTR-T or 0.5 mg mL^{-1} of OPD-HCl. Both films and tablets were analyzed using the proposed UPLC, HPTLC and spectrophotometric techniques.

3. RESULTS AND DISCUSSION

Orally fast dissolving films became a novel approach to oral drug delivery system as they provide convenience and ease of use over other dosage forms. This films dissolve in the salivary fluids of the oral cavity within a minute, releasing the active drug provides a direct entry of such agents into the systemic circulation, thereby avoiding the first-pass hepatic metabolism. This is due to the large surface area of the film that wets quickly upon contact with saliva[32]. Marketed orally dissolving films products have also become available including Listerine, Chloraseptic, Triaminic and multivitamins[33].The backbone of an orally dissolving film is generally formed of a plasticizer and film forming polymer that provide the necessary elasticity and shape to the film. Kollicoat IR, a polyvinyl alcohol – polyethylene glycol graft copolymer is a new pharmaceutical excipient that was specially developed as a coating polymer for instant release tablets. The molecule is hydrophilic and thus readily soluble in water[34]. Hydroxy propyl Methyl Cellulose (HPMC E5) is known for its good film forming properties and has excellent acceptability. HPMC polymer has a high glass transition temperature and is classified according to its viscosity which affects the solubility–temperature relationship [35].

3.1.Evaluation of Films
In vitro **dissolution studies of olopatadine- HCl - Kleptose inclusion complex:** It was found that 40.76% of plain drug was dissolved after 15 minutes while a complete drug release was obtained from the inclusion complex (ratio

1:1) after the same period. The increase in drug dissolution upon complexation with Kleptose may be due to the interactions between the hydrophobic part of the drug and the non-polar cavity of Kleptose that causes dehydration of the hydrophobic drug molecule and its transfer into the cavity, thereby increasing the affinity toward water and hence increasing the dissolution. The surfactant-like properties of Kleptose can be another reason to explain the higher dissolution rate of its complexes. The third reason is that Kleptose reduces the interfacial tension between particles of drug and the dissolution medium, leading to a greater rate of dissolution [36]. Other complex ratios showed higher drug release than plain drug but not more than those made by 1:1 ratio, Fig. (2).

Experimental design: D-optimal design was applied and the material attributes and response variables were studied and related to determine the effect of each factor on the determined responses using Design Expert-9 software. The polynomial equations obtained in terms of coded factors for this response was: Y1 (KTR-T released after 3 minutes) = 48.91 + 7.49 A + 21.91 B + 9.28 C + 1.18 AB + 18.82AC + 0.034 BC − 10.98 A2 − 2.41 B2. Y2 (OPD-HCl released after 3 minutes) = 36.82 + 3.39 A + 13.63 B + 5.35 C + 0.81 AB + 8.57AC + 1.48BC + 6.00 A2 − 1.23 B2. Based on the experimental design and factor combination, a quadratic model was found to be significant for percent drug released after 3 minutes with *F* value of 84.21 and 210.61 for KTR-T and OPD-HCl of *P* value < 0.0001 respectively, which implies that model and terms are significant. The model shows a nonsignificant lack of fit of value = 1.08 for Y1 and 33.86 for Y2 as shown in table (3). The predicted R-Squared" of 0.9082 and 0.9217for Y1 and Y2 respectively was found in reasonable agreement with the "Adjusted R-Squared" of 0.9765 and 0.9905 for Y1 and Y2 respectively.

Contour plots and response surface plots are shown in Fig. (3).

The polynomial equation obtained in terms of coded factors for this response was:
Y (disintegration through 28 sec) = 32.36 − 1.06 A − 12.00 B − 2.26 C − 0.75 AB − 1.42 AC + 2.25 BC − 0.41 A2 + 8.44 B2
This equation can be used to make predictions about the response for given levels of each factor. A quadratic model was found to be significant for disintegration through 28 sec with *F* value of 154.46 *P* value< 0.0001 which implies that model and terms are significant. The model shows a nonsignificant lack of fit of value = 4.66. The predicted R-Squared" of 0.9102 was found in reasonable agreement with the "Adjusted R Squared" of 0.9871. These values confirm that the equations of the models are highly reliable (Table 4). Contour plots and response surface plots are shown in Fig. (4) Which shows the effect of factors X1, X2 and X3 on the given responses (Y).

Differential scanning calorimetry (DSC)
Thermal behaviour of KTR-T, OPD-HCl, kleptose, OPD-HCl -Kleptose inclusion complex, kollicoat IR, HPMC E5, film that contain kollicoat IR and film that contain HPMC E5 were examined using thermal analyzer. The sample size was 5 mg and the temperature range was between 30 and 300ºC. Nitrogen was used as carrier gas and DSC analysis was performed at heating rate of 10ºC/min. DSC thermogram of KTR-T **Fig. (5a)** showed one sharp endothermic peak at 168.81°C. While that of OPD-HCl, the DSC thermogram **Fig. (5b)** showed one sharp endothermic peak at 258.23 °C. While that of Kleptose **Fig. (5c)** showed one broad endothermic peaks at 109.48 °C. The OPD-HCl -Kleptose inclusion complex **Fig. (5d)** showed reduction in intensity of the endothermic peak of OPD-HCl with disappearance of the Kleptose peak at 109.48 °C; indicating complexation of drug with Kleptose. While kollicoat **Fig. (5e)** showed one endothermic peaks at

213.06°C. DSC thermogram of HPMC E5 **Fig. (5f)** showed one broad endothermic peak at 95.36°C. DSC thermogram of film that contain kollicoat **Fig.(5g)** showed three endothermic peaks at 168.23°C, 213.06°C 258.42°C. while film that contain HPMC E5 **Fig. (5 h)** showed three endothermic peaks at 95.63°C, 168.44°C and 258.17°C.this result indicates that presence of characteristic peaks of both drugs and polymers this indicates that there was no drugs interaction between two drugs and polymer or film's excipients used.

Fourier transfer Infrared spectroscopy (FTIR)

Infrared spectra of KTR-T, OPD-HCl, kleptose, OPD-HCl -Kleptose inclusion complex, kollicoat IR, HPMC E5, film that contain kollicoat IR and film that contain HPMC E5 were recorded by KBr method using IR Spectrophotometer scanning was done from 750 -4000 cm^{-1}.

The FTIR spectra of KTR-T **Fig. (6a)** showed significant band at 3352, 3400-2400, 1600 and 1475 cm^{-1} which indicates the presence of hydroxyl, Amine (N-H stretching) and Aromatic ring (C=C stretching) respectively. The FTIR spectra of OPD-HCl **Fig. (6b)** showed significant bands at 3417, 3020, 2588, 1708, 1612, and 1242 cm^{-1} which indicates the presence of hydroxyl, aromatic C-H stretching, tertiary amine salt, carbonyl groups, phenyl nucleus skeletal stretching and aliphatic C-N stretching respectively. While The prominent peaks of Kleptose are at 3363.2 cm^{-1}, 2926 cm^{-1}and 1001 cm^{-1} corresponding to C-H stretching, C-H stretching, and OH bending, respectively **Fig.(6c)**. Other peaks appear are at 1647.2 cm^{-1} for C=O (amide I band), at 1417.6 cm^{-1} for CH2 deformation, at 937.4 cm^{-1} for CH$_2$ stretching and at 856.3 cm^{-1}for C-H bending. The IR spectra of the inclusion complex **Fig. (6d)** showed that peak for tertiary amine salt of the drug was decreased and also OH stretching at 3417cm^{-1} was disappeared indicating inter molecular hydrogen bonding between OLO and Kleptose. Decreasing of several guest signals can be considered as a confirmation of the formations of the inclusion complex. While The FTIR spectra of Kollicoat IR **Fig. (6e)** showed large band between 3700-3200 cm^{-1} for OH stretching of alcohol, 2904 cm^{-1} for C-H stretching of alkyl groups and1384 cm^{-1} for C-O ether. The IR spectra of HPMC E5 **Fig. (6f)** showed bands at 3421 cm^{-1} for OH stretching of alcohol, 2931 cm^{-1} for C-H stretching of alkan, 1620 cm^{-1}for C=Cand1384 cm^{-1} for C-O ether [37]. The FTIR spectra of the fast dissolving film that contain Kollicoat IR **Fig. (6g)** showed significant bands in the functional groups region at 3410, 2924, 1620 and 1384 cm^{-1} which are the same as the characteristic bands of Kollicoat IR; so there were no interaction between drugs and Kollicoat IR. While FTIR spectra of the fast dissolving film that contain HPMC E5 **Fig. (6h)** showed significant bands in the functional groups region at 3390, 2927, 1612 and 1381 cm^{-1} which are the same as the characteristic bands of HPMC E5; so there were no interaction between drugs and HPMC E5 [38].

Evaluation of fast dissolving films: The drug content values were between 97.88 to 100.56%. The folding endurance values of the prepared films were found to be in the range from 45 to 60. Thickness found to be in the range of 0.30 to 0.33mm. Mouth dissolving time of the films was from 36 to 62 seconds. The disintegration time of the films was from 28 to 55 seconds. Surface pH found to be in the range of 6.30 to 6.86. (Table 5).

In vitro **dissolution of the prepared fast dissolving films:** Increased drugs release was observed from the formulae containing FFA kollicoat IR and increasing its concentration lead to increase drug release. However those contain HPMC as its concentration increased drugs release decreased, which was observed in F3 and F12. This can be

explained on the bases that presence of kollicoat IR increases drug release due to wetting ability and conversion of crystalline form to amorphous one which increase the dissolution rate as the drug simply dissolved along with the polymer [39]. Also the polyvinyl alcohol moiety of kollicoat provides good film forming properties and the polyethylene glycol part acts as an internal plasticizer leading to excellent flexibility. In contrast to other film formulations, the plasticizer cannot migrate because it is covalently bound in the molecule and the increase level of HPMC E5 that results in formation of high viscosity gel layer due to more intimate contact between the particles of polymer resulting in decrease in the mobility of drug particles from the swollen matrix, which leads to a decrease in the release rate[40]. The formulation F5 showed a maximum percentage drug release of 100.11% in 4 min for KTR-T and 100.88% in 9 min for OPD-HCl, **Fig (7)**.

Development of design space: Figure (8) shows the overlay plot graph of the contour plots from each response laid on top of each other for a fast dissolving film giving maximum drug release and minimum disintegration time, the suggested formula (F standard) was 0.29 g kollicoat IR and 0.01 g kollidone.

Evaluation of the optimal formula F standard: F standard was also evaluated for surface pH, folding endurance, thickness, content uniformity, disintegration, mouth dissolving times and in vitro dissolution. F standard give highest release 100.09% and 100.55% after 4 min and 9 min for KTR-T and OPD-HCl; respectively with minimum disintegration time 27 second, Table (6) and Fig.(9). Then, this optimum formula was analytically evaluated.

3.1.**Analytical application: UPLC method** [41] –The chromatographic separation of KTR-T and OPD-HCl were optimized. Different mobile phases in different ratios were studied, where best peak shape and adequate separation of the two drugs was obtained by using 0.1% *o*-phosphoric acid – acetonitrile (60:40 v /v). Different flow rates and wavelengths were tried; good resolution with sensitive detector response was obtained at 260 nm using a flow rate of 1 ml min^{-1}. Under the described parameters, the peaks of the two drugs were well resolved at retention time of 1.542 and 3.131 for OPD-HCl and KTR-T, respectively, as shown in Fig.(10).

HPTLC method [41]**-**Different mobile phases in different ratios and at different λ_{max} for detection were tried. It was found that chloroform-methanol- 2.5% ammonia (1: 9: 0.1 by volume) as a developing system followed by densitometric determination at 260 nm offered best separation and resolution. Where R_f were approximately 0.23 and 0.8 for OPD-HCl and KTR-T, respectively, Fig.(11).

Spectrophotometric method

Zero-Order and Isoabsorptive methods -The zero-order absorption spectra (D^0) of KTR-T and OPD-HCl showed marked overlapping; Fig. (12). However it allows the analysis of KTR-T in presence of OPD-HCl at 325 nm where the later showed zero crossing absorbance. For OPD-HCl, isoabsorptive method [42] was employed for the determination of total concentration of OPD-HCl and KTR-T at λ_{iso} 266 nm and hence OPD-HCl concentration was calculated by subtraction of KTR-T concentrations. Applying the following equations:
At any λ, the absorbance can be calculated from Eqs 1 – 3.

Table (1): Experimental domains and coding of the variables.

Variables	levels		
	-1	**0**	**+1**
X1 (concenteration of FFA)	0.2	0.25	0.3
X2 (concenteration of disintegrant)	0	0.006	0.012
X3 type of FFA	Kollicoat IR		HPMC E5

Table (2): Composition of fast dissolving film of ketorolac tromethamine and olopatadine-HCL as obtained from D-optimal design.

	KTR-T	OPD-HCL - Kleptose complex	OPD-HCL	HPMC E5	Kollicoat IR	Kollidone	Glycerol (mL)
F1	10	1:1	-	0.3	-	0.012	0.1
F2	10	1:1	-	0.2	-	0.012	0.1
F3	10	1:1	-	0.3	-	0	0.1
F4	10	1:1	-	-	0.25	0.012	0.1
F5	10	1:1	-	-	0.3	0.006	0.1
F6	10	1:1	-	0.2	-	0	0.1
F7	10	1:1	-	-	0.2	0.006	0.1
F8	10	1:1	-	-	0.25	0	0.1
F9	10	1:1	-	0.25	-	0.006	0.1
F10	10	1:1	-	-	0.2	0	0.1
F11	10	1:1	-	-	0.2	0.012	0.1
F12	10	1:1	-	0.3	-	0.006	0.1
F_k	10	-	-	-	0.2	-	0.1
F_o	-	-	5	-	0.2	-	0.1

N.B. all amounts in grams

F_k KTR-T film without disintegrant.

F_o OPD-HCL film without complex and disintegrant.

Fig.1: Chemical structure of ketorolac tromethamine and olopatadine-HCL.
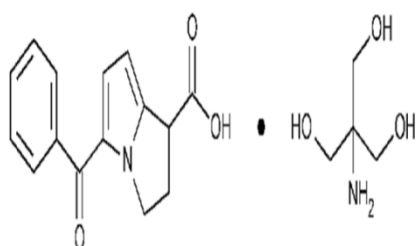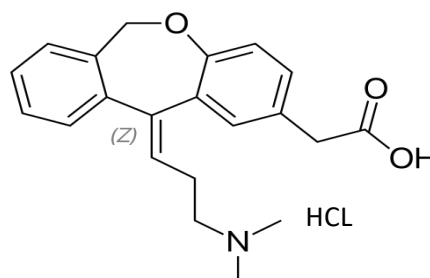
ketorolac tromethamine   **olopatadine-HCL**

Table (3): Analysis of variance for Y1 (KTR-T) and for Y2 (OPD-HCL) released after 3 minutes.

Source	Mean Square	F value	p-value Prob>F
Model (Y1)	1260.64	84.21	< 0.0001
A- Conc of FFA	500.21	33.41	0.0004
B- Conc of disintegrant	4388.76	293.16	< 0.0001
C- Type of FFA	1391.21	92.93	< 0.0001
AB	7.44	0.50	0.5008
AC	3336.62	222.88	< 0.0001
BC	0.011	7.217E-004	0.9792
A^2	485.39	32.42	0.0005
B^2	20.84	1.39	0.2719
Model (Y2)	402.21	210.61	< 0.0001
D- Conc of FFA	102.61	53.73	< 0.0001
E- Conc of disintegrant	1699.62	889.99	< 0.0001
F- Type of FFA	462.47	242.17	< 0.0001
AB	3.47	1.82	0.2148
AC	691.92	362.32	< 0.0001
BC	20.15	10.55	0.0117
A^2	144.80	75.82	< 0.0001
B^2	5.42	2.84	0.1304

Fig.2: Percent drug released from plain olopatadine-HCL and from different inclusion complexes with kleptose in different ratios.
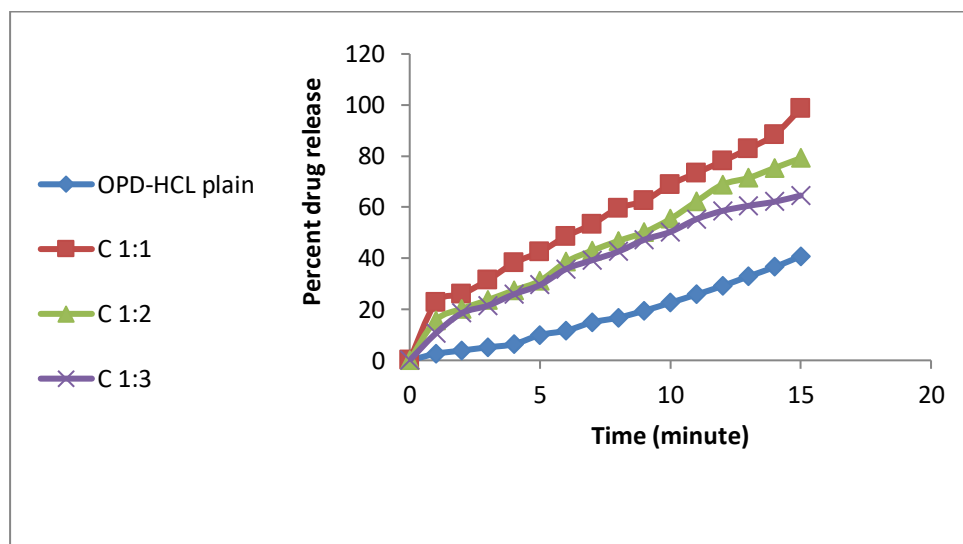
Table (4): Analysis of variance for Y (disintegration time) and Table (5): Evaluation of Ketorolac tromethamine KTR-T and OPD-HCL

Source	Mean Square	F value	p-value Prob>F
Model (Y)	219.80	154.46	< 0.0001
Conc of FFA	10.07	7.07	0.0288
Conc of disintegrant	1316.57	925.23	< 0.0001
Type of FFA	82.34	57.87	< 0.0001
AB	3.00	2.11	0.1846
AC	19.04	13.38	0.0064
BC	46.29	32.53	0.0005
A^2	0.66	0.46	0.5149
B^2	255.37	179.46	< 0.0001

	Drug content		Folding endurance	Film thickness (mm)	Mouth dissolving time (sec)	Disintegration time (sec)	Surface pH
	KTR-T	OPD-HCL					
	R%±SD						
F1	98.88±0.71	100.73±0.46	48±0.12	0.33±0.03	43±0.03	34±0.02	6.81±0.05
F2	98.91±0.51	99.14±0.58	44±0.51	0.31±0.02	36±0.04	29±0.01	6.58±0.08
F3	100.12±0.86	99.93±0.34	47±0.01	0.31±0.02	66±0.08	59±0.04	6.67±0.08
F4	98.98±0.45	99.03±0.77	58±0.11	0.30±0.01	40±0.05	29±0.01	6.74±0.04
F5	100.04±0.33	100.07±0.84	60±0.09	0.30±0.01	38±0.06	28±0.04	6.82±0.03
F6	99.22±0.77	98.94±0.65	45±0.12	0.33±0.03	62±0.04	55±0.02	6.63±0.09
F7	100.56±0.54	99.02±0.84	56±0.11	0.32±0.02	40±0.02	32±0.06	6.86±0.05
F8	100.83±0.73	101.02±0.22	57±0.01	0.32±0.02	55±0.05	47±0.03	6.71±0.03
F9	98.47±0.65	98.27±0.58	45±0.09	0.30±0.03	43±0.09	35±0.04	6.51±0.06
F10	97.88±0.23	98.15±0.84	58±0.12	0.30±0.01	46±0.09	36±0.09	6.79±0.04
F11	98.19±0.66	98.12±0.49	57±0.12	0.32±0.01	38±0.04	31±0.02	6.73±0.05
F12	98.22±0.69	100.12±0.72	49±0.01	0.32±0.02	57±0.03	48±0.06	6.62±0.05

Table (6): Evaluation of the optimal formula F standard

	F standard	
Drug content	KTR-T	OPD-HCL
	98.84	97.81
Folding endurance	62	
Film thickness(mm)	0.31	
Mouth dissolving time	39	
Disintegration time	27	
Surface pH	6.65	

Fig. (3): Response surface plots (contour and 3D) showing the effect of different independent variable on dissolution after 3 minutes (a and c: HPMC E5) and (b and d: Kollicoat IR) for ketorolac tromethamine and olopatadine-HCL, respectively.
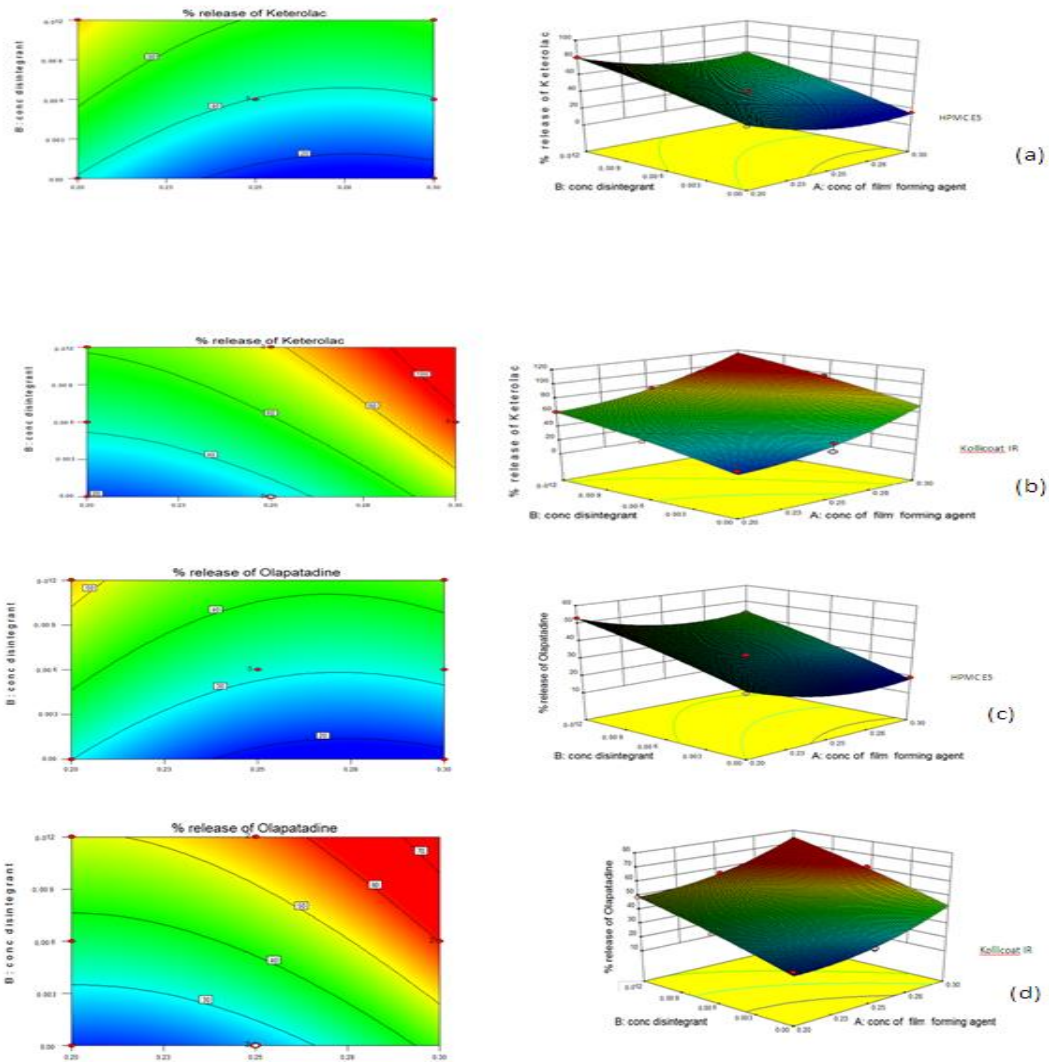
Fig. (4): Response surface plots (contour and 3D) showing the effect of different independent variable on Disintegration time. (a) HPMC E5 and (b) Kollicoat IR**.**
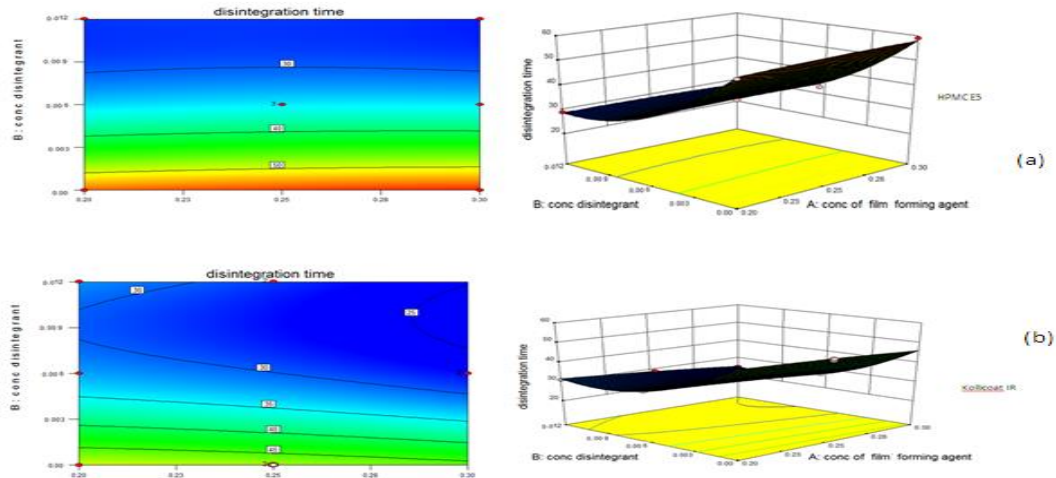

Fig.5: DSC of (a) ketorolac tromethamine, (b) olopatadine-HCl, (c) Kleptose, (d) olopatadine-HCL -kleptose inclusion complex, (e) Kollicoat, (f) HPMC E5, (g) Fast dissolving film contain kollicoat (h) Fast dissolving film contain HPMC E5
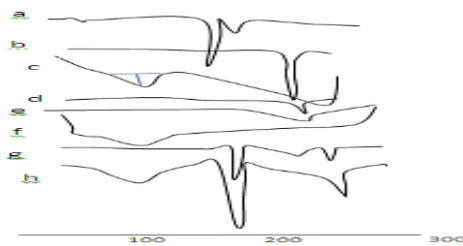
Fig 6: FT-IR spectra of(a) ketorolac tromethamine, (b) olopatadine-HCL, (c) Kleptose, (d) olopatadine-HCL -kleptose inclusion complex, (e) Kollicoat, (f) HPMC E5, (g) Fast dissolving film contain kollicoat (h) Fast dissolving film contain HPMC E5.
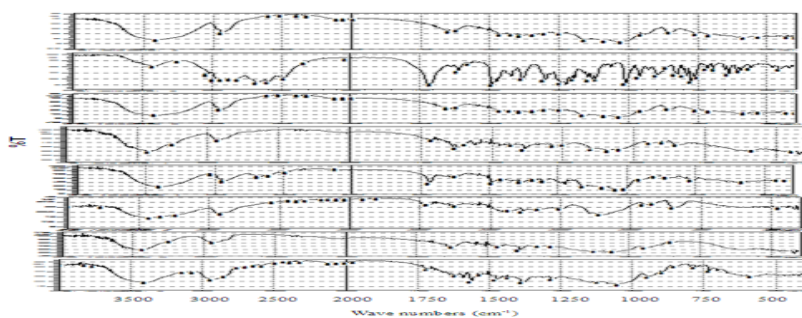
Table (7): System suitability results of the UPLC method

Parameter	KTR-T	OPD-HCL	Reference value
Number of theoretical plates (**N**)	8233	7791	The higher the value, the more efficient the column is
Resolution factor	15.66		>2
Capacity factor (K)	2.23	2.90	1–10
Selectivity factor	8.55		≥1

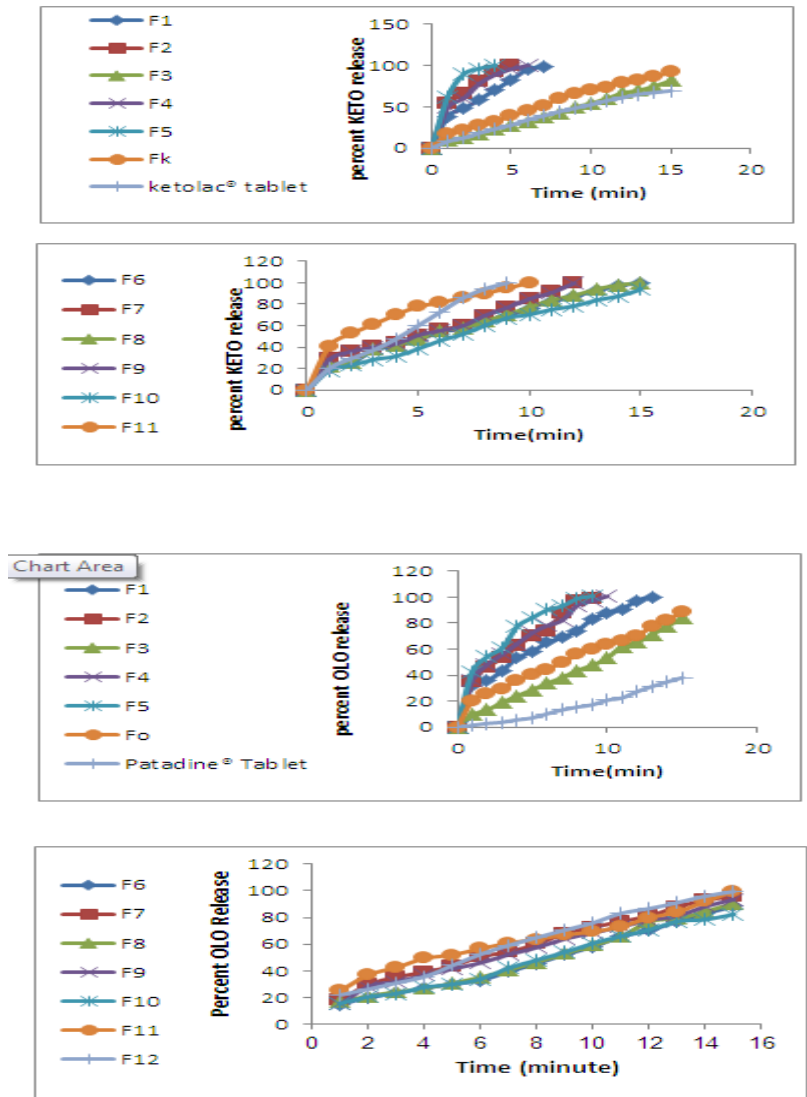**Fig.7:** Percent release of ketorolac tromethamine and olopatadine-HCl from fast dissolving films

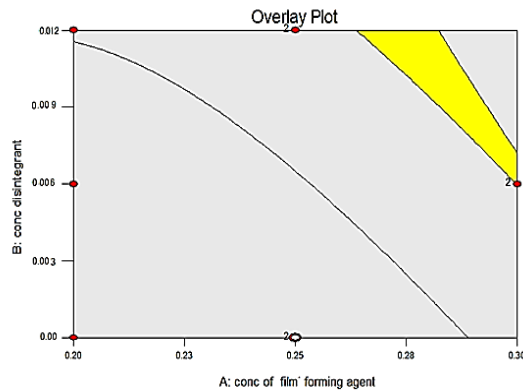**Fig. (8):** The overlay plot graph illustrating the suggested formula was 0.29 g kollicoat IR and 0.01 g kollidone.

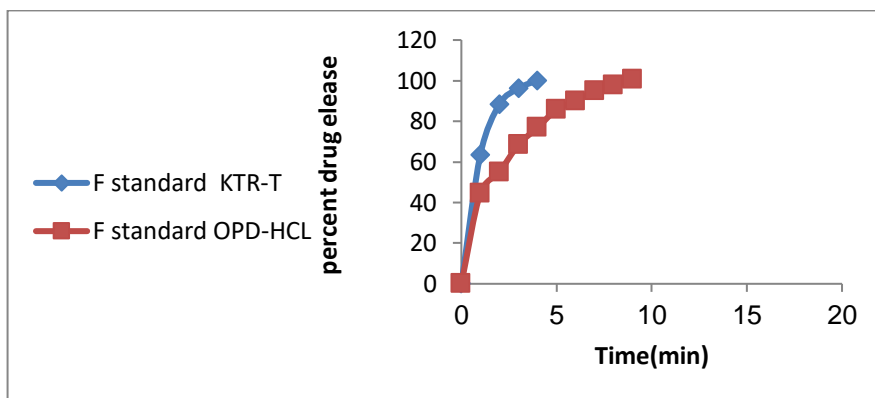**Fig.9:** Percent release of ketorolac tromethamine and olopatadine-HCl from Optimized formula F standard.

Table (8): Regression and validation parameters for the determination of ketorolac tromethamine and olopatadine-HCl by the UPLC and HPTLC methods.

	UPLC		HPTLC	
	KTR-T	OPD-HCL	KTR-T	OPD-HCL
$\lambda_{max\ (nm)}$	260nm			
Linearity range (µg mL^{-1})	1-10 µg/ mL		0.5-8 µg/ spot	1-8.5 µg/ spot
Regression parameters Slope (b) ± SD Intercept (a) ± SD Correlation coefficient (r^2)	14.5750±0.1151 -0.5880±0.1250 0.9998	7.1051±0.0517 -0.3639±0.3199 0.9998	2972.53 ±17.63 195.93 ±76.78 0.9998	874.02±5.47 590.49±25.66 0.9998
Accuracy (R %)	100.18	100.55	100.55	99.89
Precision (RSD %) Intra day Inter day (n=9)	0.58-1.16 0.81-1.21	0.17-0.69 0.33-0.97	0.36-1.01 0.46-0.68	0.20-1.66 0.48-1.95

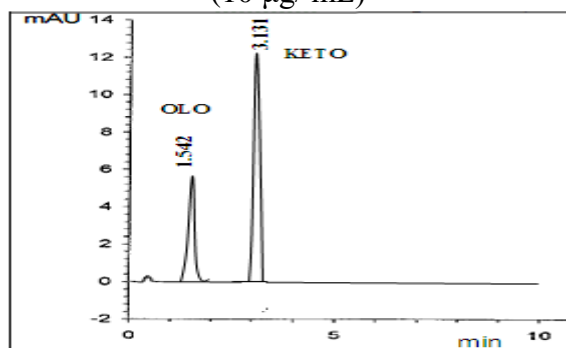**Fig. 10:** UPLC chormatogram of olopatadine-HCL (5 µg/ ml)and (b) ketorolac tromethamine (10 µg/ mL)

Table (9): Regression and validation parameters for the determination of ketorolac tromethamine and olopatadine-HCL by the UV- Spectrophotometric methods

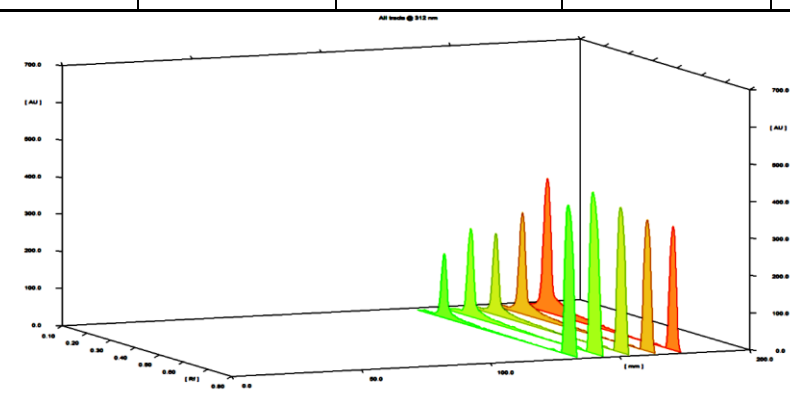|	KTR-T		OPD-HCL			
	Zero order method	DW method	Isoabsorptive method	DW method	1DR method	RD method
$\lambda_{max\,(nm)}$	325	286.4-309	266	241-255	271	262-280
Linearity range ($\mu g\,mL^{-1}$)	5-40 µg/ mL	5-40 µg/ mL	10-105 µg/ mL	10-70 µg/ mL	10-70 µg/ mL	10-70 µg/ mL
Regression parameters Slope (b) Intercept (a) Correlation coefficient (r^2)	0.0541 -0.0261 0.9992	0.0255 0.0129 0.9996	0.0081 0.0441 0.9998	0.0112 0.0213 0.9997	0.0079 0.0021 0.9993	0.1211 0.0229 0.9992
Accuracy (R %)	99.90	100.27	99.98	99.89	99.82	100.65
Precision (RSD %) Intra day Inter day (n=9)	0.60-1.40 0.65-1.91	0.39-1.57 1.11-1.62	0.62-1.89 0.95-1.89	0.20-1.66 0.48-1.95	0.80-1.92 1.27-1.93	0.07-0.38 0.11-0.71

Fig. 11: Densitogram of the lab-prepared mixtures of ketorolac tromethamine and olopatadine-HCl in different ratios

Table (10): Determination of ketorolac tromethamine and olopatadine- HCl in their laboratory prepared mixtures by the UPLC and HPTLC methods

Ratio KTR-T: OPD-HCL	UPLC method				HPTLC			
	KTR-T added (µg/mL)	OPD-HCL added (µg/mL)	% Recovery of KTR-T	% Recovery of OPD-HCL	KTR-T added (µg/mL)	OPD-HCL added (µg/mL)	% Recovery of KTR-T	% Recovery of OPD-HCL
1:1	5	5	100.21	100.14	1.5	1.5	101.96	99.23
2:1	10	5	99.49	99.88	8	4	99.82	100.18
4:1	4	1	100.73	102.24	6	1.5	99.60	98.36
1:2	5	10	98.17	99.75	0.5	1	101.40	100.17
1:4	1	4	101.94	99.27	2	8	101.22	99.28
	Mean%±SD		100.12±1.40	100.26±1.15	Mean%±SD		100.80±1.03	99.44±0.76

Table (11): Determination of ketorolac tromethamine and olopatadine- HCL in their laboratory prepared mixtures by the UV-spectrophotometric methods

Ratio KTR-T: OPD-HCL	KTR-T				OPD-HCL					
	KTR-T added (µg/mL)	OPD-HCL added (µg/mL)	Zero order method	DW method	KTR-T added (µg/mL)	OPD-HCL added (µg/mL)	Isoabsorptive method	DW method	1DR method	RD method
1:1	10	10	98.91	99.25	10	10	99.74	100.63	97.34	99.43
2:1	20	10	99.45	102.18	30	15	98.51	98.84	98.61	100.09
4:1	40	10	100.50	99.23	40	10	100.95	99.73	101.14	101.16
1:2	5	10	100.56	100.21	35	70	98.91	102.41	102.41	102.07
1:4	20	80	98.80	99.69	15	60	100.40	101.24	98.35	100.91
	Mean%±SD		99.64±0.85	100.11±1.22	Mean%±SD		99.70±1.01	100.57±1.37	99.57±2.12	100.73±1.01

Fig. 12: Absorption spectra of (—) ketorolac tromethamine 40 µg ml^{-1}, (–·–·–)olopatadine-HCL 40 µg ml^{-1} and(---) mixture of 20 µg ml^{-1} of each drug, using methanol as blank
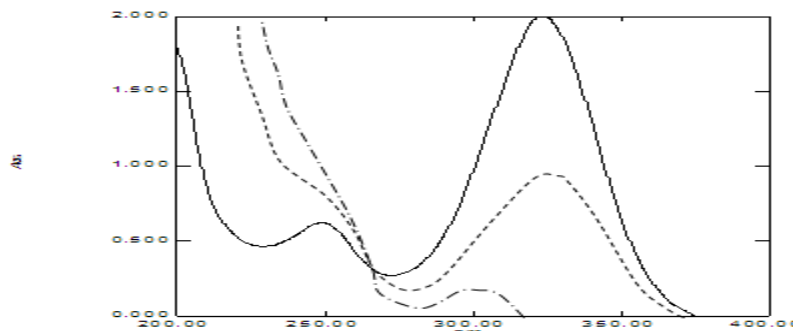
Fig. 13: Ratio spectra of olopatadine-HCl (10-70µg/ml) using 10 µg/mL ketorolac tromethamine as divisor and methanol as blank.
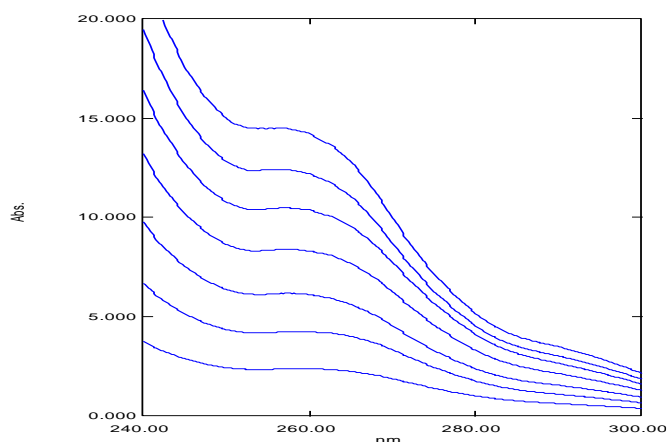

Table (12): Results obtained by UPLC and HPTLC methods compared with reported method [27] for the determination of ketorolac tromethamine and olopatadine- HCl in the prepared fast dissolving film and in market tablets.

Parameter	UPLC		HPTLC		Reported method[27]	
	KTR-T	**OPD-HCL**	**KTR-T**	**OPD-HCL**	**KTR-T**	**OPD-HCL**
	Prepared film		**Prepared film**		**Prepared film**	
Linearity	1-10	1-10	0.5-8	1-8.5	10-60	2.5-15
N	5	5	5	5	5	5
Mean%±SD	99.95±1.23	100.22±1.61	100.44±0.83	100.15±1.45	99.98±0.68	99.89±0.91
Variance	1.51	2.59	0.69	2.10	0.46	0.83
t-	0.05	0.30	0.96	0.25	-	-
F-	3.27	3.13	1.49	2.54	-	-
Standard addition	101.16±1.02	101.60±0.77	100.76±1.97	99.99±1.91	-	-
	Ketolac® tablet	**patadine® tablet**	**Ketolac® tablet**	**patadine® tablet**	**Ketolac® tablet**	**patadine® tablet**
Linearity	1-10	1-10	0.5-8	1-8.5	10-60	2.5-15
N	5	5	5	5	5	5
Mean%±SD	100.45±0.85	100.04±1.01	100.12±0.74	99.97±1.87	99.67±0.73	99.11±0.88
Variance	0.72	1.02	0.55	3.50	0.53	0.77
t-	2.00	1.55	0.97	0.93	-	-
F-	1.36	1.32	1.03	4.52	-	-
Standard addition	100.05±0.76	100.72±1.61	101.02±1.05	100.61±1.41	-	-

Table (13): Results obtained by spectrophotometric methods compared with reported method[27] for the determination of ketorolac tromethamine and olopatadine- HCl in the prepared fast dissolving film and in market tablets.

Parameter	KTR-T			OPD-HCL				
	Zero order method	DW method	Reported method[27]	Isoabsorptive method	DW method	1DR method	RD method	Reported method[27]
	Prepared film			Prepared film				
Linearity	5-40	5-40	10-60	10-105	10-70	10-70	10-70	2.5-15
N	5	5	5	5	5	5	5	5
Mean%±SD	100.48±1.02	100.50±1.34	99.98±0.68	100.66±0.88	100.36±1.3	100.33±1.2	99.35±1.12	99.89±0.9
Variance	1.04	1.80	0.46	0.77	1.69	1.44	1.25	0.83
t-	0.91	0.79	-	1.37	0.67	0.66	0.57	-
F-	2,25	3.88	-	0.94	2.04	1.74	1.51	-
Standard addition technique	99.40±1.15	100.16±1.70	-	100.48±0.70	100.21±0.63	99.00±1.43	98.86±1.60	-
	Ketolac® tablet			patadine® tablet				
Linearity	5-40	5-40	10-60	10-105	10-70	10-70	10-70	2.5-15
N	5	5	5	5	5	5	5	5
Mean%±SD	100.90±1.15	100.26±1.68	99.67±0.73	99.85±1.21	99.03±0.78	100.31±1.20	99.30±1.14	99.11±0.88
Variance	1.32	2.82	0.53	1.46	0.61	1.44	1.30	0.77
t-	2.03	0.72	-	1.11	0.15	1.81	0.30	-
F-	2.48	5.30	-	1.89	1.27	1.86	1.68	-
Standard addition technique	101.25±0.96	99.88±0.64	-	99.00±0.73	100.39±0.9	99.43±1.44	98.78±1.52	-

The theoretical t- and f- values at p= 0.05 were 2.31 and 6.39, respectively. Reported method [27] involved RP-HPLC method for simultaneous estimation of KTR-T and OPD-HCL in bulk and pharmaceutical using C18 column with UV detection at 260 nm and a mobile phase of 0.1N NaH_2PO_4 (pH 4.6): acetonitrile (50: 50 v/v), at flow rate of 1.0 mL / min.

Fig. 14: First derivative of smoothed ratio spectra of olopatadine-HCl (10 – 70 μg/ ml) using 10 μg /ml ketorolac tromethamine as divisor and methanol as blank.
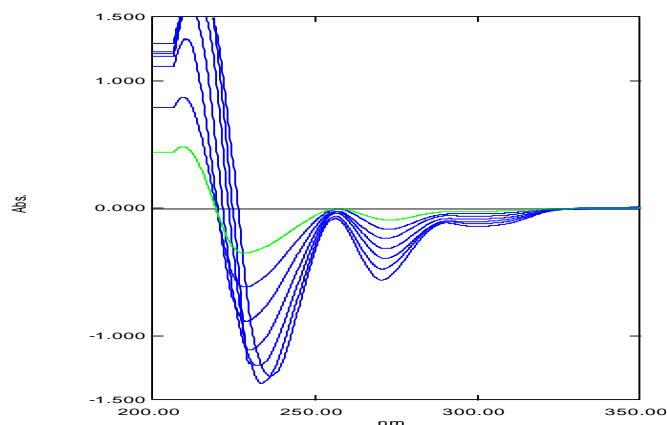
$A = A(1\%,1cm)\, bC$

………………………… (1)

Therefore, for drug 1:

$A_1 = A_1(1\%,1cm)\, b_1 C_1$

……………………… (2)

for drug 2:

$A_2 = A_2(1\%,1cm)\, b_2 C_2$

……………….……... (3)

If $C_1 = C_2$, $A_1 = A_2$ and $b_1 = b_2$

Therefore, this λ is called the isoabsorptive point and at this λ

$A_1(1\%,1cm) = A_2(1\%,1cm)$

………………..…… (4)

And since for a mixture of both drugs, the absorbance at this λ can be calculated from Eq5.

$A_M = A_1(1\%,1cm)\, C_{1M} + A_2(1\%,1cm)\, C_{2M}$

…………. (5)

As $A_1 = A_2 = A_M$ and $A_1(1\%,1cm) = A_2(1\%,1cm)$, therefore,

$A_M = A(1\%,1cm)(C_{1M} + C_{2M}) = A(1\%,1cm)(C_{TM})$ ……. (6)

$(C_{1M} + C_{2M}) = (C_{TM})$

………….……………. (7)

Where A_1, A_2 and A_M = absorbance of drug 1, drug 2 and their mixture at isoabsorptive point, and C_{1M}, C_{2M} are the concentration of the two drugs in the mixture.

Dual Wavelength method [43] **-** From the overlain spectra shown in Fig. (12), the difference between 309 and 286.4 nm was selected for the estimation of KTR-T in presence of OPD-HCl which shows the same absorbance at these wavelengths. Also, the difference between 241 and 255 nm was used for the determination of OPD-HCl in presence of KTR-T.

Derivative ratio (1 DR) method[44]**-** It was found that the spectra of KTR-T cannot accept any concentration of OPD-HCl as divisor, whereas OPD-HCl spectra can be divided by certain concentrations of KTR-T, allowing for the determination of OPD-HCl only in presence of KTR-T. At wavelengths 233, 271 and 300 nm, good linearity was observed but the % R at 271 nm was the best, which may be attributed to its higher signal to noise ratio; Fig. (13 and 14). Consequently, the peak amplitudes of the first derivative of ratio spectra are then recorded at 271 nm. Effect of delta lambda is studied; using $\Delta\lambda = 8$ nm gave best results. Different concentrations of divisor were used (5-40 μg/ml) of KTR-T 10 μg/ml of KTR-T was the best regarding average % R.

Ratio difference (RD) method[45]**-** This method comprises two critical steps. The first is the choice of the divisor and the selected divisor should compromise between minimal noise and maximum sensitivity. The second is the choice of the wavelengths at which measurements are recorded. Linear correlation was obtained between the differences in amplitudes betwwen 262 and 280 nm, against the corresponding concentration of OPD-HCl using 10 μg/ml of KTR-T as divisor.

Method Validation

1. **System suitability-** System suitability test was performed in accordance with USP[46] to ensure system performance before or during the drug analysis.

Results shown in Table 7 indicate adequate resolution

2. **Linearity-**Under the described experimental conditions, linear calibration curves between peak areas to respective drug concentration were obtained through the concentration ranges of 1-10 μg/ mL of both drugs using UPLC method and 0.5 -8 μg/ spot or 1-8.5 μg/ spot of the KTR-T and OPD-HCl, respectively for HPTLC method. The spectrophotometric methods were found to be valid over the concentration range of 5–40 μg/mL for KTR-T and 10- 105 μg/mL or 10- 70 μg/ml for OPD-HCl. Regression parameters were computed and presented in Tables 8 and 9.

3. **Accuracy and precision-** Accuracy calculated as (R%) ranged from 99.82 to 100.65% for the two drugs. Intraday precision (RSD %) ranged from 0.07 to 1.92% , while inter day precision ranged from 0.11 to 1.95% for both drugs; indicating good repeatability and reproducibility of the methods, Tables 8 and 9**.**

4. **Selectivity**-It was determined by applying the proposed methods to laboratory prepared mixtures containing different ratio of the two drugs. Good mean % recoveries of 100.12±1.40and 100.26±1.15 were obtained for KTR-T and OPD-HCL, respectively in UPLC method. While for HPTLC, % recoveries amounted to 100.80±1.03 and 99.44±0.76 for the two drugs, respectively, (Table 10). While for spectrophotometric technique, % recoveries were 99.64±0.85 and 100.11±1.22 by zero order and dual wavelength methods, respectively for KTR-T. While for OPD-HCl, % recoveries were 99.70%±1.01, 100.57%±1.37, 99.57%±2.12 and 100.73%±1.01 by isoabsorptive, dual wavelength, derivative ratio and ratio difference methods, respectively, Table 11. It is noteworthy to mention that the ratio of KTR-T: OPD-HCl in the market preparation (Accupat® eye drops) is 4:1.

Application of the proposed methods to the prepared fast dissolving film and market tablets: The proposed methods were successfully applied for analysis of both drugs in the prepared film and market tablets. The validity of the proposed method was further assessed by applying the standard addition technique. The results obtained were reproducible with acceptable SD, Tables (12 and 13). Statistical analysis of the results obtained by the proposed methods compared with a reported one[27]showed that the calculated t and F values are less than the tabulated ones indicating no significant difference between them confirming accuracy and precision at 95% confidence limit, Tables (12and 13). The proposed methods are precise, accurate, robust and fast. The two chromatographic methods are more sensitive, less time and solvent consuming. The UV-spectrophotometric methods are more simple and did not require separation of the two drugs. Therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

CONCLUSIONS

KTR-T and OPD-HCl fast dissolving film were prepared. The solubility and bioavailability of OPD-HCl was increased by complexation with kleptose while Kollidone was used to increase the release of both OPD-HCl and KTR-T from the film. D-optimal design was applied to study the effect of determined material attributes and critical process parameters on drug release from the film. The design space was determined from which, the final ratios of polymers in the film was determined and used to formulate fast dissolving film. These films were successfully analysed by UPLC, HPTLC and spectrophotometric techniques. These methods proved to be accurate and precise, thus can be

effectively applied for the routine estimation of both drugs in bulk and in their combined formulations.

REFERENCES

1. O' Neil J.M. The Merck Index, 14th Ed., Merck Research Laboratories, Merck and Co. Inc, Rahway, USA, 2006, p. 1025 and 1178.

2. Devi B., Kamatham S. S., Bhaskara R. V. and Srinivas K.; A new RP-HPLC method development and valdation for the simultaneous estimation of ketorolac tromethamine and tramadol hydrochloride in pharmaceutical dosage forms. Asian Journal of Pharmaceutical and Clinical Research., 2017; 10(5):186.

3. Razzaq S. N., Mariam I., Khan I. U. and Ashfaq M.; Development and validation of liquid chromatographic method for gatifloxacin and ketorolac tromethamine in combined dosage form. Journal of Liquid Chromatography & Related Technologies. 2012; 35(5):651-661

4. Dubey SK, Duddelly S, Jangala R.N.; Rapid and Sensitive Reverse phase High-performance Liquid Chromatography Method for Estimation of Ketorolac in Pharmaceuticals Using Weighted Regression. Indian journal of pharmaceutical sciences. 2013:89-93.

5. Qandil A.M., Tashtoush B.M., Al-Taani B.M., Al-Nabulsi S.M. and Al-Zogoul S.; Simultaneous RP-LC Determination of Ketorolac and its Piperazinyl alkyl Ester Prodrugs. Chromatographia. 2008; 67(3- 4):287-291.

6. Pardhasaradhi R.B., Suryanarayana M.V.,Venkatraman S., Krupadanam G. L.and Sastry C. S. P.; Purity evaluation of ketorolac tromethamine by HPLC. Indian Drugs. 1993; 30(4): 176-179.

7. Wu A. T. and Massey I. J.; Simultaneous determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography. J. Chromatogr. 1990; 99: 241-246.

8. NageswaraRao K, GanapatyS and LakshmanaRao A.; validated rp-hplc method for the determination of olopatadine in bulk drug and in pharmaceutical dosage form. IJPCBS. 2012; 2(4):722-728.

9. Bhatt Parth Dand Akhtar Jawed PUB.; Development and validation of stability indicating RP-HPLC method for estimation of olopatadine hydrochloride in bulk drug and it's formulations. International Journal of Pharmaceutical Sciences Review & Res.2011; 9(2):153- 158.

10. Wen-Fang H, Bao-Ping H and Li-Fen W.; Determination of Olopatadine hydrochloride in Olopatadine hydrochloride eye drops by HPLC. Food and Drug. 2010:13

11. El Yazbi F. A. , Hassan E. M. , Khamis E. F. , Ragab M. A.A. , and Hamdy M. M.A.; Development and Validation of a High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Two Binary Mixtures Containing Ketorolac Tromethamine with Phenylephrine Hydrochloride and with Febuxostat. J Chromatogr Sci. 2016; 54(5): 819–828.

12. Devarajan PV, Gore SP and Chavan SV.; HPTLC determination of ketorolac tromethamine. J Pharm Biomed Anal. 2000; 22: 679-83.

13. Vekaria H. J. * and kumar R.J.; HPTLC method for simultaneous estimation of montelukast and olopatadine in its combined dosage forms IJPSR, 2015; 6(12): 5174-78.

14. Mahajan A, Gandhi P,Pandita N, Gandhi Santosh and Deshpande P.; Validated High Performance Thin Layer Chromatographic Method for Estimation of Olopatadine Hydrochloride as Bulk drug and in Ophthalmic Solutions. International Journal of ChemTech Research. IJCRGG.2010; 2(3):372-1375.

15. Liang W, Zhou H, Liu D, Hu P and Jiang J.; Quantitative determination of Olapatadine in human plasma by high performance liquid hromatography tandem mass spectrometry. J Chinese Mass Spectrom. 2006; 27:193-197.

16. Kazuhiro F, Hiroshi M and Hiroyuki K.; Determination of Olopatadine, a new antiallergic agent, and its metabolites in human plasma by high - performance liquid chromatography with electrospray ionization tandem mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications. 1999; 731(2):345-352

17. Shingbal D. M. and Naik U. C.; Spectrophotometric determination of ketorolac tromethamine and its dosage forms using 3-methyl 2-benzothiazolinone hydrazone hydrochloride; Indian Drugs, 1997; 34(10): 608-609

18. Kamath B. V., Shivram K. and Vangani S.; Spectrophotometric determination of ketorolac tromethamine by charge transfer and ion-pair complexation; Anal.Lett. 1994; 27(1): 103-112.

19. Abdel-Razeq S. A., Marzouk M. A. and Soliman M. M.; Validated Spectrophotometric Methods for the Determination of Ketorolac Tromethamine; Egypt.J. Anal. Chem. 2013 vol.22,:114-129.

20. Sebaiy M. M, El-Henawee M. M, Abdellatef H. E. and Alshuwaili M. Kh.; Introducing FDA Validation Guidelines for the Spectrophotometric Determination of Olopatadine Hydrochloride in Pure Form and Eye Drops. Der Pharmacia Lettre, 2018; 10(1): 45-57

21. Suddhasattya D., Vikram R. Y., Swetha B, Sandeep K.D. and Murthy P.N.; Method development and validation for the estimation of Olopatadine in bulk pharmaceutical dosage forms and its stress degradation studies using UV-VIS spectrophotometric method. Int J Pharm Pharm Sci. 2010; 2(4):212-218

22. Prakash M. S. and Meena S.; Fluorophotometric determination of ketorolac tromethamine; Indian Drugs, 1996; 33(4): 149-151

23. Santhosh P., Kumar N.S., Renukadevi M., Gopalan A.I., Vasudevan T. and Lee K.P.; Enhanced electrochemical detection of ketorolac tromethamine at polypyrrole modified glassy carbon electrode; Anal. Sci. 2007; 23(4): 475-478.

24. Squella J. A., Lemus I., Sturm J. C. and Nunez Vergara L. J.; Voltammetric behaviour of

ketorolac and its HPLC-EC determination in tablets; Anal. Lett. 1997; 30(3): 553-564.

25. Sturm J. C., Canelo H., Nunez Vergara L. J. and Squella J. A.; Voltammetric study of ketorolac and its differential pulse polarographic determination in pharmaceuticals; Talanta, 1997; 44(5): 931-937.

26. Sreedhar N.Y., Sreenivasulu A., Kumar M. S. and Nagaraju M.; Voltammetric determination of olopatadine hydrochloride in bulk drug form and pharmaceutical formulations. IJPSR 2012; 3(7):2320-24

27. Satyanarayana M.V., Satyadev T.N.V.S.S.; Ramakrishna Ch. and Anuradha V., stability indicating RP-HPLC method for simultaneous determination of ketorolac tromethamine and olopatadine hydrochloride in bulk and its pharmaceutical formulations. IJRPC 2014; 4(3): 546-556.

28. Banu B.H.and Priyanka B.; RP-HPLC assay method development and validation of ketorolac and olopatadine in opthalmic liquid dosage forms JBPR, 2014; 3(5) 04-0 7.

29. Bhatt Y.J., Sharma S.K. and Multani P.J.; A validated uv spectrophotometric method for the estimation of olopatadine and ketorolac tromethamine in ophthalmic dosage form. Int.J. Pharm.Sci. Rev. and Res., 2013; 20(2):118-120.

30. Mohamed M. I., Soliman N. A. and Abd-El Rahim S. H.; Development of a Novel Ketorolac Tromethamine Sublingual Film. J. Am. Sci., 2015; 11(7): 27-37.

31. Bansal S., Bansal M. and Garg G.; Formulation and evaluation of fast dissolving film of an antihypertensive drug. IJPCBS 2013; 3(4): 1097-1108

32. Renuka M, Avani A; Formulation development of Taste-masked rapidly dissolving film of cetirizine hydrochloride. Pharm technol. 2009; 33(2): 48-56.

33. Rajni B., Sushil K., and Pravin P.; Design Optimization and In Vitro-In Vivo Evaluation of Orally Dissolving Strips of Clobazam. J.Drug Deliv., 2014; 1-15.

34. Priyanka N, Iti C, Mohd Y.; Insights into Polymers: Film Formers in Mouth Dissolving Films. Drug Invention Today, 2011; 3(12): 280-289.

35. McGinity JW, Felton L A.; An aqueous polymeric coating for pharmaceutical dosage forms, 3rd Ed., McGinity and Felton, editors, New York: Informa Healthcare, 2008; 56-57.

36. Kamal D., Kavita P., Ramana M.V., and Vinny L.; Dissolution behavior of β-cyclodextrin molecular inclusion complexes of aceclofenac. J. Pharm. Bioallied Sci., 2011; 3 (3), 417-425.

37. Ibrahim M.A.; Tenoxicam-kollicoat IR binary systems: physicochemical and biological evaluation. Poloniae Pharmaceutica Drug Research, 2014; 71 (4), 647-659.

38. Tekade B. W., Jadhao U. T., Patil V. R. and Sumit D.; Formulation and evaluation of chlorpheniramine maleat fast dissolving film as antiallergic medication. EJPMR. 2017; 4(5), 340-345.

39. Janssen S., Armas H.N., Remon J.P., and Mooter G.V.; The use of a new hydrophilic polymer, Kollicoat IR, in the formulation of solid dispersions of itraconazole, Eur. J. Pharm. Sci., 2007; 30, 288–294.

40. Darwish M.K.; Preparation and Evaluation Of Zaleplon Fast Dissolving Films Using Different Film Forming Polymers, JGTPS, 2014; 5(4): 2055 – 2060.

41. Darwish M.K., Fouad M. M., Zaazaa H. E., Abdel-Razeq S. A. and Nasr Z. A.; Formulation, optimization and simultaneous determination of atorvastatin calcium and losartan potassium in pure and bilayer tablets, JGTPS, 2014; 5(3): 1756 -1768.

42. Lotfy H.M., Amer S.M., Zaazaa H.E. and Mostafa N.S.; Spectrophotometric Methods for Quantitative Determination of Binary Mixture of Naproxen Sodium and Domperidone Maleate. Austin J Anal Pharm Chem. 2015; 2(3): 1044.

43. Lotfy., Saleh., Hassan N.Y. and Elgizawy S.M.; A comparative study of the novel ratio difference method versus conventional spectrophotometric techniques for the analysis of binary mixture with overlapped spectra, AJAC., 2012; 3: 761-769.

44. Darwish M.K., Fouad M. M., Zaazaa H. E., Abdel-Razeq S. A. and Nasr Z. A.; Spectrophotometric methods for simultaneous determination of gatifloxacin and dexamethasone in their binary mixture. IAJAPR, 2015; 5(9): 3048-3057.

45. Rashed N.S., Abdallah O. M. and Said N. S.; Development and validation of spectrophotometric methods for simultaneous determination of levocetrizine dihydrochloride and montelukast sodium in tablet dosage form. WJPPS. 2015; 4(5): 73-88.

46. The United State Pharmacopoeia 35, NF 30, Asian Ed. Rand Mc Nally, USA, 2012.