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Formulation and in-vitro evaluation of a transdermal patch loaded with letrozole

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ABSTRACT

The prime objective behind this investigation was to plan a Letrozole enclosed adhesive transdermal patch; since the transdermal route is an outright attractive option concerning its route, convenience and safety. Letrozole, a non-steroidal type II aromatase inhibitor, is reported for treating breast tumours and in postmenopausal women. In this study, few factors confined to formulation such as drug-in-adhesive, enhancers and amount of drug-loaded were investigated. The procedure used supposedly involves, the incarnation of the LET in phospholipids exploiting to a spray dryer. FTIR, X-RD, and DSC techniques which are used to evaluate entrapment efficiency were employed to LET spray-dried powder (LT-SDP). The molecule size, polydispersity file, and the EE were allegedly found to be 284.0 nm, 0.247 and 59.08%. On adding LT-SDP to a cream base with peppermint and olive oil as regular infiltration, the optimized formulation showed superior skin targeting in both *in vitro* and *in vivo* observations post-study. *In vivo* bioavailability studies showed just about four-fold increment in the plasma whereas the mean residence time and half-life were reasonably higher as compared to the LET cream in plain. The *in vivo* results observed remarkable patch concurrence with the plasma fixations anticipated from the *in vitro* infiltration. As an outpatient convenience, avoidance of gastrointestinal incompatibility provides suitability for self-administration for breast cancer prevention and treatment.

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INTRODUCTION

Many drugs show unwanted behaviour associated exclusively with a particular route of administration, and thereby the development of transdermal delivery systems became one of the recent remedies to eliminate one of the traditional dosage problems. (Chen *et al.*, 2006). Transdermal drug delivery systems are discrete dosage forms which, when applied to the intact skin ensures the controlled rate of drug delivery through the skin into the systemic circulation, (Jain, 1997). Administrating drug

through the skin often provides a slower, more controlled alternative route for release into the bloodstream escaping the first-pass metabolism. Transdermal application is believed to be an attractive method of drug administration, with listed benefits of improved pharmacological and physiological response, suitability for self-administration, and the feasibility of continuous drug delivery over up to one week, (Modamio *et al.*, 2000). Besides the belief, the transdermal patches are also user-friendly, well-located, simple to use, and avoids the drug levels fluctuations. The Drugs with short biological half-lives with a reduced therapeutic value are suitable and offers multi-day dosing, which is universally accepted as a part of improved patient compliance and thus accepted, (Ranade, 1991).

Breast cancer, currently the second most fatal disease with the women and no quantifiable impacts of it as far as personal satisfaction and worries about the potential for tumour repeat and demise ought not to be thought little of, (Piccart, 1998). Notably, estrogen is firmly associated with the development of human breast carcinoma, and the estrogen receptors are expressed by an incredible share of breast carcinomas (Funke *et al.*, 2002). Late examinations have demonstrated that estrogens are produced locally in case of breast carcinoma by a few enzymes, (Suzuki *et al.*, 2008). Among such enzymes, aromatase is commonly viewed as the most significant protein, and aromatase inhibitors are effectively utilized in the treatment of breast carcinoma in postmenopausal ladies as estrogen hardship treatment, (Marwah *et al.*, 2016).

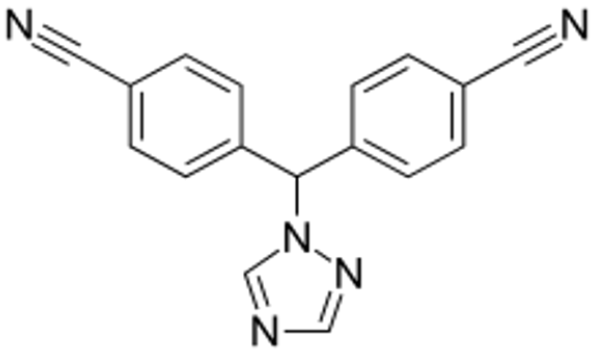

Figure 1: Chemical structure of letrozole

Due to its increased strength, endurance and specificity among the aromatase inhibitors, (Miki *et al.*, 2007), letrozole is currently recognized as the first-line drug in the endocrine treatment of estrogen-subordinate breast carcinoma in postmenopausal patients. Till date, an enormous extent (around 75% before menopause and near 100% after menopause) of the organically dynamic estrogen is

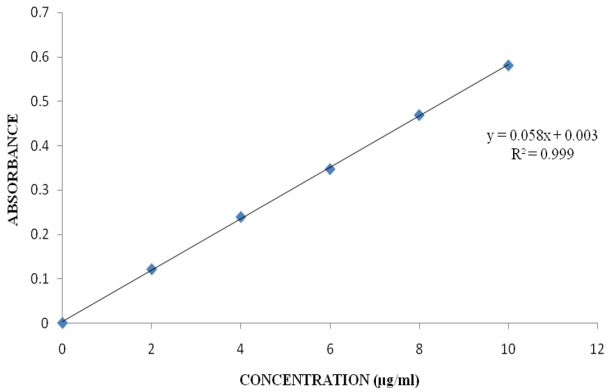

Figure 2: Standard calibration curve of letrozole

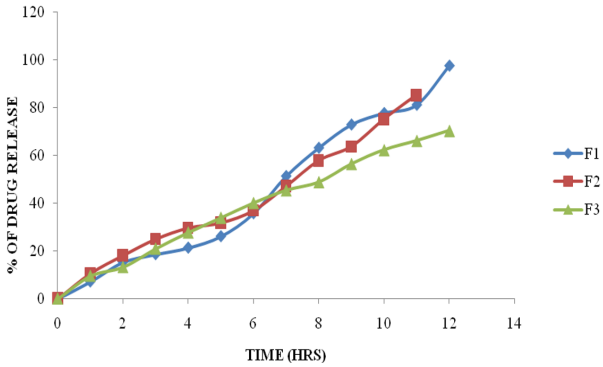

Figure 3: Cumulative % drug permeation of Letrozole patch (F1, F2, F3)

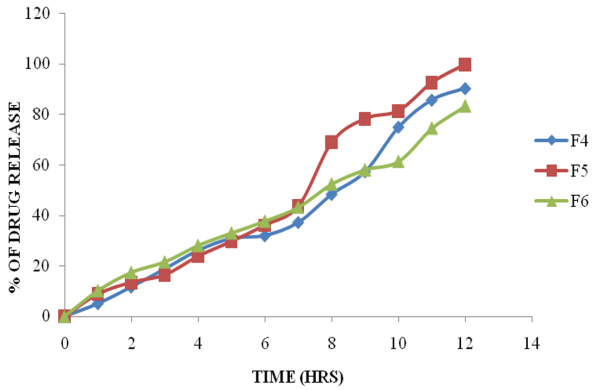

Figure 4: Cumulative % drug permeation of Letrozole patch (F4, F5, F6)

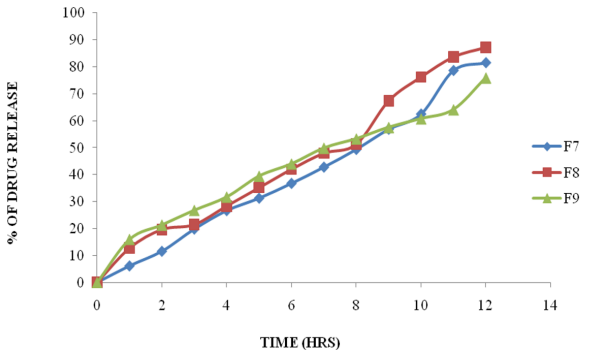

Figure 5: Cumulative % drug permeation of Letrozole patch (F7, F8, F9)

Table 1: Formulation of a transdermal patch containing letrozole.

Ingredients	Formulation chart								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Letrozole	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
HPMC K100 M	4.50	8.10	12.15	-	-	-	-	-	-
Polyvinyl alcohol	-	-	-	4.50	8.10	12.15	-	-	-
Polyvinylpyrrolidone	-	-	-	-	-	-	4.50	8.10	12.15
PEG-200 (ml)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Dimethylsulphoxide (ml)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Methanol (ml)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Table 2: Standard graph of letrozole

Concentration (μg/ml)	Absorbance (at 235 nm)
0	0
2	0.121
4	0.239
6	0.347
8	0.469
10	0.581

Table 3: Results of identification tests, melting point and solubility of letrozole

Parameters	Results
Colour	White
Odour	Odourless
Taste	Bitter
Appearance	A white powder
Melting point	184-185°C
Solubility in distilled water	0.0403 mg/ml
Solubility in pH 7.4 phosphate buffer	78.3 mg/ml

Table 4: Results of physicochemical parameters of all prepared patches of letrozole.

Formulation Code	Average weight (mg)	Thickness (mm)	Folding endurance	Flatness (%)	Flatness (%)	% Drug Content
F1	73±2.02	0.041 ±0.002	82 ± 0.14	98	Transparent	96.2 ± 2.10
F2	76 ±4.12	0.045±0.006	85 ± 2.10	100	Transparent	99.11 ± 0.45
F3	75 ±1.21	0.050±0.002	86 ± 3.17	97	Transparent	99.65 ± 2.71
F4	75 ±5.41	0.046±0.006	82 ± 3.11	98	Transparent	96.01 ± 2.24
F5	72 ±7.11	0.047±0.001	81 ± 2.34	100	Transparent	97.31 ± 3.73
F6	75 ±3.05	0.043±0.005	89 ± 2.15	99	Transparent	98.35 ± 0.59
F7	72 ±6.79	0.045±0.003	83 ± 2.36	99	Transparent	96.11 ± 1.24
F8	78 ±2.41	0.044±0.002	85 ± 2.04	100	Transparent	98.65 ± 1.57
F9	75 ±5.14	0.049±0.004	81 ± 2.96	98	Transparent	99.12 ± 2.54

Table 5: In vitro drug permeation of letrozole containing different concentrations of HPMC K 100 M, polyvinylalcohol and Polyvinylpyrrolidone

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	7.14	10.64	9.57	5.12	8.85	10.18	6.14	12.72	15.98
2	15.26	18.14	13.26	11.69	13.47	17.39	11.56	19.61	21.33
3	18.58	24.98	20.93	18.82	16.44	21.54	19.79	21.43	26.78
4	21.31	29.64	27.72	26.11	23.86	28.07	26.62	28.32	31.71
5	26.10	31.78	33.90	30.87	29.71	32.97	31.22	35.11	39.46
6	35.71	36.97	40.10	31.98	36.11	37.65	36.72	41.94	43.99
7	51.29	47.14	45.39	37.24	43.75	43.17	42.73	47.89	49.87
8	63.12	57.87	48.83	48.36	68.93	52.35	49.31	51.21	53.31
9	72.86	63.75	56.44	57.33	78.24	57.96	56.78	67.34	57.56
10	77.67	75.24	62.29	74.98	81.39	61.31	62.40	76.14	60.76
11	81.12	85.33	66.14	85.71	92.59	74.46	78.52	83.58	64.14
12	97.49	-	70.36	90.27	99.81	83.22	81.42	87.12	75.97

Table 6: Kinetics data of F5 letrozole patch

Time (t)	Cumulative (%) Release Q	Log (T)	Log (%) Release	Log (%) Remaining	Release Rate (Cumulative % Release / T)	Peppas Log Q/100	% Drug Remaining
0	0	-	-	2.000	-	-	100
1	8.85	0.000	0.947	1.960	8.850	-1.053	91.15
2	13.47	0.301	1.129	1.937	6.735	-0.871	86.53
3	16.44	0.477	1.216	1.922	5.480	-0.784	83.56
4	23.86	0.602	1.378	1.882	5.965	-0.622	76.14
5	29.71	0.699	1.473	1.847	5.942	-0.527	70.29
6	36.11	0.778	1.558	1.805	6.018	-0.442	63.89
7	43.75	0.845	1.641	1.750	6.250	-0.359	56.25
8	68.93	0.903	1.838	1.492	8.616	-0.162	31.07
9	78.24	0.954	1.893	1.338	8.693	-0.107	21.76
10	81.39	1.000	1.911	1.270	8.139	-0.089	18.61
11	92.59	1.041	1.967	0.870	8.417	-0.033	7.41
12	99.81	1.079	1.999	-0.721	8.318	-0.001	0.19

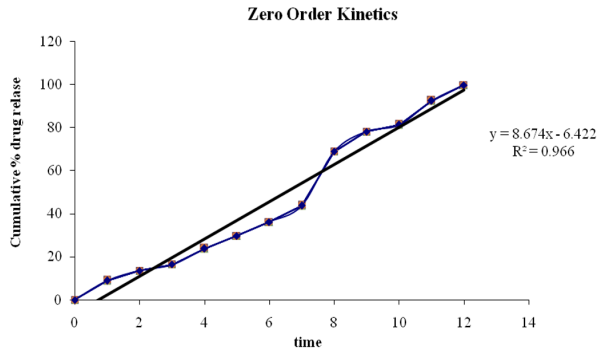

Figure 6: Graph of Zero-order kinetics

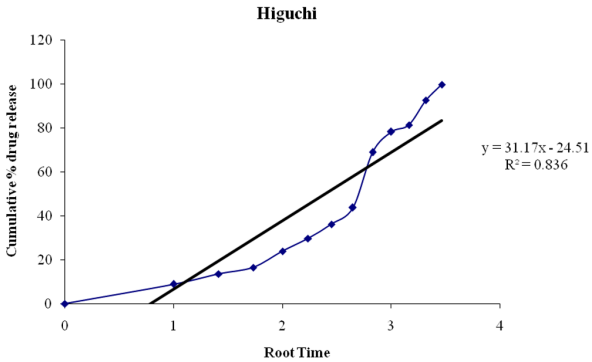

Figure 7: Graph of Higuchi release kinetics

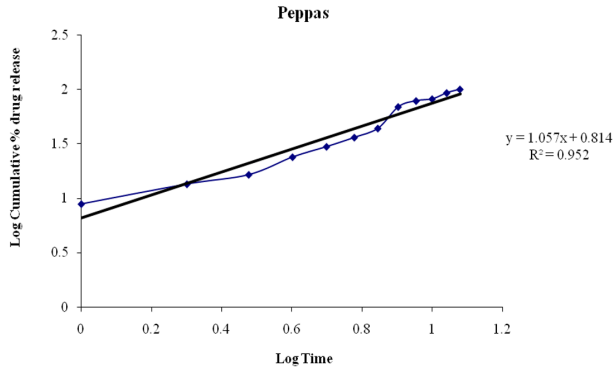

Figure 8: Graph of Peppas release kinetics

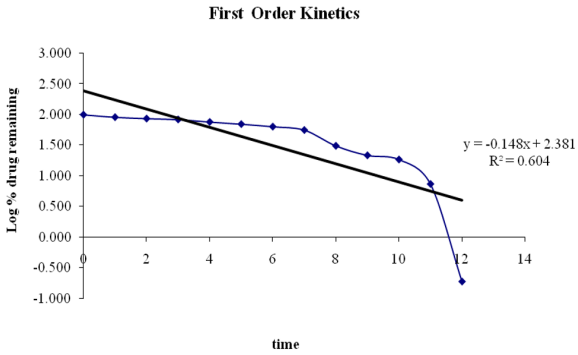

Figure 9: Graph of First-order release kinetics

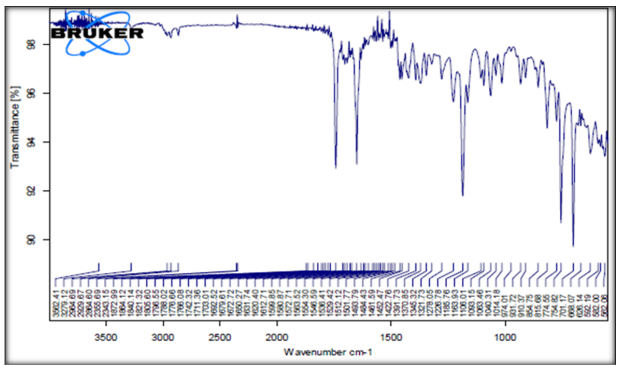

Figure 10: FTIR Spectrum of pure Letrozole drug

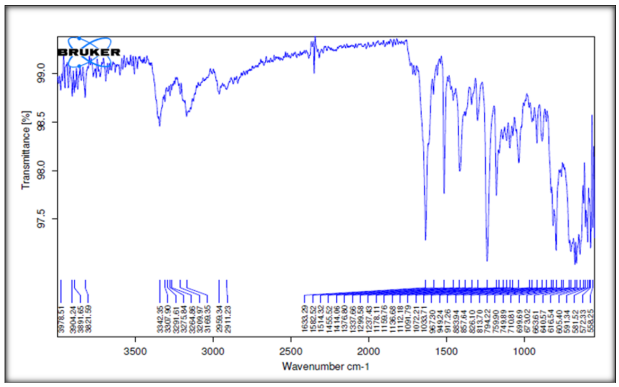

Figure 11: FTIR of Optimized formulation

delivered locally in the breast carcinoma, (Labrie *et al.*, 2003). Oral tablets are the only commercial dosage form of letrozole available at present which is to be taken once a day making patient compliance a vital issue. Therefore it needs some attention (Piccart, 1998). For the reasons mentioned, the percutaneous administration has been studied as a way with prime focus on letrozole, (Ke *et al.*, 2005). Luckily, letrozole has a few ideal physicochemical properties, with low sub-atomic weight (MW 285.10), great lipophilicity (log $K_{o/w}$ 5 1.73, as obtained in a past trial) and just a little day by day portion (2.5 mg/day) is required.

As of not long ago, there has been next to no data about the transdermal conveyance of letrozole, (Suzuki *et al.*, 2005). Permeation enhancers play an important reason to be in TDDS to improve the diffusion of the active ingredient, (Tan and Pfister, 1999) and a lot of classic enhancers exert a significant enhancing result. (Labrie *et al.*, 1997). The chemical structures of letrozole are shown in Figure 1.

MATERIALS AND METHODS

Materials

The following reagents were used as purchased without further purification: Letrozole (Procured from Sigma Laboratories Bangalore, India; provided by Sura labs, Dilsukhnagar, Hyderabad.); HPMCK100M (Hetero Labs, Hyderabad, India), Polyvinyl Alcohol (Hetero Labs. Hyderabad, India), Polyvinylpyrrolidone (Accord Labs, Secunderabad), PEG-200 (ml) (Merck Specialities Pvt Ltd), Dimethylsulphoxide (ml) (Merck Specialities Pvt Ltd), Methanol (ml) (Merck Specialities Pvt Ltd).

FT-IR study

The IR spectrum of the pure Letrozole sample was recorded and spectral analysis performed by placing a dry sample of the drug after mixing and triturating with dry potassium bromide.

Analytical method development for letrozole

Determination of λ max

A 100mg of letrozole was precisely weighed and was dissolved in 35ml methanol. The solution was then diluted using phosphate buffer (pH 7.4) to 100 ml, (stock solution-I). 10ml solution from the stock solution I was taken and volume made up to 100ml with phosphate buffer to get 100 μg/ml concentrations (stock solution-II). 10 ml solution from stock II was taken, and volume made up to 100 ml with a buffer to get 10 μg/ml. 10 μg/ml solution was scanned from 200-400nm.

Construction of calibration curve

A 100mg of letrozole was precisely weighed and was dissolved in 35ml methanol and this diluted using phosphate buffer (pH 7.4) to 100 ml. (stock solution-I). 10ml solution from the stock solution I was taken and volume make up to 100ml with phosphate buffer to get 100 μg/ml concentrations (stock solution-II). It was further diluted with phosphate buffer pH 7.4 to get solutions in the fixation scope of 2,4,6,8 and 10 μg /ml. The absorbances of these solutions were determined spectrophotometrically at 235 nm.

Preparation of transdermal patches containing Letrozole

Single or in mix polymers were correctly measured and separated in an individual dissolvable solvent and a later cast in a Petri-dish with mercury on a plain surface and left to dry overnight at room temperature. The matrix-type transdermal patches containing letrozole were prepared using different groupings of HPMC K100 M, Polyvinyl Alcohol and, Polyvinylpyrrolidone. These polymers in different centres were separated in the different solvents. By then, the medicine was incorporated step by step in the polymeric course of action and blended on the appealing stirrer to get a uniform solution. PEG-200 was used as a plasticizer, and then the solution was poured on the Petri dish having a surface area of 78 cm and dried at room temperature followed by which the patches were cut into 2x2 cm^2 patches where 8 mg was the drug joined for each 2x2 cm^2 patch, Table 1 **.**

Physicochemical characterization of transdermal patches

The different physicochemical portrayals of transdermal patches are as per the following.

Weight variation

The three disks of 2*1cm^2 were cut and burdened electronic parity for the weight variety test. This test was done to check the consistency of weight and have a check on the variation among different batches, (Jayaprakash *et al.*, 2010).

Drug content Determination

The readied tranquillize contained patches indicated surface territory (2 cm2) were cut and broken up in (5% of methanol contained) 100ml of pH 7.4 phosphate cradle, and vivaciously shaken for 12hrs, and then sonicated for 15 minutes, centrifuged at 5000 rpm for 30 min. A Polymeric arrangement containing the medication was sifted through 42 number Whatman filter paper, and 1ml of the filtrate was taken in a test tube and weakened multiple times with a similar dissolvable and by utilizing a twofold pillar UV-Visible spectrophotometer to decide sedate substance at λ max 235 nm. The regarded fake treatment fix was taken as a clear arrangement. (Adrain *et al.*, 2002).

Flatness

A transdermal fix ought to have a smooth surface and ought not to tighten with time. This can be exhibited with evenness study. For levelness assurance, one strip is cut from the middle and two from each side of patches. The length of each strip is estimated, and various long is estimated by deciding per cent tightening. Zero per cent choking is equal to 100 per cent evenness.

Thickness

Vernier calliper's with least check 0.001mm was used, and the thickness of films was estimated at five distinct locales, and the average of five readings was taken with standard deviation.

Folding endurance

A piece of film (4x3 cm) was cut equally and over and again collapsed at a similar spot till it broke. The occasions the film could be collapsed at a similar spot without breaking gave the specific benefit of collapsing perseverance, (Yener *et al.*, 2010).

***In-vitro* drug diffusion study**

The in vitro investigation of medication saturation through the semi penetrable layer was performed utilizing a dispersion cell. The altered cell with a higher limit (25 ml) is employed to keep up sink condition. This membrane was mounted between the donor and receptor compartment of a dissemination cell.

The transdermal fix was set on the layer and secured with aluminium foil. The receptor compartment of the dispersion cell was loaded up with isotonic phosphate cradle of pH 7.4. The hydrodynamics in the receptor compartment was kept up by blending with an attractive dab at consistent rpm, and the temperature was kept up at $37\pm0.5^{\circ}$C.

The dispersion was done for 12 h, and 1 ml test was pulled back at a time frame h. The receptor stage was recharged with an equivalent volume of phosphate cradle at each example withdrawal. The examples were examined for sedate substance spectrophotometrically at 235 nm.

Drug release kinetics

Dispersion information of over two techniques was fitted in Zero requests, First request and Higuchi conditions. The instrument of medication discharge was dictated by utilizing Higuchi condition.

Zero-Order Kinetics

Zero requests as a full measure of Percentage tranquillize discharged versus time, $C = K_0t$, where, K_0 is the zero-request rate consistent communicated in units of fixation/time, and t is the time in hours. A diagram of fixation versus time would yield a straight line with an incline equivalent to K_0 and catch the starting point of the tomahawks.

First-order kinetics

First request as total log level of log (%) combined medication remaining versus time, $\text{Log } C = \text{Log } C_0 - kt/2.303$ where, C_0 is the underlying convergence of medication, k is the principal request consistent, and t is the time.

Higuchi Model

Higuchi's model as an aggregate level of medication discharged versus a square foundation of time, $Q = K t^{1/2}$, where, K is the consistent mirroring the structure factors of the framework and t are the time in hours. Subsequently, tranquillize discharge rate is corresponding to the equal of the square foundation of time.

Korsmeyer Peppas equations

Korsmeyer Peppas condition used to decide the instrument of medication discharge structure the polymer framework of the tablet. Log a combined level of medication discharged versus Log time, and the type n was determined through the incline of the straight line. $M_t/M_\infty = Kt_n$, where M_t/M_∞ is the fragmentary solute discharge, t is the discharge time, K is an active, consistent quality of the medication/polymer framework, and n is an example that describes the instrument of the arrival of tracers. For round and hollow lattice tablets, if the example $n = 0.45$, at that point the medication discharge system is Fickian dissemination, and on the off chance that $0.45 < n < 0.89$, at that point, it is non-Fickian or bizarre dispersion. An example estimation of 0.89 is demonstrative of Case-II Transport or run of the mill zero-request discharge, (Pang *et al.*, 1988).

RESULTS AND DISCUSSION

FT-IR study

The compatibility studies of letrozole with excipients indicate no characteristic visual changes and no additional peaks were observed during FT-IR studies. Figure 10 and Figure 11.

Analysis of drug

The λmax of letrozole was seen as 235 nm. The obtained absorbance against different standard concentration and a calibration curve of letrozole are displayed in Table 2 and Figure 2.

Evaluation of Patch

Nine formulations underwent trials to achieve the winning matrix type Letrozole transdermal patches. Various physical parameters such as appearance, melting point and solubility are mentioned in Table 3.

The formulations F1 to F9 were changing in thickness when contrasted with different plans which are because of variety in the polymer focus, which confirms the rise in polymer concentration increases the thickness of the patch. For all other formulations, it was seen as in between 0.041 ± 0.007 to 0.051 ± 0.004 mm.

All formulations from F1 to F 9 shows weight variation in between 72 ± 6.79 to 78 ± 2.41 mg. Folding endurance from formulations F1 to F9 was found to be in between 81 ± 2.34 to 89 ± 2.15, which can withstand the foldings of the skin. All formulations showed % drug content from 96.01 ± 2.24 to 99.65 ± 2.71. Table 4

In vitro dispersion study

All the formulation *in vitro* diffusion study was carried out by using Franz type diffusion cell under specific condition such as temp maintained at 32 ± 0.5^oC. The diffusion was carried out for 12 h, and 5 ml sample was withdrawn at an interval of 1 h, Table 5 .

Cumulative % drug permeation of Letrozole patch (F1, F2, F3)

The formulations F1 to F3 were prepared by different concentrations of HPMC K100 M (4.5, 8.10, 12.15mg) the drug release or drug permeation from the patch was dependence on the concentration of polymer in the matrix. At low polymer concentration, the drug permeation is more within 12 hours. It was the total amount of drug. It was permeated Table 6 and Figure 3.

Cumulative % drug permeation of Letrozole patch (F4, F5, F6)

The 8.10 mg concentration of polymer showed maximum drug released at 12 hours 99.81%. The 4.5mg concentration of polymer was showed maximum drug release 90.27 at the desired time. Hence in these three formulations, F5 formulation showed total drug release at the desired time. (Figure 4)

The formulations F7 to F9 were prepared by different concentrations of Polyvinylpyrrolidone (4.5, 8.10, 12.15mg) the drug release or drug permeation from the patch was dependence on the concentration of polymer in the matrix. The 8.10mg (F8) concentration of polymer showed maximum

drug release 87.12 within 12 hours. The 4.5mg (F7) concentration of polymer showed maximum drug released at 12 hours, 81.42 %. The 12.15mg (F9) concentration of polymer showed less drug release 75.97 at 12 h. Among all nine formulations, F5 formulation showed good drug permeation from the patch. Among all, *in vitro* evaluation parameters F5 formulation passed all evaluation parameters.

Kinetic models for letrozole

Different models were tried for clarifying the energy of medication discharge, to dissect the system of the medication discharge rate energy of the structure of the measurements, the obtained information was fitted into zero-request, first-request, Higuchi, and Korsmeyer-Peppas discharge model. Figures 5, 6, 7, 8 and 9**.** From the above information, the improved detailing adhered to Zero-request energy model guideline.

CONCLUSIONS

In the current examination, an endeavour has been made to structure and build up the detailing of Letrozole patches utilizing various kinds of polymers by dissolvable dissipation strategy and mercury substrate technique. The medication utilized is the best-read for treatment in rewarding hormonally-responsive bosom disease after a medical procedure. Letrozole was effectively planned as controlled discharge transdermal patches, which forestalls the recurrence of the organization and gives great patient consistence. From the trial results got, F5 detailing has been chosen as the best definition among the various plans. The *in-vitro* medicate dispersion concentrates from the detailing were seen as continued discharge. All the assessment boundaries got from the best plan were seen as palatable. The information got from the in-vitro discharge considers were fitted to different active models like zero request, first request, Higuchi model, and Pappas model. From the motor information, it was discovered that medication discharge follows Zero-request model discharge by dispersion strategy from the polymer. In light of the perceptions, it very well may be reasoned that the endeavour of definition and assessment of the Letrozole patches was seen as useful in the arrival of the medication for an all-encompassing time of 12 hrs.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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