FORMULATION AND EVELUATION OF WOUND HEALING SPRAY LOADED WIITH AGERATUM CONYZOIDES FOR WOUND HEALING ACTIVITIES

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Abstract:-

In the present research work wound healing psray was developed for an effective treatment of healing wounds using *Ageratum conyzoides* as model drug. The spray were prepared by The gel formulations were made by continuously stirring a moderately-speed magnetic stirrer with a weighed quantity of the polymers Carbopol 940(01gm), HPMC (0.5gm), and xanthan gum in water. The vesicles were characterized on the basis of entrapment efficiency, vesicle size, Zeta potential, morphological Characterization of ufasomes, SEM. The Spray were evaluated on the basis of Physical appearance,flame extention back, density, PH, viscosity, leak test spray patteren, In vitro drug release studies, and stability Studies.

Keywords: - Ageratum conyzoides, spray, antiseptic, aerosol.

Introduction:-

Pharmaceutical improvement aims to provide a high-quality product and a production process that consistently produces the product's intended overall performance. The manufacturing and research statistics and experience in pharmaceutical development provide medical knowledge to support the current state of the design field, specifications, and production controls. The oral route of management is losing popularity because to current technical developments in the field of drug shipping (such as the creation of functionalized biopolymers) and the increasing need for more localized delivery to minimize aspect outcomes[1]. Oral medicine administration has a compelling promise with skin delivery. The body is shielded from external threats by the skin, which serves as the body's first line of defense[2].

Although the skin in different parts of the body varies widely in thickness, the underlying structure is always the same. Drug administration through the skin can be generally divided into two categories: transdermal and dermal (topical). Dermal delivery, on the one hand, describes the process of administering a medication directly to the skin's surface, where it acts on the site of action to produce a greater concentration of the medication locally and less systemic exposure[3]. Conversely, transdermal delivery describes the movement of a medication from the skin's surface into the bloodstream in order to reach therapeutic concentrations. Active ingredients have long been administered locally through the skin, and this method is becoming more and more common for systemic distribution via the development of transdermal patches[4]. The growth in chronic skin disorders, the need for targeted administration, and improved patient compliance may be the primary causes of the growing interest in transdermal delivery[5]. Because the skin has a huge surface area, topical and transdermal administrations of a medication offer substantial therapeutic potential[6]. Additionally, transdermal administration minimizes significant variations in plasma concentration, particularly with repeated dosage, by enabling regulated release of the medication into internal areas[7-8]. By maintaining a constant blood level, serious adverse effects may be prevented. Transdermal administration has several benefits, chief among them being the avoidance of the gastrointestinal (GI) tract and first-pass metabolism, which enhances the drug's bioavailability. This approach is a reasonable method to enable patients to self-administer in a painless and comfortable manner. when a patient is unconscious, nauseated, or too young to take medication orally, give the medication to them instead. Topical and transdermal treatments have a very high approval rate overall, as seen by their expanding market. It is expected that the value of skin delivery, which was valued at about $12.838 billion in 2020, will increase to $13.457 billion by 2026 at a compound annual growth rate greater than other delivery routes[9]. Actually, the US market has approved the therapeutic use of more than 36 transdermal products and 16 active medicinal components[10]When sprayed on the skin, a liquid pharmaceutical mixture known as a spray transforms into a mist. Large portions of the body may be treated with this technique of drug delivery, which can also help people with pain problems who find it exceedingly uncomfortable to massage medication into their skin. It can also relieve pain and irritation. It can demonstrate a quick healing effect on wounds, be applied by the patient with ease as a self-medication, and prevent the first pass metabolism. There are a lot of wound healing products on the market. There isn't yet a common topical therapy for wound healing.

MATERIALS AND METHOD USED: -

4.1 List of drug and excipient: -

S.No.	Name	Manufacturer
1.	Ageratum conyzoides	Jackson laboratory pvt. Ltd.
2.	Tween 80	India glycols limited Indore

3.	Sodium dihydrogen phosphate dehydrate	Advanced inorganic new Delhi
4.	Carbopol 940	Akhil healthcare center sayajigaanj
5.	HPMC	Raksh bio science private limited Chennai
6.	Ethanol	PVS scientific Chennai
7.	Propylene glycol	Adarsh chemicals Chennai
8.	Methyl paraben	Nobel pharma & chemicals Chennai
9.	Dimethyl sulfoxide	Grasse internation Chennai

Table1 :- List of materials used

- **List of equipment used :-**

Sr. no.	Name	Manufacutrer
1.	Digital Weighing Balance	Shimadzu, Japan
2.	UV/VIS Spectrophotometer	Shimadzu, Japan
3.	Magnetic stirrer	Remi Equipment Mumbai
4.	Melting Point Apparatus	Remi Equipment Mumbai
5.	pH Meter	Ohaus, USA
6.	Infrared Spectrophotometer (FTIR)	Perkin Elmer Germany
7.	Water bath	Sunshine Scientific Equipment Delhi
8.	Brook Field Viscometer	Dolphin Pharmacy Instruments Pvt. Ltd Mumbai

Table 2:- list of equipment

Process for formulation:-

Take a 30ml of ethanol, ageratum conyzoides powder was dissolved. The gel formulations were made by continuously stirring a moderately-speed magnetic stirrer with a weighed quantity of the polymers Carbopol 940(01gm), HPMC (0.5gm), and xanthan gum in water. After that, it was spread out over a conical flask with a flat bottom and left to dissolve overnight at room temperature without stirring the mixture to create a thick, transparent mass. Then, a thick, transparent gel mass containing ethanol was combined with it. The spray's pH was changed with the use of dimethylsulfoxide. Lastly, methyl paraben 0.3gm preservative was gradually added while being stirred constantly. To prepare the topical spray of Ageratum conyzoides, mix 100 ml of water with a stirrer for 30 minutes.

Particle size (Zeta Potential) :

Using a Zetasizer (Nano-ZS90, Malvern Instruments, UK), the spray samples were suspended in Milli-Q water and screened for particle size at 25 °C. The sample analysis was conducted using the disposable cuvettes. The mean ± standard deviation for the three replicates 175 was given as the result.

4.8 Scanning Electron Microscope (SEM): Ageratum conyzoides can be captured on scanning electron micrographs using a scanning electron microscope. For this aim, a Hitachi S-4800 scanning electron microscope is utilized. Using double-sided adhesive tape, the microspheres were constructed directly on the SEM sample stub and coated with 200 nm thick gold layer at 0.001 Torr of decreased pressure. [11]

4.9 Evaluation of topical spray:-

4.9.1 PHYSICOCHEMICAL CHARACTERISTICS

4.9.1.1 Flame extension and flash back:

On the lab platform, a burner was lit on fire. A one-meter scale was positioned above the platform of the next burner. Formulations were sprayed into a flame for around four seconds. Flame extension and flame flashback were measured over scale with the use of the ruler. Every measurement was made three times.[12]

4.8.1.2 Pressure test:

Every container was arranged such that it was upright. The liquid was extracted from the dip tube and valve by pressing the actuator. The pressure gauge was inserted in lieu of the actuator. The valve was activated by pressing the gauge, and each aerosol container's propellant pressure was recorded using the pressure gauge.[13]

4.8.1.3 Density:

Weighed was a dried and empty pycnometer. It was filled in using the sample. To prevent the thermometer from expanding from the heat of the hand, the pyrometer was held by the neck and one or two thicknesses of paper were placed between the fingers and the bottle. By dividing the resulting weight of the liquid by its volume in the pycnometer, the correct density value was determined. [14]

4.8.1.4 pH:

With the use of a digital pH meter, the optimal solution pH of the spray was established. With the aid of phosphate buffer pH 4.0, 7.0, and 9.0, the pH meter was calibrated prior to measuring the pH of the improved formulation.

After that, a tiny glass beaker containing around 30 ml of spray solution was filled, the pH meter electrode was dipped into it for a minute, and the pH was recorded. Each formulation's pH was measured three times, and the mean results were computed. [15]

Since the pH of the topical spray formulations ranges from 5 ± 0.2 to 7.5 ± 0.2, which is within the skin's natural pH range, there won't be any skin irritation.

4.8.1.5 Viscosity:

Using an Oswald viscometer, the solution's viscosity for topical spray was measured. The Brook Field Viscometer was filled with 30 ml of spray solution. Time was used to measure the solution's flow from point A to point B in the viscometer. At least three readings were assigned. The spray solution's viscosity was assessed in comparison to the water's viscosity.[16]

4.8.1.6 Leakage test of Ageratum conyzoides topical spray:

The leakage test was carried out in compliance with USP protocol. The date and time were recorded to the closest half hour for nine aerosol canisters. Weighed to the closest milligram, each container was identified as W1. Before the second weight was recorded as W2, the containers were let to stand upright at a temperature of 25.0 ± 2.0 ºC for a minimum of three days. Each container's leakage rate, expressed in milligrams per year, was computed using the following formula:

(365) (24/T) (W1 – W2), where T is the test period, expressed in hours. [17]

4.8.1.7 Spray pattern of Ageratum conyzoides topical spray:

Onto absorbent paper, a spray formulation containing 10 mg of sudan red dye was applied for two seconds. Five centimetres was maintained as the distance between the target and the container. By spraying the concentrate horizontally, the spray pattern was assessed. D max. and D min. were computed to find the ovality ratios.[18]

4.8.1.8 Spray angle of Ageratum conyzoides topical spray:

To get the plume angle, ageratum conyzoides spray was sprayed for five seconds, and then a picture was taken. The angle at which the valve releases the material captured in the picture and measured to ascertain the plume angle.

It was discovered that the spray angle had a significant impact on the deposition pattern on diseased skin. Spray angle was discovered to be between 17 and 21. This indicates that the ratios of co-solvent to propellant and drug concentration to propellant had no discernible effect on spray angle.

Particle size of Ageratum conyzoides topical spray:

There were no particles in all formulations when observed under microscope**.**

Assay Percentage

Drug content

Method of analysis by UV Spectrophotometrically

Standard Solution-

In order to produce the standard solution, 10 mg of Ageratum conyzoides standard were dissolved in 100 mL of methanol and mixed for 10 minutes using a magnetic stirrer in a 100 ml volumetric flask. Aliquots of 10 ml were pipetted out of this stock solution into a 100 ml volumetric flask and brought up to mark with methanol to get a concentration in the range of 10 µg/ml.

Test Solution-

Aliquots of 10 ml were pipetted into a 100 ml volumetric flask and brought up to mark with methanol to achieve a concentration in the range of 10 µg/ml after the 1 ml of samples were dissolved in 100 ml of ethanol by swirling on a magnetic stirrer for 10 min. Each example solution was made three times over. All solutions were filtered via 0.45-µm-pore nylon syringe filters before to application.

Procedure:

- A UV/Vis double beam spectrophotometer was used to scan the resultant solution.
- Absorbance was measured with a UV spectrophotometer at 288 nm.
- Analyze the test solution's and the reference solution's absorption of Ageratum conyzoides.
- Determine the Ageratum conyzoides amount by comparison.[108]

4.9 In vitro study: -For this purpose a, dialysis bag method was used (molecular weight cut off 10,000 Da) method was used wherein 1ml of spay was taken inside the dialysis bag which was then tied form both eds. This bag containing spray was then placed in beaker containing 50ml of release media having pH 6.8. These beaker were placed on magnetic stirrer Maintained at 37^0C /100rpm. During incubation. Ageratum conyzoides was released from the spray and cross the dialysis membrane to reach into the external media. At regular time intervals, 1ml of external release medium was withdrawn and replaced with same amount of fresh medium. Amount and percent cumulative release from the spray the release curve were fitted into different kinetic models such as zero order, first order, Higuchi model, Hixon-Crowell, and beaker Lonsdale model. Best goodness of fit was determined from the linear regression co-efficient obtained from the model fitting in data.

4.9.1 Drug release from kinetic study:-

To know the release kinetics, the data obtained from in vitro profile was fitted into various model like.

- Zero order of kinetic model:- cumulative percent drug release v/s time
- First order kinetic model:- log cumulative percent drug remaining v/s time
- Higuchi's model:- cumulative percent drug release v/s square root of time
- Korsmeyer Peppas model:- log cumulative percent drug release v/s log time

4.9.1.1 Zero order kinetics:-

It describes the system in which the drug release rate is independent of its concentration.

$$\mathbf{Q_t = Q_0 + K_0 t \ldots\ldots (1)}$$

Where, Qt = amount of drug dissolved in time t

Q_0 = initial amount of drug in solution which is often 0

K_0 = zero order release constant

If the release pattern obeys zero order, then the plot of Q_t v/s t will give a straight line with a slope of K_0 and an intercept at 0.

4.9.1.2 First order kinetics:-

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\mathbf{\log Q_t = \log Q_0 + K_t/2.303 \ldots\ldots (2)}$$

Where
Q_t = Amount of drug released in time t
Q_0 = initial amount of drug in the solution
K = first order of kinetic
If the release pattern obeys first order, then the plot of log Q_0-Q_t v/s t will be straight line with a slope of Kt/ 2.303 and an intercept at t = log Q_0.

4.9.1.3 Higuchi's model:-

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$\boxed{\mathbf{Q = KH.t1/2}} \qquad (3)$$

Where
Q = amount of drug released at time t
D = diffusion coefficient of the drug in the matrix
A = total amount of drug in unit volume of matrix
C_s = the solubility of drug in the diffusion medium
e = porosity of matrix.
ṭ = tortuosity
t = time (hrs.) at which Q amount of drug is released.
Equation may be simplified if one may assume that De, Cs and A are constant. Then equation becomes:
$Q = K.t^2$ ……(4)

When the data is plotted according to equation i.e. cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

4.9.1.4 Korsmeyer-peppas model (power law):-

The power law describes the drug release from the polymeric system in which the release deviates from fickian diffusion. It is expressed using following equations;

M_t/ M_α = ktn …….. (4)

Log M_t/M_α = LOG K = NLOG T …….(5)

Where
Mt & M_α = cumulative amounts of drug release at time t at infinity
K = constant incorporating structure and geometrical characteristics of the system
N = exponent determining the mechanism of drug release to characterize the release mechanism, the dissolution data ($M_t/ M_\alpha < 0.6$) are evaluated.
A plot of M_t/ M_α/ v/s log t will be linear with slope n and intercept value of log k. antilog of k gives the value of k. peppas used the n value in order to characterize different release mechanism as shown below

'n' value	**Drug release**
<5	Fickian
0.5<n<1	Non- Fickian
>1	Case 2transport

T**able 3:- Release mechanism**

4.10 Stability study:- The formulation stability study was studied by storing at two different temperature and humidity (Rh 25± 2^0C, 37±2^0C) . They were accepted visually organoleptic properties and all other charactestics are studied.

5.1 Result and discussion:-

Result of preformulation study of the drug:-

5.1.1 Organoleptic properties:-

Organoleptic properties of the Ageratum conyzoides found to be as per I.P. monograph. The organoleptic properties of Ageratum conyzoides were found in given table.

Sr. no.	**Propeties**	**Inferences**
1.	Colour	Green
2.	Odour	odourless
3.	Form	Crystalline granular powder
4.	Taste	Bitter

Table 4:- organoleptic properties of drug

Discussion:- Ageratum conyzoides is typically green in colour. Ageratum conyzoides have an odorless and crystalline form is present. After various aspects of drug formulation is tasteless: this beneficial for patient acceptability. As tasteless drugs are generally easier to administration

Melting point determination:-

Sr. no.	Reference	Obsereved
1	139-140 C^0	139 ^oC

Table 5:- Melting point

Discussion:- the melting of ageratum conyzoides was found to be 139^{o}C shown in table 7. And the reference melting point of drug ageratum conyzoides is 139 -140 ^{o}C which is range of the pure drug. Hence are no any impurities in the drug sample.

Determination of maximum wavelength:-

The wavelength of maximum absorption is distinct for each drug and it is determined using UV spectrophotometer. 10mg of the pure drug was dissolved in 100ml of distilled water then 5ml is pipette out from this solution into 10ml standard flask and made up to the volume . the λmax was determined by using UV spectrophotometer. The UV-Visible absorbance spectra were recorded at the wavelength between 200-600 nm every 1hr interval for the next 5 hours.

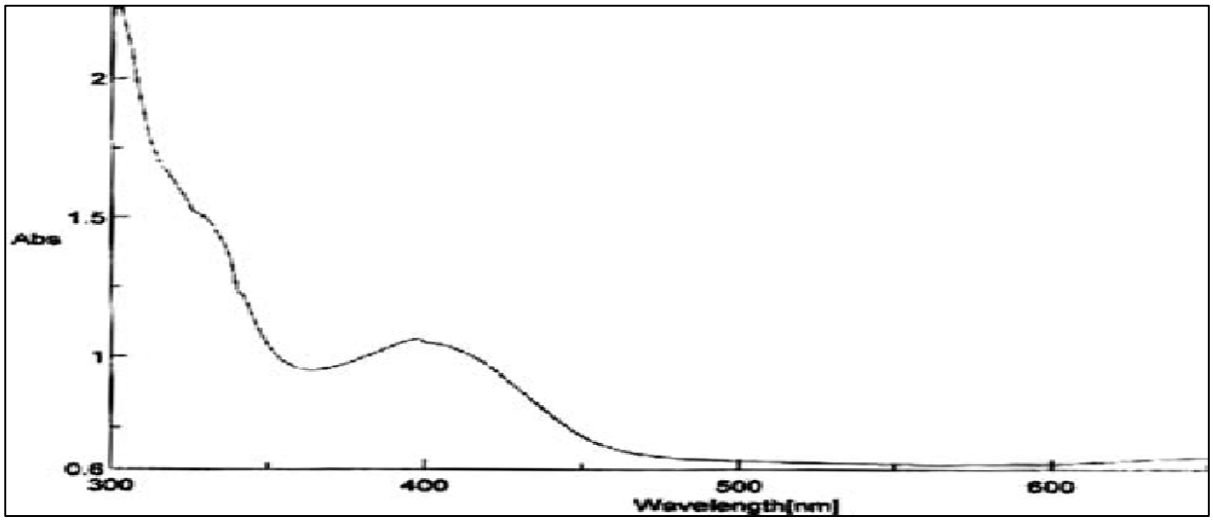

Fig1:- UV spectrum of ageratum conyzoides

Name of Drug	Absorption maxima (λmax)	
	Obsereved	Refernce
Ageratum conyzoides	397nm	400nm

Table 6:- UV absorption (λmax)

Discussion:-

The UV spectrum of Ageratum conyzoides shown absorption peak at 397 nm, indicating significant absorption at this wavelength as shown in table 5.3, while the Reference absorption wavelength is 400 nm, which is similar to observed wavelength. This characteristic indicates the drug sample is pure and has no impurities in the drug sample.

Preparation of standard calibration curve of ageratum conyzoides:-

Calibration curve of ageratum conyzoides:

S.no.	Concentration (ug/ml)	Absorbance at 288 nm
1	5	0.157
2	10	0.314
3	15	0.474
4	20	0.625
5	25	0.790

Table 7:- calibration curve concentration

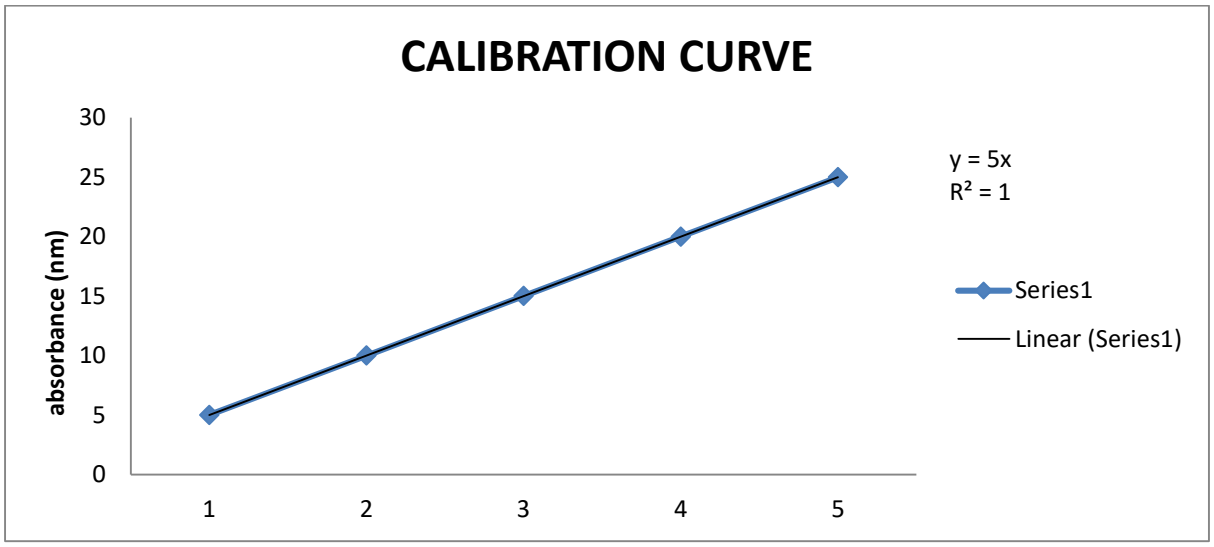

Fig 2:- Calibration curve of ageratum conzoides

Discussion:- the calibtration curve of ageratum conyzoides was obtained using 5 to 25 concentration Of ageratum conyzoides in ethanol. The absorbance was measured at 228nm. The calibration curve of ageratum conyzoides as shown in graph indicated the regression equation y= 5x and R^2= 1, which shows good linearity as shown in the fig no. 25.

5.1.5 Determination of solubility of drug:-

Sr. no.	solvent	solubility (mg/ml)
1	Water	3.65
2	Hydrochloric acid	4.65
3	methanol	2.55
4	ethanol	33.50.

Table 10:- solubility of the drug

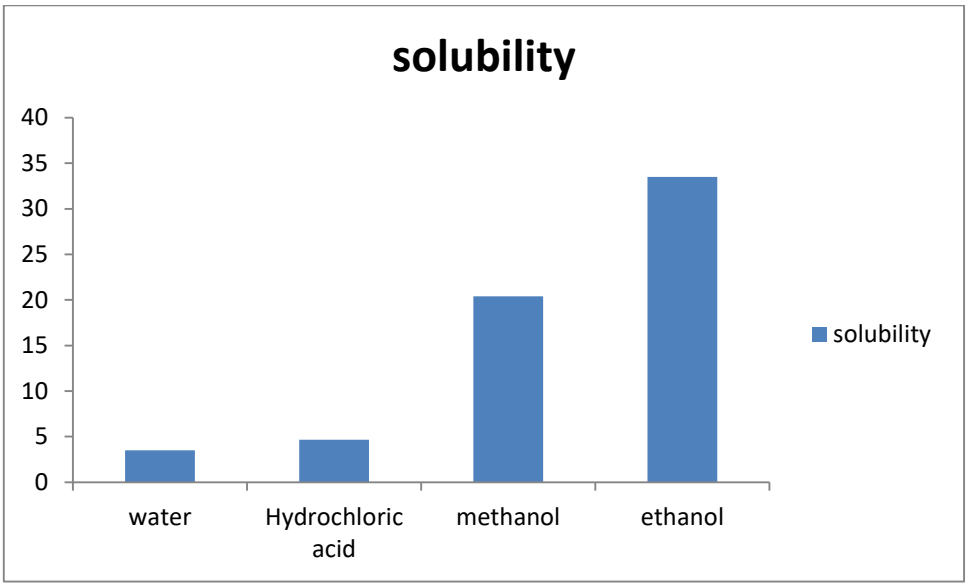

Fig 3:- solubilty of the drug

DISCUSSION:- Ageratum conyzoides exhibits varying solubility across solvents: high in ethanol (33.3), moderate soluble in methanol (20.4), less soluble in hydrochloric acid(4.65), water (3.5), are show in figure no. 26

5.1.6 FTIR studies of Drug:-

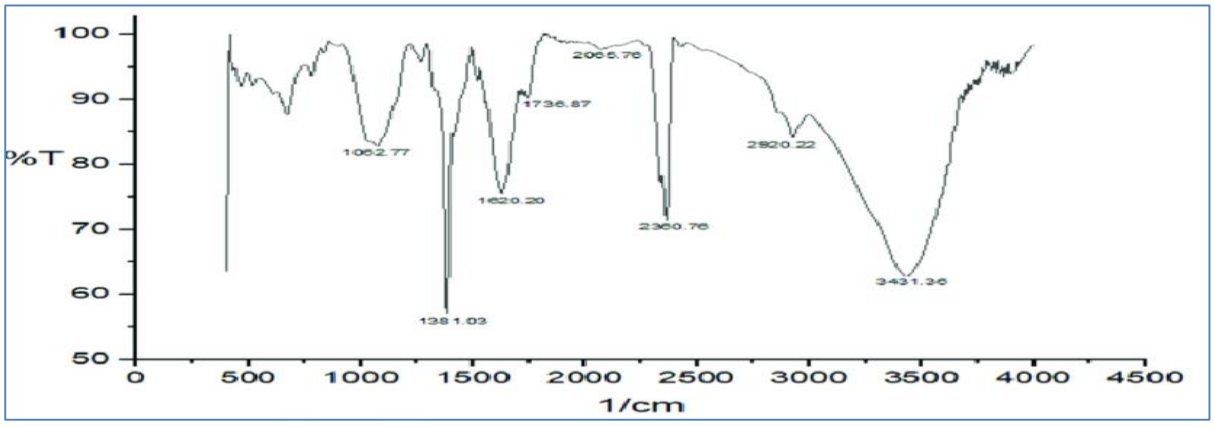

Fig 4:- FTIR of the drug

Functionalgroup	Appearance (intensity)	Compound	Frequency Range	Ageratum
O-H stretch	Strong, broad	Alcohol	3550-3200	3431.36
C-H stretch	Medium	Alkane	3000-2840	2920.22
C-H bending	Weak	Aromatic compound	2000-1650	2065.76
C=C stretch	Strong	Alkene	1648-1638	1736.87

C=C stretch	Medium	Alkene	1662-1626	1620.20
N-O asymmete	Strong	Nitro compound	1550-1475	1500.55
C-C stretch	Medium	Aromatic	1500-1475	1421.40
O-H Bending	Medium	Phenol	1390-1310	1363.28
C-N stretch	Medium	Aliphaticamines	1250-1020	1381.03
S=O stretch	Strong	Sulfoxide	1070-1030	1062.77
C-H Bending	Strong	1,2,3-tridistributed	780±20	902.46 778.22 700.38
C-Br stretch	Strong	Halo compound	690-515	500.23
C-I stretch	Strong	Halo compound	600-500	440.78

Table 8:- FTIR of drug

Discssion:- the FTIR spectrum of the Ageratum conyzoides were shown in the figure 27 and table 11

5.2 Formualtion of ageratum conyzoides:-

Ingredients	**AC 1**	**AC 2**	**AC 3**	**AC 4**	**AC 5**	**AC 6**	**AC 7**
Ageratum conyzoides Powder	1%	1%	1%	1%	1%	1%	1%
Ethanol	30%	30%	30%	30%	30%	30%	30%
Carbopol 940	0.01%	0.02%	0.03%	0.04%	0.01%	0.02%	0.03%
HPMC	0.5%	1%	1.5%	2%	0.5%	1%	1.5%
Propylene glycol	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Methyl paraben	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%

Ingredients	AC 1	AC 2	AC 3	AC 4	AC 5	AC 6	AC 7
Tween 80	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Sodium Dihydrogen Phosphate Dihydrate	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Dimethylsulfoxide	0.001%	0.001%	0.001%	0.001%	0.001%	0.001%	0.001%
3Distilled Water	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100 ml	q.s. 100ml	q.s. 100ml	q.s. 100ml

Table 9:- formulation table AC1-AC7

Zeta potential:-

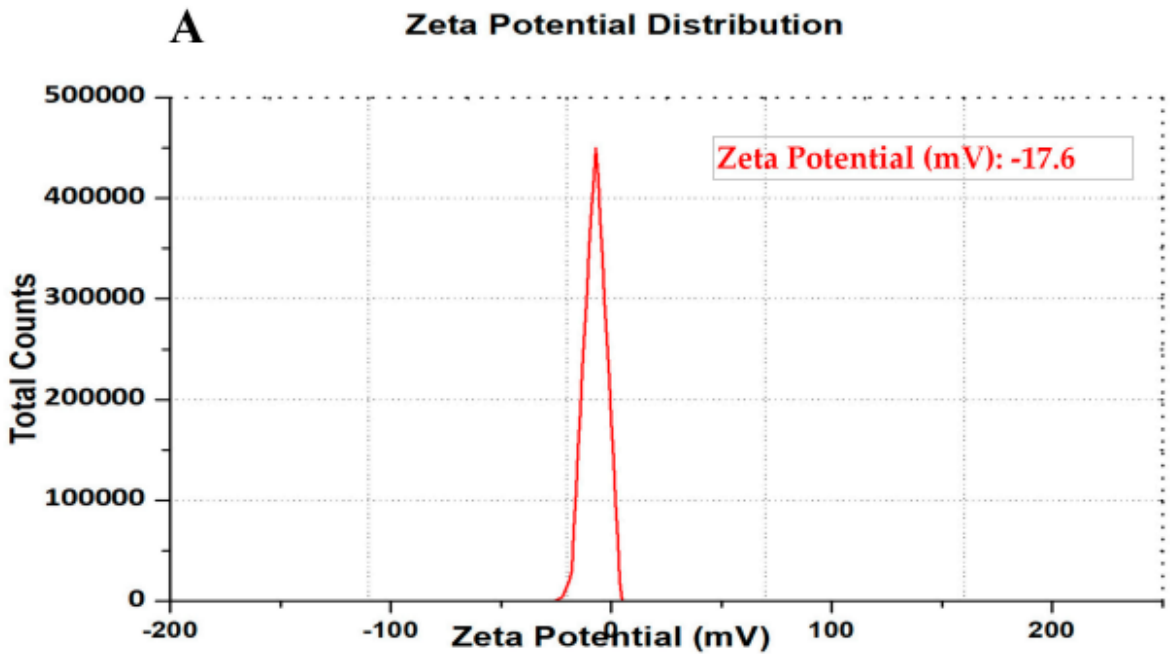

FIGURE 5: -Zeta potential of ageratum conyzoides

Discusion:- demonstrated high zeta potential of AC4 -17.6±1.5 is likely due to a optimal values such as lipid composition, inclusion of charged molecules, the pH and iconic strength of the dispersion medium, presence of stabilizing agent, and the preparation methods used. The type and concentration of lipids used in might leads to a higher surface charge density.

In contrast, while other formulations shows low zeta potential such as in is likely due to suboptimal values such as AC1-16..2±1.8, AC2 -17.2±0.7, AC3 -16.5±0.4, AC5-15.8±1.5, AC6 -16.5±0.4 AC7 -16.7±0.5

The best zeta potential was in the N4 -17.6±1.5 ensures better colloidal stability, reduces risk of aggregation, and contributes to improved performance and longer shelf life of the formulation.

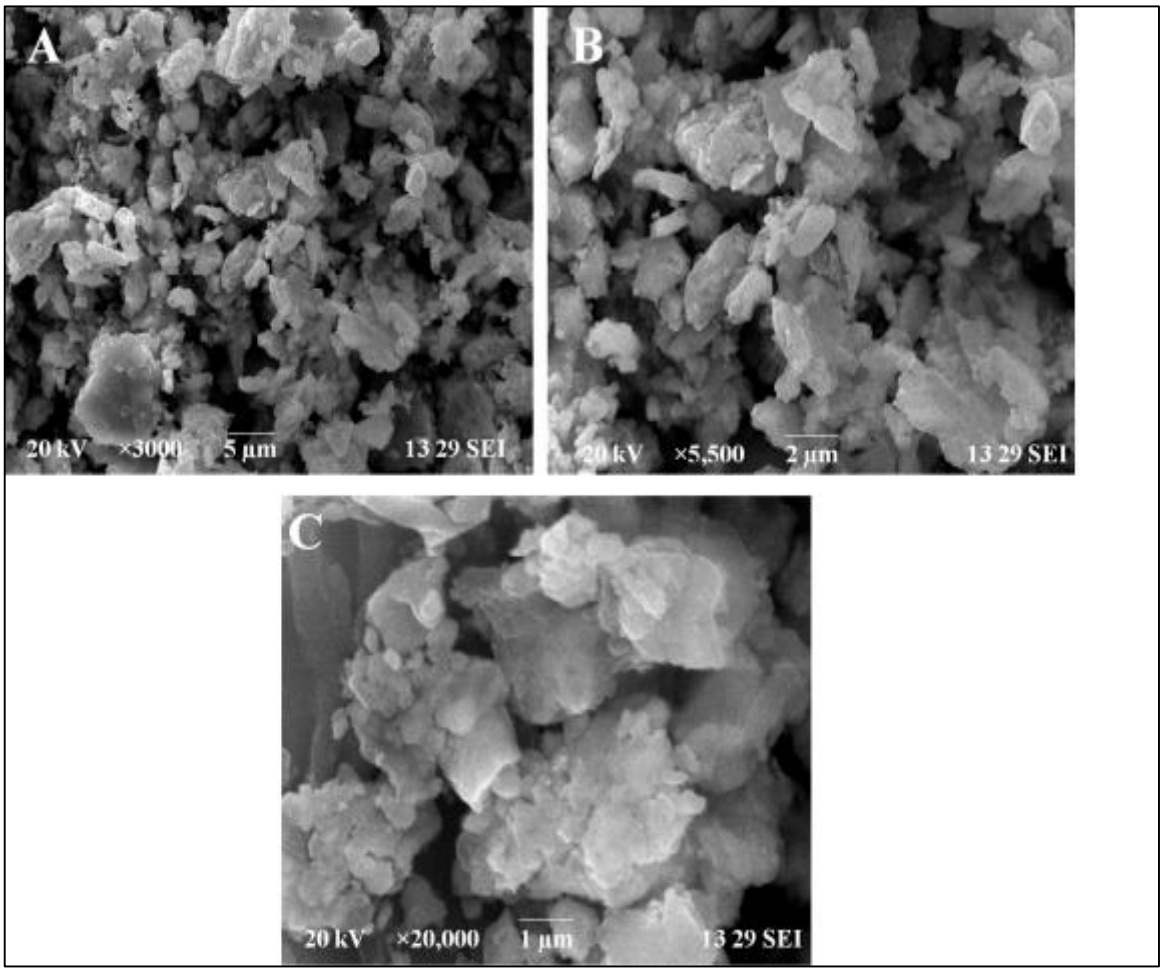

FIGURE 6:- scanning electron microscopic image of Ageratum conyzoides

Discussion:- The AC4 show the proper image of the ageratum conyzoides. The preparation methods for AC4 is probably designed to produce and maintain spherical shape were shown in Figure 28 . The high zeta potential of AC 4 results in strong repulsive force, reducing aggregation . While, the other formulations like AC 1, AC3, AC 5 likely contain an inferior mix of rod like structures. Variation in preparation conditions such as temperature, hydration rate, or lack of sufficient shear forces could lead to rod like structures. Lower zeta potential results in weak repulsive forces , leading to more aggregation and less control over shapes. Hence, The Best Representation of scanning electron microcopy was found in AC 4 which shows proper spherical shape and less aggregation of particles.

5.3 Evaluation of topical spray :-

Evaluation of topical spray for pH, Viscosity, Flame extension and Vapor pressure Spray pattern leak test and drug contents										
Batch no.	**pH**	**Viscosity (cp)**	**Flame extension and flash back**		**Density**	**Vapor pressure (BAR)**	**Spray pattern (cm)**	**Spray angle**	**Leakage test**	**Drug content (%)**
			Flame extension (cm) using	**Flame flash back (cm)**						
AC1	6.7	3.48	48.22	24	0.907	5.33	3.1	19	No	98.44
AC2	6.4	3.77	62.88	12	0.901	5.60	2.6	19	No	99.42
AC3	5.4	4.02	54.36	11	0.902	5.55	2.7	18	No	97.14
AC4	6.8	3.48	44.83	18	0.903	5.33	2	17	No	99.97
AC5	6.12	4.16	69.72	15	0.901	5.53	3.4	12	yes	98.58
AC6	7.12	4.02	50.45	13	0.903	5.55	2.6	19	No	98.32
AC7	6.87	3.48	52.73	16	0.902	5.45	3.1	18	No	98.71

Table 10:- evaluation of spray

discussion:- On the basis of this results , it can be inferred that the ageratum conyzoides topial formulation no. Ac-1. AC-6 pH fall which is out of range and AC-1 AC-3 AC-8 show leakage and AC-7 spray amgle is poor all other formulation show good appearance and passes all the tests with in limits.

5.4 In vitro study of topical spray:- In vitro study of the preparation spray we can prepare 7 formulation of the spray loaded with ageratum conyzoides . then we can study on these all formulation prepare with different value of the excipient then the formulation can show the maximum release of the drug in time. and other formulation are show lower percentage of the drug from its formulation with in particular time. The percentage of drug shown in below table:

Sr. No.	Time(hrs)	AC1	AC2	AC3	AC4	AC5	AC 6	AC 7
1	0	6.25±0.112	7.12 ±0.135	5.45±0.123	4.56±0.122	5.22±0.136	4.25±0.163	6.45±0.112
2	0.25	18.25±0.235	19.26±0.231	23.32±0.555	28.12±0.336	20.11±0.265	20.33±0.564	25.25±0.235

Table 11:- Percentage of drug formulation AC4

In vitro kinetic release:-

time (hrs)	Squar root of time (h) ½	Log time	Cumulative % of drug release	Log cumulative % drug release	Cumulative drug remaining	log Cumulative % of drug ramianing
0	0	0	0	0	0	0
0.25	0.6	-0.601	25.68	0.675	72.30	1.976
0.25	-0.703	-300	27.44	1.434	70.56	1.877
1	1	0	29.66	1.445	75.65	1.754
2	1.45	0.305	35.14	1.454	67.55	1.615
3	1.74	0.455	45.12	1.544	55.44	1.544
4	2.23	0.698	60.55	1.645	35.22	1.96
6	3.25	0.753	63.21	1.742	30.23	1.88
8	3.27	0.855	65.22	1.852	29.11	1.79
10	3.44	1	73.12	1.956	25.41	1.64

Mean±SD, n=3

Table 13:- in vitro kinetic release

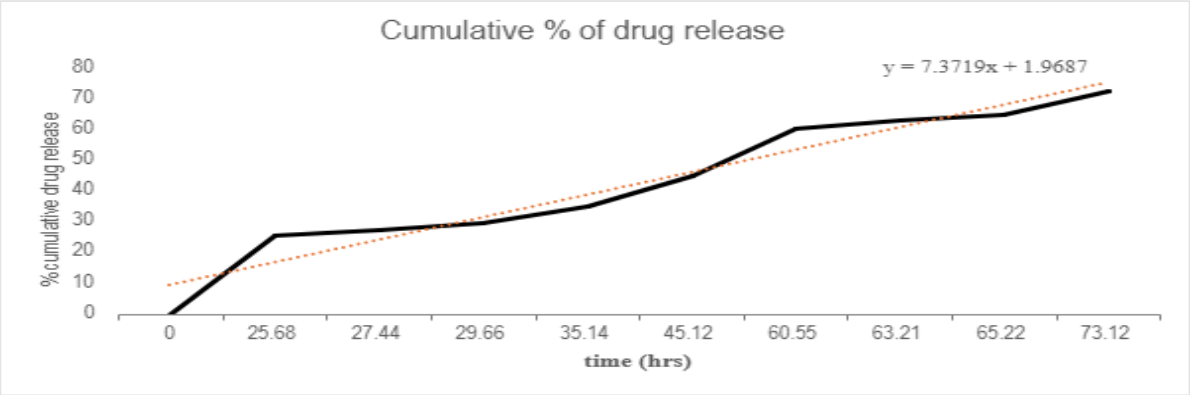

Figure 7:- Zero order of kinetics of formulation AC4

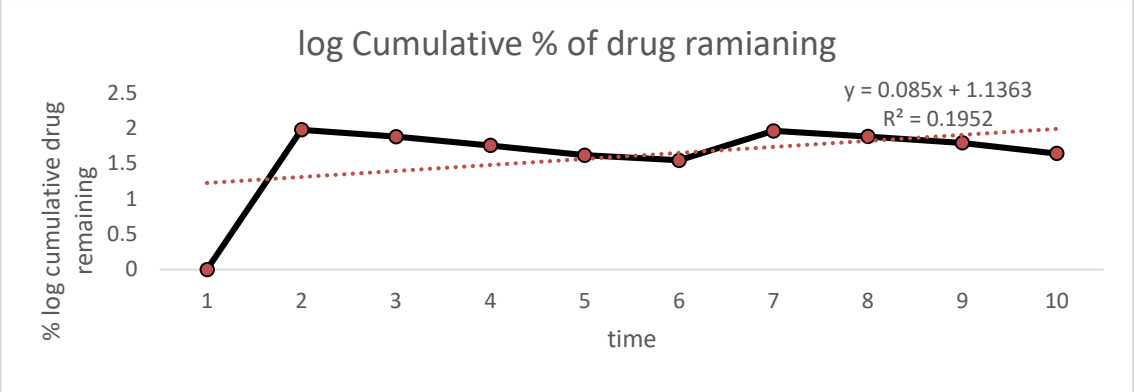

Figure 8 :- First order of kinetic of formulation AC4

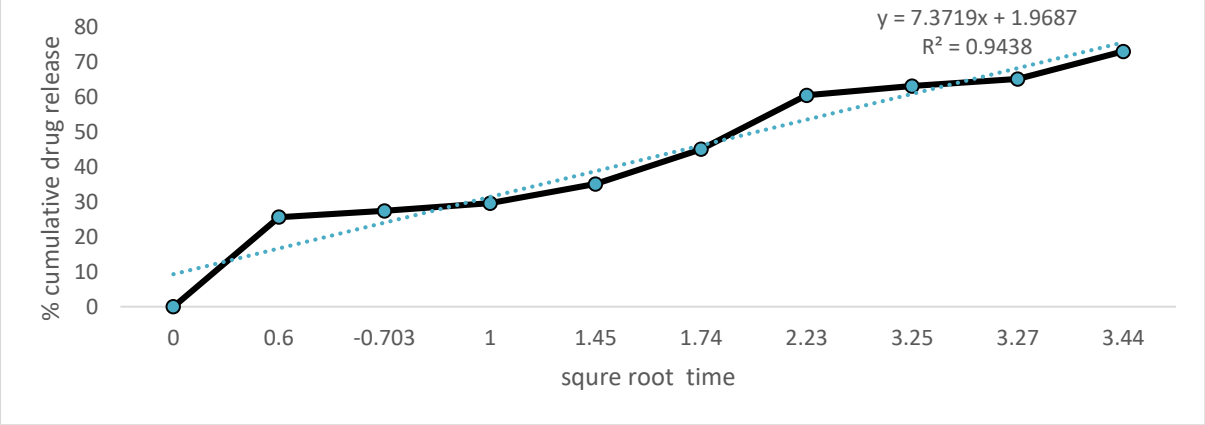

Figure 9 :- Higuchi model of formulation AC4

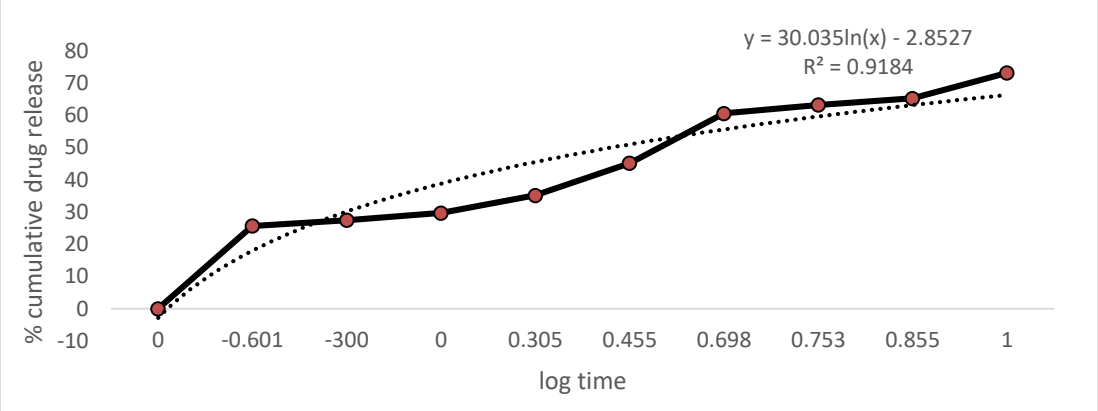

Figure10 :-Korsmeyer- peppas model of formulation AC4

Sr. No.	kinetic model	correlation of coefficient value (R^2)
1.	Zero order	----
2.	First order	0.1952
3.	Higuchi model	0.9438
4.	Korsmeyer- peppas model	0.9184

Table14:- kinetic equation parameter of AC4 formulation

Discussion:- In case R2 value was calculated from the graph and reported in table 16 and figure 35 to 38. Considering the determination cofficients, higuchi model (R^2= 0.9438) to best fit the release data . this demonstrations that ageratum conyzoides were loaded in the spray formulation and the drug was released from the spray by immediate mechanism.

5.5 Stability study of the ageratum conyzoides topical spray:-

Stability testing is done to give proof of how a drug's or product's quality changes over time under the effect of environmental variables including light, humidity, and temperature. The produced topical spray's stability was tested over 180 days at intervals of 30 days. The optimal formulation was maintained at 25±2ºC and 40°C ± 2/RH70% ± 5, and its organoleptic qualities, pH, and drug content were assessed. The formulation was stable, according to the results, and no appreciable modifications were noticed. Studying the stability of topical spray.

Stability Study of topical spray for pH, Viscosity, Flame extension and Vapor pressure Spray pattern leak test and drug contents										
Batch no.	**pH**	**Viscosity (cp)**	**Flame extension and flash back**		**Density**	**Vapor pressure (BAR)**	**Spray pattern (cm)**	**Spray angle**	**Leakage test**	**Drug content (%)**
			Flame extension (cm) using LPG	**Flame flash back (cm) using LPG**						

AC1	6.7	3.48	48.22	24	0.907	5.33	3.1	19	No	98.44
AC2	6.4	3.77	62.88	12	0.901	5.60	2.6	19	No	99.42
AC3	5.4	4.02	54.36	11	0.902	5.55	2.7	18	No	97.14
AC4	6.8	3.48	44.83	18	0.903	5.33	2	17	No	99.97
AC5	7.12	4.02	50.45	13	0.903	5.55	2.6	19	No	98.32
AC6	6.87	3.48	52.73	16	0.902	5.45	3.1	18	No	98.71
AC7	6.12	4.16	69.72	15	0.901	5.33	3.4	12	Yes	98.55

Table 15:- stability study of drug

Table : Stability study of drug content of Spray formulation AC 4

Duration(days)	**Percentage of drug content at 4±2ºC**	**Percentage of drug content at 25±2ºC/65%±5%RH**	**Percentage of drug content at 40±2ºC/75%±5%RH**
0	99.97±0.543	99.97±0.545	99.78±0.119
30	99.97±0.528	99.97±0.524	99.78±0.111
60	99.97±0.509	99.97±0.498	99.78±0.108
90	99.97±0.501	99.97±0.477	99.78±0.102

120	99.95±0.490	99.95±0.465	99.77±0.098
150	99.86±0.461	99.89±0.432	99.76±0.083
180	99.85±0.446	99.85±0.405	99.85±0.072

Table 16:- stability study of the AC 4

Table : Stability study of penetration of spray formulation AC4

Duration(days)	**Penetration time at 4±2ºC**	**Penetration time at 25±2ºC/65%±5%RH**	**Penetration time time at 40±2ºC/75%±5%RH**
0	4.19±0.09	4.19±0.10	4.08±0.10
20	4.19±0.09	4.27±0.09	4.01±0.09
40	4.19±0.09	4.30±0.09	5.00±0.09
60	4.19±0.09	4.33±0.09	5.29±0.07
80	4.19±0.09	4.36±0.8	5.62±0.05
100	4.19±0.09	4.40±0.8	5.69±0.04
120	4.19±0.09	4.44±0.04	5.70±0.02

Table 17:-stablity study of spray AC4

Table : Stability studies of in vitro drug release of drug from spray formulation AC4 at 25ºC

Time (min)	Cumulative drug release at 25±2ºC	
	0days	**180days**
0	0	0
5	30.89 ±0.09	30.89 ±0.09
10	50.9± 0.08	50.9± 0.08
15	60.89± 0.071	60.89± 0.071
20	70.5± 0.065	70.5± 0.065
25	80.01± 0.081	80.01± 0.081
30	94.9± 0.051	94.19± 0.051

Table 18:- stability study comulative release of AC4

Table: Stability studies of in vitro drug release of spray formulation AC4 40±º2C

Time (min)	Cumulative drug release at 40±2ºC	
	0days	**180days**
0	0	0
5	30.89 ±0.09	30.89 ±0.09
10	50.9± 0.08	50.9± 0.08
15	60.89± 0.071	60.89± 0.071
20	70.5± 0.065	70.5± 0.065
25	80.01± 0.081	80.01± 0.081
30	94.9± 0.051	94.9± 0.051

Table 19:-stability study for in vitro release

Discussion:-

The stability study of the formulation spray should be studied the best formulation AC4 shows the best result in stability studies conducted AC4 at different temperatures over period of 180 days revealed robust stability characteristics crucial for its pharmaceutical application. Throughout the study AC4 exhibited no significant changes in visual appearance maintaining its clarity and homogeneity across all conditions. Notably, the formulation demonstrated exceptional drug content stability retaining 99.97% of its initial drug concentration throughout the entire study period. This consistent drug release is indicative of the formulation's ability to provide reliable therapeutic efficacy over time which is essential for its intended use in topical spray formulations. Temperature variation did not adversely affect the stability of AC4, underscoring it resilience to environmental factors. These findings validate the formulation design approach taken and highlight AC4.

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