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FORMULATION, DEVELOPMENT AND EVALUATION OF ANTICANCER POLYHERBAL FORMULATION

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ABSTRACT

Ayurveda provides major information about medicinal plants. But yet a large number of medicinal plants are not screened scientifically for their therapeutic activity against certain diseases. There is a growing demand of the natural sources as they are the major source for active therapeutic agent who has provided many potent and active therapeutic agents to cure various diseases. The present studies indicate the formulation and evaluation herbal tablet formulation. It helps to understand the compression (pre and post) studies of tablet. As well as it is also beneficial to understand the standardization and stability of tablet with suitable excipients.

KEYWORD: Herbal tablet formulation, Development, Evaluation, Standardization and stability study of tablets.

INTRODUCTION

Herbal formulation shall mean a dosage form consisting of one or more herbs or processed herb(s) in specified quantities to provide specific nutritional, cosmetic benefits, and/or other benefits meant for use to diagnose, treat, and mitigate diseases of human beings or animals and/or to alter the structure or physiology of human beings or animals.

Herbal dosage form can be categorized into two types as follows [1, 2, 3]:

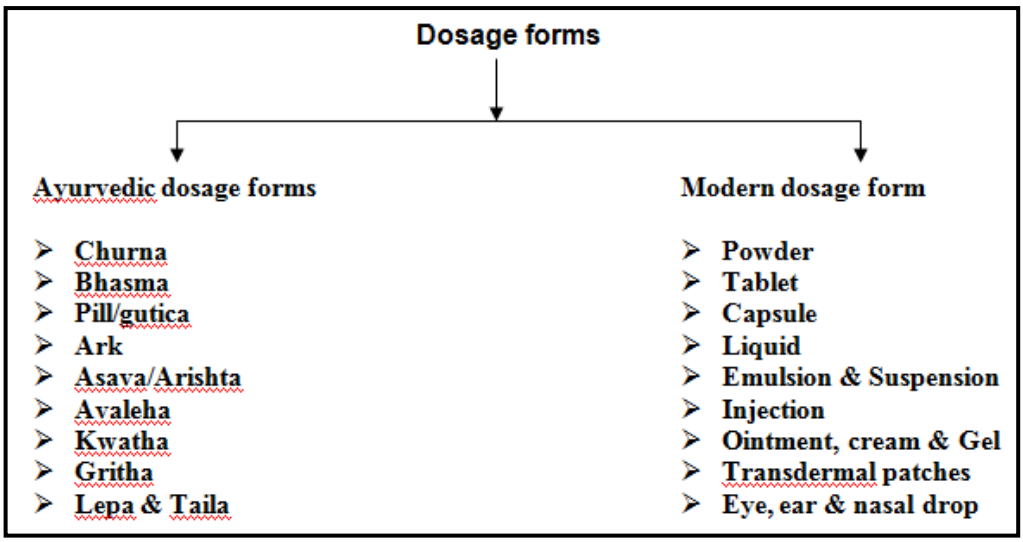


Figure No. 1: Different Dosage Forms

For non-cytotoxic drugs the oral route is most commonly used because of its convenience and, for most drugs, efficiency of absorption. Oral formulation is to be preferred in the first place for its convenience and its potential to improve patient quality of life. In addition, cytostatic therapies that require protracted drug administration may be facilitated by an oral formulation. Finally, from economic perspectives oral administration is attractive, as it eliminates the costs for hospitalization and infusion equipment supplies. Despite these obvious advantages only few cytostatics are used by the oral route. Patient non-compliance and insufficient bioavailability are noted as limitations of oral treatment strategies. Many anti-tumour agents have poor bioavailability as a result of limited aqueous solubility, degradation in gastro-intestinal fluids and/or affinity for intestinal and liver cytochrome P450 (CYP3A4) and P-glycoprotein (P-gp), which serve to protect the body from xenobiotics. The substantial interpatient variability in bioavailability after oral administration represents another major limitation. The feasibility of an oral anticancer agent is dependent primarily on its oral bioavailability and its local toxicity. Optimizing oral formulation therefore means overcoming bioavailability constraints due to intestinal drug transporting and/or metabolizing systems, such as CYP3A4 and P-gp. Co-administration of specific inhibitors of these systems may be an important progress in this setting, resulting in increased bioavailability of otherwise poorly absorbed agents.

2. MATERIAL AND METHODS:

2.1 Preparation of Tablets:

2.1.1 Material Used in the preparations:

The following materials that were either AR/LR grade or the best possible grade available were used as supplied by the manufacturer without further purification or investigation.

Table No. 1: Materials used in the preparation.

Name	Supplier of Material
Isopropyl alcohol	Qualigens Fine Chemicals, Mumbai
Microcrystalline Cellulose pH 102	Alkem Labs Ltd. Mumbai
Magnesium stearate	Loba Chem. Ltd, Mumbai.

2.1.2 Equipment and instruments used in the preparations:

Table No. 2: Equipment and instruments used in the preparation.

Sr. No.	Name	Manufacturer
1.	Electronic Balance	Jupitor Scientific Co. Tamil Nadu
2.	FTIR 8300 Spectrometer	Shimadzu Scientific Instruments, Mumbai.
3.	Hot air oven	Kumar Industries, Bombay
4.	Single punch tablet machine	Labpress MEKCIPLAB Enterprises
5.	Friability Test Apparatus	Electrolab Enterprises, Mumbai
6.	Disintegration test apparatus	Electrolab Enterprises, Mumbai
7.	Vernier caliper	Jashbin Enterprises, Mumbai

2.1.3 Preformulation Studies:

Preformulation is defined as phase of research and development process where physical, chemical and mechanical properties of a new drug substance are characterized alone and when combined with excipients, in order to develop stable, safe and effective dosage form.

A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design, or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development. Hence, preformulation studies on the obtained sample of drug for identification and compatibility studies were performed[4].

Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy studies and HPTLC were used for the evaluation of physicochemical compatibility and interactions, which helps in the prediction of interaction of the drug with polymers, diluents and lubricants used in case tablet formulations. Positive interactions sometimes have a beneficial effect as far as desired release parameters are concerned. The earlier investigations recommended that the ratio of drug to excipients used in study was 1:5 for diluents, 3:1 for binders or disintegrants, 5:1 for lubricants and 10:1 for colorants etc, but it is observed that 1:1 ratio of drug excipients maximizes the possibility of interaction and helps in easier detection of incompatibilities. Therefore, in the present study 1:1 ratio was used for preparation of physical mixtures and analyzed for compatibility studies[5].

2.1.4 Fourier transform infrared (FTIR):

FTIR studies are very helpful in the evaluation of drug–polymer interaction studies. If there is any incompatibility between the drugs and excipients, these can be predicted by changes in the functional peaks (characteristic wave numbers).

Diffuse reflectance technique was used (400 to 4000 cm^{-1}), drug and various polymers were thoroughly mixed with 300 mg of potassium bromide, compressed and the spectrum was obtained by placing the thin pellet in light path[6].

2.2 Formulation and Preparation of Tablet 6:

Two methods may be used to disperse drug and additives in the retardant base.

2.2.1 Solvent Evaporation Technique:

In which a solution or dispersion of drug and additives is incorporated. The solvent is removed by evaporation.

2.2.2 Compression Technique:

This involves the compression of granules, which may be prepared by wet granulation or by granulation technique or direct compression of a blend of drug release retardant material and other additives.

Many processing steps have been eliminated in the manufacture of some pharmaceutical tablets as a result of the development of directly compressible excipients, wherein powdered drug can be directly mixed with the excipients and then immediately compressed into a finished tablet. This is the method of choice in tablet manufacture, where the process may be employed to produce a high quality finished product. Direct compression offers the most expeditious method of manufacturing tablets because it utilizes the least handling of materials, involves no drying step and is thus the most energy-efficient method, and is also the fastest. This is most economical method of tablet production. The direct-compression processing technique offers simplicity, economy, and the potential for high-volume output. Equipment considerations are minimal, since typically only mixing and compression equipment are required. Facilities designed exclusively for this type of processing can concentrate on efficient methods for bulk granulation processing and transfer to dosage-form production. Control of the physical characteristics of excipients if the oral solid dosage form is made by direct compression can also be important.

2.2.3 Formulation of Tablets:

The composition of a tablet core containing plant extracts (in varying concentration) are shown in Table 12. The plant extracts dispersed in isopropyl alcohol evenly. Microcrystalline cellulose and magnesium stearate were passed through sieve no. 80 separately. Four different formulations with various concentrations were prepared by keeping the amount of microcrystalline cellulose at 150 mg and magnesium stearate at 1%. Microcrystalline cellulose is added in the dispersed extracts. The granules were lubricated with magnesium stearate and compressed on a 12-station tablet machine in which only 1 station were used, weighing 400 mg using 10 mm flat punches to a hardness of 3.5-7 Kg/ cm^2. Tableting was performed under a compression force of 1 tone using Hydraulic press. A batch of 50 tablets was prepared for all formulations.

Figure No. 2: Tablet Compression Machine.

Table No. 3: Composition of tablet (400 mg)

Name of Components (mg)	**F1**	**F2**	**F3**	**F4**
A. squamosa extract	1.94	1.94	1.94	1.94
A. indica extract	39.6	79.2	39.6	39.6
C. longa extract	79.2	39.6	39.6	39.6
E. officinalis extract	39.6	39.6	79.2	39.6
M. oleifera extract	39.6	39.6	39.6	79.2
Microcrystalline cellulose	195	195	195	195
Magnesium stearate	1%	1%	1%	1%

2.3 Evaluation of Tablet:

2.3.1 Pre-Compression Parameters [7, 8]:

The methods to measure certain granulation characteristics have been developed to monitor granulation suitability for tableting.

Good flow properties are essential for the transport of the material through the hopper into and through the feed frame and into the dies.

2.3.1.1 Angle of repose:

Angle of repose is defined as the maximum angle possible between the surface of the pile of powder and the horizontal plane.

The angle of repose is designated by θ and given by equation:

$$\text{Tan } \theta = r/h \text{ or } \theta = \text{Tan}^{-1}\, r/h$$

Where;

h = height of the pile, cm.

r = radius of the base of the pile, cm.

The lower the angle of repose, better the flow properties, when granules are placed

in the hopper and allowed to slide down into the die for compression. It forms a pile. The angle of repose may be calculated by measuring the height (h) of the pile and the radius of the base (r) with ruler. Different ranges of flowability in terms of angle of repose are given in Table No.4.

Table No. 4: Evaluation of Flowability.

Sr. No.	Flowability	Angle of Repose
1.	Excellent	$25\text{-}30^0$
2.	Good	$30\text{-}35^0$
3.	Fair	$35\text{-}37^0$
4.	Poor	$37\text{-}45^0$
5.	Very Poor	Above 45^0

Application:

During tableting, improper flow of granules from the hopper leads to under-fill or over-fill in the die cavity. As a result, tablets will have under-weight or over weight, weight variation further affects the content uniformity and dose precision. It also creates problems of hardness and friability during compression.

2.3.1.2 Bulk Density and tapped density[9]:

The Bulk density denotes the total density of the materials as it exists. The bulk volume includes the true volume. Volume of inter-particle spaces and intra particle pores. The bulk density of a powder depends primarily on particle size distribution, particle shape, and the tendency of the particles to adhere to one another.

Method:

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2 gm of powder from each formula, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulas:

$$\text{LBD} = \frac{\text{Weight of the Powder}}{\text{Volume of the packing}}$$

$$\text{TBD} = \frac{\text{Weight of the powder}}{\text{Tapped volume of the packing}}$$

Application: Bulk density property is used in-

- Checking the uniformity of bulk chemicals.
- Selecting the size of container, mixing apparatus for the production.
- Determining the proper size of the packing material

$$\text{Hausner ratio} = \frac{\text{TBD}}{\text{LBD}}$$

2.3.1.3 Compressibility Index:

Whether the powder is porous or non porous, the total porosity expression for the calculation remains the same. The compressibility index of the granules was determined by Carr's compressibility index. Different ranges of flowability in terms of Carr's index are given in Table No.5.

$$\text{Carr's Index (\%)} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100$$

Table No. 5: Evaluation of Flowability.

Sr. No.	Carr's index	Type of Flow
1.	5-15	Excellent
2.	12-16	Good
3.	18-21	Fair
4.	23-25	Poor
5.	33-38	Very Poor

2.3.1.4 Hausner ratio:

It is another parameter to check compressibility of powder. Different ranges of flowability in terms of Hausner ratio are given in Table No. 6. Hausner ratio was determined by following formula:

Table No. 6: Evaluation of Flowability

Ratio	Interpretation	Equivalent to carr's index
1.25	Good flow	20%
>1.25	Poor flow	33%

Applications:

Compressibility index provide information about hardness, disintegration, tablet porosity etc., there dissolution and release of drugs.

2.3.2 Post-Compression Parameters[10, 11, 12]:

2.3.2.1 General appearance of Tablets:

Tablets were examined under a lens for the shape and color of the tablet, its overall elegance, uniformity, consistency, surface texture, odor, taste, etc.

2.3.2.2 Thickness and Diameter Test:-

Thickness and diameter test permits accurate measurement and provides information on the variation between tablets. Ten tablets were taken and the thickness and diameter was measured using a Vernier caliper. The tablet thickness and diameter should be controlled within a ± 5% variation of a standard value.

2.3.2.3 Weight Variation Test:

The USP weight variation test is run by weighing 20 tablets individually. Calculating the average weight and comparing the individual tablet weight to the average. The tablet meet the USP test, if not more than 2 tablets are outside the

percentage limit and if no tablet differs by more than 2 times the percentage limit.

Table No. 7: Weight variation tolerance for uncoated tablets

Average weight of tablet (mg.)	Maximum percentage difference allowed
130 or Less	10
130 – 324	7.5
More than 324	5

2.3.2.4 Hardness Test:

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto Hardness Tester. The force needed to disrupt them by crushing in kg/cm^2 expresses it. Six tablets were randomly picked from each formulation and the mean and standard deviation values were calculated.

2.3.2.5 Friability Test for uncoated tablets:

It is the phenomenon whereby tablet surfaces are damaged and/or show evidence of lamination or breakage when subjected to mechanical shock or attrition. The friability of tablets was determined using Electrolab Friabilator. It is expressed in percentage (%). Six tablets were initially weighed ($W_{initial}$) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W_{final}). The % friability was then calculated by:

$$F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

% friability of tablets less than 1% are considered acceptable.

2.3.2.6 Disintegration Test for uncoated tablets:

This test determines whether tablets disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions. Disintegration is defined as that state in which no residue of the tablet or capsule remains on the screen of the apparatus or, if a residue remains, it consists of fragments of insoluble coating of the tablets or of capsule shells or is a soft mass with no palpable core.

Procedure:

One tablet was introduced into each tube and, a disc was added to each tube. The assembly was suspended in the beaker containing distilled water and the apparatus was operated for the specified time. Temperature of the liquid was maintained the at 37° ±1°C. When all six tablets have disintegrated time is measured.

2.3.3 Standardization of Formulation[13]:

In recent years, there has been great demand for plant derived products in developed countries. Due to lack of infrastructures, skilled manpower, reliable methods and stringent regulatory laws most of these manufacturers produce their product on very tentative basis.

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable,

specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization is an essential measurement for ensuring the quality control of the herbal drugs. "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also means adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations. "Evaluation" of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration.

Standardization of herbal drugs is not an easy task as numerous factors influence the bioefficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and to rationalize the combination in case of polyherbal drugs.

The herbal formulation in general can be standardize schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. After all the routine physical, chemical and pharmacological parameters are to be checked for all the batches to select the final finished product and to validate the whole manufacturing process.

2.3.3.1 HPTLC Method[14, 15]:

Traditionally TLC has been widely used for the analysis of medicinal plants and it's included as a method for identification in monograph of herbal drugs in most pharmacopoeias throughout the world. The HPTLC plates are prepared from optimized adsorbent layers and extremely even surfaces. These plates offer greater separation efficiency through smaller plate heights than the conventional TLC plates. Shorter analysis time, detection limits in the nanogram range with UV absorption detection and in the picogram range with fluorometric detection are some additional advantages with these plates. The greater efficiency of these plates demonstrated by fact that they can provide typically about 4,000 theoretical plates over a distance of 3 cm in 10 minutes compared with 2,000 theoretical plates over 12 cm in 25 minutes for conventional TLC plates.

Steps involved in HPTLC:

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer prewashing
4. Layer preconditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spot
8. Scanning
9. Documentation of chromatic plate

Advantages of HPTLC:

1. Lower analysis time
2. Low maintenance cost
3. Simple sample preparation
4. No prior treatments for solvents like filtration and degassing
5. Low mobile phase consumption per sample
6. Visual detection possible
7. No interference from previous analysis – no contamination

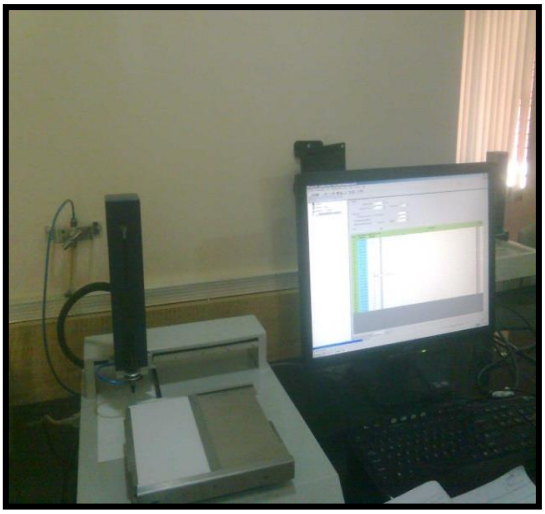


Figure No. 3: sample applicator

Figure No. 4: UV Chamber

Chemicals and reagents used:

Curcumin and Gallic acid standard were procured from SD Fine Chemical Ltd. and Thomas Baker (Chemical) Pvt. Ltd. Silica gel $60F_{254}$ TLC plates (20x10 cm, layer thickness 0.2 mm, E. Merck) were used as a stationary phase. All chemicals and reagents were of analytical grade and obtained from Qualigens Fine chemicals. Prepared formulation F_3 was used for analysis.

Instruments used:

The instruments used in the present study were Camag HPTLC system comprising of Camag Linomat V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC scanner 3, Camag WinCATS software, Camag Twin trough chamber (20x10 cm). Ultrasonicator was used for extraction of the drugs from the tablets.

Preparation of standard solution:

10 mg of each Curcumin and Gallic acid were weighed separately transferred in two different 10ml volumetric flasks. Both the drugs were dissolved in 5 ml of methanol solvent by vigorous shaking and then volume was made up to mark with methanol to obtained final concentration of 1 mg/ml of each component. Out of that 0.5 ml pipette out and transferred to 10 ml volumetric flask and volume was made up to mark with methanol to get 50 ng/ml solution.

Application of standard solution:

Linomat, Automatic TLC Sampler (ATS4), Samples are applied as bands by spray-on technique using following parameters:

Distance from lower edge of plate in cm for TTC	08
Minimum distance from left and right edge of plate in mm	10
Minimum space in mm between bands / spot* in mm	04
Band length in mm	08

Neither Linomate 5 nor ATS4 allows programming of distance between bands/spot. Therefore distance between tracks (center to center) and band length

must be chosen in order to meet the minimum distance requirements. For spot application volume and application speed have to be determined empirically.

Preparation of developing solvent:

Developing solvent consisting of more than one component are prepared by measuring the required volume of chloroform(7.5 ml),ethyl acetate (6 ml),formic acid (0.5 ml) and transferred into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content.

Development of plate:

Plates are developed in a saturated Twin Trough Chamber according to the following procedure:

The appropriate volume (20 ml for 20x10 cm TTC) of developing solvent was prepared. The chamber was opened and correctly sized (20 x10 cm) piece of filter paper was placed in rear trough. The developing solvent was poured in chamber so that the filter paper thoroughly wetted and adheres to rear wall of TTC. The chamber was tilted on the side (about 45^0) so that all solvent volume in both troughs equalizes. Then the chamber was set on the bench, the lid was replaced and chamber was allowed to equilibrate for 5 min. The desired developing distance (60 mm from lower edge of the plate) was marked with a pencil on the right edge of the plate. Then the lid of the plate was slide off the side. The plate was inserted into front trough.

They should face the filter paper and the back of the plate is resting against front wall of TTC. The lid was replaced. The plate was developed to the mark. The lid was opened and the plate was removed. Then the plate was dried (vertically in the direction of chromatography) 5 min. in a stream of cold air. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber is dried and, if necessary also cleaned.

Development of plates:

Each plate was developed under UV 254 nm for both the drugs.

Labeling plates:

Each plate is given an individual identification number (ID), which will be written in pencil in the top right corner. The ID includes project number, dash, year, month, day, dash and a consecutive number each day.

Quantitative evaluation:

The quantitative evaluation was performed with the TLC Scanner 3 using WinCATS software. The analysis files are labeled to reflect the plate ID and any additional descriptive information if multiple evaluations under different condition are performed.

2.3.3.2 Method Validation [16, 17]:

This method was validated as per the ICH guidelines. The method validation parameters checked were linearity, precision, and reproducibility, limit of detection and limit of quantification.

Calibration curves of Curcumin and Gallic acid:

A stock solution of curcumin and gallic acid (1mg/ml) was prepared in methanol. Different volumes of stock solution, 2, 4,6,8,10,12 and 14 were spotted in duplicate on TLC plate to obtain concentrations of 300,400,500,600 and700 ng per spot of curcumin and 100,200,300,400,500,600 and 700 ng per spot of gallic acid, respectively. The data of peak height/area versus drug concentration were treated by linear least-square regression.

Precision:

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (100 ng per spot of curcumin and gallic acid). The samples were analyzed on same day and on the next day.

Limit of Detection and Limit of Quantification:

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was calculated using the following formula:

$$LOD = \frac{3.3 \times \text{Standard Deviation of the}}{\text{Y-intercept}}$$

The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOQ was calculated using the following formula:

$$LOQ = \frac{10 \times \text{Standard Deviation of the}}{\text{Y-intercept}}$$

Reproducibility:

As per ICH guideline reproducability of sample application and measurement of peak area were carried out using sixteen replicates of the same spot (100 ng per spot of curcumin and gallic acid).

Tablet Analysis:

Twenty tablets were weighed accurately and ground to fine powder and dissolved in 100 ml Methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatman filter paper No. 41 and transferred to 10 ml volumetric flask and volume was made up to 10 ml with Methanol. Required dilutions were made to get desired concentrations of curcumin and gallic acid.

Recovery studies:

The accuracy of proposed method was evaluated by addition of standard drug solution to pre-analysed tablet sample solution at three different concentration level at 80, 100 and 120% of linearity of both the drug.

2.3.4 Stability Studies [18, 19]:

Stability of a drug has been defined as the ability of a particular formulation, in a specific container to remain within its physical, chemical, therapeutic and toxicological specifications.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions, retest periods and shelf life to be established.

ICH specifies the length of study and storage conditions:

Long term testing 25^{o}C ±2^{o}C / 60 % RH±5 % for 12 months

Accelerated testing 40^{o}C ±2^{o}C / 75 % RH±5 % for 3 months

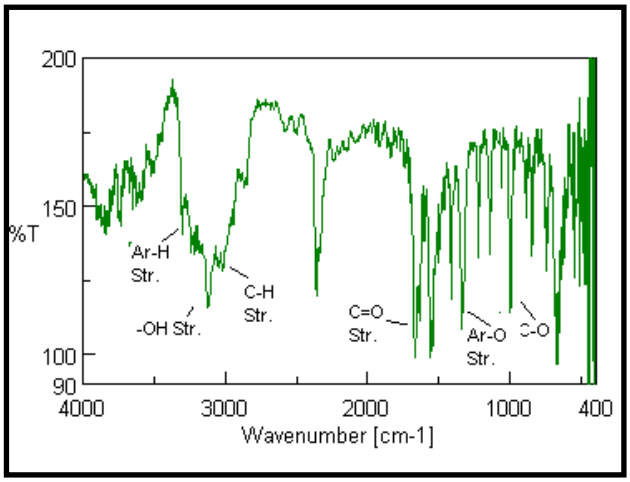
In the present study, stability studies were carried out at 25^{o}C / 60 % RH and 40^{o}C / 75 % RH for a specific time period up to 30 days for all formulations.

3. RESULT AND DISCUSSION:

3.1 Preformulation Studies:

3.1.1 Compatibility Studies:

Drug excipient compatibility studies were carried out prior to the preparation of tablet, to check whether any compatibility related problems are associated between drug and excipients used in the formulations. While no new bands or shift in characteristic peaks appeared, thus indicating compatibility between drug and excipients. IR spectra are shown below:



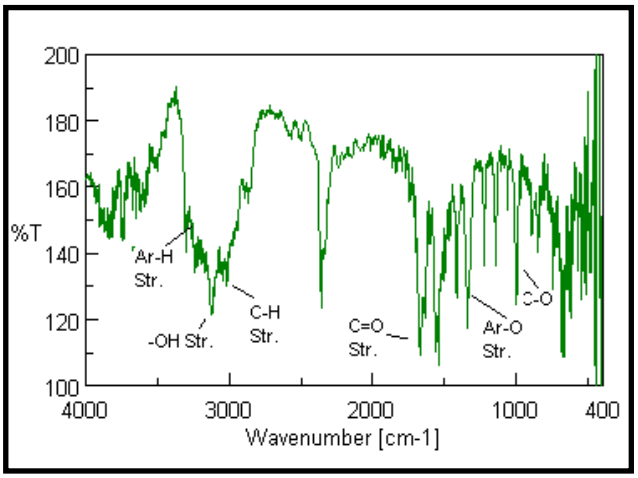
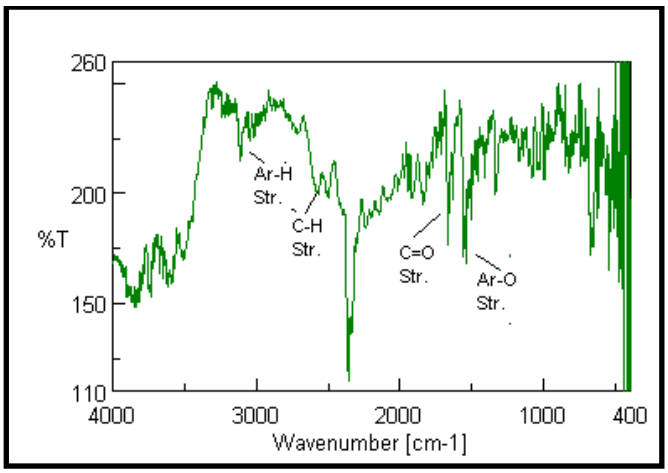


Figure No. 5&6 IR spectra of *A. squamosa* & *A. indica*: MCC



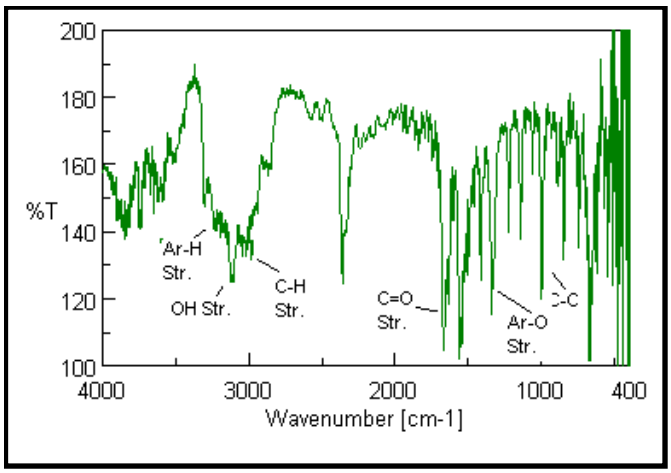


Figure No. 7&8 IR spectra of *C. longa* & *E. officinalis*: MCC

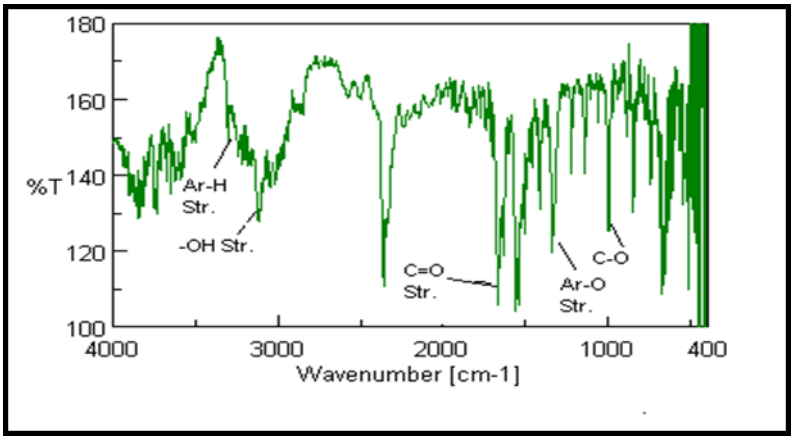


Figure No. 8 IR spectra of *M. oleifera*: MCC

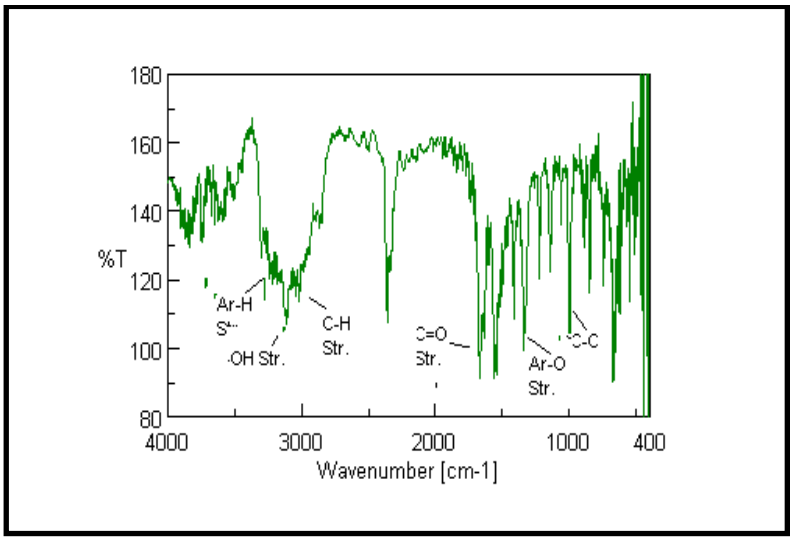


Figure No. 9 IR spectra of Formulation

3.2 Evaluation of Tablet:

3.2.1 Pre-Compression Parameters:

3.2.1.1 Angle of Repose:

The results for angle of repose are recorded in Table No. 8. As mentioned earlier, if the granules shows angle of repose values between 25-30° they are considered as excellent flowability granules and if they shows the values between 30-35°, then granules are of good flowability. The powder mix of F3 formulation is found to have excellent flow property. The angle of repose calculated is 27.14°.

3.2.1.2 Bulk Density:

The granules of F3 formulations were evaluated for Loose Bulk Density (LBD) and Tapped Bulk Density (TBD). The results of LBD and TBD are found to be 0.275±0.0005% and 0.317±0.004% respectively. The results are recorded in Table No. 8.

3.2.1.3 Compressibility Index:

The result of compressibility index (%) is found to be 13.16±0.92% while Hausner ratio is found to be 1.149±0.01.The direct compressible granulations have shown excellent compressibility index values ranging around 13.16±0.92%. Generally compressibility index values up to 15%

result in good to excellent flow properties.
The results are recorded in Table No.8.

Table No. 8: Pre-Compression Parameters of F3

Sr. No.	Angle of Repose	LBD (gm/ml)	TBD (gm/ml)	Carr's Index (%)	Hausner ratio
1.	28.81	0.275	0.314	12.4	1.14
2.	27.92	0.276	0.322	14.2	1.16
3.	24.70	0.276	0.317	12.9	1.148
Mean ± SD	**27.14±2.16**	**0.275±0.0005**	**0.317±0.004**	**13.16±0.92**	**1.149±0.01**

3.2.2 Post-Compression Parameters:

3.2.2.1 General Appearance of Tablets:

Randomly selected tablets from batch examined under lens showed circular shape and yellowish green color for F3 formulations. There were little or no manufacturing defects in the tablets. Surface was elegant, smooth, and uniform.

3.2.2.2 Thickness and Diameter Test:

The results of thickness and diameter in mm for uncoated tablets are shown in Table No. 9. Tablet mean thickness (n=4) were almost uniform in the formulations. Tablet mean diameter (n=10) were also found to be uniform in F3 formulations.

3.2.2.3 Weight Variation Test:

The weight variation for the formulations is shown in Table No.9. All the tablets passed weight variation test as the average % weight variation was within the pharmacopoeial limits of ±5%. The weight of all the tablets was found to be uniform with low standard deviation values.

3.2.2.4 Hardness Test:

The hardness of all the tablets was maintained within 3.5 to 7 kg/cm^2. The mean hardness values (n=6) were measured for all the formulations using a Monsanto hardness tester. The results are tabulated in Table No. 9. Uniformity is depicted from low standard deviation.

3.2.2.5 Friability Test:

Another measure of tablet's strength is friability. The values of friability test are given in Table No. 9. The percent friability for the formulation was below 1%, indicating that the friability is within the prescribed limits. The results of friability test indicate that the tablets possess good mechanical strength.

3.2.2.6 *In vitro* Disintegration Studies:

The uncoated tablets are when subjected for disintegration test, found to be complying with compendial specifications. Table No. 9 shows the results of disintegration test.

Table No. 9: Post-Compression Parameters of F3

Sr. No.	Diameter in mm	Thickness in mm	Weight In mg/tab	Hardness in kg	Friability %	Disintegration Time (in seconds)
1.	10	04	0.399±0.005	4.1±0.2	0.501	10.98±0.045

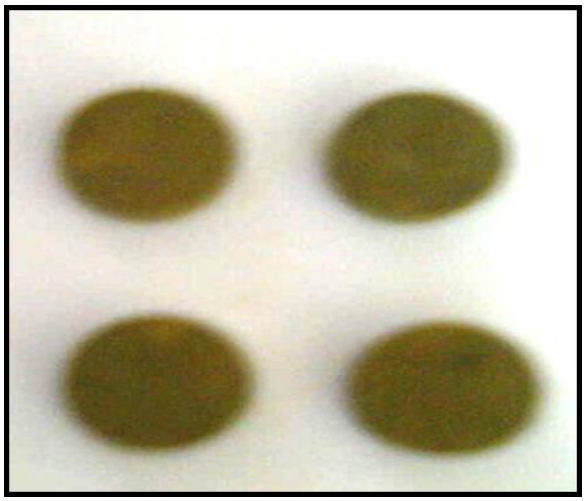
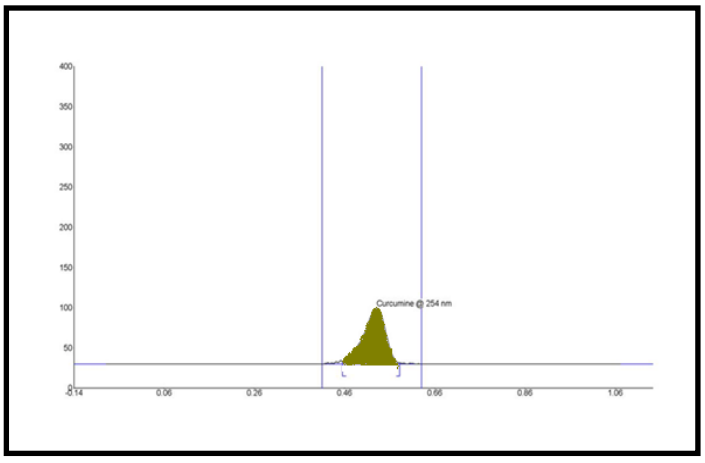


Figure No. 10 F3 Formulation

3.2.3 Standardization of formulation:

3.2.3.1 Calibration curves of curcumin:

The linear regression data for the calibration curves (*n*=3) as shown in Table 10 showed a good linear relationship over the concentration range 300-700 ng per spot with respect to peak area.



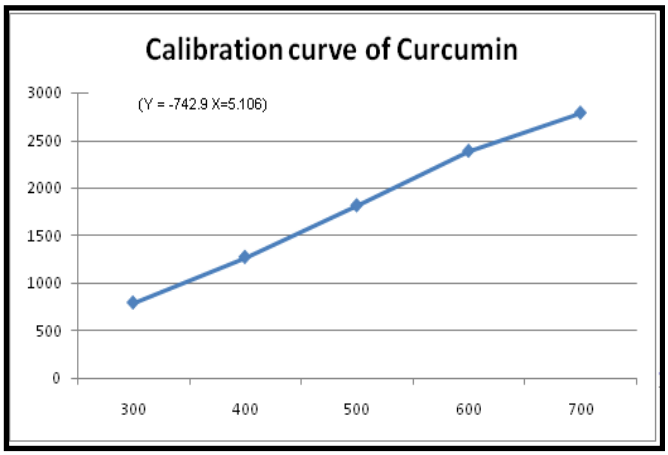


Figure No. 11 & 12: Typical chromatogram & Calibration curve of Curcumin

Table No. 10: Observation table for calibration curve of curcumin

Amount(ng)	Area
300	791.71
400	1271.08
500	1813.60
600	2386.55
700	2786.77

3.2.3.2 Calibration curves of gallic acid:

The linear regression data for the calibration curves (n=3) as shown in Table 11 showed a good linear relationship over the concentration range 100-700 ng per spot with respect to peak area.

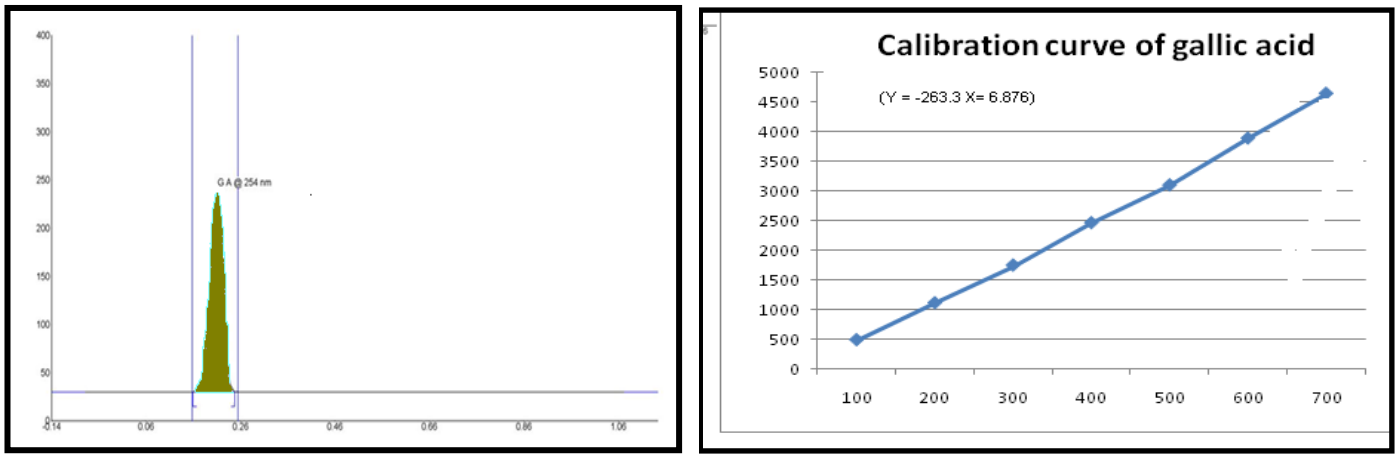


Figure No. 13 & 14: Typical chromatogram & Calibration curve of gallic acid

Table No. 11: Observation table for calibration curve of gallic acid

Amount(ng)	Area
100	490.50
200	1115.75
300	1750.52
400	2465.46
500	3103.13
600	3893.30
700	4647.91

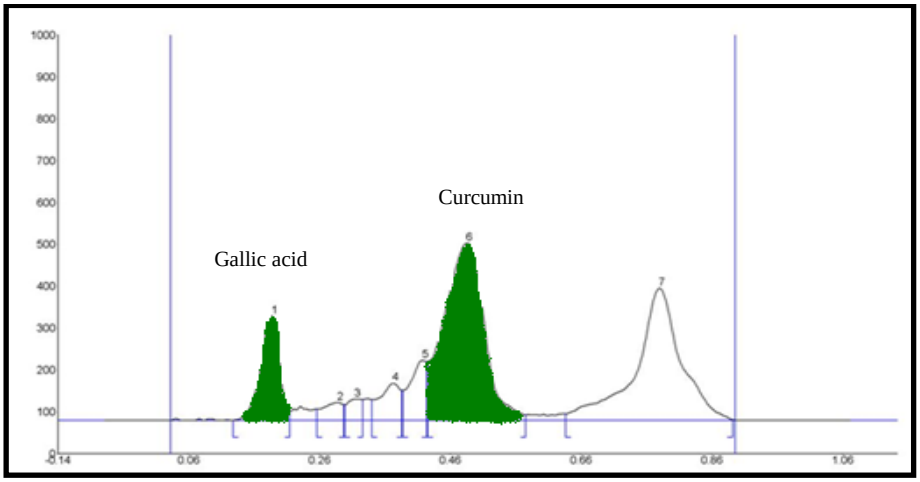


Figure No. 15: Typical chromatogram of Formulation (F3)

3.2.4 Method Validation:

3.2.4.1 Precision:

The repeatability of sample application and measurement of peak area were expressed in terms of %RSD and results are depicted in Table No. 12 and 13, which revealed inter-day and intraday variation of curcumin and gallic acid at concentration of 100 ng per spot.

Table No. 12: Observation table for Interday Precision

	Interday precision				
Component	**Mean area**	**% found**	**S.D**	**%RSD**	**S. E**
Curcumin	3277.6	97.74	18.54	0.56	0.96
Gallic acid	4598.8	98.94	2.55	0.05	0.08

Table No. 13: Observation table for Intraday Precision

	Intraday precision				
Component	**Mean area**	**% found**	**S.D**	**%RSD**	**S. E**
Curcumin	1162.1	99.14	0.62	0.05	0.18
Gallic acid	1893.6	99.05	1.4	0.07	0.12

LOD and LOQ:

The calibration curve in this study was plotted between amount of analyte versus average response (peak area) and the regression equation was obtained (Y = -742.9 X=5.106) and (Y = -263.3 X= 6.876) with a regression coefficient of 0.998 and 0.999 respectively. Detection limit and quantification limit was calculated by the method as described and shown in Table No.14.

Table No. 14: Method validation

Parameter	**Curcumin**	**Gallic acid**
Linearity range(ng/spot)	300-700	100-700
Slope	5.106	6.876
Intercept	742.9	263.3
Coefficient of correlation	0.99863	0.99904
Limit of Detection	100 ng/spot	33.33 ng/spot
Limit of Quantitation	300ng/spot	100ng/spot
Reproducibility	97.5 %	98.86 %

Tablet analysis:

Table No. 15: Analysis of tablet

Drugs	**R_f**	**Amount found**	**% drug found**
Curcumin	0.55	40.60mg	102.54
Gallic acid	0.26	77.38mg	97.70

Recovery studies:

The analyzed samples were spiked with extra 80, 100 and 120% of the standard curcumin, gallic acid and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Table No. 16: Recovery studies of curcumin and gallic acid

Level of % Recovery	**Amount present(mg/tab)**		**Amount of standard added(mg/tab)**		**Total amount recovered(mg)**		**% Recovery***	
	C.L. Extract	**E.O. extract**	**C.L. extract**	**E.O. extract**	**Curcumin**	**Gallic acid**	**Curcumin**	**Gallic acid**
80	39.6	79.2	31.68	63.36	74.13	139.48	104	97.84
100	39.6	79.2	39.6	79.2	78.80	155.69	99.5	98.29
120	39.6	79.2	47.52	95.04	85.58	176.74	98.24	101.46

Table No. 17: Statistical validation

Component	**Mean**	**Standard Deviation**	**Coefficient of Variation**	**Standard Error**
Curcumin	100.58%	3.02	0.37	0.28
Gallic acid	99.19%	1.97	1.98	0.11

Stability Studies:

Formulation kept at 25^{o}C / 60 % RH and 40^{o}C / 75 % RH was found to stable meeting all of its predetermined specification. Here it was checked only for relevant compendia specifications for tablets and *in vitro* degradation studies. There was no change in appearance and average weight was 400 mg.

4. CONCLUSION:

From the obtained results, it can be concluded that there is no interaction between excipients and drug. All the polymers used are compatible with the drug under the proposed method of fabrication. F3 formulation was found to be significantly effective than other formulation in all the screening models for cancer. This suggests that F3 component in a specific proportion exerted remarkable synergistic cytotoxic action on the target cells. Evaluation parameters like hardness and friability indicated that the tablets so prepared were mechanically stable and complied with necessary pharmacopoeial specifications.

Percentage weight variation and disintegration test for uncoated tablets also complies with the pharmacopoeial standards. The proposed HPTLC method was found to be rapid, simple and accurate for quantitative estimation of Curcumin and Gallic acid in polyherbal formulations. The recovery values of curcumin and gallic acid were found to be about 100.58% and 99.18% respectively, which shows the reliability and suitability of the method. The lowest detectable limit was found to be 100 ng/spot and 33.33 ng/spot. Thus method can be employed for routine analysis. The stability study indicates it is safe and stable formulation.

5. REFERENCES:

1. Agrawal SS, Paridhavi M. Herbal Drug Technology. 1st ed. Universities press; 2007, p.570 and 645.
2. NCI's Web site at http://www.cancer.gov/cancerinfo/copingand from NCI's Cancer Information Service (http://www.cancer.gov/help).
3. Anonymous: The Indian Pharmacopoeia. Ghaziabad: The Controller of Publications, Government of India; 3: 2007, p.1380.
4. Shaik Mohd. Asif. Design and Characterization of Colon Targeted Nimesulide double coated Tablets for the Prophylaxis of Colon Cancer. K. L. E. Society's college of pharmacy, Belgaum: 2006.
5. Anonymous: The Indian Pharmacopoeia. I, II: New Delhi: The Controller of Publications, Government of India; 1996, p.A-53, A-54 and A-89.
6. Prabu SL, Shirwaikar AA, Shirwaikar A, Ravikumar G, Kumar A, Jacob A. Formulation and evaluation of oral sustained release of Diltiazem Hydrochloride using rosin as matrix forming material. ARS Pharmaceutica 2009; 50 (1): 32-42.
7. Reddy AN. Formulation of sustained release matrix tablets using hydrophilic and hydrophobic polymers. Rajiv

Gandhi University of health sciences, Karnataka.

8. Patra CN et al, Improvisation of the flowability and compressibility of *Azadirachta indica* Bark powder for the processing into tablet dosage form. International Journal of Green Pharmacy 2007; 1(3-4): 16-19.
9. Kamble SS, Jadhav VM, Kadam VJ. Development and Validation of HPTLC method to detect 3-Hydroxy Androstane [16, 17-C] (6'methyl, 2'-1-Hydroxy –isopropene-1-yl) 4, 5, 6 H Pyran in Polyherbal Formulation. International Journal of Pharma Research and Development 2009; 7: 1-13.
10. Ansari MJ, Ahmad S, Kohli K, Ali J, Khar RK. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis 2005; 39: 132–38.
11. Shaik Mohd. Asif. Design and Characterization of Colon Targeted Nimesulide double coated Tablets for the Prophylaxis of Colon Cancer. K. L. E. Society's college of pharmacy, Belgaum: 2006.
12. Palani V, Senthilkumaran RK, Govindasamy S. Biochemical evaluation of antitumor effect of Muthu Marunthu (A herbal formulation) on experimental fibrosarcoma in rats. Journal of Ethnopharmacology 1999; 65: 257–65.
13. Panchawat S, Singh K, Rathore DR, Sisodia SS, Nema RK. Standardisation and evaluation of herbal drug formulations. 2009.
14. Costanzo SJ. HPTLC. Journal of Chemical Education1984; 61: 1015-18.
15. Helfmann E. History of Chromatography. A Laboratory Handbook of Chromatographic and Electrophoretic Methods.3rd ed. New York: p. 1-13.
16. Coker PS et al, Phytomedicine, 2003, 133-38 with modifications taken from Ferrigni NR et al, Journal of Natural Products 1982; 45 (6): 679 – 86.
17. Mclaughlin JL, Rogers LL, Anderson JE. The use of biological assay to evaluate botanicals. Drug Information Journals 1999; 32: 513-24.
18. Anonymous: Herbal Pharmacopoeia. I: A Joint Publication of Regional Research Laboratory and Indian Drug Mfg's Association; 1998, p. 64-71.
19. ICH Harmonised Tripartite Guideline Stability testing of new drug substances and products, Q1A (R2), Current *Step 4* versions, 2003, 1-24.