3 MATERIALS AND METHODOLOGY

UV-Visible spectrophotometry is champion amongst most significant part of time used strategy in pharmaceutical examination. It incorporates measuring measure of brilliant or clear radiation devoured by substance in game plan. Instrument which measure extent, or limit of extent, of force of two light emanations in U.V-Visible area are called Ultraviolet-Visible spectrophotometers.

In subjective examination, regular blends can be recognized by usage of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric examination is used to take in measure of sub-nuclear species fascinating radiation. Spectrophotometric method is essential, brisk, sensibly specific and correlated to little measures of blends. key law that speaks to quantitative spectrophotometric examination is Beer - Lambert law.

Mix's law: It communicates that force of light outflow monochromatic radiation decreases exponentially with amount of holding particles. In that capacity, absorbance is in respect to core interest.

Lambert's law: It communicates that force of light emanation monochromatic radiation lessens exponentially as it experiences medium of homogeneous thickness. mix of these two laws yields Beer-Lambert law.

Ale Lambert law: When light emanation is experienced direct cell containing answer of anabsorbing substance, decline of force of light may happen. Logically, Beer-Lambert law is conveyed as

\[ A = a b c \]

Where, \( A \)= absorbance or optical thickness
\( a \)= absorptivity or annihilation coefficient
\( b \)= path length of radiation through illustration (cm)
\( c \)= concentration of solute in plan.
Estimation of remedial substance using spectrophotometer may did by arranging game plan in clear dissolvable and measuring it's absorbance at suitable wavelength. wavelength normally picked is wavelength of most prominent maintenance ($\lambda_{\text{max}}$), where little pass in setting wavelength scale has little effect on measured absorbance. Ideally, obsession should be accustomed to give absorbance of about 0.9, around which precision and exactness of estimations are perfect.

Test of single section test, which contains other engaging substances, is then figured from planned absorbance by using one of three key philosophy. They are, usage of standard absorptivity regard, arrangement chart and single or twofold point regulation. In standard absorptive quality methodology, usage of standard (1%, 1 cm) or E qualities are used as piece of solicitation to center its absorptivity. It is valuable in circumstances where it is troublesome or expensive to get example of reference substance. In arrangement graph technique, absorbances of different standard courses of action of reference substance at obsessions wrapping example centers are measured and change outline is produced. accumulating of analyte in illustration course of action is examined from graph as center identifying with absorbance of plan. single point organization system incorporates estimation of absorbance of example course of action and of standard game plan of reference substance. convergance of substances in example is found out from comparing relationship that exists amidst absorbance and center (Davidson AG., 2002). Extremely cutting edge tried and true and speedy liquid chromatographic (LC) division systems are transformed into essential in various business undertakings like pharmaceuticals, agrochemicals, hues, petrochemicals, trademark things and others. It requires to great degree remarkable gadget which fuses going with.

1. To awesome degree careful edge blenders.
2. HPLC high weight pumps with to great degree reliable stream.
3. Outstanding high exactness, low dissipating, HPLC test valves.
4. High capability HPLC areas with unmoving squeezing materials.
5. High affectability low disseminating HPLC locators.
6. Quick data acquirement structures.
7. Low disseminating interfacing tubes for valve to segment and fragment to identifier.

UV/Vis spectrophotometers, including diode bunch identifiers, are most for most part used discoverers. Fluorescence spectrophotometers, differential refractometers, electrochemical identifiers, mass spectrometers, light diffusing discoverers, radioactivity locators or other outstanding markers may in like manner be used. Locator includes travel through cell mounted toward end of fragment. light outflow radiation experiences stream cell and into discoverer. As blends elute from segment, they experience phone and hold radiation, achieving quantifiable essentialness level changes. Adjusted (mercury light), variable (deuterium or highpressure xenon light), and multi-wavelength discoverers are comprehensively available. Present day variable wavelength pointers can be adjusted to change wavelength while examination is in headway. Multi-wavelength identifiers measure absorbance at two or more wavelengths in meantime. In diode display multi-wavelength markers, industrious radiation is experienced example cell, and after that decided into its constituent wavelengths, which are only recognized by photodiode bunch. These identifiers secure absorbance data over entire UV-perceptible scope, hence outfitting analyst with chromatograms at various, selectable wavelengths, spectra of eluting tops moreover peak goodness. Differential refractometer locators measure refinement between refractive rundown of adaptable stage alone and that of versatile stage containing chromatographic blends as it ascends out of segment.

Frequently, there is bigger time period between date of presentation of medication into business sector and date of its incorporation in pharmacopeias. This happens as result of conceivable vulnerabilities in nonstop and more extensive utilization of these medications, report of new toxicities, and improvement of patient resistance and presentation of better medications by contenders.
In such cases standard scientific strategies for these medications may not be accessible in Pharmacopeias. It gets to be fundamental, in this way, to grow new explanatory system for such medications. Additionally quality is vital in every item or administration in pharmaceuticals as it includes life. Investigation which helps in discovering spatial plan of ion as in particle and vicinity or position of certain natural useful gathering in given compound. What’s more surface examination assumes imperative part in material studies to get surface related physical properties, for example, geography, profundity profiling, introduction of atom and so forth. Concoction examination has some fundamental strides like, decision of system, testing, preparatory specimen treatment, partitions, last estimation and appraisal of results. It is with first step viz. decision of system, consideration ought to be practiced to choose best possible instrument to do productive examination.

Wrong choice as of right now will prompt good for nothing examination. Analytical methods are broadly classified as Physical, Chemical and Instrumental analysis. Physical observation includes description of compound, measurements of its dimension (shape, size), colour, odour etc. Chemical analysis includes titrimetric analysis of compound such as potentiometry, iodometry, argentometry, permagnometry etc. Instrumental methods of chemical analysis have become backbone of experimental Chemistry. Method development is done for new products and existing products. Approval is characterized as archived proof, which gives high level of certification that particular procedure, will reliably create item meeting its foreordained determination and quality traits (Loftus B. T. also, Nash R. A, 2003). Approval of explanatory system is characterized as procedure by which it is built up, by research facility ponders, that execution attributes of strategy meet necessities for proposed scientific application.

Acceptance of logical techniques is procedure of deciding suitability of given approach for giving helpful scientific information. Acceptance is formal and deliberate evidence that system follows prerequisites for testing item when
watching characterized strategies. Technique approval is essentially concerned with distinguishing proof of wellsprings of potential blunders and evaluation of potential slips in system. Exactness of scientific technique is characterized as "the level of closeness of test outcomes got by that strategy to genuine quality" that implies decided estimation of analyte in specimen compares to genuine worth. Precision may be measured in distinctive ways and technique ought to be suitable to grid. Exactness of systematic technique communicates closeness of assention (level of dissipate) between progression of estimations acquired from different inspecting of same homogeneous example under endorsed conditions. Exactness of systematic technique is typically communicated as change, standard deviation or coefficient of variety of progression of estimations. Intermediate exactness communicates inside of research centers varieties: diverse days, distinctive investigators, diverse hardware, and so on. Degree to which middle of road accuracy ought to be set up relies on upon circumstances under which technique is proposed to be utilized. Candidate ought to set up impacts of irregular occasions on exactness of investigative technique. Ordinary varieties to be concentrated on incorporate days, experts, hardware, and so forth. It is not viewed as important to examine these impacts exclusively. Utilization of test configuration (grid) is supported. Reproducibility communicates exactness between labs (collective studies, generally connected to institutionalization of technique). Reproducibility is surveyed by method for between lab trials. Reproducibility ought to be considered if there should arise occurrence of institutionalization of expository system, for occasion, for incorporation of methods in pharmacopeias. This information are not piece of promoting approval dossier. Reproducibility communicates exactness between diverse conditions: more often than not in distinctive research centers from examples taken from same homogenous bunch of material. For these rules, basic appraisal of repeatability will be acceptable. Exactness of scientific system is normally communicated as difference, standard deviation or coefficient of variety of arrangement of estimations. At least 5 duplicate example determinations ought to be made.
together with straightforward factual appraisal of results, including percent relative standard deviation. Expression optimize is regarded as creation of ideal, successful or purposeful as likely and strategy can be regarded like technique to discover those standards of dependent variable. Ahead of such experimentation when conducted at before final preparation phase, assured troubles occur. By means of broadcasting numerous designs as well as Analysis of variance can answer such trouble. subsequent severe difficulty can happen through novel added ingredients and novel establishment factors, of which qualitative or quantitative properties are not recognized and nor they are expected. Another impediment is that, formulated yield, in scrupulous, dosage forms have to obey rules to numerous necessities, extremely frequently challenging. formulator has to deal with such objectives and decide negotiation. more difficulty is short of imminent in equilibrium among wanted and previous understanding to carry out sufficient optimization lesson and enhancement in acquaintance gained through such lesson. It must be focused that in presentation of optimization lesson, establishment scientist can as well be factor, dependable preceding knowledge and acquaintance is prime requirement. Investigational plan is mathematical plan that provides or gives position of variation of variables. figure and outline of such plan points inside investigational area lie on integer of possessions that have to be anticipated. As per amount of factors, their levels, probable communications and regulation of sculpt, range of investigational design are taken. Every research can be shown as end inside investigational area, end being distinct by it’s harmonize in liberty. It is investigational plan that employs dimensional factor gap at curve of plan gap. Factorial plans are employed in investigations where influence of diverse circumstances on selection for concurrent examination of influence of numerous factors as well as their relations. easiest factorial plan is two factorial plans, in which two conditions are measured every at two levels, results in to four investigations that are located in 2-dimensional factor gap at curve of rectangle. If three factors are taken, every in two levels, eight investigations are required that are located at curves of orthogonal cube. amount of investigations
is demonstrated through $2n$, where “$n$” is considered as amount of factor. If amount of factors and levels are big, then quantity of investigations required to total factorial plan is huge. To decrease amount of investigations, partial factorial plans can be employed for example half of creative numeral of investigations through full factorial plan. It is partial factorial plan having $K = m*4$ investigation to examine single variables in which $K$ is integer of variables at same time $m$ is amount of levels. Improved plan which includes compensation of factorial plan or partial factorial plan as well as star plan is regarded as central composite plan established by Box and Wilson. It is having imbedded factorial or partial factorial plan through middle ends which is amplified among collection of star ends which permit evaluation of curve. It is prepared of Box Behnken plan is investigation plans or outcome plan organized By George Box and Donald Behnken in 1960, for aim of optimization study. Plan is regarded orthogonal reasonable unfinished wedge plan. It can be crack into position of unfinished wedge that revenue each consequence is not anticipated in all slab, but each factor result is calculated equivalent integer of periods through impartial separation above diverse slabs. Its "absent curves" may be helpful when investigator should evade joint factor boundaries. This possession protects possible beating of information in those issues. Box Behnken plan is quadratic reaction surface advance. Box Behnken plan purposely chosen because, it needs smaller amount of investigational runs as well as fewer periods and hence gives distant extra price valuable method in contrast to conservative methods of preparing and optimizing formulations. Do not contain axial points in addition they make sure that every factor is not at all concurrently located at their uppermost or lowly levels.

Hence such plans are helpful in eliminating research carried out beneath tremendous situation for which unacceptable findings can happen. Simplex Lattice plans are employed to discover inner and borders of simplex. Quantity of factors examines its magnitude. Outline of plan points in factor gap and their figure rely on amount of replica which is postulated. Points have been dispersed systematic above factor gap, resulting in network. Factors can be proscribed correctly and accurately, coefficients of replica formulas can be considered with
no trouble. For purpose of grouping of ingredients in preparation of dosage form, particular plans have been consequent, depending on grouping constraints. Portion cannot be pessimistic, and amount of portions of ingredients should be identical to 1. Imperative characteristic is that integer of coefficients to be examined is abridged. Combination constraint has outcomes for investigational plans. Factors cannot be selected liberally. In two ingredient grouping, merely one portion can be selected, whereas in ingredient grouping simply two portions and goes on. residual portion finishes figure to one that gives dimension decrease. In brief reasons for development of newer methods of drugs analysis are:

- The medication or medication mix may not be official in any pharmacopeias.
- A fitting expository method for medication may not be accessible in writing because of patent regulations.
- Analytical systems may not be accessible for medication as plan excipients.
- Analytical systems for medication in mix with different medications may not be accessible.

Option logical technique is proposed by candidate for utilization rather than administrative systematic method. Security testing structures imperative piece of procedure of medication item advancement. reason for soundness testing is to give prove on how nature of medication substance or medication item differs with time affected by mixed bag of ecological components, for example, temperature, moistness, and light, and empowers suggestion of capacity conditions, retest periods, and timeframes of realistic usability to be set up. The two primary parts of medication item that assume vital part in timeframe of realistic usability determination are test of dynamic medication, and degradants created, amid soundness study. Cutting edge techniques for decision for quantitative examination are UV, HPLC, GC, GCMS, LCMS and HPTLC, which
are very advanced. Chromatographic routines are normally utilized as part of administrative labs for subjective and quantitative examination of medication substances, drug items, crude materials and natural examples all through all periods of medication advancement, from exploration to quality control. HPLC is quickest developing diagnostic strategy for investigation of medications. Its effortlessness, high specificity, and extensive variety of affectability make it perfect for examination of numerous medications in both measurement shapes and organic liquids. Rapid growth of HPLC has been facilitated by development of reliable, moderately priced instrumentation and efficient columns. HPTLC is classical separative technique that has enjoyed wide spread popularity particularly in analysis of complex mixtures of natural origin. Now-a-days HPTLC is turning into routine investigative method because of its preferences of low working expense, high specimen throughput, and requirement for least example clean-up. significant point of preference of HPTLC is that few specimens can be run at same time utilizing little amount of versatile stage not at all like HPLC, in this way bringing down examination time and expense per investigation. Day by day numbers of new drugs are introduced into market. Frequently, there is bigger time period between date of presentation of medication into business sector and date of its incorporation in pharmacopeias. This happens as result of conceivable vulnerabilities in nonstop and more extensive utilization of these medications, report of new toxicities, and improvement of patient resistance and presentation of better medications by contenders. In such cases standard scientific strategies for these medications may not be accessible in Pharmacopeias.

It gets to be fundamental, in this way, to grow new explanatory system for such medications. Additionally quality is vital in every item or administration in pharmaceuticals as it includes life. It is Branch of Chemistry that picks nature and character of substance and its piece. In mid-20th centaury, there were just four perceived branches of Chemistry to be specific, Biochemistry, Physical chemistry, Inorganic and Organic Chemistry. Its centrality created, and at same time, absorbed techniques and capacities from all other four branches so by
1950s, exact science was at long last perceived as branch of Chemistry in its own benefit. The Qualitative analysis identifies nature of substance, and if it is mixture, nature of components present. The Quantitative analysis determines elemental composition of substance and quantitative distribution of each component. Investigation which helps in discovering spatial plan of ion as in particle and vicinity or position of certain natural useful gathering in given compound. What's more surface examination assumes imperative part in material studies to get surface related physical properties, for example, geography, profundity profiling, introduction of atom and so forth. Concoction examination has some fundamental strides like, decision of system, testing, preparatory specimen treatment, partitions, last estimation and appraisal of results. It is with first step viz. decision of system, consideration ought to be practiced to choose best possible instrument to do productive examination. Wrong choice as of right now will prompt good for nothing examination. Analytical methods are broadly classified as Physical, Chemical and Instrumental analysis. Physical observation includes description of compound, measurements of its dimension (shape, size), colour, odour etc. Chemical analysis includes titrimetric analysis of compound such as potentiometry, iodometry, argentometry, permagnometry etc. Instrumental methods of chemical analysis have become backbone of experimental Chemistry. Security testing structures imperative piece of procedure of medication item advancement. reason for soundness testing is to give prove on how nature of medication substance or medication item differs with time affected by mixed bag of ecological components, for example, temperature, moistness, and light, and empowers suggestion of capacity conditions, retest periods, and timeframes of realistic usability to be set up. Two primary parts of medication item that assume vital part in timeframe of realistic usability determination are test of dynamic medication, and degradants created, amid soundness study. For analysis of these drugs different analytical methods are routinely being used. One of most exploited methods for analysis of drugs is spectroscopy; which may be defined as method of analysis that embraces measurement of absorption by chemical
species of radiant energy at definite and narrow wavelength, approximating monochromatic radiation.

It is one of valuable techniques in pharmaceutical analysis is defined as method of analysis, which deals with measurement of spectra. Spectrophotometry is branch, which embraces measurement of absorption of radiation energy of definite and narrow wavelength approximating monochromatic radiations by chemical species. absorption of electromagnetic radiation of definite and narrow wavelength range by molecules, ions and atoms employed in analytical field includes ultraviolet, visible, infrared and atomic absorption spectroscopy. Spectroscopic method is simple, rapid, moderately specific and applicable to compound.

This method is material to figure centralization of part of interest found in blend containing it alongside some undesirable meddling segment. Absorbance distinction between two purposes of blend spectra is straightforwardly corresponding to centralization of analytic regardless of interferent. In this method two wavelengths were selected (\(\lambda_1, \lambda_2\)) where drug showing equal absorbance (or difference between absorbance is zero) and drug B showing some response. Then different concentrations of drug and drug B are prepared to confirm that at all different concentrations of drug difference between absorbance at two selected wavelengths (\(\lambda_1, \lambda_2\)) remain zero, and at all different concentration of drug B difference between absorbance at two selected wavelength (\(\lambda_1, \lambda_2\)) showing linear response. So calibration curve is prepared for absorbance difference vs. concentration of drug B (Absorbance difference is zero for drug A). Similarly for estimation of drug A, two wavelengths were selected where drug B showing same absorbance (difference between absorbance is zero) and drug showing linear response.

System is in light of isolating range for blend into standard spectra for each of examinations and to acquire range that is autonomous of analyte fixation utilized as divisor. utilization of institutionalized spectra as divisors minimizes trial slips. exact decision of standard divisors and working wavelengths is basic for few
reasons. Proportion spectra subsidiary licenses utilization of wavelengths relating to greatest or least furthermore utilization of separation between back to back most extreme and least. The vicinity of part of maxima and minima in proportion spectra subsidiary since these wavelengths give open door for determination of these mixes in vicinity of other dynamic mixes and excipients that perhaps meddled with assay.

AUC strategy is applicable where there is no sharp peak or when wide spectra are procured. It incorporates check of joined estimation of absorbance regarding wavelength between two chose wavelengths $\lambda_1$ and $\lambda_2$. Range figuring handling thing ascertains region bound by bend and even pivot. Level hub is chosen by entering wavelength run over which region must be computed. This wavelength extent is chosen on premise of rehashed perception in order to get linearity between range under bend and fixation. Alignment bend was developed by plotting fixation versus AUC. In cutting edge pharmaceutical industry, superior fluid chromatography (HPLC) is major and necessary scientific device connected in all phases of medication disclosure, improvement, and generation. It is perfect for examination of numerous medications in both dose shapes and natural liquids because of its effortlessness, high specificity and extensive variety of affectability. It is one method of chromatography, one of most utilized explanatory systems. Standard of HPLC is realized that determining force of chromatographic segment increments with section length and number of hypothetical plates per unit length, although there are limits to length of column due to problem of peak broadening. Chromatographic procedure can be characterized as division strategy including mass-exchange in middle of stationary and portable stage. HPLC uses fluid portable stage to independent segments of blend. Stationary stage can be fluid or strong stage. These parts are initially broken up in dissolvable, and afterward compelled to move through chromatographic section under high weight. In segment, blend isolates
3.1 INSTRUMENTS AND APPARATUS:

- Shimadzu UV-1800, UV-Visible double beam Spectrophotometer with matching pair of 1 cm quartz cuvettes (Software –UV Probe, Version 2.42). spectral bandwidth is 0.5 nm.
- Chromatographic analysis was carried out on LC-2010 CHT series, Auto injection system, temperature controller (system controller and UV detector, LC solution software was used to acquire and process data.
- Electronic analytical balance (Shimadzu)
- pH meter (Systronic)
- Sonicator (Sonica Ultrasonic Cleaner)
- 0.45 µm Nylon 66 (N66) 47 mm membrane filter paper
- Volumetric flask – 10, 25, 50, 100 ml (Borosil)
- Pipettes – 1, 2, 5, 10 ml (Borosil)
3.2 CHEMICALS AND REAGENTS:

- Procurement of standard drugs, marketed formulation and Chemicals.
  
  a. Standard APIs were procured from Vaibhav Analytical Laboratories, Ahmedabad. Marketed formulation used i.e. Tablets for all three combinations were procured from local market.
  
  b. Methanol of A.R. Grade, Chemco Chemicals Ltd., India.
  
  c. HPLC grade Methanol, Water, Acetonitrile and other chemicals used in Mobile phase preparation(Rankem, RFCL chemicals Pvt Ltd.)

3.3 UV SPECTROSCOPIC METHODS

3.3.1 SIMULTANEOUS ESTIMATION OF THC AND DKP

The mixture of THC (THC) and DKP (DKP) is present in ratio of 1:6.25 (THC: DKP). The absorption spectra of pure drugs and their dosage form were recorded between 200-400 nm using methanol as solvent. Methanol was used as common solvent for preparation of solutions.

3.3.1.1 Area Under Curve Method

Selection of wavelength range for estimation

Suitable dilutions of THC and DKP were prepared separately in methanol to get final conc. 10 µg/ml for both THC and DKP. Both drug solutions were scanned in range of 200-400 nm and overlain spectrum was studied. After repeated observations of response wavelength ranges were selected for estimation of THC and DKP further area was integrated between selected wavelength ranges and estimation was carried out.

Preparation of standard solution

Preparation of standard stock solution of THC (1000 µg/ml):
Accurately weighed quantity 10 mg of THC was moved into 10 ml volumetric jar, broke up and weakened up to stamp with methanol. This gave stock arrangement having quality of 1000 µg/ml.

**Preparation of working standard solution of THC (100 µg/ml):**

100 µg/ml of THC arrangement was arranged by weakening 2.5 ml of stock arrangement up to imprint with methanol in 25 ml volumetric flask.

**Preparation of standard stock solution of DKP (1000 µg/ml):**

Accurately weighed quantity 10 mg of DKP was transferred into 10 ml volumetric flask, dissolved what's more, weakened up to stamp with methanol. This will give stock arrangement having quality of 1000 µg/ml.

**Preparation of working standard solution of DKP (100 µg/ml):**

100 µg/ml of DKP solution was prepared by diluting 2.5 ml of stock solution up to mark with methanol in 25 ml volumetric flask.

**Preparation of combined working standard solution containing THC and DKP:**

Precisely measured amount of tablet powder comparable to 4 mg of THC and 25 mg of DKP were exchanged to 100 ml volumetric carafe, broke down in adequate measure of methanol and weakened up to check with methanol to give convergance of 40 µg/ml of THC and 250 µg/ml of DK

**Preparation of calibration curve**

Alignment bend for THC comprised of distinctive amassings of standard THC arrangement extending from 4-20 µg/ml. arrangements were arranged by exchanging 0.4, 0.8, 1.2, 1.6 and 2.0 ml aliquots of working standard arrangement of THC (100 µg/ml) into arrangement of 10 ml volumetric flagons and volume was conformed to check with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for THC. For DKP Calibration bend running from 5-45 µg/ml. aliquots from DKP (100 µg/ml) 0.5, 1.5, 2.5, 3.4 and 4.5 ml were moved into arrangement of 10 ml volumetric jars and volume was changed in accordance
with imprint with methanol to get last conc. of 5, 15, 25, 35 and 45 µg/ml for DKP.

Examining wavelength extents chose for estimation of zone of THC and DKP were 365-375 nm (λ1-λ2) and 250-260 nm (λ3-λ4) individually and region were coordinated between these chose wavelengths ranges for both medications, which indicated straight reaction with expanding fixation. Adjustment bend of range versus fixation was plotted and straight-line compared.

**Analysis of marketed formulation**

20 tablets were measured; typical weight was determined then controlled. measure of powder relative to 4 mg THC and 25 mg DKP was moved in 100 ml volumetric carafe. Methanol (50 ml) was added to it and sonicated for 20 min. game plan was filtered through Whatman channel paper No. 41 and store was washed with methanol. Washing and filtrate were united in another 100 ml volumetric flask and volume was adjusted up to engrave with methanol. above plan was suitably debilitated with methanol to get last storing up of 4 µg/ml of THC and 25 µg/ml of DKP.

For synchronous determination using zone under twist (AUC) procedure, test course of action of meds were weighed in extent of 200-400 nm, each was measured at 365-375 (λ1-λ2) and 250-260 (λ3-λ4) for assessment of THC and DKP independently. measures of THC and DKP display in test plans were controlled by fitting response into backslide scientific articulation procured from arrangement curves of THC and DKP.

Concentration of THC and DKP was acquired by taking after mathematical treatment as follows:

\[ C_{THC} = \frac{A_{1.x1}Y_1 - A_{1.x2}Y_2}{a_{2.x1}Y_1 - a_{1.x2}Y_2} \]  \hspace{1cm} (1)

\[ C_{DKP} = \frac{A_{1.x2} - A_{2.x1}}{a_{2.x1}Y_1 - a_{1.x2}Y_2} \]  \hspace{1cm} (2)
Where,

\( C_{\text{THC}} \) = Concentrations of THC,
\( C_{\text{DKP}} \) = Concentrations of DKP,
\( A_1 \) = Area of mixture at 365-375 nm,
\( A_2 \) = Area of mixture at 250-260 nm,
\( aX_1 \) = Absorptivity value of THC at 365-375 nm,
\( aX_2 \) = Absorptivity value of THC at 250-260 nm,
\( aY_1 \) = Absorptivity value of DKP at 365-375 nm,
\( aY_2 \) = Absorptivity value of DKP at 250-260 nm.

**Validation of AUC Method**

**Linearity and range**

Linearity is communicated regarding relationship co-efficient of direct relapse investigation.

Linearity response was controlled by separating 5 free levels of arrangement curve in extent of 4-20 µg/ml for THC and 5-45 µg/ml for DKP. impressive measure of arrangement twist of locale versus obsession and relationship coefficient and backslide line scientific articulations for THC and DKP choose precision.

**Accuracy**

**Preparation of Sample solution:**

Twenty tablets were measured; powdered and measure of powder tantamount to 4 mg of THC and 25 mg of DKP was measured and moved into 100 ml of volumetric carafe, Methanol (50 ml) was added to it and sonicated for 20 min. plan was isolated through Whatman channel paper No. 41 and volume was adjusted up to engrave with methanol (40 µg/ml of THC and 250 µg/ml of DKP).

Pipette out 1 ml of above course of action in 10 ml volumetric carafe and debilitated to stamp with methanol (4 µg/ml of THC and 25 µg/ml of DKP).
measure of THC and DKP was figured at each of three level and % recoveries were enlisted (80%, 100% and 120%)

**Precision**

**Repeatability**

From Sample course of action aliquot was traded to distinctive 10 ml volumetric flask and debilitated up to stamp with methanol such that it gives convergance of 4 µg/ml of THC and 25 µg/ml of DKP. Each center was prepared Six times. zone of each game plan was measured at picked wavelengths and % CV was registered.

**Intraday exactness**

Test arrangements containing 4, 12, 20 µg/ml of THC and 5, 25, 45 µg/ml of DKP were broke down three times on same day and % CV was computed.

**Interday exactness**

Test arrangements containing 4, 12, 20 µg/ml of THC and 5, 25, 45 µg/ml of DKP wereanalyzed on three distinct days and % CV was computed.

**Breaking point of discovery**

From linearity twist numerical proclamation, SD of catches (response) was learned. By then LOD was measured by using numerical expressions given as a major aspect of fragment.

Most distant purpose of LOD of solution was processed by using join examinations appointed by ICH guidelines:

\[
\text{LOD} = 3.3 \times \sigma/S,
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve
**Limit of quantification**

From linearity curve equation, standard deviation (SD) of intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section.

The limit of quantification (LOQ) of drug was calculated by using following equations designated by International Conference on Harmonization (ICH) guideline:

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = standard deviation of Intercept  
\(S\) = Mean slope of calibration curve

### 3.3.1.2 Absorption Correction Method
#### Selection of wavelength for estimation of THC and DKP

Suitable dilutions of THC and DKP were prepared separately in methanol to get final conc. 10 µg/ml for both THC and DKP. Both drug solutions were scanned in range of 200-400 nm and overlain spectrum was studied. Two wavelengths for one drug were selected where absorbance of that drug is same on both wavelengths \(\lambda_1\) and \(\lambda_2\). Now, these two wavelengths should be selected such that at first wavelength \((\lambda_1)\) other drug should show zero absorbance (No interference) and second wavelength \((\lambda_2)\) where both drugs should show considerable absorbance. For estimation in marketed formulation first drug can be directly estimated at \(\lambda_1\) without interference of other drug and another drug can be estimated after correction of absorbance at \(\lambda_2\). After selection of wavelengths for both drugs, simultaneous estimation of THC and DKP can be carried out in marketed formulation.

### Preparation of standard solution

**Preparation of standard stock solution of THC (1000 µg/ml):**

Accurately measured sum 10 mg of THC was moved into 10 ml volumetric container, broke down and debilitated up to check with methanol. This gave stock game plan having nature of 1000 µg/m
Preparation of working standard solution of THC (100 µg/ml):
100 µg/ml of THC arrangement was arranged by weakening 2.5 ml of stock arrangement up to imprint with methanol in 25 ml volumetric flask.

Preparation of standard stock solution of DKP (1000 µg/ml):
Precisely measured amount 10 mg of DKP was moved into 10 ml volumetric carafe, broke up and weakened up to stamp with methanol. This will give stock arrangement having quality of 1000 µg/ml.

Preparation of working standard solution of DKP (100 µg/ml):

100 µg/ml of DKP arrangement was arranged by weakening 2.5 ml of stock arrangement up to imprint with methanol in 25 ml volumetric flask.

Preparation of combined working standard solution containing THC and DKP

Precisely measured amount of tablet powder proportional to 4 mg of THC and 25 mg of DKP were exchanged to 100 ml volumetric jar, broke down in adequate measure of methanol and weakened up to stamp with methanol to give centralization of 40 µg/ml of THC and 250 µg/ml of DKP.

Preparation of calibration curve

Alignment bend for THC comprised of diverse centralizations of standard THC arrangement running from 4-20 µg/ml. arrangements were arranged by exchanging 0.4, 0.8, 1.2, 1.6 and 2.0 ml aliquots of working standard arrangement of THC (100 µg/ml) into arrangement of 10 ml volumetric cups and volume was changed in accordance with imprint with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for THC. For DKP Calibration bend going from 5-45 µg/ml. aliquots from DKP (100 µg/ml) 0.5, 1.5, 2.5, 3.4 and 4.5 ml were moved
into arrangement of 10 ml volumetric cups and volume was changed in
accordance with imprint with methanol to get last conc. of 5, 15, 25, 35 and 45 µg/ml for DKP.

**Analysis of marketed formulation**

Twenty tablets were measured; normal weight was computed then controlled. amount of powder proportionate to 4 mg THC and 25 mg DKP was moved in 100 ml volumetric flagon. Methanol (50 ml) was added to it and sonicated for 20 min. arrangement was sifted through Whatman channel paper No. 41 and buildup was washed with methanol. Washing and filtrate were consolidated in another 100 ml volumetric carafe and volume was balanced up to imprint with methanol. above arrangement was suitably weakened with methanol to get last convergance of 4 µg/ml of THC and 25 µg/ml of DKP.

For concurrent determination utilizing Absorption amendment system, test arrangement of medications was checked in scope of 200-400 nm. absorbance was measured at 375 nm and 240 nm for evaluation of THC and DKP individually. measures of THC and DKP exhibit in test arrangements were controlled by utilizing after mathematical statement.

\[
C_{THC} = \frac{(375 \text{ nm})}{(1\%, 1\text{cm})} \quad \text{375 nm} \\
C_{DKP} = \frac{C_{ADKP}(240 \text{ nm})}{(1\%, 1\text{cm})} \quad 240 \text{ nm} \\
CA_{DKP}(240 \text{ nm}) = (240 \text{ nm}) - A_{THC(375 \text{ nm})} \\
A_{THC(375 \text{ nm})} = C_{THC} \times (1\%,1\text{cm})375 \text{ nm}
\]

Where,

- \(C_{THC}\) = Concentration of THC
- \(C_{DKP}\) = Concentration of DKP
- \(A_{(375 \text{ nm})}\) = Absorbance of mixture at 375 nm
- \(A_{(240 \text{ nm})}\) = Absorbance of mixture at 240 nm
- \(CA_{DKP}(240 \text{ nm})\) = Corrected absorbance of DKP at 240 nm
- \(A_{THC(375 \text{ nm})}\) = Absorbance of THC at 375 nm.

**Validation of Absorbance Correction Method**

**Linearity and range**
Linearity is communicated as far as connection co-effective of straight relapse examination.

The linearity reaction was controlled by investigating 5 autonomous levels of adjustment bend in scope of 4-20 µg/ml for THC and 5-45 µg/ml for DKP. considerable measure of alignment bend of absorbance versus focus and connection coefficient and relapse line comparisons for THC and DKP decided.

Accuracy

Preparation of Sample solution:
Twenty tablets were measured; powdered and amount of powder equal to 4 mg of THC and 25 mg of DKP was measured and moved into 100 ml of volumetric jar, Methanol (50 ml) was added to it and sonicated for 20 min. arrangement was sifted through Whatman channel paper No. 41 and volume was balanced up to imprint with methanol (40 µg/ml of THC and 250 µg/ml of DKP).

Pipette out 1 ml of above arrangement in 10 ml volumetric cup and weakened to stamp with methanol (4 µg/ml of THC and 25 µg/ml of DKP). measure of THC and DKP was figured at each of three level and % recuperations were processed (80%, 100% and 12

Precision

Repeatability

From Sample arrangement aliquot was exchanged to different 10 ml volumetric carafe and weakened up to check with methanol such that it gives centralization of 4 µg/ml of THC and 25 µg/ml of DKP. Every fixation was readied Six times. absorbance of every arrangement was measured at chose wavelengths and % CV was calculated.

Intraday precision
Sample solutions containing 4, 12, 20 µg/ml of THC and 5, 25, 45 µg/ml of DKP were analyzed three times on same day and % CV was calculated.

**Interday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and 5, 25, 45 µg/ml of DKP were analyzed on three different days and % CV was calculated.

**Limit of detection**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was figured. At that point LOD was measured by utilizing scientific expressions given as part of segment.

The cutoff of recognition (LOD) of medication was ascertained by utilizing accompanying mathematical statements assigned by International Conference on Harmonization (ICH) rule:

\[
LOD = 3.3 \times \frac{\sigma}{S},
\]

Where, \(\sigma\) = standard deviation of Intercept

\(S\) = Mean slope of calibration curve

**Limit of quantification**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was figured. At that point LOQ was measured by utilizing scientific expressions given as part of area.

The breaking point of evaluation (LOQ) of medication was ascertained by utilizing accompanying comparisons assigned by International Conference on Harmonization (ICH) rule:

\[
LOQ = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = standard deviation of Intercept
S = Mean slope of calibration curve

3.3.2 SIMULTANEOUS ESTIMATION OF THC AND ETD

The mixture of THC (THC) and ETD (ETD) is present in ratio of 1:37.5 (THC:ETD). absorption spectra of pure drugs and their dosage form were recorded between 200-400 nm using methanol as solvent. Methanol was used as common solvent for preparation of solutions.

3.3.2.1 First order Derivative spectroscopic method

Selection of Zero Crossing Point (ZCP) for estimation of THC and ETD

It is technique for decision for estimation of medications in consolidated measurement structure in light of its capacity to determine two covering spectra and disposing of lattice impedances or obstructions because of vague shoulder on side of assimilation band, which is impractical by whatever other UV spectroscopic systems. To begin with request subordinate strategy was observed to be more precise as it is more delicate to littlest changes in focus. It has advantage that it takes out otherworldly impedance from one of two medications while evaluating other medication by selecting zero intersection point in subordinate spectra of every medication at chose wavelength. Each of the Zero request spectra were then changed over to their individual first request Derivative Spectra utilizing inbuilt programming and Zero Crossing point (ZCP) chose. choice of ZCP ought to be done such that at zero intersection purpose of one medication other medication shows extensive absorbance. After determination of ZCP for both medications, synchronous estimation of THC and ETD can be completed in promoted detailing.

Preparation of standard solution

Arrangement of standard stock arrangement of THC (1000 µg/ml):

Exactly measured sum 10 mg of THC was moved into 10 ml volumetric glass, separated and debilitated up to stamp with methanol. This gave stock plan having nature of 1000 µg/ml.
Arrangement of working standard arrangement of THC (10 µg/ml):

10 µg/ml of THC arrangement was arranged by weakening 0.25 ml of stock arrangement up to stamp with methanol in 25 ml volumetric jar.

Readiness of standard stock arrangement of ETD (1000 µg/ml):

Absolutely measured sum 10 mg of ETD was moved into 10 ml volumetric glass, separated and debilitated up to check with methanol. This will give stock course of action having nature of 1000 µg/ml.

Arrangement of consolidated working standard arrangement containing THC and ETD

Unequivocally measured measure of tablet powder indistinguishable to 4 mg of THC and 150 mg of ETD were traded to 100 ml volumetric container, separated in satisfactory measure of methanol and debilitated up to check with methanol to give centralization of 4 µg/ml of THC and 150 µg/ml of ETD.

Arrangement of adjustment bend

Modification twist for THC involved particular convergances of standard THC game plan going from 2-6 µg/ml. game plans were masterminded by trading 2.0, 3.0, 4.0, 5.0 and 6.0 ml aliquots of working standard course of action of THC (10 µg/ml) into game plan of 10 ml volumetric carafes and volume was changed as per engraving with methanol to get last conc. of 2, 3, 4, 5 and 6 µg/ml for THC. For ETD Calibration twist reaching out from 75-175 µg/ml. aliquots from ETD (10 µg/ml) 0.75, 1.0, 1.25, 1.5 and 1.75 ml were moved into course of action of 10 ml volumetric containers and volume was adjusted to stamp with methanol to get last conc. of 75, 100, 125, 150 and 175 µg/ml for ETD. Every one of the Zero solicitation overlain spectra were then changed over to their specific first demand Derivative Spectra and estimation is done on suitable ZCP for each of THC and ETD.

Examination of promoted detailing
20 tablets were measured; typical weight was discovered then filled. measure of powder practically identical to 8 mg THC and 300 mg ETD was moved in 100 ml volumetric container. Methanol (50 ml) was added to it and sonicated for 20 min. plan was isolated through Whatman channel paper No. 41 and development was washed with methanol. Washing and filtrate were united in another 100 ml volumetric container and volume was adjusted up to engrave with methanol. above game plan was suitably debilitated with methanol to get last hoarding of 4 µg/ml of THC and 150 µg/ml of ETD. Absorbance was measured at picked ZCP and measures of THC and ETD in tablets were registered by suggesting scientific proclamation.

VALIDATION OF FIRST ORDER DERIVATIVE UV METHOD

Linearity and range

Linearity is communicated as far as relationship co-productive of straight relapse investigation.

The linearity reaction was dictated by investigating 5 free levels of adjustment bend in scope of 2-6 µg/ml for THC and 75-175 µg/ml for ETD. ton of alignment bend of absorbance versus fixation and relationship coefficient and relapse line mathematical statements for THC and ETD decide

Accuracy

Preparation of Sample solution:

It was carried out to determine suitability and reliability of proposed method. Accuracy was determined by calculating % Recovery. amount of THC and ETD was calculated at each of three level and % recoveries were computed at three different levels (80%, 100% and 120%). For THC accuracy study performed on 4 µg/ml concentration and 150 µg/ml for ETD.

Precision

Repeatability
From Sample arrangement aliquot was exchanged to different 10 ml volumetric flagon and weakened up to stamp with methanol such that it gives centralization of 4 µg/ml of THC and 150 µg/ml of ETD. Every fixation was readied Six times. absorbance of every arrangement was measured at chose wavelengths and % CV was ascertained.

**Intraday precision**

Sample solutions containing 3, 4, 5 µg/ml of THC and 100, 125, 150µg/ml of ETD were analyzed three times on same day and % CV was calculated.

**Interday precision**

Sample solutions containing 3, 4, 5µg/ml of THC and 100, 125, 150 µg/ml of ETD were analyzed on three different days and % CV was calculated.

**Limit of detection**

From linearity bend comparison, standard deviation (SD) of captures (reaction) was figured. At that point LOD was measured by utilizing scientific expressions given as part of area.

Point of confinement of recognition (LOD) of medication was ascertained by utilizing accompanying mathematical statements assigned by International Conference on Harmonization (ICH) rule:

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S},
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Limit of quantification**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was figured. At that point LOQ was measured by utilizing numerical expressions given as part of area.
The farthest point of measurement (LOQ) of medication was ascertained by utilizing accompanying mathematical statements assigned by International Conference on Harmonization (ICH) rule:

\[ \text{LOQ} = 10 \times \frac{\sigma}{S} \]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

3.3.2.2 Area Under Curve Method
Selection of wavelength range for estimation

suitable amassings of THC and ETD were arranged independently in methanol to get last conc. 10 µg/ml for both THC and ETD. Both medication arrangements were examined in scope of 200-400 nm and overlain range was contemplated. After rehashed perceptions of reaction wavelength extents were chosen for estimation of THC and ETD further territory was incorporated between chose wavelength reaches and estimation was done.

Preparation of standard solution

Preparation of standard stock solution of THC (1000 µg/ml):

Precisely measured amount 10 mg of THC was moved into 10 ml volumetric carafe, broke down and weakened up to stamp with methanol. This gave stock arrangement having quality of 1000 µg/ml

Preparation of working standard solution of THC (10 µg/ml):

10 µg/ml of THC arrangement was arranged by weakening 0.25 ml of stock arrangement up to imprint with methanol in 25 ml volumetric flask.

Preparation of standard stock solution of ETD (1000 µg/ml):
Precisely measured amount 10 mg of ETD was moved into 10 ml volumetric cup, disintegrated and weakened up to stamp with methanol. This will give stock arrangement having quality of 1000 µg/ml

**Preparation of combined working standard solution containing THC and ETD**

Precisely measured amount of tablet powder proportionate to 4 mg of THC and 150 mg of ETD were exchanged to 100 ml volumetric cup, disintegrated in adequate measure of methanol and weakened up to check with methanol to give centralization of 4 µg/ml of THC and 150 µg/ml of ETD.

**Preparation of calibration curve**

Adjustment bend for THC comprised of diverse convergances of standard THC arrangement extending from 2-6 µg/ml. arrangements were arranged by exchanging 2.0, 3.0, 4.0, 5.0 and 6.0 ml aliquots of working standard arrangement of THC (10 µg/ml) into arrangement of 10 ml volumetric carafes and volume was conformed to stamp with methanol to get last conc. of 2, 3, 4, 5 and 6 µg/ml for THC. For ETD Calibration bend running from 75-175 µg/ml. aliquots from ETD (10 µg/ml) 0.75, 1.0, 1.25, 1.5 and 1.75 ml were moved into arrangement of 10 ml volumetric cups and volume was conformed to check with methanol to get last conc. of 75, 100, 125, 150 and 175 µg/ml for ETD. Inspecting wavelength extents chose for estimation of territory of THC and ETD were 251-261 nm (λ₁-λ₂) and 274-284 nm (λ₃-λ₄) separately and zone were incorporated between these chose wavelengths ranges for both medications, which indicated direct reaction with expanding focus. Alignment bend of zone versus fixation was plotted and straight-line comparised

**Analysis of marketed formulation**

Twenty tablets were measured; normal weight was ascertained then fueled. amount of powder equal to 8 mg THC and 300 mg ETD was moved in 100 ml volumetric jar. Methanol (50 ml) was added to it and sonicated for 20 min.
arrangement was sifted through Whatman channel paper No. 41 and buildup was washed with methanol. Washing and filtrate were consolidated in another 100 ml volumetric cup and volume was balanced up to imprint with methanol. above arrangement was suitably weakened with methanol to get last centralization of 4 µg/ml of THC and 150 µg/ml of ETD.

For synchronous determination utilizing zone under bend (AUC) strategy, test arrangement of medications were examined in scope of 200-400 nm range was and ETD individually. measures of THC and ETD introduce in test arrangements were dictated by fitting reaction into relapse mathematical statement acquired from adjustment bends of THC and ETD.

The concentration of THC and ETD was obtained by following equations:

\[ C_{THC} = \frac{A_2 aY_1 - A_1 aY_2}{aX_2 aY_1 - aX_1 aY_2} \]  

\[ C_{ETD} = \frac{A_1 aX_2 - A_2 aX_1}{aX_2 aY_1 - aX_1 aY_2} \]  

Where,

- \( C_{THC} \) = Concentrations of THC,
- \( C_{ETD} \) = Concentrations of ETD,
- \( A_1 \) = Area of mixture at 251-261 nm,
- \( A_2 \) = Area of mixture at 274-284 nm,
- \( aX_1 \) = Absorptivity value of THC at 251-261nm,
- \( aX_2 \) = Absorptivity value of THC at 274-284nm.
VALIDATION OF AUC METHOD

Linearity and range

Linearity is communicated regarding relationship co-productive of direct relapse investigation.

The linearity reaction was dictated by breaking down 5 free levels of alignment bend in scope of 2-6 µg/ml for THC and 75-175 µg/ml for ETD. great deal of adjustment bend of territory versus fixation and connection coefficient and relapse line mathematical statements for THC and ETD decide

Accuracy

Preparation of Sample solution:

It was completed to focus suitability and dependability of proposed system. Precision was dictated by figuring % Recovery. measure of THC and ETD was figured at each of three level and % recuperations were registered at three unique levels (80%, 100% and 120%). For THC exactness study performed on 4 µg/ml focus and 150 µg/ml for ETD.

Precision

Repeatability

From Sample arrangement aliquot was exchanged to different 10 ml volumetric jar and weakened up to stamp with methanol such that it gives centralization of 4 µg/ml of THC and 150 µg/ml of ETD. Every fixation was readied Six times. range of every arrangement was measured at chose wavelengths and % CV was ascertained.

Intraday precision
Sample solutions containing 3, 4, 5 µg/ml of THC and 100, 125, 150 µg/ml of ETD were analyzed three times on same day and % CV was calculated.

**Interday precision**

Sample solutions containing 3, 4, 5 µg/ml of THC and 100, 125, 150 µg/ml of ETD were analyzed on three different days and % CV was calculated.

**Limit of detection**

From linearity bend comparison, standard deviation (SD) of captures (reaction) was ascertained. At that point LOD was measured by utilizing scientific expressions given as part of area. breaking point of location (LOD) of medication was computed by utilizing accompanying mathematical statements assigned by ICH rule:

\[
LOD = 3.3 \times \frac{\sigma}{S},
\]

Where, \(\sigma\) = standard deviation of Intercept

\(S\) = Mean slope of calibration curve

**Limit of quantification**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was figured. At that point LOQ was measured by utilizing scientific expressions given as part of area. point of confinement of evaluation (LOQ) of medication was figured by utilizing accompanying comparisons assigned by ICH rule:

\[
LOQ = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = standard deviation of Intercept

\(S\) = Mean slope of calibration curve

3.3.3 SIMULTANEOUS ESTIMATION OF THC AND LNC
The mixture of THC (THC) and LNC (LNC) is present in ratio of 1:1 (THC: LNC). Absorption spectra of pure drugs and their dosage form were recorded between 200-400 nm using methanol as solvent. Methanol was used as common solvent for preparation of solutions.

3.3.3.1 Area Under Curve Method
Selection of wavelength range for estimation of THC and LNC
The suitable convergences of THC and LNC were arranged independently in methanol to get last conc. 10 µg/ml for both THC and LNC. Both medication arrangements were filtered in scope of 200-400 nm and overlain range was mulled over. After rehashed perceptions of reaction wavelength reaches were chosen for estimation of THC and LNC further territory was incorporated between chose wavelength extents and estimation was done.

Preparation of standard solution

Preparation of standard stock solution of THC (1000 µg/ml):
Accurately weighed quantity 10 mg of THC was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol. This gave stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of THC (100 µg/ml):
100µg/ml of THC solution was prepared by diluting 2.5 ml of stock solution up to mark with methanol in 25 ml volumetric flask.

Preparation of standard stock solution of LNC (1000 µg/ml):
Accurately weighed quantity 10 mg of LNC was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol. This will give stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of LNC (100 µg/ml):
100µg/ml of LNC solution was prepared by diluting 2.5 ml of stock solution up to mark with methanol in 25 ml volumetric flask.

**Preparation of combined working standard solution containing THC and LNC**

Precisely measured amount of tablet powder comparable to 40 mg of THC and 40 mg of LNC were exchanged to 100 ml volumetric flagon, broke down in adequate measure of methanol and weakened up to check with methanol to give centralization of 4 µg/ml of THC and 4 µg/ml of LNC.

**Preparation of calibration curve**

Alignment bend for THC comprised of distinctive centralizations of standard THC arrangement going from 4-20 µg/ml. arrangements were arranged by exchanging 0.4, 0.8, 1.2, 1.6 and 2.0 ml aliquots of working standard arrangement of THC (100 µg/ml) into arrangement of 10 ml volumetric flagons and volume was changed in accordance with imprint with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for THC. Also for LNC, Calibration bend running from 4-20 µg/ml. aliquots from LNC (100 µg/ml) 0.4, 0.8, 1.2, 1.6 and 2.0 ml were moved into arrangement of 10 ml volumetric cups and volume was acclimated to stamp with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for LNC.

**Analysis of marketed formulation**

Twenty tablets were measured; normal weight was ascertained then fueled. amount of powder proportional to 8 mg of THC and 8 mg of LNC was moved in 100 ml volumetric cup. Methanol (50 ml) was added to it and sonicated for 20 min. arrangement was sifted through Whatman channel paper No. 41 and deposit was washed with methanol. Washing and filtrate were joined in another 100 ml volumetric cup and volume was balanced up to imprint with methanol. above arrangement was suitably weakened with methanol to get last centralization of 4 µg/ml of THC and 4 µg/ml of LNC.
For synchronous determination utilizing zone under bend (AUC) strategy, test arrangement of medications were examined in scope of 200-400 nm, range was measured at 365-375 nm (λ1-λ2) and 285-295 nm (λ3-λ4) for evaluation of THC and LNC individually. measures of THC and LNC display in test arrangements were controlled by fitting reaction into relapse mathematical statement got from alignment bends of THC and LNC.

The concentration of THC and LNC was obtained by following equations:

\[
C_{THC} = \frac{A_2 aY_1 - A_1 aY_2}{aX_2 aY_1 - aX_1 aY_2} \tag{1}
\]

\[
C_{LNC} = \frac{A_1 aX_2 - A_2 aX_1}{aX_2 aY_1 - aX_1 aY_2} \tag{2}
\]

Where,

\(C_{THC}\) = Concentrations of THC,

\(C_{LNC}\) = Concentrations of LNC,

\(A_2\) = Area of mixture at 285-295 nm,

\(aX_1\) = Absorptivity value of THC at 285-295 nm,

\(aY_1\) = Absorptivity value of THC at 365-375 nm.

VALIDATION OF AUC METHOD

Linearity and range
Linearity is communicated as far as relationship co-proficient of straight relapse examination. Linearity reaction was controlled by breaking down 5 free levels of adjustment bend in scope of 4-20 µg/ml for THC and LNC. great deal of adjustment bends of zone versus focus and relationship coefficient and relapse line mathematical statements for THC and LNC decided.

**Accuracy**

**Preparation of Sample solution:**

It was done to focus suitability and unwavering quality of proposed strategy. Precision was dictated by figuring % Recovery. measure of THC and LNC was ascertained at each of three level and % recuperations were registered at three distinct levels (80%, 100% and 120%). For both medications THC and LNC exactness study performed on 4 µg/ml.

**Precision**

**Repeatability**

From Sample arrangement aliquot was exchanged to different 10 ml volumetric jar and weakened up to stamp with methanol such that it gives convergance of 4 µg/ml for both THC and LNC. Every fixation was readied Six times. region of every arrangement was measured at chose wavelength rangeess and % CV was computed.

**Intraday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were separately analyzed three times on same day and % CV was calculated.

**Interday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were analyzed on three different days and % CV was calculated.

**Limit of detection**

From linearity bend comparison, standard deviation (SD) of captures (reaction) was figured. At that point LOD was measured by utilizing scientific expressions
given as part of segment. farthest point of location (LOD) of medication was computed by utilizing accompanying mathematical statements assigned by ICH rule:

\[ \text{LOD} = 3.3 \times \sigma / S, \]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Limit of quantification**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was ascertained. At that point LOQ was measured by utilizing numerical expressions given as part of segment. utmost of evaluation (LOQ) of medication was figured by utilizing accompanying comparisons assigned by ICH rule:

\[ \text{LOQ} = 10 \times \sigma / S \]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**3.3.3.2 First order Derivative spectroscopic method**

**Selection of Zero Crossing Point (ZCP) for estimation of THC and LNC**

Derivative spectroscopy is method of choice for estimation of drugs in combined dosage form because of its ability to resolve two overlapping spectra and eliminating matrix interferences or interferences due to indistinct shoulder on side of absorption band, which is not possible by any other UV spectroscopic methods. First order derivative method was found to be more accurate as it is more sensitive to smallest changes in concentration. It has advantage that it eliminates spectral interference from one of two drugs while estimating other drug by selecting zero crossing point in derivative spectra of each drug at selected wavelength. All Zero order spectra were then converted to their respective 1\textsuperscript{st} order Derivative Spectra using inbuilt software and Zero Crossing point (ZCP) selected. selection of ZCP should be done such that at zero crossing point of one drug other drug shows considerable absorbance. After selection of ZCP for both drugs, simultaneous estimation of THC and LNC can be carried out in marketed formulation.
Preparation of standard solution

Preparation of standard stock solution of THC (1000 µg/ml):

Precisely measured amount 10 mg of THC was moved into 10 ml volumetric carafe, disintegrated and weakened up to stamp with methanol. This gave stock arrangement having quality of 1000 µg/ml.

Preparation of working standard solution of THC (100 µg/ml):

100µg/ml of THC solution was prepared by diluting 2.5 ml of stock solution up to mark with methanol in 25 ml volumetric flask.

Preparation of standard stock solution of LNC (1000 µg/ml):

Precisely measured amount 10 mg of LNC was moved into 10 ml volumetric flagon, disintegrated and weakened up to stamp with methanol. This will give stock arrangement having quality of 1000 µg/ml.

Preparation of working standard solution of LNC (100 µg/ml):

100µg/ml of LNC solution was prepared by diluting 2.5 ml of stock solution up to mark with methanol in 25 ml volumetric flask.

Preparation of combined working standard solution containing THC and LNC

Precisely measured amount of tablet powder identical to 40 mg of THC and 40 mg of LNC were exchanged to 100 ml volumetric flagon, broke down in adequate measure of methanol and weakened up to stamp with methanol to give amassing of 4 µg/ml of THC and 4 µg/ml of LNC.

Preparation of calibration curve

Alignment bend for THC comprised of diverse convergances of standard THC arrangement going from 4-20 µg/ml. arrangements were arranged by exchanging 0.4, 0.8, 1.2, 1.6 and 2.0 ml aliquots of working standard
arrangement of THC (100 µg/ml) into arrangement of 10 ml volumetric cups and volume was changed in accordance with imprint with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for THC. Correspondingly for LNC, Calibration bend going from 4-20 µg/ml aliquots from LNC (100 µg/ml) 0.4, 0.8, 1.2, 1.6 and 2.0 ml were moved into arrangement of 10 ml volumetric flacons and volume was changed in accordance with imprint with methanol to get last conc. of 4, 8, 12, 26 and 20 µg/ml for LNC. All Zero request overlain spectra were then changed over to their separate first request Derivative Spectra and estimation is done on suitable ZCP for each of THC and LNC.

Analysis of marketed formulation

Twenty tablets were measured; normal weight was computed then controlled. amount of powder identical to 8 mg of THC and 8 mg of LNC was moved in 100 ml volumetric cup. Methanol (50 ml) was added to it and sonicated for 20 min. arrangement was sifted through Whatman channel paper No. 41 and buildup was washed with methanol. Washing and filtrate were consolidated in another 100 ml volumetric jar and volume was balanced up to imprint with methanol. above arrangement was suitably weakened with methanol to get last convergance of 4 µg/ml of THC and 4 µg/ml of LNC. Absorbance was measured at chose ZCP and measures of THC and LNC in tablets were figured by alluding mathematical statement of straight line.

VALIDATION OF FIRST ORDER DERIVATIVE METHOD

Linearity and range

Linearity is communicated as far as connection co-effective of straight relapse investigation. linearity reaction was controlled by investigating 5 autonomous levels of adjustment bend in scope of 4-20 µg/ml for THC and LNC. considerable measure of alignment bend of absorbance versus fixation and relationship coefficient and relapse line comparisons for THC and LNC decided.

Accuracy
Preparation of Sample solution:

It was completed to focus suitability and unwavering quality of proposed system. Exactness was controlled by computing % Recovery. measure of THC and LNC was figured at each of three level and % recuperations were processed at three distinct levels (80%, 100% and 120%). For both medications THC and LNC precision study performed on 4 µg/ml.

**Precision**

**Repeatability**

From Sample arrangement aliquot was exchanged to different 10 ml volumetric carafe and weakened up to stamp with methanol such that it gives centralization of 4 µg/ml for both THC and LNC. Every focus was readied Six times. absorbance of every arrangement was measured at chose wavelengths and % CV was ascertained.

**Intraday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were separately analyzed three times on same day and % CV was calculated.

**Interday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were analyzed on three different days and % CV was calculated.

**Limit of detection**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was ascertained. At that point LOD was measured by utilizing scientific expressions given as part of segment.

The point of confinement of location (LOD) of medication was ascertained by utilizing accompanying mathematical statements assigned by International Conference on Harmonization (ICH) rule:
LOD = 3.3 X σ/S,

Where, σ = standard deviation of Intercept

\[
S = \text{Mean slope of calibration curve}
\]

**Limit of quantification**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was ascertained. At that point LOQ was measured by utilizing numerical expressions given as part of segment.

The point of confinement of measurement (LOQ) of medication was figured by utilizing accompanying comparisons assigned by International Conference on Harmonization (ICH) rule:

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, σ = standard deviation of Intercept

\[
S = \text{Mean slope of calibration curve}
\]

3.4 **RP-HPLC METHODS WITH FORCED DEGRADATION STUDIES AND QBD APPROACH FOR ROBUSTNESS STUDY**

3.4.1 **SIMULTANEOUS ESTIMATION OF THC AND DKP**

**Selection of detection wavelength**

The affectability of HPLC strategy that uses UV identification relies on legitimate determination of recognition wavelength. perfect wavelength is particular case that gives great reaction for medication that is to be recognized. In present study drug arrangement of 10µg/ml THC & 10µg/ml DKP was, in this manner, arranged in Methanol: Sodium Phosphate Buffer (70:30 v/v) pH 4.5. These medication arrangements were then checked in UV area of 200-400 nm and overlain ranges were recorded.
Selection of chromatographic condition

Fitting choice of HPLC technique relies on way of example (ionic, ionizable or impartial atom), its sub-atomic weight and solveny. medication chose for present study is polar in nature and thus either turned around stage or particle pair or particle trade chromatography can be utilized. Turned around stage HPLC was chosen for starting detachments in light of its straightforwardness and suitability.

To upgrade chromatographic conditions, impact of chromatographic variables, for example, versatile stage pH, stream rate, and dissolvable proportion were considered. subsequent chromatograms were recorded and chromatographic parameters, for example, limit variable, unbalanced component and segment proficiency were ascertained. conditions that gave best determination, symmetry and limit component were chosen for estimation.

Selection of flow rate of mobile phase

After numbers of trial flow rate of mobile phase was optimizes as 1.0 ml/min, showing best resolution.

Selection of elution mode

Converse stage chromatography was picked in light of its prescribed utilization for ionic and moderate to polar mixes. It is basic, helpful as well as better performs as far as effectiveness, dependability and reproducibility. C18 section was chosen on grounds that it is minimum polar contrasted with C4 and C8 segments. C18 section permits eluting polar mixes all more rapidly in correlation to non-polar mixes. Notwithstanding this, UV finder is utilized, which permits simple discovery of mixes in UV straightforward natural solvents. 250 x 4.6 mm section of 5µm particles pressing was favored as beginning stage for system improvement. Slope mode was picked because of straightforwardness in application and power as for more segment soundness.

Optimize Chromatographic conditions
Stationary phase: RP C\textsubscript{18} (250×4.6mm) 5µ

Mobile phase : Methanol: Sodium Phosphate Buffer (70:30 v/v)

pH : 4.5

Flow rate : 1.0 ml/min

Wavelength : 280 nm

**Preparation of standard solution**

**Standard stock solution of THC**

Accurately weighed THC (100 mg) was transferred to 100 ml volumetric flask and dissolved in methanol and diluted up to mark with methanol to give stock solution having strength 1 mg/ml (1000 µg/ml).

**Working standard solution of THC**

100 µg/ml of THC working standard solution was prepared by diluting 1 ml of stock solution with methanol to 10 ml with methanol.

**Standard stock solution of DKP**

Accurately weighed DKP (100 mg) was transferred into 100 ml volumetric flask and dissolved in methanol and diluted up to mark with methanol to give stock solution having strength 1 mg/ml (1000 µg/ml).

**Working standard solution of DKP**

100 µg/ml of DKP working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with methanol.

**Preparation of Calibration Curve for THC and DKP**
Calibration curve for THC

The adjustment bend was developed with five fixations extending from 5-40 µg/ml for THC. arrangements were arranged by exchanging 0.5, 1, 2, 3 and 4 ml aliquots of working standard arrangement of THC (100 µg/ml) into arrangement of 10 ml volumetric jars and volume was acclimated to stamp with methanol to get last amassing of 5, 10, 20, 30, 40 µg/ml. Every one of arrangements was infused. information of top range versus focus was dealt with by direct slightest square relapse examination.

Calibration curve for DKP

The alignment bend was built with five fixations extending from 30-240µg/ml for DKP. arrangements were arranged by exchanging 0.3, 0.6, 1.2, 1.8 and 2.4 ml aliquots of standard stock arrangement of DKP (1000 µg/ml) into arrangement of 10 ml volumetric cups and volume was acclimated to stamp with methanol to get last centralization of 30, 60, 120, 180, 240 µg/ml. Every one of arrangements was infused. information of crest territory versus fixation was dealt with by direct minimum square relapse investigation.

Preparation of binary mixtures of THC and DKP

Precisely measured 100 mg THC and 600 mg of DKP were exchanged to 100 ml volumetric cup. It was disintegrated with adequate methanol and weakened up to stamp with methanol to give amassing of 1000 µg/ml of THC and 6000 µg/ml of DKP. 2.5 ml of this arrangement was further weakened to 25 ml with methanol to get 100 µg/ml of THC and 600 µg/ml of DKP. Above arrangement was weakened further to get focus scope of 5, 10, 20, 30 and 40 µg/ml for THC and 30, 60, 120, 180 and 240 µg/ml for DKP.

FORCED DEGRADATION STUDY:

In order to establish whether analytical method for assay was stability-indicating, pure active pharmaceutical ingredient (API) and tablet content of THC & DKP was subjected to various stress conditions to conduct forced degradation
studies. Stress studies were carried out under conditions of acid/base hydrolysis, oxidation, Thermal as mentioned in ICH Q1A (R2). UV light degradation of drug substances and drug product was performed in solid state as mentioned in ICH Q1B.

**Acid Degradation:**

To different 10 ml volumetric flask, 1 ml stock solutions of THC and DKP were taken and 2 ml of 1M HCl for THC and DKP was added respectively. Both flasks were heated at 60°C for 0 min, 30 min, 1 hr and 2 hr and allowed to cool to room temperature. Solutions were neutralized with 1 M NaOH and diluted up to mark with mobile phase. Appropriate aliquots were taken from above solutions and diluted with mobile phase to obtain final concentration of 20 µg/ml THC and 120 µg/ml DKP separately and in mixture.

**Alkali hydrolysis:**

To distinctive 10 ml volumetric flagon, 1 ml stock arrangements of THC and DKP were taken and 2 ml of 1 M NaOH for THC and DKP was included separately. Both cups were warmed at 60°C for 0 min, 30 min, 1 hr and 2 hr and permitted to cool to room temperature. Arrangements were killed with 1 M HCl and weakened up to imprint with versatile stage. Suitable aliquots were taken from above arrangements and weakened with versatile stage to get last amassing of 20 µg/ml THC and 120 µg/ml DKP independently and in blend.

**Oxidative stress degradation:**

To perform oxidative anxiety corruption, suitable aliquots of stock arrangements of THC and DKP were taken in two diverse 10 ml volumetric jars and 2 ml of 3 % hydrogen peroxide was included separately. Both jars were warmed in water shower at 60 C for 0 min, 30 min and 1 hr and permitted to cool to room temperature and weakened up to imprint with versatile stage. Suitable aliquots were taken from above arrangements and weakened with versatile stage to get
last centralization of 20 µg/ml THC and 120 µg/ml DKP independently and in blend.

**Photolytic Degradation:**

**Condition (1): In UV-Light for 30 min.**

Systematically 10mg of unadulterated examples of THC and DKP were uncovered in UV-light at 80°C for 30 min independently in 10 ml volumetric flagons. solids were permitted to cool and broke down in few ml of methanol. Volumes were made up to imprint with methanol. Arrangements were further weakened by portable stage taking suitable aliquots in 10 ml volumetric carafe to acquire last centralization of 20 µg/ml THC and 120 µg/ml DKP independently and in blend.

**Condition (2): In Sun-Light for 30 min.**

Logically 10mg of unadulterated examples of THC and DKP were uncovered in Sun-light for 30 min independently in 10 ml volumetric jars. solids were permitted to cool and broke down in few ml of methanol. Volumes were made up to imprint with methanol. Arrangements were further weakened by versatile stage taking proper aliquots in 10 ml volumetric cup to get last convergence of 20 µg/ml THC and 120 µg/ml DKP independently and in blend.

**Thermal Degradation:**

Systematically 10mg of immaculate examples of THC and DKP were uncovered in Hot Air Oven at 60°C for 30 min independently in 10 ml volumetric cups. solids were permitted to cool and broke down in few ml of methanol. Volumes were made up to imprint with methanol. Arrangements were further weakened by versatile stage taking suitable aliquots in 10 ml volumetric jar to acquire last centralization of 20 µg/ml THC and 120 µg/ml DKP independently and in blend.

All response arrangements were infused in fluid chromatographic framework and chromatograms were recorded
VALIDATION

System suitability studies

The system suitability was evaluated by five replicate analyses of THC and DKP mixture at concentration of 20µg/ml of THC and 120µg/ml of DKP. Column efficiency (Numbers of Theoretical Plates), Resolution and Peak Asymmetry (Tailing Factor) were calculated for standard solutions.

Linearity

Suitable aliquots of THC and DKP working standard arrangements were taken in distinctive 10 ml volumetric cups and weakened up to imprint with versatile stage to acquire last centralizations of 5, 10, 20, 30 and 40 µg/ml for THC and 30, 60, 120, 180 and 240 µg/ml for DKP. arrangements were infused utilizing 20 µL settled circle framework and chromatograms were recorded. Adjustment bends were built by plotting normal crest territory versus focuses and relapse mathematical statements were registered for both medication.

Precision

Result should be expressed as relative standard deviation (RSD) or co-efficient of variance (% CV).

Repeatability

Repeatability was carried out by estimating response of 20µg/ml of THC and 120µg/ml of DKP, six times and results are reported in terms of relative standard deviation.

Intra-day precision

Mixed solutions containing THC (10, 20, 30 µg/ml) and DKP (60, 120, 180 µg/ml) were analyzed three times on same day and % CV was calculated.
**Inter-day precision**

Mixed solutions containing THC (10, 20, 30 µg/ml) and DKP (60, 120, 180 µg/ml) were analyzed three times on three different days and % CV was calculated.

**Accuracy**

It was done to focus suitability and unwavering quality of proposed system. Exactness was dictated by ascertaining % Recovery. Twenty tablets were measured and powdered. Powder equal to 20 mg of THC and 120 mg of DKP was measured and moved into 100 ml of volumetric jar, standard was included premise of 80%, 100% and 120 % and weakened up to check with portable stage. measure of THC and DKP was figured at each of three level and % recuperations were processed at three unique levels (80%, 100% and 120%). For THC exactness study performed on 20 µg/ml focus and 120 µg/ml for DKP.

**Limit of detection**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was figured. At that point LOD was measured by utilizing scientific expressions given as part of segment. breaking point of recognition (LOD) of medication was ascertained by utilizing accompanying mathematical statements assigned by ICH rule:

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S},
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Limit of quantification**
From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was computed. At that point LOQ was measured by utilizing numerical expressions given as part of segment. farthest point of measurement (LOQ) of medication was figured by utilizing accompanying comparisons assigned by ICH rule:

\[
LOQ = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma \) = standard deviation of Intercept
\( S \) = Mean slope of calibration curve

**Robustness**

Robustness and Ruggedness of method was determined by subjecting method to slight change in method condition, individually, the:

- Pump flow rate,
- PH Change.
- Mobile Phase Composition change

Three replicates were made for same concentration (20\( \mu \)g/ml of THC and 120\( \mu \)g/ml of DKP). % CV was calculated.

**Specificity**

Specificity is capacity of technique to gauge analyte reaction in vicinity of its potential pollutions and corruption items. Generally utilized excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into pre measured amount of medications. chromatogram was taken by fitting weakenings and amounts of medications were resolved.

**ANALYSIS OF TABLET FORMULATION**

Twenty tablets were weighed. Normal weight was figured and tablet powder proportionate to around 4 mg THC and 25 mg DKP was moved in 100 ml
volumetric cup. Methanol (50 ml) was added to above jar and carafe was sonicated for 15 minutes. Arrangement was sifted utilizing whatman channel paper No.41 and volume was made up to imprint with versatile stage. Fitting volume of aliquot was exchanged to 10 ml volumetric jar and volume was made up to imprint with versatile stage to get answer containing 20 µg/ml of THC and 125 µg/ml of DKP. arrangement was sonicated for 10 min. It was infused according to above chromatographic conditions and top ranges were recorded. measurements were done by keeping these qualities to straight line comparison of alignment bend.

3.4.2 SIMULTANEOUS ESTIMATION OF THC AND ETD

Selection of detection wavelength

The affectability of HPLC technique that uses UV discovery relies on fitting choice of location wavelength. perfect wavelength is one that gives great reaction for medication. affectability of HPLC technique that uses UV location relies on fitting determination of discovery wavelength. perfect wavelength is particular case that gives great reaction for medication that are to be distinguished. In present study drug arrangement of 10 µg/ml THC & 10 µg/ml ETD was, accordingly, arranged in Acetonitrile: Phosphate cushion (60:40 v/v) pH acclimated to 5.0 by orthophosphoric corrosive. These medication arrangements were then examined in UV area of 200-400 nm and overlain ranges were recorded. that are to be distinguished. In present study drug arrangement of 10 µg/ml THC & 10 µg/ml ETD was, in this way, arranged in Acetonitrile: Phosphate support (60:40 v/v) pH changed in accordance with 5.0 by orthophosphoric corrosive. These medication arrangements were then checked in UV district of 200-400 nm and overlain ranges were recorded.

Selection of chromatographic condition

Legitimate choice of HPLC strategy relies on way of specimen (ionic, ionizable or nonpartisan particle), its sub-atomic weight and dissolvability. medication chose for present study is polar in nature and henceforth either turned around
stage or particle pair or particle trade chromatography can be utilized. Switched stage HPLC was chosen for beginning partitions due to its effortlessness and suitability.

To upgrade chromatographic conditions, impact of chromatographic variables, for example, portable stage pH, stream rate, and dissolvable proportion were contemplated. Subsequent chromatograms were recorded and chromatographic parameters, for example, limit element, deviated component and section proficiency were figured. Conditions that gave best determination, symmetry and limit variable were chosen for estimation.

**Selection of flow rate of mobile phase**

After numbers of trial flow rate of mobile phase was optimizes as 1.0 ml/min, showing best resolution.

**Selection of elution mode**

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to polar compounds. It is not only simple, convenient but also better performs in terms of efficiency, stability and reproducibility. $C_{18}$ column was selected because it is least polar compared to $C_4$ and $C_8$ columns. $C_{18}$ column allows eluting polar compounds more quickly in comparison to non-polar compounds. In addition to this, UV detector is used, which allows easy detection of compounds in UV transparent organic solvents. 250 x 4.6 mm column of 5µm particles packing was preferred as starting point for method development. Gradient mode was chosen due to simplicity in application and robustness with respect to longer column stability.

**Optimize Chromatographic conditions**

Stationary phase: RP $C_{18}$ (250x4.6mm) 5µ

Mobile phase : Acetonitrile: Phosphate buffer (60:40 v/v)

PH: pH adjusted to 5.0 by orthophosphoric acid
Flow rate : 1.0 ml/min

Wavelength : 259 nm

Preparation of standard solution

Standard stock solution of THC

Precisely measured THC (100 mg) was exchanged to 100 ml volumetric carafe and broke up in methanol and weakened up to check with methanol to give stock arrangement having quality 1 mg/ml (1000 µg/ml).

Working standard solution of THC

100 µg/ml of THC working standard arrangement was arranged by weakening 1 ml of stock arrangement with methanol to 10 ml with methanol.

Standard stock solution of ETD

Precisely measured ETD (100 mg) was moved into 100 ml volumetric cup and broke down in methanol and weakened up to check with methanol to give stock arrangement having quality 1 mg/ml (1000 µg/ml).

Working standard solution of ETD

100 µg/ml of ETD working standard arrangement was arranged by weakening 1 ml of stock answer for 10 ml with methanol.

Preparation of Calibration Curve for THC and ETD

Calibration curve for THC

The calibration curve was constructed with five concentrations ranging from 10-50 µg/ml for THC. solutions were prepared by transferring 1, 2, 3, 4 and 5 ml aliquots of working standard solution of THC (100 µg/ml) into series of 10 ml volumetric flasks and volume was adjusted to mark with methanol to get final concentration of 10, 20, 30, 40 and 50 µg/ml. All solutions were injected. data of
peak area versus concentration were treated by linear least square regression analysis.

**Calibration curve for ETD**

The calibration curve was constructed with five concentrations ranging from 50-250µg/ml for ETD. The solutions were prepared by transferring 0.5, 1, 1.5, 2.0 and 2.5 ml aliquots of standard stock solution of ETD (1000 µg/ml) into series of 10 ml volumetric flasks and volume was adjusted to mark with methanol to get final concentration of 50, 100, 150, 200, 250µg/ml. All solutions were injected. Data of peak area versus concentration were treated by linear least square regression analysis.

**Preparation of binary mixtures of THC and ETD**

Accurately weighed 30 mg THC and 150 mg of ETD were transferred to 100 ml volumetric flask. It was dissolved with sufficient methanol and diluted up to mark with methanol to give concentration of 300 µg/ml of THC and 1500 µg/ml of ETD. Above solution was diluted further to get concentration range of 10, 20, 30, 40 and 50 µg/ml for THC and 50, 100, 150, 200 and 250 µg/ml for ETD.

**FORCED DEGRADATION STUDY:**

With specific end goal to set up whether systematic technique for examine was security demonstrating, immaculate dynamic pharmaceutical fixing (API) and tablet substance of THC & ETD was subjected to different anxiety conditions to direct constrained corruption studies. Stress studies were done under states of corrosive/base hydrolysis, oxidation, Thermal as said in ICH Q1A (R2). UV light corruption of medication substances and medication item was performed in strong state as specified in ICH Q1B.

**Acid Degradation:**
To different 10 ml volumetric flask, 1 ml stock solutions of THC and ETD were taken and 2 ml of 1 M HCl for THC and ETD was added respectively. Both flasks were heated at 60ºC for 0 min, 30 min, 1 hr and 2 hr and permitted to cool to room temperature. Arrangements were killed with 1 M NaOH and weakened up to stamp with portable stage. Suitable aliquots were taken from above arrangements and weakened with portable stage to acquire last convergance of 20 µg/ml THC and 100 µg/ml ETD independently and in blend.

**Alkali hydrolysis:**

To diverse 10 ml volumetric flagon, 1 ml stock arrangements of THC and ETD were taken and 2 ml of 1 M NaOH for THC and ETD was included individually. Both flagons were warmed at 60ºC for 0 min, 30 min, 1 hr and 2 hr and permitted to cool to room temperature. Arrangements were killed with 1 M HCl and weakened up to stamp with versatile stage. Fitting aliquots were taken from above arrangements and weakened with portable stage to acquire last amassing of 20 µg/ml THC and 100 µg/ml ETD independently and in blend.

**Oxidative stress degradation:**

To perform oxidative anxiety corruption, suitable aliquots of stock arrangements of THC and ETD were taken in two diverse 10 ml volumetric carafes and 2 ml of 3 % hydrogen peroxide was included separately. Both cups were warmed in water shower at 60 oC for 0 min, 30 min and 1 hr and permitted to cool to room temperature and weakened up to stamp with versatile stage. Fitting aliquots were taken from above arrangements and weakened with portable stage to get last centralization of 20 µg/ml THC and 100 µg/ml ETD independently and in blend.

**Photolytic Degradation:**

**Condition (1): In UV-Light** for 30 min.

Analytically 10mg of pure samples of THC and ETD were exposed in UV-light at 80º C for 30 min separately in 10 ml volumetric flasks. solids were allowed to
cool and dissolved in few ml of methanol. Volumes were made up to mark with methanol. Solutions were further diluted by mobile phase taking appropriate aliquots in 10 ml volumetric flask to obtain final concentration of 20 µg/ml THC and 100 µg/ml ETD separately and in mixture.

**Condition (2): In Sun-Light** for 30 min.

Symptomatically 10mg of unadulterated examples of THC and ETD were revealed in Sun-light for 30 min freely in 10 ml volumetric carafes. solids were allowed to chill and severed in few ml of methanol. Volumes were made up to stamp with methanol. Plans were further debilitated by adaptable stage taking suitable aliquots in 10 ml volumetric jug to get last storing up of 20 µg/ml THC and 120 µg/ml ETD independently and in blend.

**Thermal Degradation:**

Analytically 10mg of pure samples of THC and ETD were exposed in in Hot Air Oven at 60°C for 30 min separately in 10 ml volumetric flasks. solids were allowed to cool and dissolved in few ml of methanol. Volumes were made up to mark with methanol. Solutions were further diluted by mobile phase taking appropriate aliquots in 10 ml volumetric flask to obtain final concentration of 20 µg/ml THC and 120 µg/ml ETD separately and in mixture.

All reaction solutions were injected in liquid chromatographic system and chromatograms were recorded.

**VALIDATION**

**System suitability studies**

The system suitability was evaluated by five replicate analyses of THC and ETDmixture at concentration of 30µg/ml of THC and 150µg/ml of ETD. column efficiency (Numbers of Theoretical Plates), Resolution and Peak Asymmetry (Tailing Factor) were calculated for standard solutions.

**Linearity**
Appropriate aliquots of THC and ETD working standard solutions were taken in different 10 ml volumetric flasks and diluted up to mark with mobile phase to obtain final concentrations of 10, 20, 30, 40 and 50 µg/ml for THC and 50, 100, 150, 200 and 250 µg/ml for ETD. solutions were injected using 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both drugs.

**Precision**

Result should be expressed as relative standard deviation (RSD) or co-efficient of variance (% CV).

**Repeatability**

Repeatability was carried out by estimating response of 30 µg/ml of THC and 150 µg/ml of ETD, six times and results are reported in terms of relative standard deviation.

**Intra-day precision**

Mixed solutions containing THC (20, 30, 40 µg/ml) and ETD (100, 150, 200 µg/ml) were analyzed three times on same day and % CV was calculated.

**Inter-day precision**

Mixed solutions containing THC (20, 30, 40 µg/ml) and ETD (100, 150, 200 µg/ml) were analyzed three times on three different days and % CV was calculated.

**Accuracy**

It was carried out to determine suitability and reliability of proposed method. Accuracy was determined by calculating % Recovery. Twenty tablets were weighed and powdered. Powder equivalent to 30 mg of THC and 150 mg of ETD was weighed and transferred into 100 ml of volumetric flask, standard was
added on basis of 80%, 100% and 120% and diluted up to mark with mobile phase. amount of THC and ETD was calculated at each of three level and % recoveries were computed at three different levels (80%, 100% and 120%). For THC accuracy study performed on 30 µg/ml concentration and 150 µg/ml for ETD.

**Limit of detection**

From linearity curve equation, standard deviation (SD) of intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section. limit of detection (LOD) of drug was calculated by using following equations designated by ICH guideline:

\[
LOD = 3.3 \times \sigma / S,
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Limit of quantification**

From linearity curve equation, standard deviation (SD) of intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section. limit of quantification (LOQ) of drug was calculated by using following equations designated by ICH guideline:

\[
LOQ = 10 \times \sigma / S
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Robustness**

Robustness and Ruggedness of method was determined by subjecting method to slight change in method condition, individually, the:

- Pump flow rate,
• pH Change.

• Mobile Phase Composition change

Three replicates were made for same concentration (30µg/ml of THC and 150µg/ml of ETD). % CV was calculated.

**Specificity**

Specificity is ability of method to measure analyte response in presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into pre weighed quantity of drugs. Chromatogram was taken by appropriate dilutions and quantities of drugs were determined.

**ANALYSIS OF TABLET FORMULATION**

Twenty tablets were weighed. Normal weight was ascertained and tablet powder proportionate to around 8 mg THC and 300mg ETD was moved in 100 ml volumetric flagon. Methanol (50 ml) was added to above jar and flagon was sonicated for 15 minutes. The arrangements was separated utilizing whatman channel paper No.41 and volume was made up to stamp with portable stage. Proper volume of aliquot was exchanged to 10 ml volumetric flagon and volume was made up to check with versatile stage to get arrangement containing 30 µg/ml of THC and 150 µg/ml of ETD. arrangement was sonicated for 10 min. It was infused according to above chromatographic conditions and crest ranges were recorded. measurements were completed by keeping these qualities to straight line mathematical statement of adjustment bend.

**3.4.3 SIMULTANEOUS ESTIMATION OF THC AND LNC**

**Selection of detection wavelength**

The affectability of HPLC system that uses UV identification relies on fitting choice of recognition wavelength. perfect wavelength is particular case that gives great reaction for medication that is to be recognized. In present study
drug arrangement of 10µg/ml THC & 10µg/ml LNC was, accordingly, arranged in Ammonium Acetate Buffer: Acetonitrile (65:35 v/v) pH 6.5. These medication arrangements were then checked in UV district of 200-400 nm and overlain ranges were recorded.

**Selection of chromatographic condition**

Fitting choice of HPLC strategy relies on way of specimen (ionic, ionizable or unbiased particle), its sub-atomic weight and dissolvability. medication chose for present study is polar in nature and consequently either turned around stage or particle pair or particle trade chromatography can be utilized. Switched stage HPLC was chosen for introductory partitions on account of its straightforwardness and suitability.

To streamline chromatographic conditions, impact of chromatographic variables, for example, versatile stage pH, stream rate, and dissolvable proportion were mulled over. subsequent chromatograms were recorded and chromatographic parameters, for example, limit element, unbalanced element and segment proficiency were ascertained. conditions that gave best determination, symmetry and limit component were chosen for estimation.

**Selection of flow rate of mobile phase**

After numbers of trial flow rate of mobile phase was optimizes as 1.0 ml/min, showing best resolution.

**Selection of elution mode**

Opposite stage chromatography was picked in light of its prescribed utilization for ionic and moderate to polar mixes. It is basic, helpful as well as better performs regarding proficiency, steadiness and reproducibility. C18 section was chosen on grounds that it is minimum polar contrasted with C4 and C8 segments. C18 segment permits eluting polar mixes all more rapidly in correlation to non-polar mixes. Notwithstanding this, UV finder is utilized, which permits simple discovery of mixes in UV straightforward natural solvents. 250 x
4.6 mm segment of 5µm particles pressing was favored as beginning stage for system advancement. Inclination mode was picked because of straightforwardness in application and power concerning longer section security.

**Optimize Chromatographic conditions**

- **Stationary phase**: RP C₁₈ (250×4.6mm) 5µ
- **Mobile phase**: Ammonium Acetate Buffer: Acetonitrile (65:35 V/v)
- **PH**: 6.5
- **Flow rate**: 1.0 ml/min
- **Wavelength**: 295 nm

**Preparation of standard solution**

**Standard stock solution of THC**

Precisely measured THC (100 mg) was exchanged to 100 ml volumetric carafe and broke up in methanol and weakened up to imprint with methanol to give stock arrangement having quality 1 mg/ml (1000 µg/ml)

**Working standard solution of THC**

100 µg/ml of the working standard arrangement was arranged by weakening 1 ml of stock arrangement with methanol to 10 ml with methanol

**Standard stock solution of LNC**

Precisely measured LNC (100 mg) was moved into 100 ml volumetric cup and broke down in methanol and weakened up to imprint with methanol to give stock arrangement having quality 1 mg/ml (1000 µg/ml).

**Working standard solution of LNC**

100 µg/ml of LNC working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with methanol.
Preparation of Calibration Curve for THC and LNC

**Calibration curve for THC**

The alignment bend was developed with five fixations extending from 10-50 μg/ml for THC. arrangements were arranged by exchanging 1, 2, 3, 4 and 5 ml aliquots of working standard arrangement of THC (100 μg/ml) into arrangement of 10 ml volumetric flacons and volume was changed in accordance with imprint with methanol to get last amassing of 10, 20, 30, 40 and 50μg/ml. Every one of arrangements were infused. information of crest range versus focus were dealt with by direct slightest square relapse investigation.

**Calibration curve for LNC**

The alignment bend was built with five focuses going from 20-100μg/ml for LNC. The arrangements were arranged by exchanging 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of standard stock arrangement of LNC (1000 μg/ml) into arrangement of 10 ml volumetric carafes and volume was changed in accordance with imprint with methanol to get last fixation of 20, 40, 60, 80 and 100μg/ml. Every one of arrangements were infused. information of crest territory versus fixation were dealt with by straight minimum square relapse examination.

**Preparation of binary mixtures of THC and LNC**

Precisely measured 40 mg THC and 40 mg of LNC were exchanged to 100 ml volumetric jar. It was broken down with adequate methanol and weakened up to check with methanol to give convergance of 400 μg/ml of THC and 400 μg/ml of LNC. Above arrangement was weakened further to get fixation scope of 10, 20, 30, 40 and 50 μg/ml for THC and 20, 40, 60, 80 and 100μg/ml for LNC.

**FORCED DEGRADATION STUDY:**

With specific end goal to build up whether scientific system for examine was security demonstrating, unadulterated dynamic pharmaceutical fixing (API) and tablet substance of THC & LNC was subjected to different anxiety conditions to
direct constrained corruption studies. Stress studies were done under states of corrosive/base hydrolysis, oxidation, Thermal as specified in ICH Q1A (R2). UV light corruption of medication substances and medication item was performed in strong state as specified in ICH Q1B.

**Acid Degradation:**

To distinctive 10 ml volumetric flagon, 1 ml stock arrangements of THC and LNC were taken and 2 ml of 1 M HCl for THC and LNC was included individually. Both cups were warmed at 60ºC for 0 min, 30 min, 1 hr and 2 hr and permitted to cool to room temperature. Arrangements were killed with 1 M NaOH and weakened up to imprint with versatile stage. Fitting aliquots were taken from above arrangements and weakened with versatile stage to acquire last centralization of 30 µg/ml of THC and 60 µg/ml of LNC independently and in blend.

**Alkali hydrolysis:**

To diverse 10 ml volumetric carafe, 1 ml stock arrangements of THC and LNC were taken and 2 ml of 1 M NaOH for THC and LNC was included individually. Both cups were warmed at 60ºC for 0 min, 30 min, 1 hr and 2 hr and permitted to cool to room temperature. Arrangements were killed with 1 M HCl and weakened up to imprint with versatile stage. Suitable aliquots were taken from above arrangements and weakened with versatile stage to acquire last centralization of 30 µg/ml of THC and 60 µg/ml of LNC independently and in blend.

**Oxidative stress degradation:**

It is performed by fitting stock arrangements aliquots of THC and LNC were taken in two specific 10 ml flagon and 02 ml of 03 % hydrogen peroxide was
joined freely. Both holders were warmed in water shower at 60°C for 0 min, 30 mins and 60 mins and permitted to cool to room temperature and incapacitated up to etch with adaptable stage. Suitable aliquots were made from above courses of move and weakened with adaptable stage to get last convergance of 30 µg/mL of THC and 60 µg/mL of LNC independently and in mixture.

**Photolytic Degradation:**

**Condition (1):** In **UV-Light** for 30 min.

Experimentally 10mg of flawless samples of THC and LNC were revealed in UV-light at 80°C for 30 min autonomously in 10 ml volumetric jugs. solids were allowed to cool and separated in few ml of methanol. Volumes were made up to engrave with methanol. Plans were further debilitated by flexible stage taking legitimate aliquots in 10 ml volumetric flask to secure last hoarding of 30 µg/ml THC and 60 µg/ml LNC freely and in mixture.

**Condition (2):** In **Sun-Light** for 30 min.

Experimentally 10mg of perfect samples of THC and LNC were revealed in Sun-light for 30 min autonomously in 10 ml volumetric carafes. solids were allowed to cool and separated in few ml of methanol. Volumes were made up to engrave with methanol. Plans were further debilitated by compact stage taking suitable aliquots in 10 ml volumetric glass to procure last accumulating of 30 µg/ml THC and 60 µg/ml LNC freely and in mixture.

**Thermal Degradation:**

Consistently 10mg of unadulterated samples of THC and LNC were revealed in Hot Air Oven at 60°C for 30 min freely in 10 ml volumetric containers. solids were allowed to chill and severed in few ml of methanol. Volumes were made up to engrave with methanol. Game plans were further debilitated by compact
stage taking fitting aliquots in 10 ml volumetric jug to get last hoarding of 30 µg/ml THC and 60 µg/ml LNC autonomously and in mix.

All reaction courses of action were implanted in liquid chromatographic structure and chromatograms were recorded.

VALIDATION

System suitability studies

Structure suitability was evaluated by five replicate examinations of THC and LNC mix at centralization of 40µg/ml of THC and 40µg/ml of LNC. portion profitability (Numbers of Theoretical Plates), Resolution and Peak Asymmetry (Tailing Factor) were determined for standard course of action.

Linearity

Suitable aliquots of THC and LNC working standard courses of action were taken in different 10 ml volumetric containers and debilitated up to engrave with flexible stage to procure last gathering of 10, 20, 30, 40 and 50 µg/ml for THC and 20, 40, 60, 80 and 100 µg/ml for LNC. courses of action were mixed using 20 µL settled circle system and chromatograms were recorded. Arrangement curves were constructed by plotting ordinary peak locale versus obsessions and backslide examinations were handled for both pharmaceuticals.

Precision

Result should be expressed as relative standard deviation (RSD) or co-efficient of variance (% CV).

Repeatability

Repeatability was completed by evaluating reaction of 40 µg/ml of THC and 40 µg/ml of LNC, six times and results are accounted for as far as relative standard deviation.

Intra-day precision
Mixed solutions containing THC (20, 30, 40 µg/ml) and LNC (40, 60, 80 µg/ml) were analyzed three times on same day and % CV was calculated.

**Inter-day precision**

Mixed solutions containing THC (20, 30, 40 µg/ml) and LNC (40, 60, 80 µg/ml) were analyzed three times on three different days and % CV was calculated.

**Accuracy**

It was completed to focus suitability and unwavering quality of proposed strategy. Exactness was dictated by ascertaining % Recovery. Twenty tablets were measured and powdered. Powder equal to 4 mg of THC and 4 mg of LNC was measured and moved into 100 ml of volumetric flag on, standard was added on premise of 80%, 100% and 120 % and weakened up to check with portable phase. measure of THC and LNC was ascertained at each of three level and % recuperations were processed at three distinct levels (80%, 100% and 120%). For THC exactness study performed on 40 µg/ml focus and 40 µg/ml for LNC.

**Limit of detection**

From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was ascertained. At that point LOD was measured by utilizing numerical expressions given as part of section. utmost of location (LOD) of medication was computed by utilizing accompanying mathematical statements assigned by ICH rule:

\[
LOD = 3.3 \times \sigma/S,
\]

Where, \( \sigma \) = standard deviation of Intercept

\( S \) = Mean slope of calibration curve

**Limit of quantification**
From linearity bend mathematical statement, standard deviation (SD) of captures (reaction) was computed. At that point LOQ was measured by utilizing numerical expressions given as part of section. cutoff of evaluation (LOQ) of medication was computed by utilizing accompanying mathematical statements assigned by ICH rule:

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = standard deviation of Intercept

\(S\) = Mean slope of calibration curve

**Robustness**

Robustness and Ruggedness of method was determined by subjecting method to slight change in method condition, individually, the:

- Pump flow rate,
- PH Change.
- Mobile Phase Composition change

Three replicates were made for same concentration (30µg/ml of THC and 60µg/ml of LNC). % CV was calculated.

**Specificity**

Specificity is capacity of strategy to gauge analyte reaction in vicinity of its potential pollutions and corruption items. Normally utilized excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into pre measured amount of medications. chromatogram was taken by suitable weakening and amounts of medications were resolved.

**ANALYSIS OF TABLET FORMULATION**

20 tablets were weighed. Ordinary weight was figured and case powder equivalent to around 40 mg THC and 40mg LNC was moved in 100 ml
volumetric carafe. Methanol (50 ml) was added to above container and flask was sonicated for 15 minutes. Courses of action was isolated using Whatman channel paper No.41 and volume was made up to engrave with compact stage. Suitable volume of aliquot was traded to 10 ml volumetric container and volume was made up to engrave with flexible stage to procure answer containing 40 µg/ml of THC and 40 µg/ml of LNC. Course of action was sonicated for 10 min. It was imbued by chromatographic conditions and peak domains were recorded. Assessments were finished by keeping these qualities to straight line numerical articulation of arrangement bend.

3.5 COMPARISON OF DEVELOPED METHODS

3.5.1 THC AND DKP

Statistical comparison of developed Area Under Curve Method, Absorption Correction Method and RP-HPLC by One way ANOVA

Three systems, Area Under Curve Method, Absorption Correction Method and RP-HPLC were produced for estimation of THC and DKP in Bulk and tablet measurements structure. To look at proposed routines ANOVA test was connected. F_{calculated} ought to be not exactly F_{critical} and then it can be reasoned that every one of the three systems have no huge distinction.

Measurable examination of created Area Under Curve Method and Absorbance Correction Method via Pair t-test

t-test is in view of t-dissimilation and is viewed as proper test for judging centrality of test mean or for judging noteworthiness of distinction between method for two specimens if there should arise an occurrence of little sample(s) when populace difference is not known.

Correlation of created Area Under Curve Method and Absorbance Correction Method was finished via Pair t-test. t figured ought to be less than critical and then it can be reasoned that both systems have no noteworthy distinction.

3.5.2 THC AND ETD
Measurable correlation of developed First Order Derivative Spectroscopic Method, Area Under Curve Method and RP-HPLC by One way ANOVA

Three strategies, First Order Derivative Spectroscopic Method, Area Under Curve Method and RP-HPLC were made for estimation of THC and ETD in Bulk and tablet estimation structure. To consider proposed schedules ANOVA test was associated. Fcalculated should be not precisely Fcritical and after that it can be derived that every one of the three systems have no discriminating refinement.

Measurable examination of grew First request Derivative Method Area Under Curve Method via Pair t-test

t-test is in perspective of t-flow and is seen as fitting test for judging significance of example mean or for judging basics of complexity between systems for two cases in occasion of little sample(s) when people variance is not known.

Relationship of developed First ask for Derivative spectroscopic system and Area Under Curve was done by means of Pair t-test. t found out should be not precisely tcritical and after that it can be assumed that both strategies have no tremendous differentiation.

3.5.3 THC AND LNC

Measurable examination of created Area Under Curve Method, First Order Derivative Spectroscopic Method and RP-HPLC by One way ANOVA

Three techniques, Area Under Curve Method, First Order Derivative Spectroscopic Method and RP-HPLC were created for estimation of THC and LNC in Bulk and tablet dose structure. To think about proposed strategies ANOVA test was connected. Fcalculated ought to be not exactly Fcritical and then it can be reasoned that every one of the three strategies have no critical contrast.
Factual correlation of created Area Under Curve Method and First request Derivative Method via Pair t-test

The t-test is in perspective of t-scattering and is seen as fitting test for judging basics of case mean or for judging enormity of refinement between strategies for two examples if there ought to be event of little sample(s) when masses contrast is not known.

Examination of made Area Under Curve Method and First ask for Derivative Method was done through Pair t-test. t figured should be not precisely t basic and a short time later it can be construed that both schedules have no essential difference.