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Characterization of vitamin-E and K Vitamin for Hydrophobicity through HPTLC method

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ABSTRACT:
This work was aimed at determination of log P values of natural compound of great medicinal value. We have selected Fat Soluble vitamins such as A and D3 this work. As reported, vitamins are essential for physiology of the body. The bioavailability of Fat Soluble Vitamins A, D3, E and K are said to be low due to hydrophobic nature. In this study hydrophobicity was determined via log P value by employing HPTLC methods. Some of Review Literature vitamin E Predicted Log P value was 12. Vitamin E was elute using Mobile phase Hexane–ethyl acetate (9: 1) in HPTLC method. this method 2 elute vitamin E Determined Log P value was found to be 3.4 to 3.5. It may be suitable for emulsion formulation.Some of Review Literature Vitamin K Predicted Log p Value was 9.7 its to much high. Vitamin K was elute using Mobile Phase: Hexane–ethyl acetate: CHCl3 (5: 3: 2) in HPTLC method. Determined Log P value was 2.0 to 2.5. It may be Suitable for emulsion formulation and other Dosage formulation.

Keyword: LogP, Fat-Soluble Vitamin-E,K and HPTLC Method

I. INTRODUCTION:

Chromatography has grown in significance and popularity to become a leading type of analysis in instrumental analytical chemistry. Chromatography is the science of separation used either for identification or quantification of chemical substances. HPTLC is the improved method of TLC in more optimized way. It is also known as planar chromatography or Flat-bed chromatography. High performance thin layer chromatography (HPTLC) basically depends upon the full capabilities of thin layer chromatography (TLC).As it is useful in analysis of qualitative method and it combines the art with quickness at a moderate cost of chromatography. Modern TLC is widely known and practical as HPTLC, which can only be performed on precoated layers, using instrumentation and mainly for the purpose of quantification. HPTLC began around 1975 with the introduction of high efficiency, commercially precoated plates, which are smaller (10 x 10 or 10 x 20 cm), have a thinner (0.1-0.2 mm) layer composed of sorbent with a finer mean particle size (5-6 µm) and a narrower particle size distribution or classification (4-8 µm), and are developed over shorter distances (about 3-7 cm) compared to classical TLC plates, which are generally 20 x 20 cm with a 0.25 mm layer containing particles with an average size of 10-12 µm (3-20 µm range). HPTLC plates provide improved resolution, shorter analysis time, higher detection sensitivity, and improved in situ quantification. Among the modern analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks, because of its suitability for high-throughtput screening, sensitivity and reliability in quantification of analytes at nanogram levels. It is one of the most applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environment analysis, and other areas.

HPTLC PRINCIPLE AND WORKING

It is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. Separation may result due to adsorption by both phenomenon. It is depending upon the nature of adsorbent used on plates and solvents system used for development.

The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The components with more affinity towards the stationary phase travels slower and the components with lesser affinity towards the stationary phase travel faster.

HPTLC take place with high speed capillary flow range of the mobile phase. There are three main steps that includes,

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1. Sample to analysed to chromatogram layer volume precision and suitable position are achieved by use of suitable instrument.
2. Solvent migrates the planned distance in layer by capillary action in this process sample separated in its components.
3. Densitometer is used for scanning separation tracks with light beam in visible or UV region.

PROTOCOL USED FOR DRUG ANALYSIS

1. Selection of Chromatographic Layer
2. Sample and Standard Preparation
3. Activation of Precoated plates
4. Application of Sample and Standard
5. Selection of Mobile Phase
6. Preconditioning (Chamber Saturation)

SELECTION OF CHROMATOGRAPHIC LAYER:

Precoated plates — different support materials — different sorbents available 80 % of analysis — silica gel 60F — Basic substances, alkaloids and steroids- Aluminium oxide amino acids, dipeptides, sugars and alkaloids — cellulose. Non-polar substances, fatty acids, carotenoids, cholesterol- RP2, RP8 an RP18.

SAMPLE AND STANDARD PREPARATION: METHYLENE CHLORIDE:

To avoid interference from impurities and water vapours. Solvents used are Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:10:1), Methylene chloride: Methanol (1:1), 1% Ammonia or 1 % Acetic acid.

Dry the plates and store in dust free atmosphere. A good solvent system is one that does not put anything on the solvent front, but moves all components of the mixture off the baseline. Between Rf 0.15 and 0.85 range, the peaks of interest should be resolved. The elution power of the mobile phase depends on a property called eluent strength.

ACTIVATION OF PRECOATED PLATES:

Plates exposed to high humidity or kept on hand for long time to be activated. By placing in an oven at 110-120^{o}C for 30 minutes. Prior to spotting Aluminium Sheets should be kept in between two glass plates and placing in oven at 110-120^{o}C for 15 minutes.

Application Of Sample And Standard:

Mainly concentration range is 0.1 µg/µl above this causes poor separation. Automatic applicator- nitrogen gas sprays sample and standard from syringe on TLC plates as bands. Band wise application better separation high response to densitometer. With sufficiently high concentration of analyte, Pharmaceutical preparation is simply dissolved in a suitable solvent that will solubilize the analyte. It is a critical step of application of the sample and to obtain good resolution for quantification in HPTLC. Sample application techniques depend on factors like the type of workload, sample matrix and time constraints.

SELECTION OF MOBILE PHASE:

Poor grade of solvent used in mobile phase preparation was found to decrease resolution, Rf reproducibility and spot definition. Sorbent layer mobile phase and the chemical properties of the analyte should be chosen. Using 3 or 4 components in mobile phase should be avoided as it is often difficult to get the reproducible ratios of different components.

Preconditioning (Chamber Saturation)

Chamber saturation has a pronounced influence on the separation profile. Time required for the saturation depends on the mobile phase. If plates are introduced into the unsaturated chamber, during the course of development, the solvent evaporates from the plate mainly at the solvent front; hence it result in increased Rf values.If tank is saturated prior to the development, solvent vapours soon get uniformity distributed throughout the chamber. As soon as the plate is kept in such a saturated chamber, it soon gets pre-loaded with solvent vapours hence less solvent is required to travel a particular distance, resulting in lower Rf values. But in some cases depending on their polarity saturation and non-saturation of chambers are required. Filter paper lining for 30 min prior to development in saturation chamber leads to uniform distribution of solvent vapours and less solvent require for the sample to travel.

DETECTION AND VISUALIZATION:

Under detection of UV light is first step and is non-destructive. Spots of fluorescent compounds can be seen at 254 nm i.e. short wave length. Non- UV absorbing compounds being visualised by using 0.1% iodine solution. If individual component does not respond to UV, then derivatization is needed with visualizing agent. By

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quenching of fluorescence due to UV light (200-400 nm) detection of separated compounds on the sorbent layers is enhanced. This process is commonly known as fluorescence quenching. Visualization at UV 254 nm: F254 should be described as phosphorescence quenching. In this instance, after the source of excitation is removed the fluorescence stays for a short period. It is longer than 10 seconds but, very short lived.

DERIVATIZATION:

Derivatization is a procedural technique that modifies functionality of an analyte's to enable chromatographic separations. Derivatization can be performed either by spraying the plates with a suitable reagent.

Hydrophoblic Natural Drugs:

- Many of phytochemicals are reported to be hydrophobic.
- To understand basic issues with oral absorption of such hydrophobic drugs, index of hydrophobicity is essential.
- Log p value of such compounds will help us to understand
- Solubility
- Permeability
- Insights appropriate formulation development

Examples:

1. Log p > 5- suitable for emulsion
2. Log P < 5-solubilization effects needed such as buffering co solvating complextion
3. Log < 0- Ideal for IV Administration

LOG-PIMPORTANCE:

- The lipophilicity of an organic compound is usually described in terms of a partition coefficient,
- Log p, which can be defined as the ratio of the concentration of the unionized compound, at equilibrium, between organic and aqueous phases: one of the solvents is water and an another is a non-polar solvent used, The log P value is a measure of lipophilicity..Log P values have been studied in approximately 100 organic liquid–water systems.

➢ **LOGP IMPORTANCE AND SOUBILITY:**

Log P Value	Solubility
<0	Highly soluble, poor permeability
0-2	Good solubility, good permeability
2-3	Minor problems in solubility
3-6	Permeability is good, solubility is very poor emulsion formulation approach is good
>6	Toxic, not suitable for biological applications

II. MATERIAL AND METHOD:

HPTLC CHROMATOGRAPHIC CONDITION:

MOBILE PHASE COMPOSITION ON THE RETENTION FACTORS FOR VITAMINS

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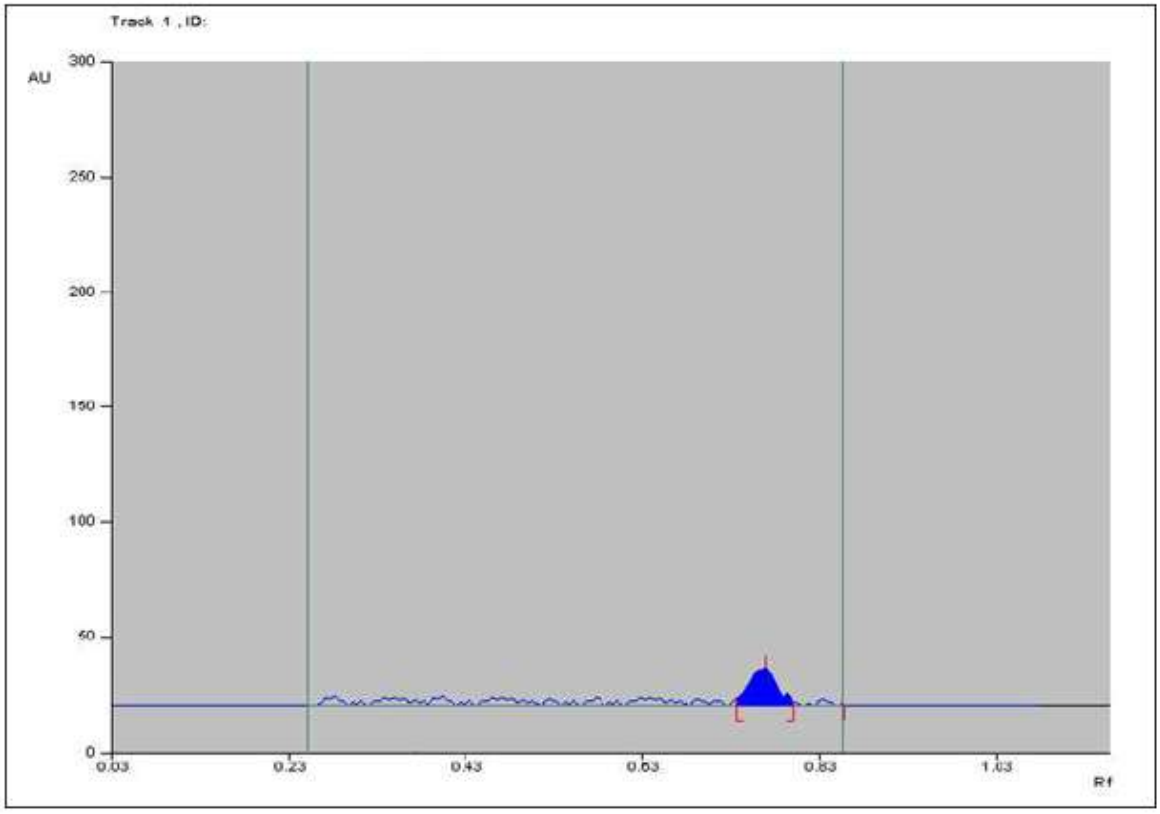
Sr No.	Vitamin	Mobile Phase	Chamber Saturation time	Distance Travel	Rf Value	Peak Area
1	E	Hexane–ethyl acetate (9: 1)	30 min	80 mm	0.78 ± 0.03	782.5±5.25
2	K	Hexane–ethyl acetate: CHCl3 (5: 3: 2)	30 min	80 mm	0.48 ± 0.02	1505.5±10.15

III. RESULT AND DISCUSSION:

HPTLCCHAROMATOGRAPHY:

VITAMIN E:



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Sr. No	Mobile Phase	Observation	TLC Plate
1	Toluene: Methanol (4:6)	Might be run with mobile phase because band is not visible on plate	
2	Ethyl acetate: Toluene	Band does move from site of the application but Rf value more than 0.85	

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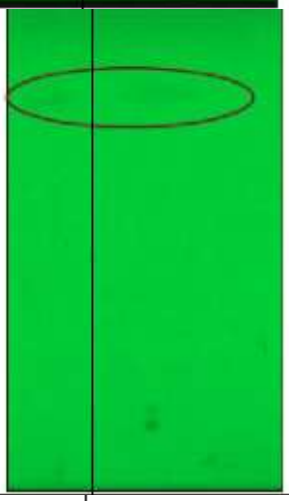
3	Ethyl acetate: Acetonitrile	Band do not visible on TLC plate	
			
4	Hexane–ethyl acetate (9: 1)	Band observed	

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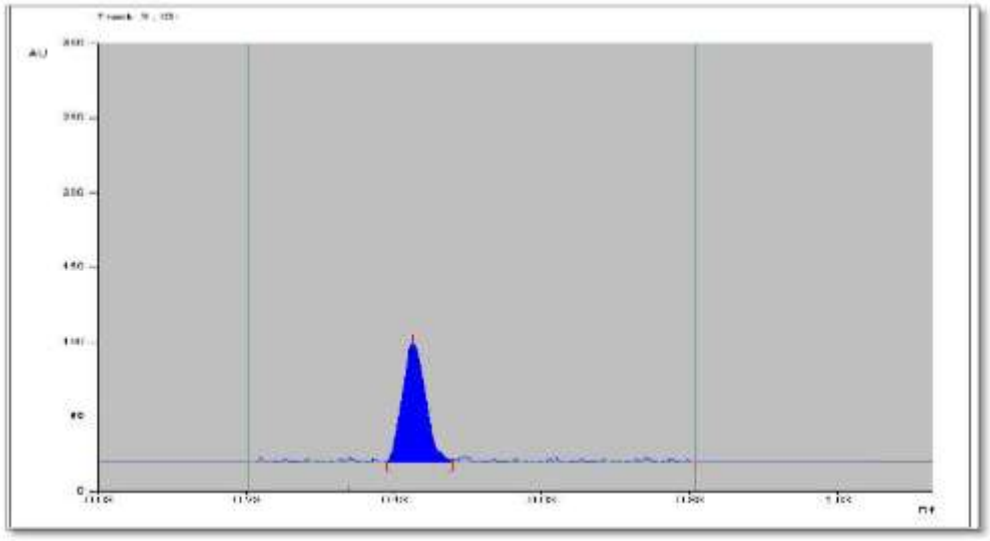
VITAMIN-K



Optimization of Mobile phase:

Sr.No	MobilePhase	Observation	TLC Plate
1	Toluene: Methanol(8:2)	Might be run with mobile phase, bandis visible on plate	

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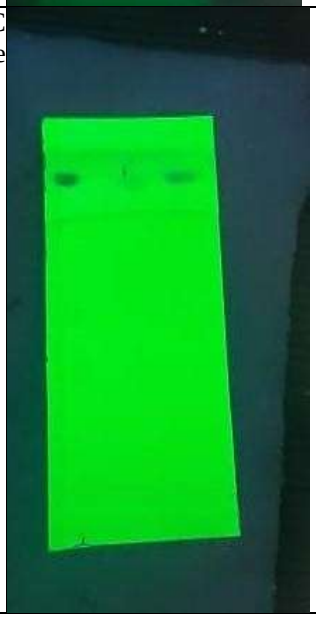
2	Ethyl acetate: Toluene	Band does move from site of the application but spread	
3	Ethylacetate: CHCl3	Band do visible on TLC plate but run with mobile phase	

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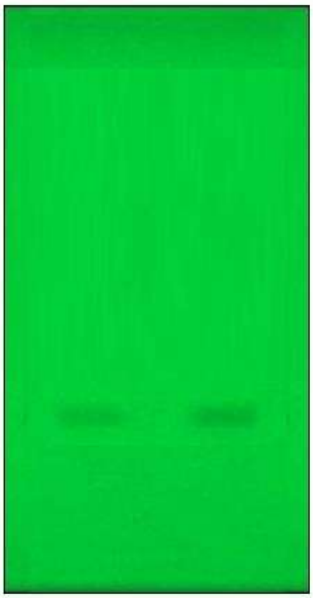
4	Hexane–ethyl acetate:CHCl3(5:3:2)	Band observed	

CALCULATION:

SR.NO	COMPOUNDS	RETENTIONTIME	K'	LOGKI	LOG PVALUE	LOGKI	DETERMINELOGP
1	VITAMINE	0.78	2.9	13.5	12.2	1.130333768	3.430196701
2	VITAMINK	0.48	1.4	6	9.7	0.77815125	2.065677065

IV. CONLCUSION:

- The natural compounds present in food ingredients have good biological activities and even some of natural compounds are used in Ayurveda system of medicine for treatment of various type of diseases in human and animals. Plant based produces such has fruits, vegetables and seeds contain certain chemicals to sustain against decay during storage.
- Natural chemicals are excellent biological activities potential suffer from drawback of having not been able to replicate in vitro activities in to in vivo pharmacological activities.
- Some analysis presented in the literature that lack of absorption, poor metabolic, poor stability, rapid elimination from the body contribute to observed inaction in the body.
- Some of Review Literature vitamin E Predicted Log P value was 12. Vitamin E was elute using Mobile phase Hexane–ethyl acetate (9: 1) in HPTLC method. this method 2 elute vitamin E Determined Log P value was found to be 3.4 to 3.5. It may be suitable for emulsion formulation.
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