International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1937-1944 www.ijprajournal.com ISSN: 2249-7781

Characterization of Vitamin-A and D_3 vitamin for Hydrophobicity through HPTLC Method

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Submitted: 10-08-2023 Accepted: 20-08-2023

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, D_3, 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. Vitamin A was elute using Mobile Phase Hexane: CHCl3 (8:2) in HPTLC Method. Determine Log P value was 0.5. Vitamin A not suitable in HPTLC method because determined Log P value was Very Low. Vitamin D3 was elute using Mobile Phase Hexane: CHCl3 (7:3) in HPTLC Method and Determine Log P value 2.88 to 3.00 in HPTLC Method. HPTLC Method was suitable any other dosage formulation.

Key word: Log P, Fat-Soluble vitamin-A, D_3 and HPTLC Method

I. INTRODUCTION:

PROBLEM WITH NATURAL PRODUCTS:

The synthesis of natural products is too difficult – the structures are too complex

Natural products structures span the range from very simple to extremely complex, if derived from a plant which grows in a remote tropical location, physical access for a recollection may be difficult, or the plant may only produce quantities of the desired compound under certain environmental or ecological conditions.

A marine organism may require an expensive expedition, especially if the animal grows in deep waters or in regions with strong or unpredictable currents. Even when one has a microbial culture in hand, the factors that induce production of the metabolite may be poorly understood. Pharmaceutical companies clearly prefer predictable, controllable sources, and for commercial viability, solutions must be found that accommodate the vagaries of natural product production.

Natural product samples have most often been tested as whole fermentation broths, or as crude extracts of plants and marine organisms. Once a hit has been confirmed in biological screening, the extract must be fractionated to isolate the active compounds, and this process typically requires that bioassays be conducted at each level of purification. Thus the length of time required conducting the bioassay and reporting the results, and the number of separation cycles needed to obtain pure compounds, are factors which dictate the time it takes to process a natural product hit. Even when cycles are made on a weekly basis using a rapid bioassay, it is unusual for a natural product extract hit to yield a pure compound after less than a month's work.

Other factors that may impend the selection of natural products are:

- Instability of compounds
- Adulteration
- Difficult separations
- Unreliability of bioassays

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

1. Solubility
2. Permeability
3. 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

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LOG-P IMPORTANCE:

- 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.

- **LOG P 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

HPTLC: HIGH PERFORMACE THIN LAYER CHROMATOGRAPHY:

High Performance Thin Layer Chromatography (HPTLC) is the most powerful advanced Technique and versatile separation. It consists of chromatographic layers of utmost separation efficiency and the application of instrumentation for all steps related in the procedure include accurate sample application, standardized reproducible chromatogram development and software-controlled evaluation.

HPTLC is a concept that includes a widely standardized methodology based on qualitative and quantitative analysis. High Performance Thin Layer Chromatography (HPTLC) is advantages in comparison to other separation techniques.

PRINCIPLE OF HIGH PERFORMACE THIN LAYER CHROMATOGRAPHY:

HPTLC having similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e, the principle of separation is adsorption. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The components with the lesser affinity towards the stationary phase travels faster. Thus the components are separated on a chromatographic plate.

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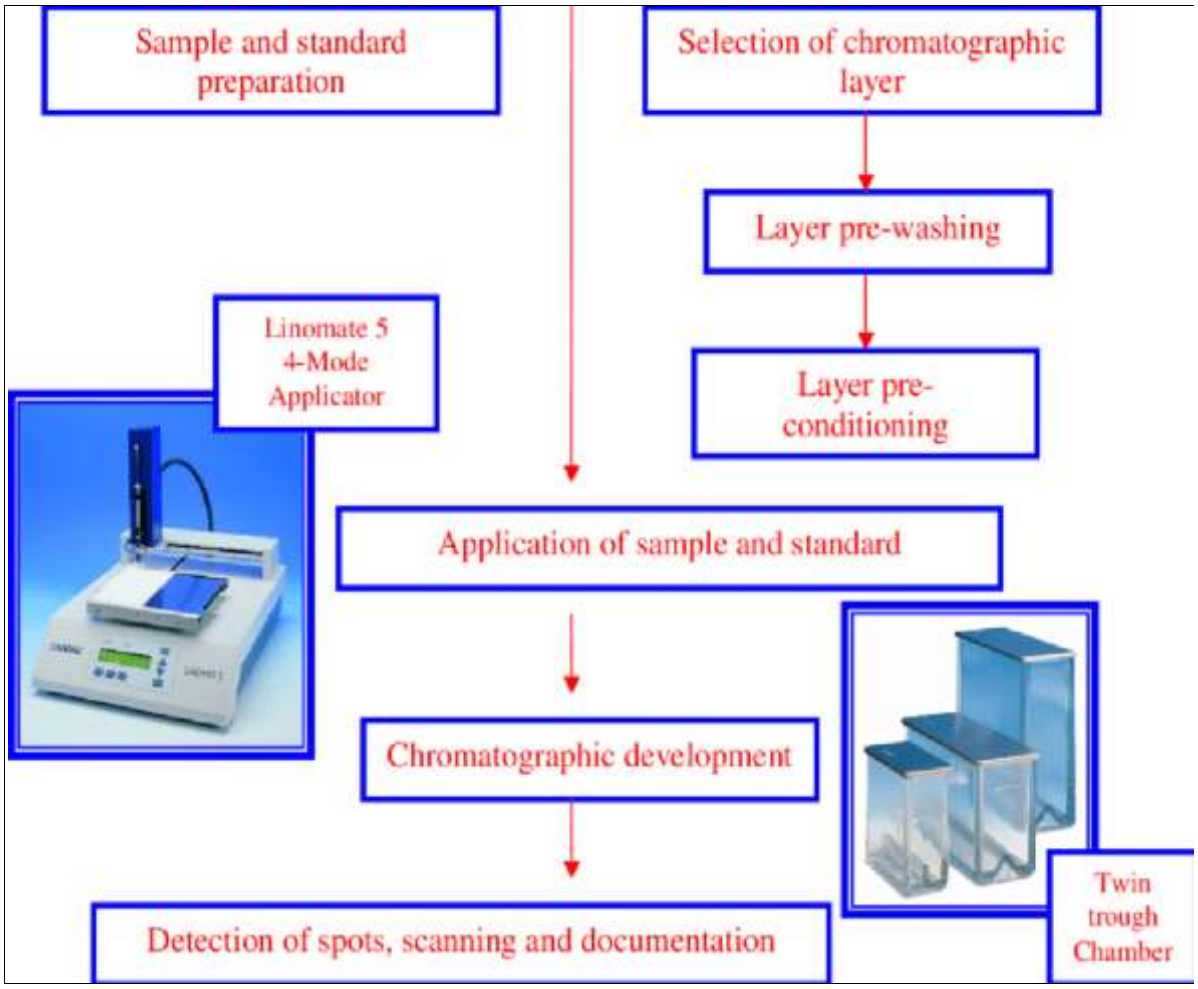
STEPS INVOLVING IN HPTLC:



First step for Selection of the Stationary Phase during method development. Stationary phase selection should be based on the type of compounds. HPTLC uses smaller plates and size for 10*20 cm with significantly decreased development distance (typically 6 cm) and analysis time (7–20 min).

HPTLC plates provide information about improved resolution, higher detection sensitivity, and improved in quantification and It commonly used for industrial pharmaceutical quantitative analysis. Mobile Phase Selection and Optimization phase. The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyse.

SAMPLE PREPARATION AND APPLICATION:

A good solvent system is one that moves all components of the mixture off the baseline but does not put anything on the solvent front. The peaks of interest should be resolved between Rf 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength which is related to the polarity of the mobile phase components. The more nonpolar the compound, the faster it will elute (or the less time it will remain on the stationary phase) and the more polar the compound.

CHROMATOGRAM DEVELOPMENT (SEPARATION)

Chromatogram development is the most important step in the HPTLC procedure and HPTLC plates are developed in twin-trough chambers, or horizontal-development chambers.

In general, saturated twin-trough chambers fitted with filter paper offer the best reproducibility. Twin-through chamber avoids solvent vapor preloading and humidity. Detection- Detection of

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separated compounds on the sorbent layers is enhanced by quenching of fluorescence due to UV light (ranged normally at 200-400 nm). This process is commonly called Fluorescence quenching.

PREWASHING

The mainly purpose of the pre washing is to remove impurities in sample. It includes water vapours and other volatile substances from the atmosphere and It exposed in the environment. Silica gel 60F is most widely used sorbent. The major disadvantage of this sorbent is that it containing iron as impurity. This iron impurity is removed by using Methanol: Water in the ratio of 9:1.

Some of the common methods using in pre-washing for HPTLC Method.

a] Ascending method
b] Descending method
c] Continuous method

ACTIVATION OF PLATES

Freshly opened box of HPTLC plate does not require activation. Plates are exposed to high humidity after plates activate. Plates are placed in oven at 110˚c-120˚c for 30 min before sample application.

PRE-CONDITIONING

Also called chamber saturation.
Un-saturated chamber causes high Rf values

SAMPLE APPLICATION

Sample application can be done by using
1] Capillary tubes
2] Micro syringes
3] Micro bulb pipettes
4] Automatic sample applicator

POST CHROMATOGRAPHIC STEPS

1] Detection
2] Photo Documentation
3] Densitometry Measurements

1] DETECTION

Detection of under UV light is first choice – non destructive Non UV absorbing compounds like ethambutol, dicylomine etc- dipping the plates in 0.1% iodine solution.

2] DENSITOMETRY MEASUREMENTS

Measure visible, UV absorbance or Fluorescence. Convert the spot\band into chromatogram consisting of peaks.

II. MATERIAL AND METHOD:

HPTLC CHROMATOGRAPHIC CONDITION:

MOBILE PHASE COMPOSITION ON THE RETENTION FACTORS FOR VITAMINS

Sr No.	Vitamin	Mobile Phase	Chamber Saturation time	Distance Travel	Rf Value	Peak Area
1	A	Hexane: CHCl3 (8:2)	30 min	80 mm	0.34 ± 0.02	890.5±7.95
2	D	Hexane: CHCl3 (7:3)	30 min	80 mm	0.63 ± 0.03	1250.5±8.80

III. RESULT AND DISCUSSION:

HPTLC CHAROMATOGRAPHY:

Sr. No	Mobile Phase	Observation	TLC Plate

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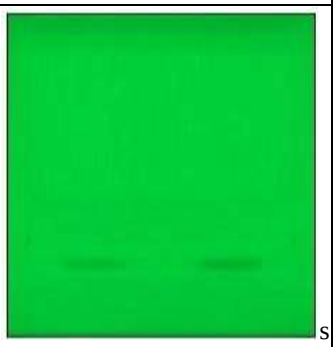
1	CHCl3: Methanol	Might be run with mobile phase because band is not visible on plate	
2	Hexane: Ethyl acetate	Band does not move from site of the application	
3.	Hexane: n-butanol	Band does not move from site of application	
4.	Hexane: CHCl3 (8:2)	Band observed	 s

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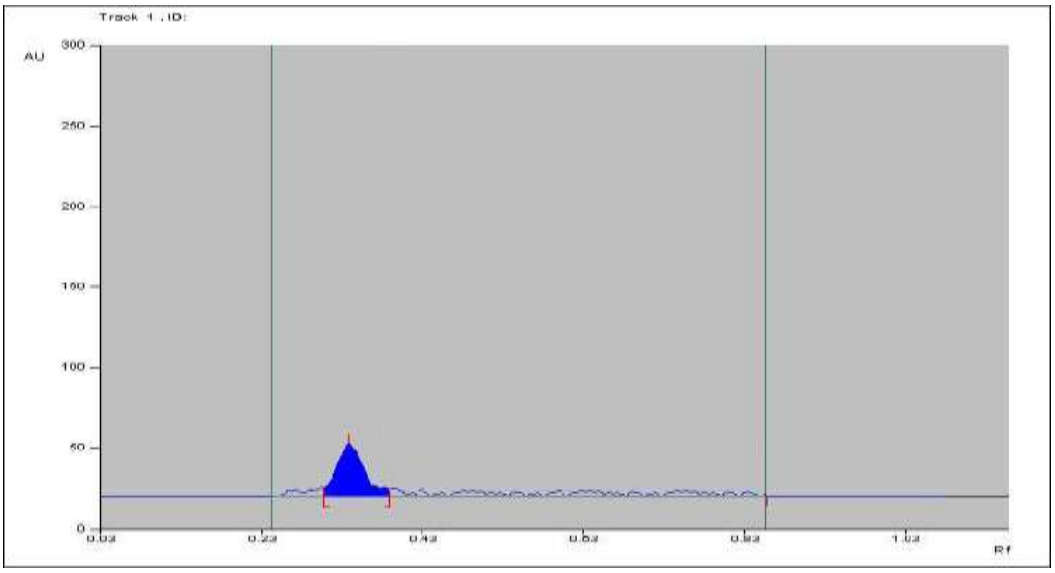
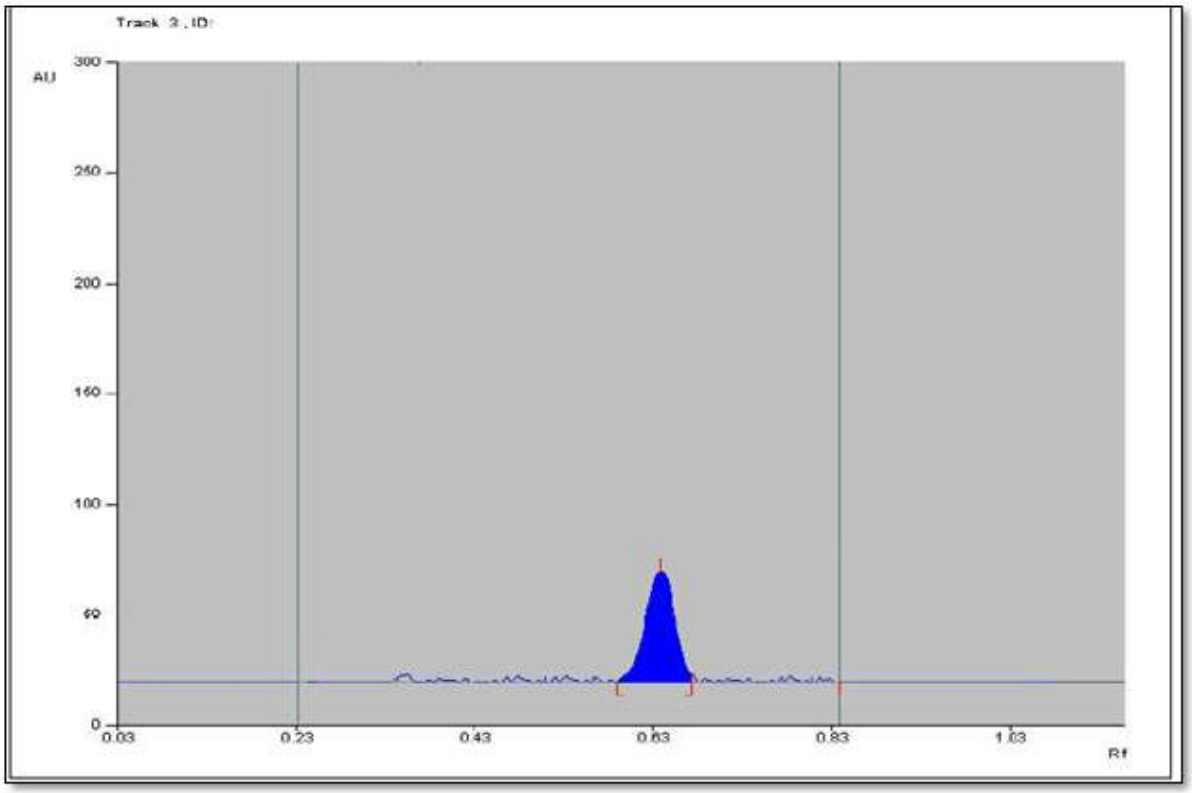
VITAMIN A:



VITAMIN-D_3



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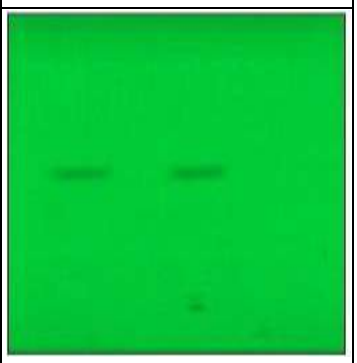
Optimization of Mobile phase:

Sr. No	Mobile Phase	Observation	TLC Plate
1	CHCl3: Toluene	Might be run with mobile phase because band is not visible on plate	
2	Hexane: Ethyl acetate	Band does move from site of the application but Rf value less than 0.15	
3	Hexane: Acetonitrile	Band do not visible on TLC plate	
4	Hexane: CHCl3 (7:3)	Band observed	

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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.
- Vitamin A was elute using Mobile Phase Hexane: CHCl3 (8:2) in HPTLC Method. Determine Log P value was 0.5. Vitamin A not suitable in HPTLC method because determined Log P value was Very Low.
- Vitamin D3 was elute using Mobile Phase Hexane: CHCl3 (7:3) in HPTLC Method and Determine Log P value 2.88 to 3.00 in HPTLC Method. HPTLC Method was suitable any other dosage formulation.

REFERENCES:

[1]. "Vitamin-D_3" information from Drug. Update www.Drug.Update.com
[2]. "Vitamin-A" drug info (data base available on internet) http://pubchem.nabi.nlm.nih.gov/compound/retional
[3]. Hong Yin et al "Method for preparing stable-type vitamin-A Microcapsules continuously", **2014**
[4]. Fang Fang Zhang et al, "Health effects of vitamin and mineral supplements",**2020**,1-5
[5]. Valtteri Virtanen et al, "Partition Coefficients (logP) of Hydrolysable Tannins", **2020** Aug; 25(16),1-3
[6]. Ali A. Albahranin et al, **"**Fat-Soluble Vitamins: Clinical Indications and Current Challenges for Chromatographic Measurement" ,**2016** Feb; 37(1),10-15
[7]. Priya Reddy et al, "Biochemistry, Fat Soluble Vitamins" **2022**, September 19, 1-5
[8]. Sima Taheri et al., "A literature review on beneficial role of vitamins and trace elements: Evidence from published clinical studies **2017**; Volume (67),
[9]. Karin Amrein et al., "Vitamin D deficiency 2.0: an update on the current status worldwide" European Journal of clinical nutrition, **2020** (74), 1498–1513
[10]. Rana A. Youness et al., "Fat-soluble vitamins: updated review of their role and orchestration in human nutrition throughout life cycle with sex differences", **2022**