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Characterization of Fat-Souble Vitamin for Hydrophobicity through HPLC method

Rana Twinkle*, Dr. G. B Sonara
1 Vidhyadeep Institute of Pharmacy, Anita, Surat
2 OPJS University, Churu, Rajasthan

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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, D3, E and K for this work. As reported, vitamins are essential for physiology of the body. The bioavailability of Fat Souble 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 HPLC methods.Vitamin A the log p value ranged between 6.17 to 6.20 in HPLC method. Vitamin D3 is not suitable for HPLC method because Vitamin D3 is very Hydrophobic nature. Vitamin E Determined LogP value was ranged between 3.4 to 3.5 in HPLC. Vitamin K was Determined Log P value 1.9 to 2.0 in HPLC method. It may be Suitable for emulsion formulation and other Dosage formulation. The true values of log p of Vitamin A,E and K differed from predicted values reported in literature, hence further exploration needed in this regard.

Key word: Log p, Fat-Soluble vitamin-A,D3, E and K

I. INTRODUCTION:[1-10]

Since the civilization, humans have utilized natural products extracted from plants for their medicinal purpose, with the earliest records from the origins of Ayurveda in India using natural products have been traced back to around 5,000 BCE. The first recorded forms of Ayurveda as medical texts evolved from the Vedas, Ayurvedic proponents like charaka and sushruta contributed with 314 and 516 drugs respectively.

Natural Compounds use for various type of Diseases in human and animal and Natural Products have good Biological Activities and health benefit. Natural products have evolved over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Natural product have most of human being uses because natural products have not any adverse effect in the body.. The earliest work on cultivation and classification of plants has much to do with their use in the treatment of many different types of human ailments and up until the identification of active principles and their synthesis on an industrial scale until the end of the nineteenth century, natural products were the principal source of medicines.

NATURAL PRODUCTS:

Natural products have always been an important source of pharmaceuticals, although synthetic chemistry has also produced many new bioactive substances and have considerably expanded the number of compounds available for tests, there are still a relatively high number of natural products and their derivatives among the best selling drugs and there has been a renewed interest in natural products as a source of pharmaceuticals. It has been recently shown that the types of natural products that show biological activity are quite different from their synthetic counterparts and statistical evaluations show a clear difference between the structural properties of natural products and synthetic compounds. As the degree of differences between natural products of different sources is also very high and there many sources of natural products are relatively untapped, interest in developing active principles from biodiversity will probably continue for a long time. A natural product is a chemical compound produced by a living organism. In broader sense, natural products include any substance produced by life.

Components as lead compounds for drug discovery.

Natural products can be prepared by chemical synthesis. Chemical synthesis is mainly divided into 2 types of synthesis.

- **Semi-synthesis/Partial synthesis:** Semi synthesis or partial chemical synthesis is a type of chemical synthesis that uses chemical compounds isolated from natural sources (e.g plant material) as the starting materials to produce other novel compounds

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with distinct chemical and medicinal properties.

- **Total Synthesis:** the total synthesis of natural products is a non-commercial research activity, aimed at deeper understanding of the synthesis of particular natural product and the development of fundamental new synthetic methods.

Natural products have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets.
Natural products have also been extended for commercial purposes like cosmetics, dietary supplements and foods produced from natural sources without added artificial ingredients.
Natural products have played a key role in pharma research, as many medicines are either natural products or derivatives thereof. Indeed, it is estimated that about 40% of all medicines is either natural products or their semisynthetic derivatives

Natural products are defined as naturally occurring compounds that are end products of secondary metabolism; often, they are unique compounds for particular organisms or classes of organisms.

PROBLEM WITH NATURAL PRODUCTS:
The synthesis of natural products is too difficult – the structures are too complex

Natural products structures spans 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

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.

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LOG P IMPORTANCE AND SOUBILITY:

LogP 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

- PKa and Log P measurements are parameters for use in understanding the behaviour of drug molecules.
- The Partition Coefficient is very useful parameter which mainly used in combination with the pKa to predict the distribution of a drug compound in a biological system. Partition coefficient (log P_{oct}), is a common parameter of the biological partition behaviour of drug molecules.
- Like example, drug absorption and log P_{oct} are directly related because of passive diffusion across the cell membrane.
- The Log *P* value is below 0 or above 5, drug candidates have intestinal and central nervous system (CNS) low solubility and poor oral bioavailability.
- The shake-flask method and RP-HPLC method are main used determine partition coefficients.
- Reversed-phase liquid chromatography (RP-HPLC) mainly used to estimate Log P_{oct}. all Methods are correlations with Log P_{oct}, even for very hydrophobic compounds.

The importance advantage of an HPLC-based measurement is that the partition coefficients can be obtained from time measurements instead of concentration determination. The result, this is retention time (tR) is independent of the compound concentration or amount injected into the chromatographic system. And Impurities and low solubility does not effect of the measurement of the compound. Other importance used Reversed-phase liquid chromatography (RP-HPLC) method is very little compound required and the Log P of a mixture of several compounds can be obtained from a single injection. HPLC-based method is some disadvantages such as short linear range of Log Poct and retention time.

Characterisation of Fat Souble Vitmain by chromatographic hydrophobicity:[10-15]

Once the active compounds are obtained in pure form, they can be subjected to structure elucidation.

The key technique for this is NMR, which make it possible to establish the connectivity of all hydrogen and carbon atoms in a molecule. Serving a very important complementary role is high resolution mass spectrometry (MS), which is capable of providing precise mass measurements that identify the molecular formula of the compound.

It is often possible to fully elucidate the structure of an unknown molecule using these two techniques alone.

Other spectroscopic techniques such as UV, IR and optical rotation serve ancillary roles, though they may become critical in specific cases.

An alternative technique for structure elucidation is x-ray crystallography, which has a long history in natural product structure elucidation. It is still an important technique, especially for determining the absolute configuration of complex chiral molecules. The obvious limitation is that the compound studied must exist in a crystalline form.

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If the native compound cannot be persuaded to crystallize, it can be derivatized with a variety of modifiers in an attempt to improve its ability to form crystals. Application of robotics to automatically generate many small scale crystallization experiments has increased the ability to find workable crystallization conditions

Some of the **Non chromatographic techniques** are mentioned below.

1. Immunoassay
2. Phytochemical screening assay
3. Fourier-transform infrared spectroscopy (FTIR)

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants.

A variety of analysis techniques for the quantification of total and isolated curcuminoids in different matrices have been reported, especially spectrophotometric methods for the determination of total curcuminods. However, this approach cannot be used to quantify individual curcuminoids, so various liquid chromatographic methods have been developed for this purpose. High-pressure liquid chromatography with UV detection (HPLC–UV) is the most common method for the determination of curcuminoids and curcumin in turmeric samples, biological samples, or dosage forms. Due to the very labile characteristics of curcuminoids, C18 columns are preferred for HPLC analysis.

For optimum oral absorption:

a. Molecular should be adequately soluble in physiological relevant buffers.
b. Should have good permeability.
c. Should have metabolically stability.
d. Majorly unionized in GIT Fluids.

II. MATERIAL AND METHOD:

Vitamins-A Chromatographic Condition: Mobile phase :(70:30v/v)(ACN:WATER)

Parameter	Condition
Method	Gradient reverse phase technique
Stationary phase	Purospher STAR C-18 (250nm X 4.6nm, 5µm)
Mobile phase	Water: ACN (30:70v/v)
Flow rate	1.0ml/min
Column Temperature	25◦C
Injection Volume	20µl
Wavelength	340nm
Total Run Time	2 hours

Vitamins-A Chromatographic ConditionMobilephase (30:70v/v)(AMMONIUMINACATATE:ACN)

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Parameter	Condition
Method	Gradient reverse phase technique
Stationary phase	SPD-M40 High Sensitivity PDA Detector Column: C18 Column ,5u,4.6 X 250mm
Mobile phase	Methanol: Water (80:20)
Flow rate	1ml/min
Column Temperature	25◦C
Injection Volume	10 µL
Wavelength	340nm
Total Run Time	30 Min

Vitamins-D_3 Chromatographic Condition

Parameter	Condition
Method	Gradient reverse phase technique
Stationary phase	SPD-M40 High Sensitivity PDA Detector Column: C18 Column ,5u,4.6 X 250mm
Mobile phase	Acetonitrile: Water (80:20)
Flow rate	1ml/min
Column Temperature	25◦C
Injection Volume	10 µL
Wavelength	290nm
Total Run Time	30 Min

Vitamins-E Chromatographic Condition

Parameter	Condition
Method	Gradient reverse phase technique
Stationary phase	SPD-M40 High Sensitivity PDA Detector Column: C18 Column ,5u,4.6 X 250mm
Mobile phase	Methanol: Water (80:20)
Flow rate	1ml/min
Column Temperature	25◦C
Injection Volume	10 µL
Wavelength	292nm
Total Run Time	30 Min

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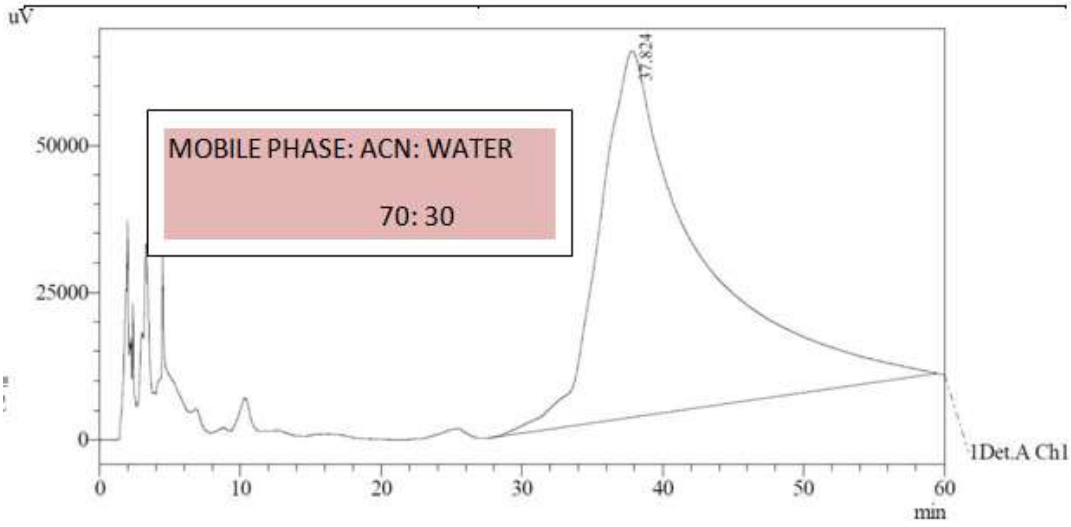
Vitamins-K Chromatographic Condition

Parameter	Condition
Method	Gradient reverse phase technique
Stationary phase	SPD-M40 High Sensitivity PDA Detector Column: C18 Column ,5u,4.6 X 250mm
Mobile phase	Acetonitrile: Water(80:20)
Flow rate	1ml/min
Column Temperature	25◦C
Injection Volume	10µL
Wave length	254nm
Total Run Time	30Min

III. RESULT AND DISCUSSION:

HPLC CHROMATOGRAPHY OF VITAMIN-A(Method-I)



COMPOUND	RETENTIONTIME
VITAMIN-A	37.824

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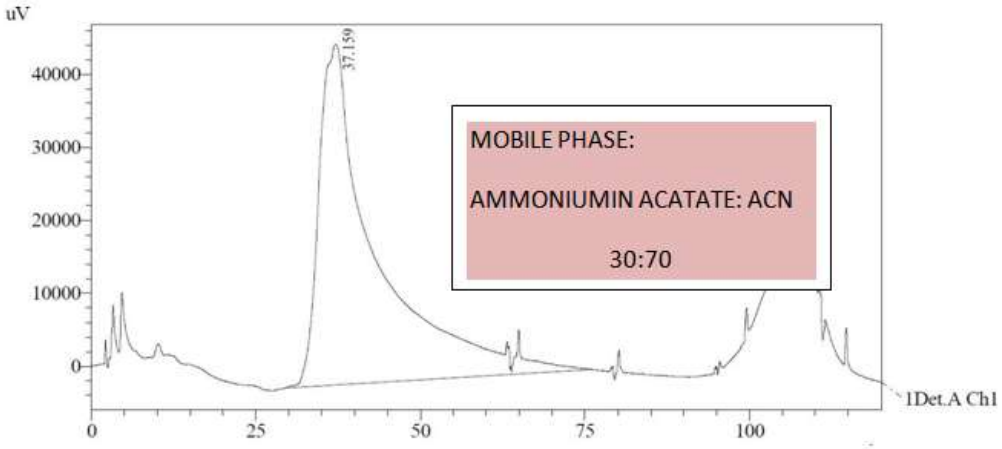
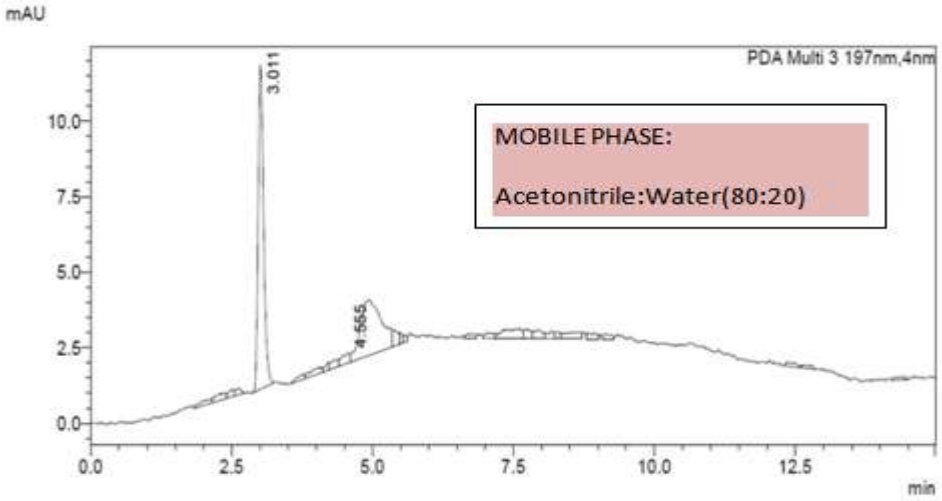
HPLC CHROMATO GRAPHY OF VITAMIN-A
(Method-II)



COMPOUND	RETENTIONTIME
Vitamin-A	37.159

HPLC CHROMATOGRAPHY OF VITAMIN-D_3



COMPOUND	RETENTIONTIME
Vitamin D_3	3.011

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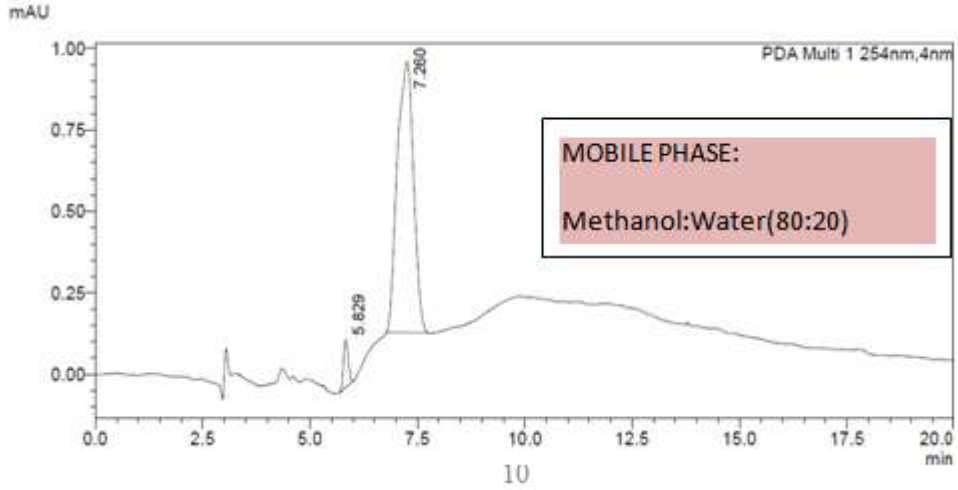
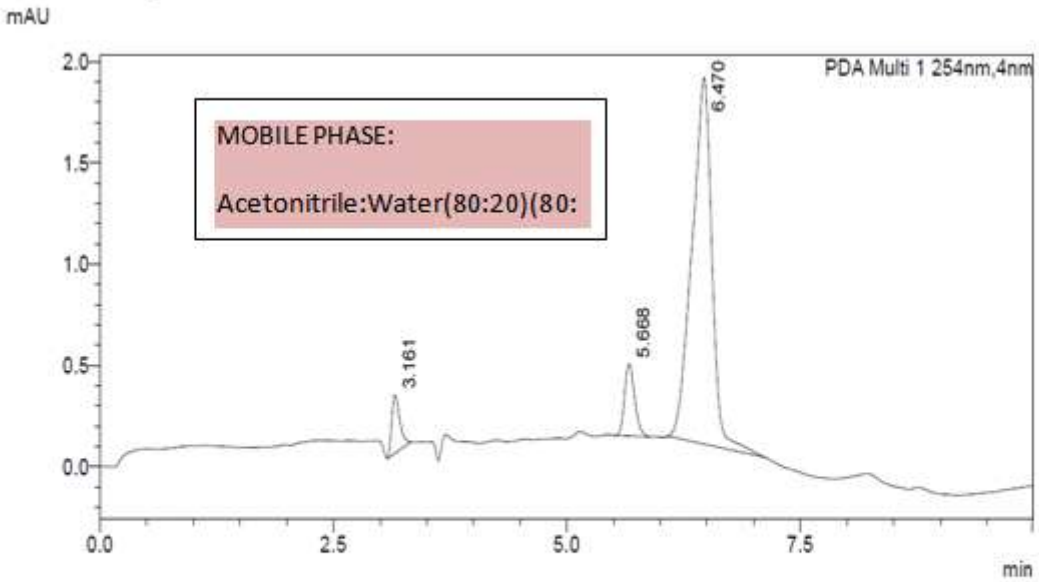
HPLC CHROMATOGRAPHY OF VITAMIN-E



10

COMPOUND	RETENTIONTIME
VitaminE	7.260

HPLC CHROMATOGRAPHY OF VITAMIN-K



COMPOUND	RETENTIONTIME
VitaminK	6.470

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LOG P VALUE FAT SOUBLE VITAMIN DETERMINATIONS

Compounds	Method-1 (70:30v/v) (ACN :water)	Method-4 (70:30v/v) (ACN:Ammonium acetate) (pH- 4)	Method-5 (80:20 v/v) (Methanol: Water)	Method-6 (80:20 v/v) (Acetanitrile: Water)
VITAMIN-A	6.17	6.20	-	-
VITAMIN-D_3	-	-	-	0.22
VITAMIN-E	-	-	2.13	-
VITAMIN-K	-	-	1.90	-

IV. RESULT AND DICUSSION

- Fat Soluble vitamin present in food ingredients has good biological activities and even some of natural compounds are used in Ayurvedic 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.
- 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.
- The some of the phytochemicals such as fat soluble vitamins such as Vitamin A, D_3, E and K have been reported to be not well absorbed after oral absorption. All these Phytochemicals were reported to be hydrophobic.
- Hence in this study, an attempt was made to understand how hydrophobic are these compounds and how their hydrophobicity could impact activity in the body. HPLC based determination of logP values of these compounds was attempted in the study. The logP values of compounds are measure of their hydrophobicity.
- Vitamin A, D3, E, K were elute in HPLC Method. Vitamin A (Method-A) was elute 2 Moblie phase (ACN : water) (70:30) using HPLC Method. HPLC Method was elute using Moblie phase ammonium acetate: ACN (30:70) and determined LogP Value was 6.1 and
6.2.

- Vitamin D3 was elute using Mobile Phase Acetonitrile: Water (80:20) in HPLC Method HPLC method not suitable because LogP value was very low. As per literature suggestions, shake flask method and potentiometric titration methods would not be suitable for these Vitamin D_3 vitamin.
- Some of Review Literature vitamin E Predicted Log P value was 12.2 but Vitamin E was elute using Mobile Phase: Methanol: Water (80:20) in HPLC. vitamin E Determined LogP value was 2.13 to 2.5. 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: Acetonitrile: Water (80:20) in HPLC method and Determined LogP value was 1.90 to 2.0. Determined LogP value was 2.0 to 2.5.It may be Suitable for emulsion formulation and other Dosage formulation.

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