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CHARACTERIZATION OF NUTRITIONAL COMPONENTS HYDROPHOBICITY BY HPLC BASED CHROMATOGRAPHIC TECHNIQUE

Twinkle S Rana[*], Dr.Gautam Sonara[2], Dr. Mahesh Salaraya[2]
[1]*Shree Dhanvantary Pharmacy College, Kudsad Road, Near Railway Station, Kim (E)-394110.*
[2]C. K. Pithawalla Institute of Pharmaceutical Science and Research, Surat.

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ABSTRACT

This work was aimed at determination of log p values of natural compound of great neutitional value. We have selected resveratrol, curcumin, vitamins such as A, D_3, and E for this work. Resveratrol and curcumin have been reported to possess diverse biological activities but have poor bioavailability. As reported in literature vitamins are essential for physiology of the body. The bioavailability of resveratrol, vitamins A, D_3, E are said to be low due to hydrophobic nature. In this study hydrophobicity was determined via log p value by employing HPLC methods. The log p value of resveratrol ranged between 0.22 to 0.66, curcumin log p value ranged between 1.83 to 2.08 and for vitamin A the log p value ranged between 6.17 to 6.20. The log p value of vitamin D_3, E cannot be determined by HPLC as none of mobile phase tested eluted those molecules within 2 hours. Hence HPLC method is not suitable for those molecules. The true values of log p of resveratrol and curcumin differed from predicted values reported in literature, hence further exploration needed in this regard.

Corresponding author
Dr.Gautam Sonara
C. K. Pithawalla Institute of
Pharmaceutical Science and Research,
Surat.

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INTRODUCTION

Since civilization, humans have utilized natural products extracted from plants for their medicinal purpose. 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. Humans replied on natural product because natural products have not any adverse effect in the body, today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation

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.

Problems 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. 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. 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 impact speed are

Instability of compounds, Adulteration, Difficult separation, Unreliability of bioassay

Log P and its importance:

Log p, which can be defined as the ratio of the concentration of the unionized compound, at equilibrium, between organic and aqueous phases. Log P values have been studied in approximately 100 organic liquid–water systems. Compounds with log P values between 3 and 6 show good absorption. Log P greater than 6 or less than 3 often have poor transport characteristics. Highly non-polar molecules have a preference to reside in the lipophilic regions of membranes. Very polar compounds show poor bioavailability because of their inability to penetrate membrane barriers. Thus, there is a parabolic relationship between log P and transport.

Characterisation of natural products by chromatographic hydrophobicity:

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. It 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. Other spectroscopic techniques such as UV, IR and optical rotation serve ancillary roles, though they may become critical in specific cases. As the number of atoms in a molecule increases, structure elucidation becomes more difficult and increase in possible structures for a given formula. An alternative technique for structure elucidation is x-ray crystallography, which has a long history in natural product structure elucidation.

MATERIALS AND METHODS:

Name of chemical: Aniline, Acetanilide, Acetophenone, Benzophenone, Diphenylamine, Curcumin, Resveratrol, Vitamin-A, Vitamin-D_3 Vitamin-E, ACN, Ammonium acetate

LIST OF INSTRUMENTS:

Sr.No	Instruments	Model and company name
1	Weighing balance	AVX220 Shimadzu
2	HPLC chromatography	LC 10 AT Liquid Chromatography with Shimadzu automatic SPD 10 A UV-Visible detector, DGU 12 A Degasser, and Waters 717 plus Auto sampler
3	PH meter	EUTECH Instrument pH Tutor
4	Ultra sonicator	Trans-o-sonic ultrasonic water bath (model-D 120/1H)

HPLC CHROMATOGRAPHY FOR SAMPLE PREPARTION:

All natural components are same sample preparation. All samples prepared for (40ppm) solution.Taken 10 mg sample in dissolved 10 ml Methanol in 10 ml volumetric flask and collected 1 ml solution in dissolved 25 ml methanol in 25 ml volumetric flask.

VITAMIN-A FOR SAMPLE PREPARTION:

Taken 2 and 3 vitamin-A chewable tablets crushed in mortar pester obtained coarse powder. Collect coarse powder in 10 ml volumetric flask after added chloroform in 10 ml volumetric flask after Sonication a solution and yellow colour solution obtained.

AMMOUNIUMM ACETATE PREPARATION:

77.083g in 1000ml ⟶ 1M
77.08mg in 1000ml ⟶ 1MM
770mg in 1000ml ⟶ 10MM
10MM Ammonium acetate ⟶ 500ml and 385mg ammonium acetate in 500ml water.

HPLC CHAROMATOGRAPHY:
Log P DETERMINATION USING METHOD-1(70% ACN: 30 % WATER)
METHOD-1:

ALL STANDARAD COMPOUNDS:

MOBILE PHASE : 30%WATER/70%ACN
COLUMN :Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE : 1.0ml/min
COLUMN TEMP : 25◦C
INJECTED VOLUME : 20µl
WAVELENGH : 254nm
RUN TIME : 10 MIN

RESVERATROL

MOBILE PHASE : 30%Water/70%ACN
COLUMN :Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE : 1.0ml/min
COLUMN TEMP : 25◦C
INJECTED VOLUME : 20µl
WAVELENGH : 300nm
RUN TIME: 10 MIN

CURCUMIN

MOBILE PHASE : 30%Water/70%ACN
COLUMN :Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE : 1.0ml/min
COLUMN TEMP : 25◦C
INJECTED VOLUME : 20µl
WAVELENGH : 450nm
RUN TIME : 10 MIN

VITAMIN-A

MOBILE PHASE : 30%Water/70%ACN
COLUMN : Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE : 1.0ml/min
COLUMN TEMP : 25◦C
INJECTED VOLUME : 20µl
WAVELENGH : 340nm
RUN TIME : 1 hr

4.1 Log P DETERMINATION USING METHOD-2 (30 % AMMONIUMIN ACETATE: 70 % ACN)
METHOD-2:

ALL STANDARD COMPOUNDS

MOBILE PHASE : 30%Ammonium acetate/70%ACN
COLUMN : Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE : 1.0ml/min
COLUMN TEMP : 25◦C
INJECTED VOLUME : 20µl
WAVELENGH : 254nm
RUN TIME : 10 MIN

RESVERTROL

MOBILE PHASE	: 30%Ammonium acetate/70%ACN
COLUMN	:Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE	: 1.0ml/min
COLUMN TEMP	: 25◦C
INJECTED VOLUME	: 20µl
WAVELENGH	: 300nm
RUN TIME	: 10 MIN

CURCUMIN

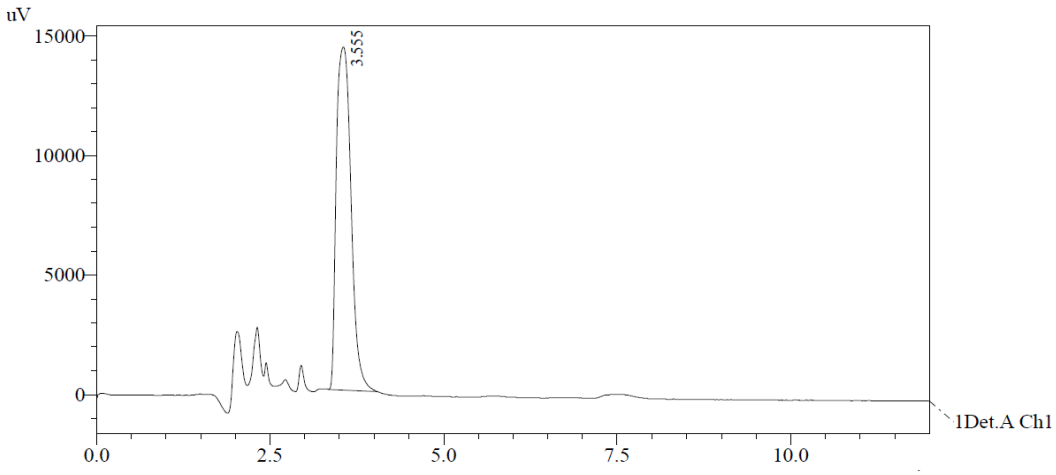
MOBILE PHASE	: 30%Ammonium acetate/70%ACN
COLUMN	: Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE	: 1.0ml/min
COLUMN TEMP	: 25◦C
INJECTED VOLUME	: 20µl
WAVELENGH	: 450nm
RUN TIME	: 10 MIN

VITAMIN-A

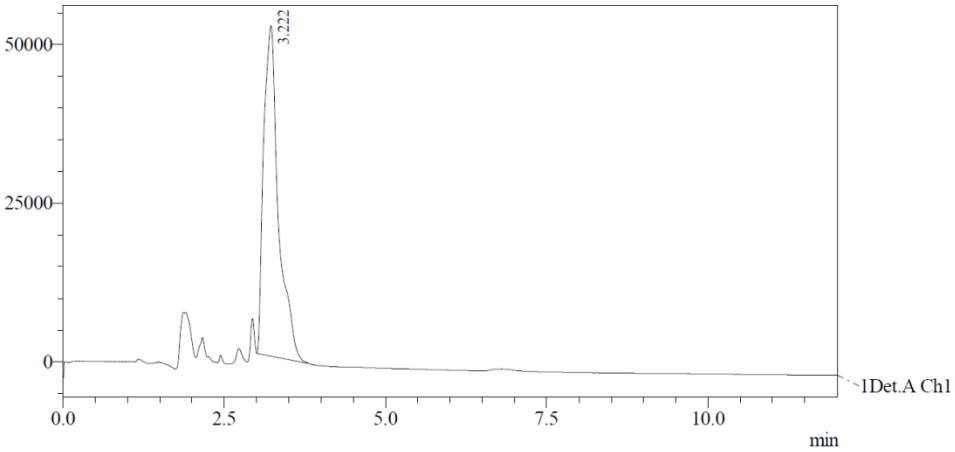
MOBILE PHASE	: 30%Ammonium acetate/70%ACN
COLUMN	: Purospher STAR C-18 (250nm X 4.6nm, 5µm)
FOLW RATE	: 1.0ml/min
COLUMN TEMP	: 25◦C
INJECTED VOLUME	: 20µl
WAVELENGH	: 340nm
RUN TIME	: 1 Hr

Log P DETERMINATION USING METHOD-1(70% ACN: 30 % WATER)

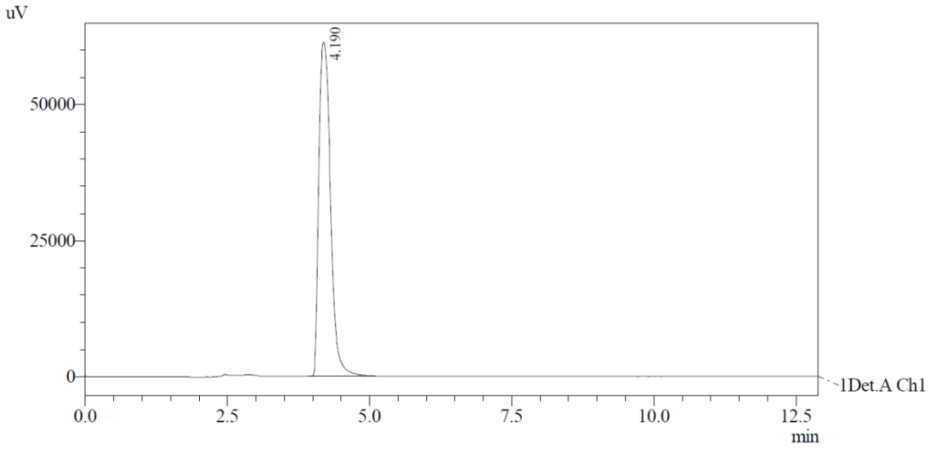
METHOD-1:
ANILINE (LOG P-0.9)



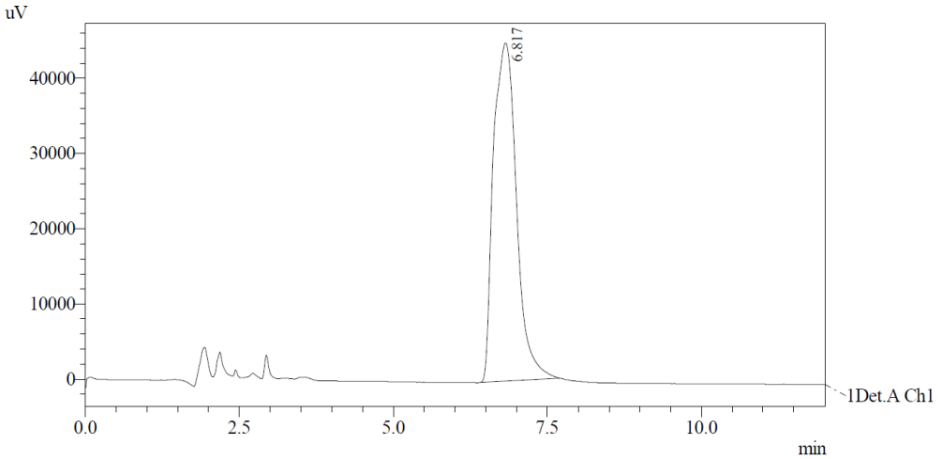
ACETANILIDE (LOG P-1)



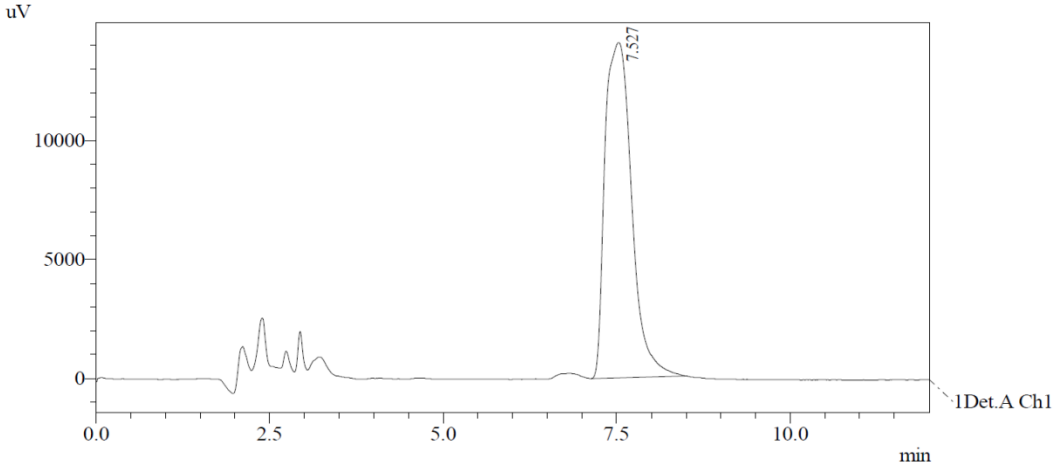
ACETOPHENONE: (LOG P-1.7)



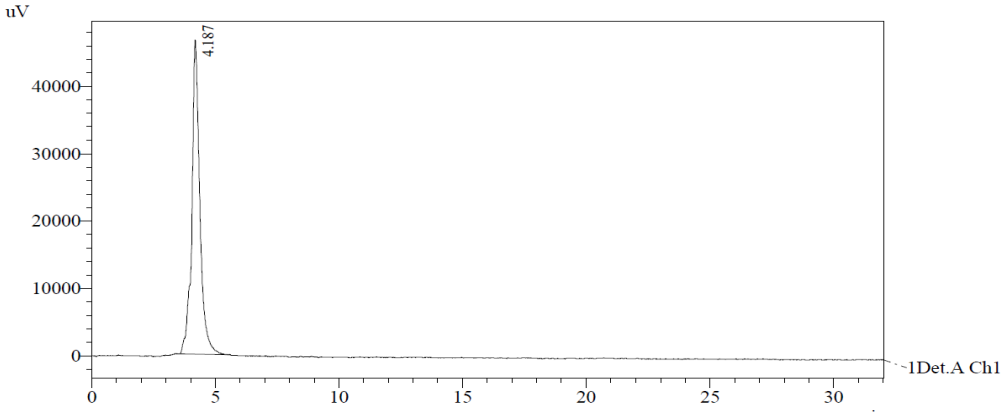
BENZOPHENONE: (LOG P-3.2)



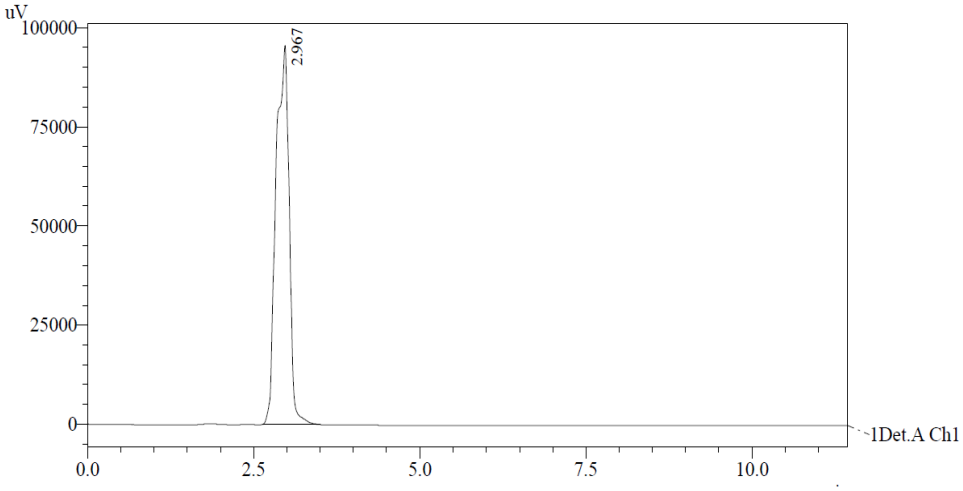
DIPHENYLAMINE (LOG P-3.4)



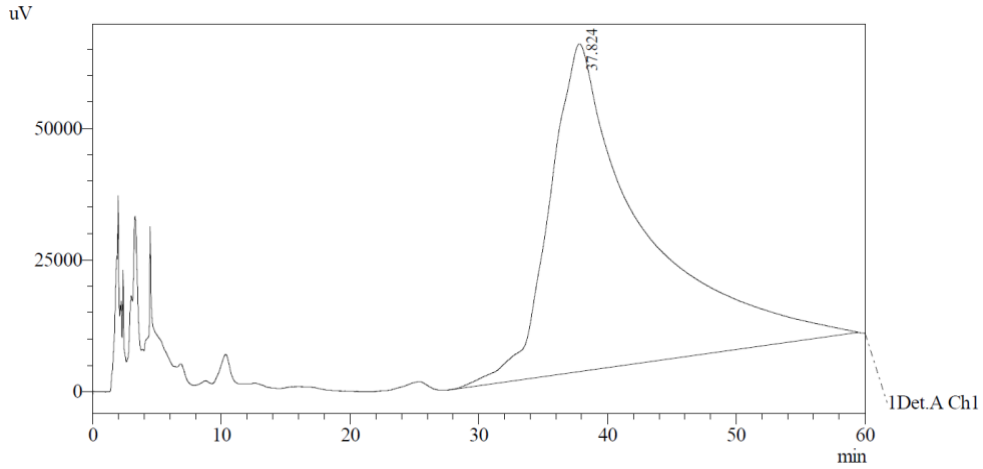
CURCUMIN:



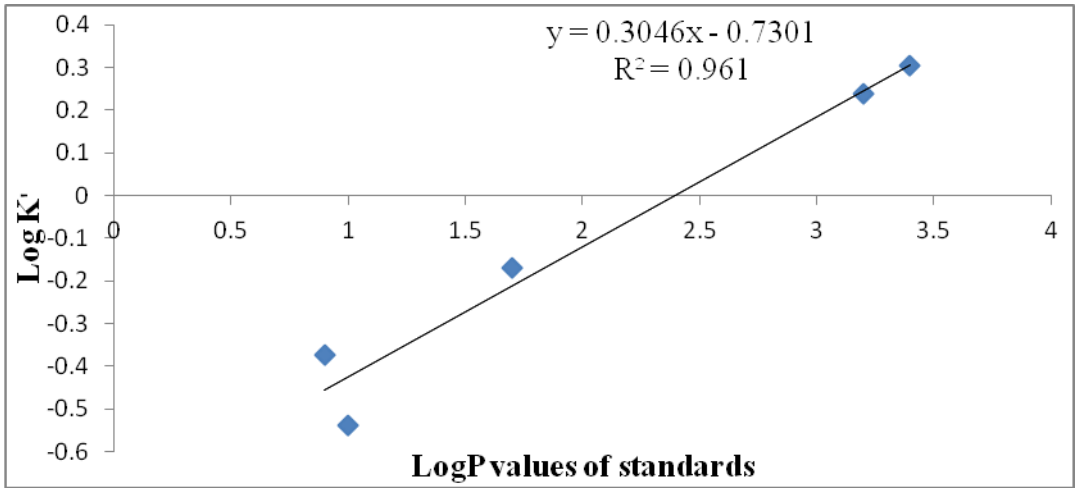
RESVERATROL:



VITANIN-A:



LOG P V/S LOG K



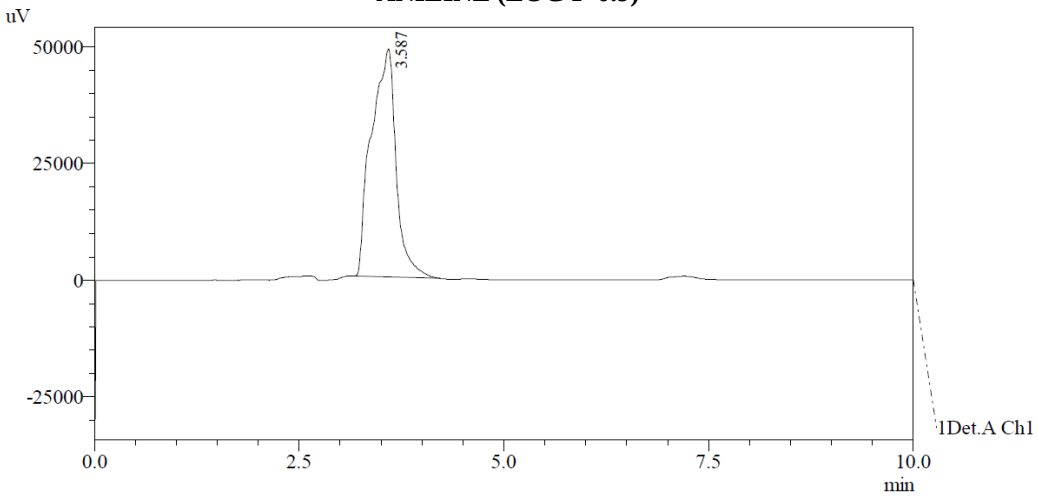
CALCULATION OF DETERMINED LOG P VALUE MOBILE PHASE :(70:30v/v) (ACN: WATER).

Compound	SD Values (Log- P)	Retention Time	K'(TR-2.5/2.5)	LOG Ki	Determined Log p value
Aniline	0.9	3.555	0.422	-0.3746	1.1668
Acetanilide	1	3.222	0.2888	-0.5394	0.6260
Acetophenone	1.7	4.190	0.676	-0.1700	1.8386
Benzophenone	3.2	6.817	1.7268	0.23724	3.1757
Diphenylamine	3.4	7.527	2.0108	03033	3.3928
Curcumin	Predict 3.2	4.187	0.6748	-0.170	1.8360
Resveratrol	Predict 3.10	2.967	0.1868	-0.7286	0.0048
Vitamin-A	Predict 6	37.824	14.1296	1.15012	6.1727

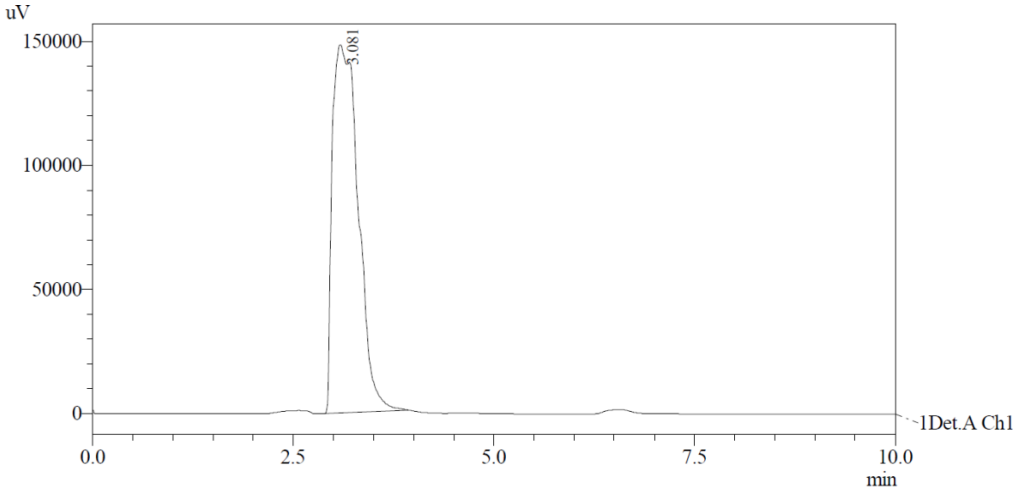
Vitamin- D_3, E Data are not obtaining after 2 hours so this is not suitable those molecules and This method is good for molecules such resveratrol and curcumin and also Vitamin D_3 and E because of too low retention time and too high elution, not eluted even after 2h.

Log P DETERMINATION USING METHOD-2 (30 % AMMONIUMIN ACETATE: 70 % ACN)

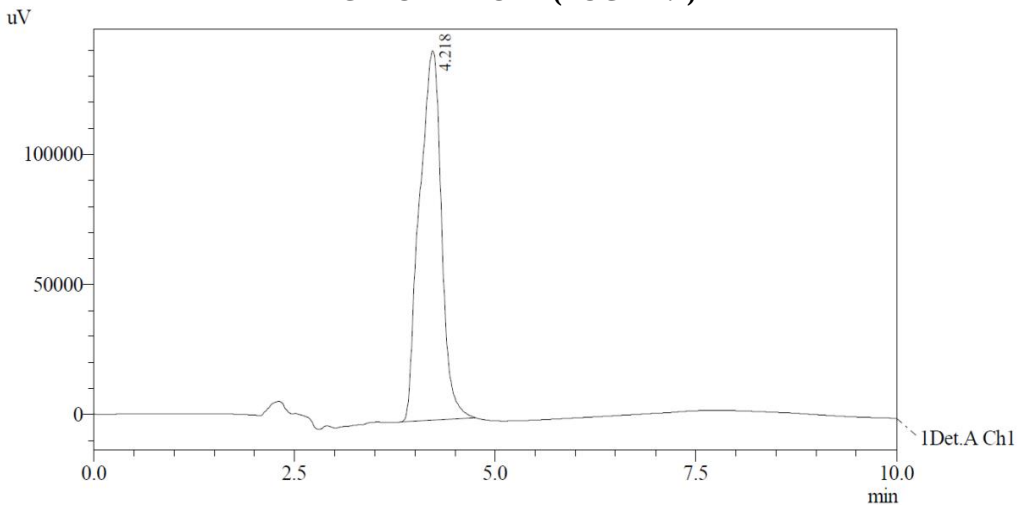
ANILINE (LOG P-0.9)



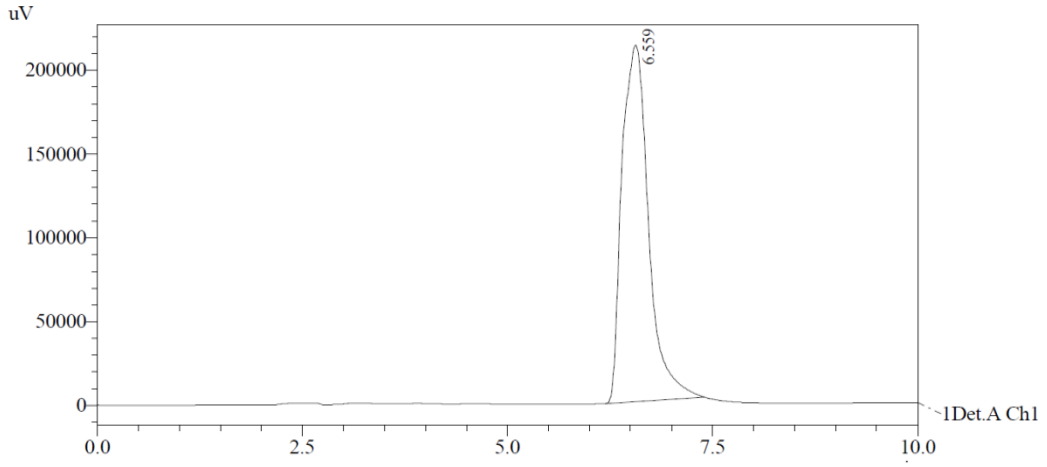
ACETANILIDE (LOG P-1)



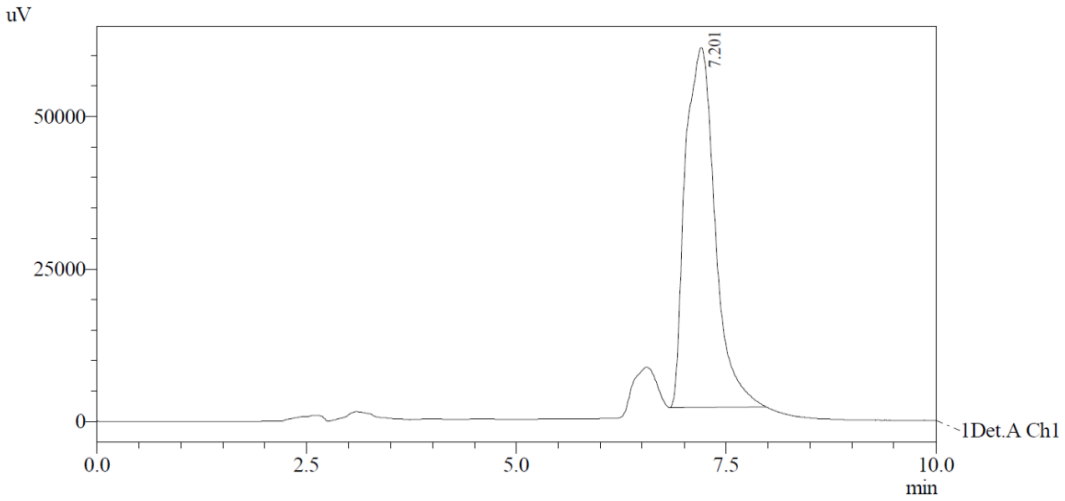
ACETOPHENONE (LOG P-1.7)



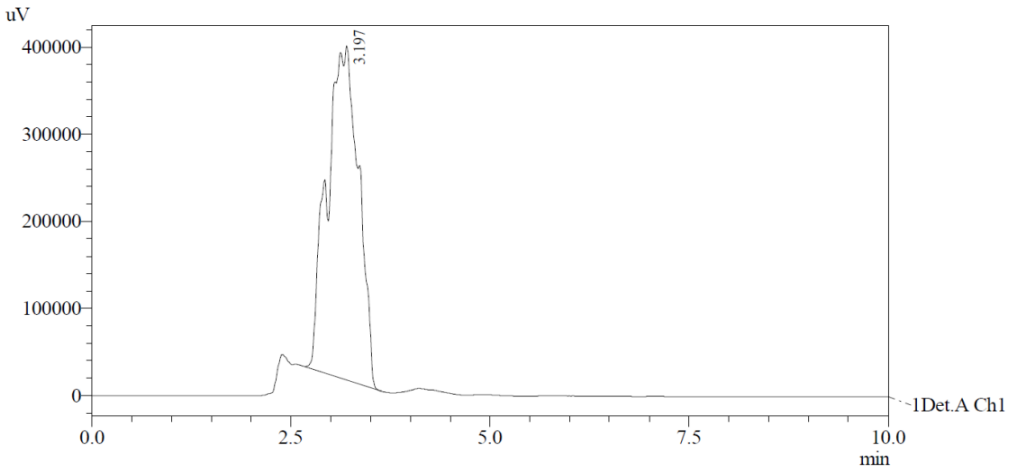
BENZOPHENONE (LOG P-3.2)



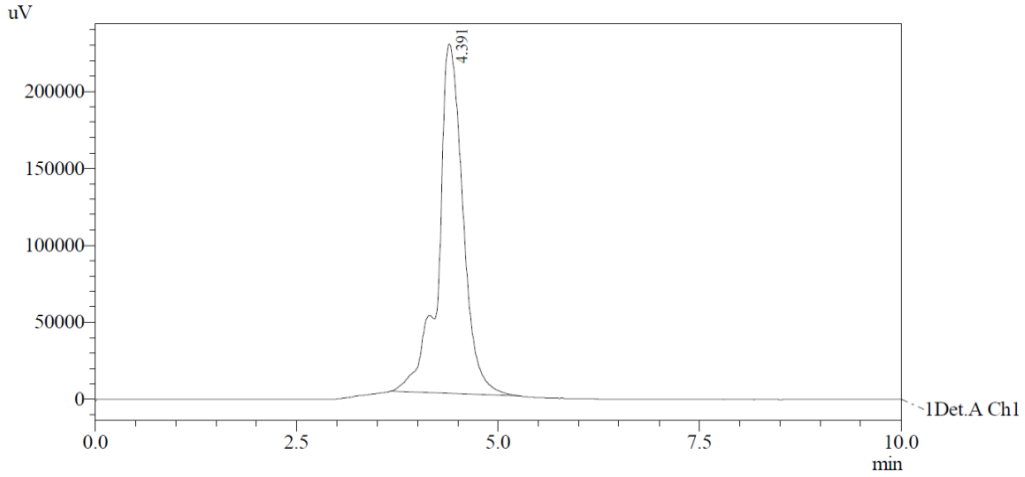
DIPHENYLAMINE (LOG P-3.4)



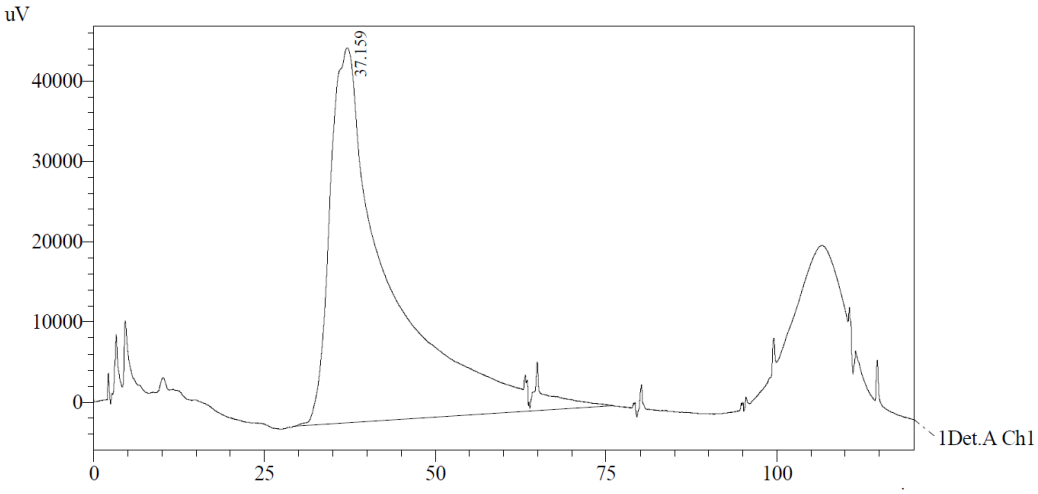
RESVERATROL



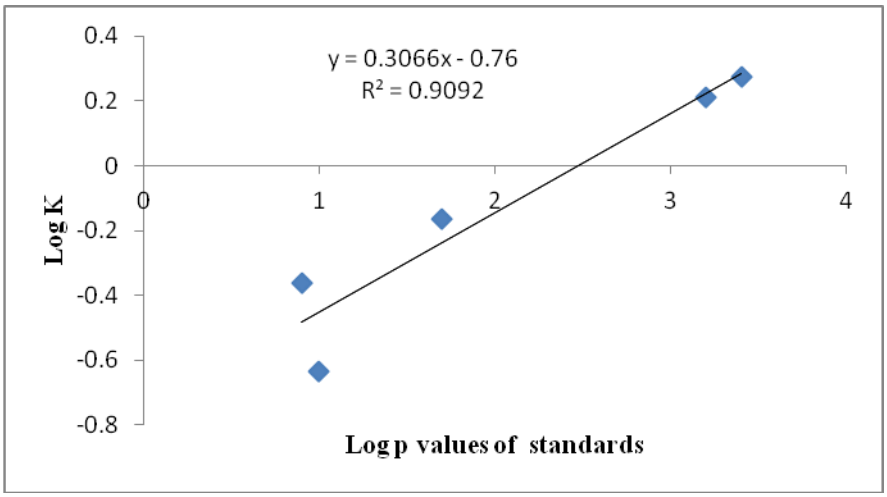
CURCUMIN



VITAMIN-A



LOG p v/s LOG K



HPLC CHARMATOGRAPHY USING MOBILE PHASE: (AMMONIUMACETATE pH 4.0: ACN) (30:70v/v)

Compounds	SD Values (LOG P)	Retention Time	K'(TR-2.5/2.5)	LOG Ki	Determined Log p values
Aniline	0.9	3.587	0.4348	-0.361	1.299
Acetanilide	1	3.081	0.2324	-0.6337	0.4117
Acetophenone	1.7	4.218	0.6872	-0.16292	1.947
Benzophenone	3.2	6.559	1.6236	0.21047	3.165
Diphenylamine	3.4	7.201	1.8804	0.27425	3.375
Curcumin	Predict 3.2	4.391	0.7564	-0.12125	2.08333
Resveratrol	Predict 3.10	3.197	0.2744	-0.5547	0.66957
Vitamin-A	Above 6	37.159	13.8636	1.1418	6.2028

Vitamin-D_3, E did not elute after 2 hours so this is not suitable those molecules.

LOG P VALUE DETERMINATIONS:

Compounds	Method-1 (70:30v/v) (ACN :water)	Method-2 (70:30v/v) (PH-4.0) (ACN: Ammonium acetate)
Resveratrol	0.004	0.66
Curcumin	1.83	2.08
Vitamin-A	6.17	6.20
Vitamin-D_3	-	-
Vitamin-E	-	-

CONCLUSION

The naturally occurring compounds present in food ingredients have demonstrated remarkable biological activities and even some of them are used in Ayurvedic system of medicine for treatment of various diseases in human and animals. Natural chemicals with excellent biological 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 depicts that lack of absorption, poor metabolic stability, rapid elimination from the body contribute to observed inaction in the body. The some of the phytochemicals such as Resveratrol, Curcumin and fat soluble vitamins have been reported to be not well absorbed after oral absorption. All these chemicals were reported to be hydrophobic. However the hydrophobicity was not clearly spelled out or graded. 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. The logP value of the compounds was determined using method 1-2. In all the methods the logP values of standard compounds matched reported values (OECD guideline). The logP value of reseveratrol and curcumin in method 1 & 2 (1.83 to 2.08) Hence it can be believed that predicted logP values may be inaccurate. On other hand, Vitamin A was eluted in method-1 and method-4 and logP value was consistent (6.1 and 6.2). Other vitamins such as Vitamin D_3 and Vitamin E did not elute in any of the method tested here even after 2 hours. Hence the HPLC method is unsuitable for these two vitamins since their logP value could be >10, HPLC method is not suitable for molecules with logP values >6. As per literature suggestions, shake flask method and potentiometric titration methods would not be suitable for these two vitamins.

Our conclusion is Resveratrol is not that hydrophobic as reported, its logP value was found be 0.6-0.66 against 3.1 (Predicted) reported in literature. Hence its insolubility in water does not translate into higher solubility in oils and is not suitable for emulsion formulation and hence other formulation techniques to need to be tested for improving absorption. Curcumin logP value was close to predicted value reported in literature 2.08 (3.2 predicted). It may be suitable for emulsion formulation. On other hand Vitamin A is too hydrophobic with logP value >6.0 and is highly suitable for emulsion formulation. The logP values of vitamin D_3 and E seems to be very high >10 and hence no suggestion on formulation can be given.

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