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CHROMATOGRAPHIC MARKER ANALYSIS - A RATIONAL APPROACH FOR QUALITY ASSESSMENT OF POLYHERBAL FORMULATION: ABHA CAPSULE

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ABSTRACT

Introduction: Ayurvedic texts describe many formulations for different ailments. Abha capsule is reputed for treating bone fracture and osteoporosis. These formulations have been considered complementary medicine or alternative to conventional medicines across the globe. These complex polyherbal formulations need science based approach toward manufacturing process and chemical standardization. **Aim:** Study was to find out a simple, accurate and sensitive HPTLC method for the detection and quantification of marker molecules of raw material and comparison with finished product for standardization. **Material:** A simple and reproducible high performance thin layer chromatography method for the determination of active component of Ayurvedic product i.e. Abha capsule has been performed for standardize formulation. **Result:** The key ingredients of Abha capsule are *Abha guggulu, Kaishore guggulu, Lakshadi guggulu* and *Hadjod*. *Guggulu* is common ingredient of all three preparations and *Hadjod* is the key ingredient of Formulation. Investigation shows Gallic acid, Piperine and gingerol is present in methanolic extract of *Abha guggulu, Kaishore guggulu* and Abha capsule. Although *Lakshadi guggulu* and Abha capsule contains Withanolide. HPTLC chromatogram of *Abha guggulu, Kaishore guggulu, Lakshadi guggulu* shows three similar peaks at Rf 0.21,0.43,0.54,0.74 which is also seen in Abha capsule which indicate presence of *guggulu* in Abha capsule. *Hadjod* extract analysis shows peaks at Rf 0.26, 0.41, 0.58, & 0.83, which also appear in extract of Abha capsule. **Conclusion:** The quality of formulation proves by presence *Hadjod*, *Guggulu* and standards like Gallic acid, Piperine, Gingerol and Withanolide in Abha capsule.

KEYWORDS: Abha Capsule, Polyherbal formulation, Phytoconstitutes, HPTLC.

INTRODUCTION

Herbal formulations have reached extensive acceptability as therapeutic agents for several diseases. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Herbal formulations have reached widespread acceptability as therapeutic agents for bone health, kidney diseases, liver diseases etc.[1] The growing use of Ayurvedic and herbals by the public is forcing moves to assess the health claims of these agents and to develop standards of quality and manufacture. Therefore quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with Ayurvedic products and also standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles.[2]

Analytical study for quality assessment of herbal drugs is paramount importance in order to justify their acceptability in the present era. An Ayurvedic preparation of medicine involves multi-step procedures. It is essential to prepare proper scientific documentation and standardization of ingredients. Analytical study ensures not only chemical constituents but also tells us about standards and quality of the drugs. Each and every drug substance has its own physical and chemical characteristics, which helps for separating it from other closely related drug. Hence, physico-chemical study of a particular sample should be carried out by the use of various parameters which helps in authentication, standardization and determination of quality of drugs. Analytical measurement encompasses two essential criteria–utility and reliability.[3]

By altruistic reasons present study an attempt was made for standardization of Abha capsule (a marketed poly

herbal formulation of Vital Care Pvt. Ltd) by employing various analytical parameters like qualitative and quantitative tests by HPTLC profiling. HPTLC technique was reported for simultaneous determination of each ingredient's marker component as well as standardization of final formulation i.e. Abha capsule.

MATERIAL AND METHODS

Test drug

A Polyherbal formulation with Brand name Abha Capsule is an Ayurvedic proprietary medicine of Vital Care Pvt Ltd, Vadaodara, Gujarat. It contains *Hadjod (Cissus quadrangularis*) and three classical formulations i.e. *Abha guggulu*,[4] *Lakshadi guggulu*,[5] and *Kaishore guggulu*,[6] as active ingredients. All the active ingredients were procured from approved vendors of manufacturer. The final capsule formulation is also collected from routine manufacturing batch.

Qualitative Estimation[7-9]

All four active constitutes of abha capsule were tested to check the presence of Alkaloids, Glycoside, Flavonoid, Saponin, Tannins/Phenolic, Sterols and Carbohydrate. Following tests were performed to identify its presence.

A) Tests for Alkaloids: Mayer's test, Dragendorff's test, Wagner's test, Hager's test.
B) Tests for Glycosides: General test, Cardiac glycosides (Keller-Kiliani test), Anthracene Glycosides (Borntrager test).
C) Tests for Flavonoid: Shinoda test (Magnesium HCl reduction test), Alkaline reagent test, Zinc HCl reduction test.
D) Test for Saponin: Frothing test, Test for steroidal saponin.
E) Test for Tannins/Phenolic: $FeCl_3$ test, Lead acetate test, Gelatin test, Acetic acid test, Silver mirror test.
F) Tests for Sterols and Triterpenoid: Libermann-Burchard test, Salkowski's test, Libermann's reaction, Sulphur test.
G) Test for Carbohydrates: Molisch's test.
H) Test for Amino acid: Ninhydrin test.

Quantitative Estimation: The Quantitative estimation of Aqueous Extract of raw material and finished product were carried out. Gallic acid, Piperine, Gingerol, Withanolide were quantified with respect to formulation by HPTLC method.

High-performance thin layer chromatography (HPTLC) based method is considered good alternative and important tool for routine analysis of drug and reduce time and cost of analysis. HPTLC fingerprint studies confirmed the results of phytochemicals screening by the presence of various colored bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds.[10] HPTLC standardization was carried out using a Hemilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60F254(Merck), 0.2 mm thickness. CAMAG HPTLC system. Methanol extract of raw material and Abha capsule finished product sample was spotted on pre-coated silica gel GF 60_{254} aluminum plate by means of Camang Linomate V. 5 µl of each extract loaded on silica gel plate and same was developed with respective solvent system as mention in table no.1 in twin trough chamber previously saturated with the above stated solvent system. After development, densitometric scan was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 and 366 nm under control of Win CATS Software (V 1.2.1. Camag).[11]

Interpretation of HPTLC chromatograph

It was done by either quantitative / qualitative means viz. in quantitative means is used for estimation of specific chemical compound into extract by comparison with standard marker compound by calibration curve method. Qualitative evaluation is used for HPTLC fingerprinting of different extracts which gives ideas about presence or absence of one more compound into different extracts or in single extract.

Table 1: Solvent system for raw material and Abha capsule.[12-16]

Sample	Ingredients	Solvent system
Hadjod	*Cissus quadrangularis* Linn.	Hexane : Ethyl acetate (1:1)
Abha Guggulu	*Piper longum, Piper nigrum* (Piperine)	Toluene: Ethyl acetate (93:7)
	Amla, Baheda, Harde (Gallic acid)	Toluene: Ethyl acetate: Formic acid: Methanol (6:6:1.4:0.6)
	Zingiber officinalis (Gingerol)	Toluene: Ethyl acetate (9:1)
	Commiphora mukul	Hexane : Ethyl acetate (1:1)
Lakshadi Guggulu	*Withania somnifera* (Withanolide)	Toluene: Ethyl acetate: Formic acid (5:5:1)
	Commiphora mukul	Hexane : Ethyl acetate (1:1)
Kishore Guggulu	*Piper longum, Piper nigrum* (Piperine)	Toluene: Ethyl acetate (93:7)
	Amla, Baheda, Harde(Gallic acid)	Toluene: Ethyl acetate: Formic acid: Methanol (6:6:1.4:0.6)
	Zingiber officinalis (Gingerol)	Toluene: Ethyl acetate (9:1)
	Commiphora mukul	Hexane : Ethyl acetate (1:1)

RESULT AND DISCUSSION

All the active ingredients of Abha capsule were subjected for the various qualitative phytochemical tests. Results are given in Table-2. Results suggested that all ingredients of Abha capsule contain Alkaloids glycoside, flavanoid, saponin, steroid and carbohydrate. Tannin is present in all the ingredients of Abha capsule except *Hadjod* extract. These all phytochemicals are present in abha capsule also which suggest that the presence of this phyto-constitutes improve the effectiveness of formulation and might be responsible for giving various pharmacological action.

Table 2: Qualitative tests for the ingredients of Abha capsule.

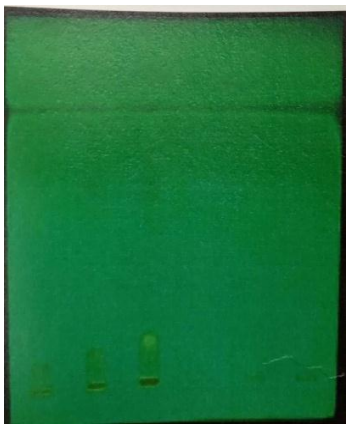
Test	Aqueous extract of				
	HE	AGP	LGP	KGP	AC
Alkaloid	+	+	+	+	+
Glycoside	+	+	+	+	+
Flavonoid	-	-	-	-	-
Tannin	-	+	+	+	+
Saponin	+	+	+	+	+
Steroid/Terpenoid	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Amino acid	-	-	-	-	-

(+): presence and (-): absent. ***HE****: Hadjod extract,* ***AGP****: Abha gugglu* powder, ***LGP****: Lakshadi guggulu Powder,* ***KGP****: Kaishore guggulu Powder,* ***AC****: Abha capsule.*

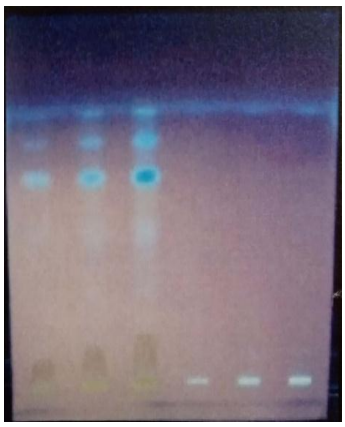
Quantitative analysis by HPTLC Fingerprinting

Hadjod Analysis

Comparative presence of *Cissus quadrangularis* (*Hadjod*) in Abha capsule by HPTLC. Stationary phase Silica gel TLC plate and mobile phase Hexane: Ethyl acetate (1:1). had given good separation peaks at at Rf 0.26, 0.41, 0.58 and 0.83



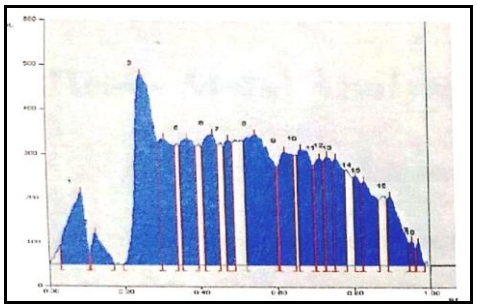
UV 254 nm



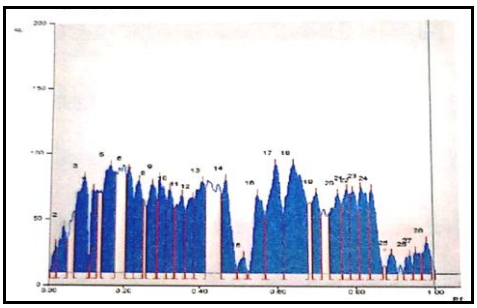
UV 366 nm

Fig. 1: HPTLC of Abha capsule extract, *Cissus quadrangularis* (*Hadjod*) extract.

Track-1: 2 µg/ml of Methanol extract of Abha capsule. Track-2: 4 µg/ml of Methanol extract of Abha capsule. Track-3: 8 µg/ml Methanol extract of Abha capsule. Track-4: 2 µg/ml Methanol extract of *Hadjod.* Track-5: 4 µg/ml of Methanol extract of *Hadjod*. Track-6: 8 µg/ml of Methanol extract of *Hadjod*.



(a)



(b)

Fig: 2 Chromatogram of *Hadjod* extract (a) & Abha capsule extract (b).

Table 3: Comparative Rf of Hadjod extract and Abha capsule by HPTLC.

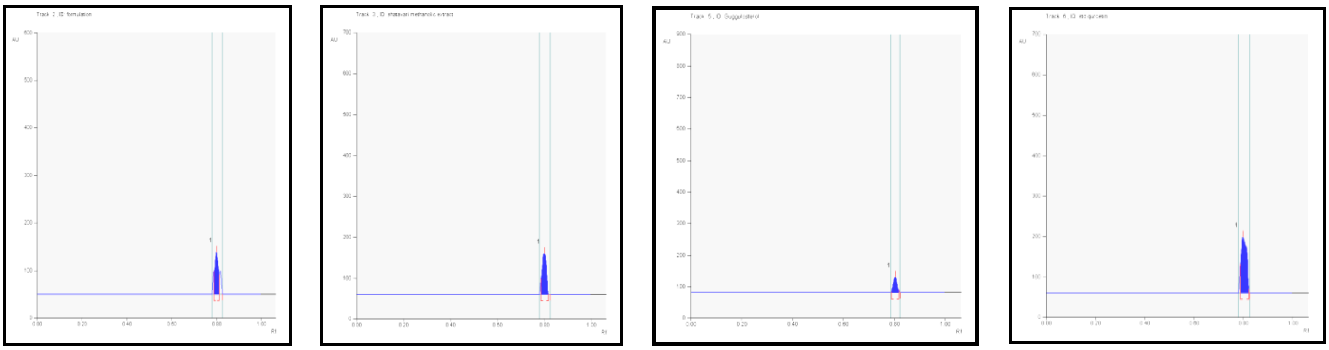
Sr. No.	Max. Rf of *Hadjod* Extract	Max. Rf of Abha capsule Extract	Peak area	
			Hadjod Extract	Abha capsule Extract
1	0.26	0.26	8317.9	827.1
2	0.4	0.41	11916	1803.2
3	0.58	0.59	32202.9	1157.8
4	0.83	0.83	7260.4	1588.5

Guggulu Analysis

Comparative presentation of Guggulu in all three *Guggulu* preparation i.e. *Abha*, *Laksha*, *Kaishore* and Abha capsule by HPTLC. Silica gel TLC plate used as stationary phase and Hexane: Ethyl acetate (1:1). as mobile phase had given good separation peaks at Rf 0.22,0.43,0.54 and 0.74

UV 254 nm **UV 366 nm**

Fig. 1: HPTLC of Abha *guggulu, Kaishore guggulu, Lakshadi Guggulu* extract and Abha capsule extract. Track-1: 4 µg/ml of Methanol extract of *Abha guggulu*. Track-2: 4 µg/ml of Methanol extract of *Lakshadi guggulu.* Track-3: 6 µg/ml Methanol extract of *Kaishore guggulu.* Track-4: 8 µg/ml Methanol extract of Abha capsule. Track-5: 2 µg/ml of Methanol extract of *Guggulu*. Track-6: 4 µg/ml of Methanol extract of *Guggulu*.



(a) **(b)** **(c)** **(d)**

Fig. 2: Chromatogram of *Abha guggulu* (a), *Laksha guggulu* extract (b), *Kaishore guggulu* (c) & Abha capsule extract (d).

Table 4: Comparative Rf of Guggulu by HPTLC.

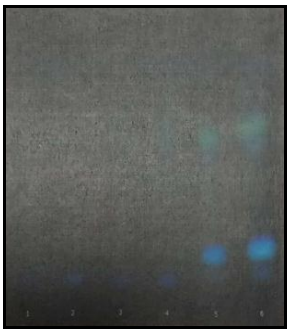
Name of sample	Max. Rf
Abha guggulu extract	0.21,0.43,0.54,0.74
Lakshadi guggulu extract	0.22,0.44,0.54,0.73
Kaishore guggulu extract	0.21,0.43,0.54,0.74
Abha capsule extract	0.22,0.43,0.54,0.74

Gallic acid Analysis

Gallic acid content of *triphala* in *abha guggulu, Kaishore guggulu* and Abha capsule by HPTLC.

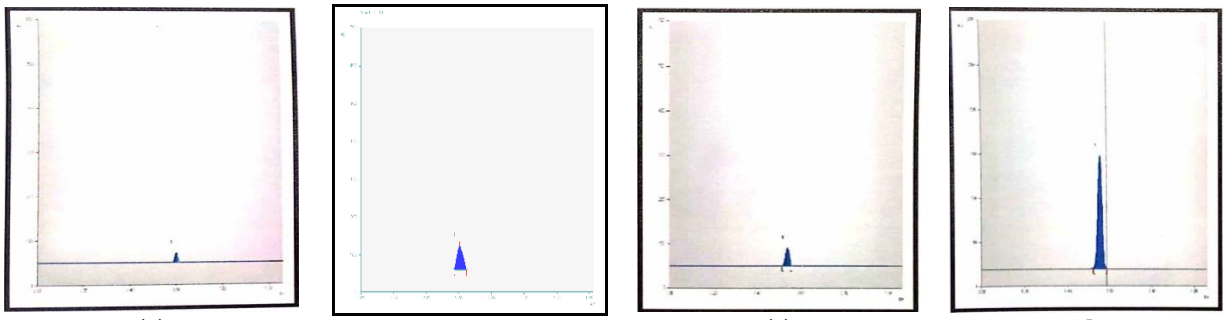
Silica gel TLC plate used as stationary phase and Toluene: Ethyl acetate: Formic acid: Methanol (6:6:1.4:0.6) as mobile phase had given good separation of gallic acid at Rf = 0.45.

UV 254 nm



UV 366 nm

Fig. 1: HPTLC of Abha capsule extract, *Abha guggulu, Kaishore guggulu* extract & Std. gallic acid. Track-1: 4 µg/ml of Methanol extract of gallic acid. Track-2: 8 µg/ml of Methanol extract of gallic acid. Track-3: 4 µg/ml Methanol extract of *Abha* capsule.Track-4: 8 µg/ml Methanol extract of *Abha* capsule Track-5: 4 µg/ml of Methanol extract of *Kaishore guggulu*. Track-6: 4µg/ml of Methanol extract of *Abha guggulu.*



(a) (b) (c) (d)

Fig. 2: Chromatogram of Abha capsule extract (a), *Abha guggulu* extract(b) *Kaishore guggulu* extract (c) & Std. Gallic acid (d).

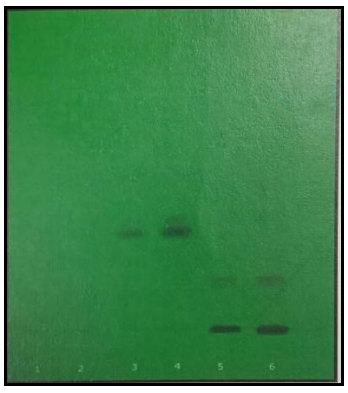
Table 5: Gallic acid content by HPTLC.

Track	Start Rf	Max. Rf	Area	Gallic acid conc. (mcg/ml)
Abha guggulu	0.42	0.44	3127.1	0.938
Kaishore guggulu	0.40	0.45	2800.5	0.838
Abha capsule	0.41	0.43	482.2	0.075

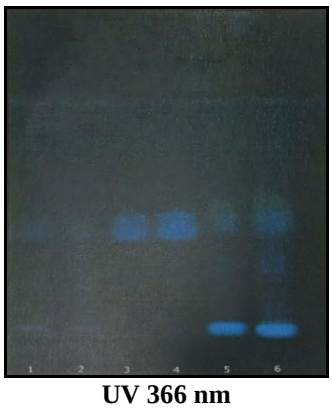
Piperine Analysis

Piperine content in *Piper longum, Piper nigrum extract* and Abha capsule extract by HPTLC.

Silica gel TLC plate used as stationary phase and Toluene: Ethyl acetate (93:7) as mobile phase had given good separation of piperine at Rf = 0.35.

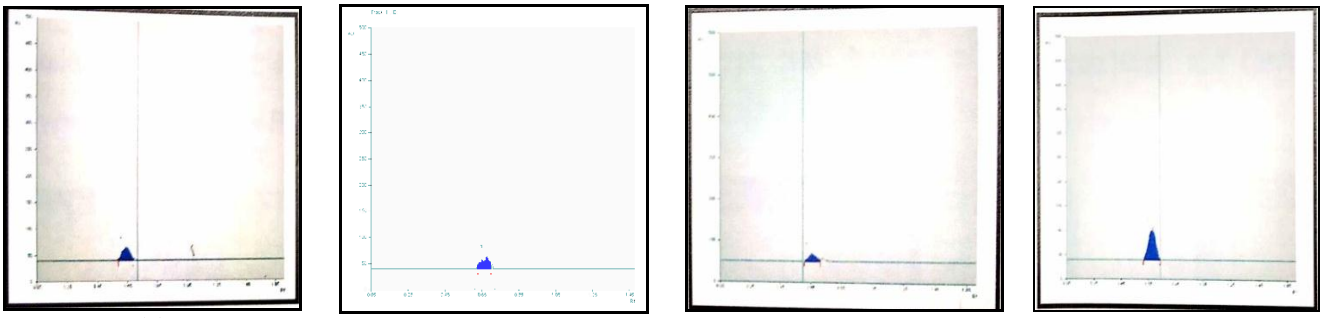


UV 254 nm



UV 366 nm

Fig. 1: HPTLC of Abha capsule extract, *Piper longum (Pippali), Piper nigrum (Marich)* extract & Std. Piperine. Track-1: 2 µg/ml of standard Piperine. Track-2: 4 µg/ml of standard Piperine .Track-3: 2 µg/ml Methanol extract of *Abha* capsule.Track-4: 4 µg/ml Methanol extract of *Abha* capsule Track-5: 4 µg/ml of Methanol extract of *Kaishore guggulu*. Track-6: 4µg/ml of Methanol extract of *Abha guggulu*



(a) (b) (c) (d)

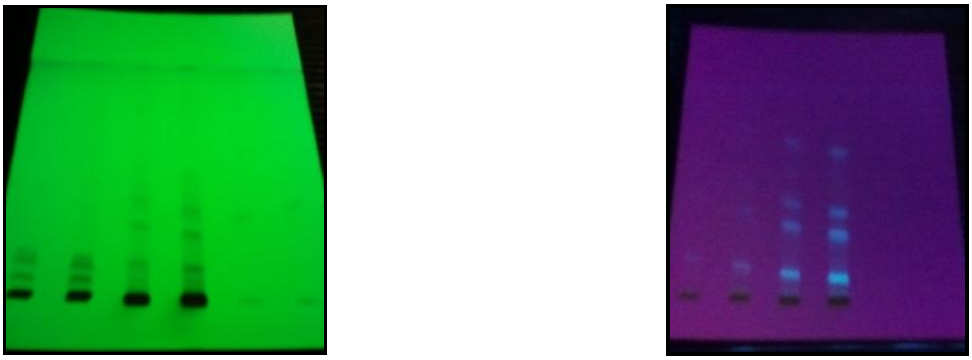
Fig. 2: Chromatogram of Abha capsule extract (a), *Abha guggulu* extract (b), *Kaishore guggulu* extract (c) & Std. Piperine (d).

Table 6: Piperine content by HPTLC.

Track	Start Rf	Max. Rf	Area	Piperine conc. (mcg/ml)
Abha guggulu extract	0.34	0.37	556.8	0.172
Kaishore guggulu extract	0.33	0.36	431.2	0.081
Abha capsule extract	0.35	0.37	263.8	0.006

Gingerol Analysis

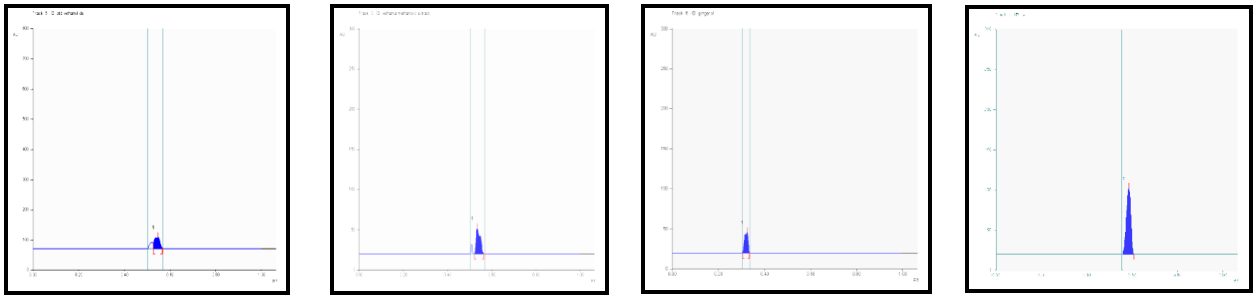
Gingerol content in *Abha guggulu*, *Kaishore guggulu* extract and Abha capsule extract by HPTLC. Silica gel TLC plate used as stationary phase and Toluene: Ethyl acetate (9:1) as mobile phase had given good separation of gingerol at Rf = 0.65.



UV 254 nm **UV 366 nm**

Fig. 1: HPTLC of Abha capsule, Zingiber officinale (Sunthi) extract & Std. Gingerol.

Track-1: 2 µg/ml of standard Gingerol. Track-2: 4 µg/ml of standard Gingerol. Track-3: 2 µg/ml Methanol extract of *Abha* guggulu. Track-4: 4 µg/ml Methanol extract of *Kaishore guggulu.* Track-5: 4 µg/ml of Methanol extract of *Abha* capsule. Track-6: 6 µg/ml of Methanol extract of *Abha* capsule.



(a) (b) (c) (d)

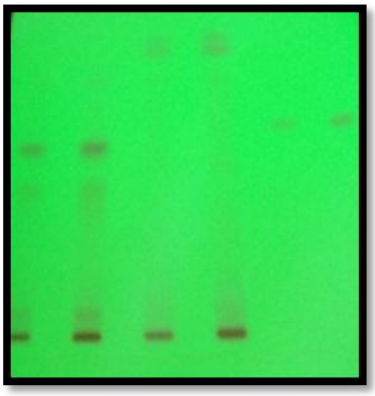
Fig. 2: Chromatogram of Abha capsule extract (a), *Abha guggulu* extract (b), *Kaishore guggulu* extract (c) & Std. Gingerol (d).

Table 7: Gingerol content by HPTLC.

Track	Start Rf	Max. Rf	Area	Gingerol conc. (mcg/ml)
Abha guggulu extract	0.57	0.62	482.1	1.15
Kaishore guggulu extract	0.63	0.65	365.2	0.87
Abha capsule extract	0.60	0.63	376.7	0.092

Withanolides Analysis

Withanolides content in *Lakshadi guggulu* extract and Abha capsule by HPTLC. Silica gel TLC plate used as stationary phase and Toluene: Ethyl acetate: Formic acid (5:5:1) as mobile phase had given good separation of withanolide at Rf = 0.55.

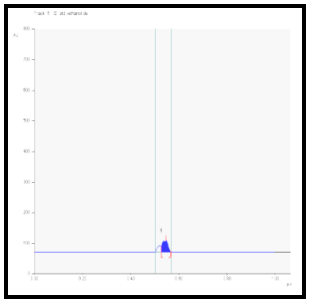


UV 254 nm

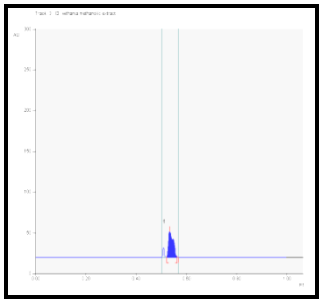
UV 366 nm

Fig. 1: HPTLC of Abha capsule extract, *Withania somnifera* extract & Std. Withanolide.

Track-1: 2 µg/ml of standard Withanolide. Track-2: 4 µg/ml of standard Withanolide. Track-3: 2 µg/ml Methanol extract of *Lakshadi guggulu.* Track-4: 2 µg/ml Methanol extract of *Lakshadi guggulu.* Track-5: 4 µg/ml of Methanol extract of Abha capsule. Track-6: 4 µg/ml of Methanol extract of Abha capsule.



(a)



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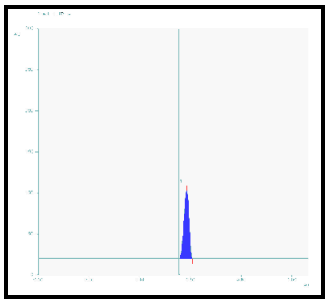

(c)

Fig: 2 Chromatogram of Abha capsule extract (a), *Lakshadi guggulu* extract(b) & Std. Withanolide (c)

Table 8: Withanolide content by HPTLC.

Track	Start Rf	Max. Rf	Area	Withanolide (mcg/ml)
Lakshadi guggulu extract	0.59	0.60	2205.1	1.398
Abha capsule extract	0.59	0.60	960.5	0.110

The quantification of various active constitutes like Gallic acid, Piperine, Gingerol, and Withanolides, as well as presence of *Guggulu* and *Hadjod* in Abha capsule were measured by superimposing overlaying the UV absorption spectrum of the sample with that from the reference standard using the CAMAG TLC scanner-3. Result showed that all the active constitute are present in respective extract and in Abha capsule. Quantitatively measured data is summarized in Table 3 to 8. Qualitative analysis show that *Hadjod* is one of the main ingredient of Abha capsule, analysis reveals four Rf i.e. 0.26, 0.41, 0.58, & 0.83 were seen both *Hadjod* extract of Abha capsule, which proves quality of Abha capsule. Guggulu is main ingredients of all three *guggulu* preparation i.e. *Abha guggulu*, *Kaishore guggulu*, *Lakshadi guggulu* study shows that HPTLC chromatogram of *Abha guggulu*, *Kaishore guggulu*, *Lakshadi guggulu* shows three similar peaks at Rf 0.21,0.43,0.54,0.74 which is also seen in Abha capsule which indicate identification of *guggulu* in final product Abha capsule.

Triphala combination of three herbs combination i.e. *Amla- Baheda- Haritaki* contains gallic acid as a standard constituent. *Abha guggulu* and *Kaishore*

guggulu contains *Triphala* so, standardization of these ingredients with gallic acid reveals *Abha guggulu, Kaishore guggulu* and Abha capsule having 0.938, 0.838and 0.075 mcg/ml respectively. *Piper longum* and *Piper nigrum* contains piperine the active moiety, result of analytical study of Piperine in *Abha guggulu, Kaishore guggulu* and Abha capsule shows presence of 0.172, 0.081and 0.006 mcg/ml respectively. *Zingiber officinale* contain gingerol known for its anti-inflammatory and analgesic activity HPTLC analysis shows presence of gingerol in *Abha guggulu, Kaishore guggulu* and Abha capsule at 1.15, 0.87 and 0.092mcg/ml concentration. Withanolide the active constituent of *Ashwagandha* and it is one of the ingredients of *Lakshadi guggulu.* HPTLC study reveals that methanolic extract of *Lakshadi guggulu* and Abha capsule contains 1.398 mcg/ml and 0.110mcg/ml respectively.

CONCLUSION

Fingerprinting of herbal medicines is utilized for the authenticity and quality control of herbal medicines and herbal preparations. Chemical fingerprints obtained by chromatographic combination of qualitative fingerprinting and quantities multi-component analysis are a novel and rational method to address the key issues of quality control of herbal medicines. From the present investigation it can be concluded that the study of phytochemical analysis can be used as a first line for quality control study at industry level for raw material. As per the label claims of the Abha capsule formulation HPTLC study confirmed, the presence of gallic acid, piperine, gingerol, and withanolides, also similarity in Rf of *Hadjod* and *Guggulu* fingerprinting with the plant materials and Abha capsule. The presence of active constitutes of Abha capsule might be responsible for various pharmacological action in bone health, disease like osteoporosis as well as in bone fracture. Abha capsule contains well known Ayurvedic ingredients which are described in classical text for bone health and related disorders.

The results obtained from this study could be used for routine monitoring of raw materials, formulations and the finished product which can lead to batch to batch consistency of Ayurvedic polyherbal formulation. The quality of formulation proves by presence *Hadjod*, *guggulu* and standards like Gallic acid, Piperine, Gingerol and Withanolide in Abha capsule.

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