



Standardisation of *Darvyadi Loha* by Powder Microscopy and High Performance Thin Layer Chromatography

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ABSTRACT

Background: On an average 50% of the anemia worldwide is assumed to be due to iron deficiency. Globally Iron Deficiency Anemia (IDA) ranks number 9 among 26 risk factors. IDA is considered to be one of most prevalent form of malnutrition¹.

*Darvyadi Loha*² is considered as a safe and effective formulation for the management of *Pandu Roga* (Anemia). *Darvyadi Loha* is a herbo-mineral preparation which consists of *Darvi*, *Haritaki*, *Vibhitaki*, *Amalaki*, *Shunti*, *Maricha*, *Pippali*, *Vidanga* and *Loha Bhasma* in equal proportion. Since this medicine is administered even to children for *Pandu Roga*, the powder microscopy and HPTLC study is essential to authenticate this particular drug.

Aims and Objectives: To standardise and evaluate the different constituents present in *Darvyadi Loha* by HPTLC and powder microscopy.

Methodology: HPTLC: One gram of *Darvyadi Loha* Choorna content was dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 4 µl, 8 µl and 12µl of the above samples of were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. Rf, colour of the spots and densitometric scan were recorded.

Powder microscopy: Pinch of *Darvyadi Loha* Choorna previously sieved is put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Result: Under Short UV, 3 peaks were observed for *Darvyadi Loha*. 6 Peaks were observed under long UV for *Darvyadi Loha*. Under post derivatisation, 6 peaks were observed for *Darvyadi Loha*.

The Powder microscopy of the given sample of *Darvyadi Loha* was assessed.

Conclusion: The results of powder microscopy can be considered for standardisation of *Darvyadi Loha*. The peaks observed in HPTLC can be considered for fingerprint profile analysis.

KEYWORDS: *Darvyadi Loha*, Powder microscopy, HPTLC.

INTRODUCTION

On an average 50% of the anemia worldwide is assumed to be due to iron deficiency. Globally Iron Deficiency Anemia (IDA) ranks number 9 among 26 risk factors. IDA is considered to be one of most prevalent form of malnutrition¹. It is estimated that iron deficiency affects about 30% of the world population and about 70-90% in the developing countries, including India.²

*Darvyadi Loha*³ is considered as a safe and effective formulation for the management of *Pandu Roga* (Anemia). *Darvyadi Loha* is a herbo-mineral preparation which consists of *Darvi*, *Haritaki*, *Vibhitaki*, *Amalaki*, *Shunti*, *Maricha*, *Pippali*, *Vidanga* and *Loha Bhasma* in equal proportion. Since this medicine can be administered even to children in the management of *Pandu Roga*, the powder microscopy and HPTLC study is essential to authenticate this particular drug.

OBJECTIVES

To standardise and evaluate different constituents present in *Darvyadi Loha* by HPTLC and Powder microscopy.

MATERIALS AND METHODS

Materials required:

HPTLC: Water bath, pipette, pre-coated silica gel plate, Linomat 5 TLC applicator, vanillin-sulphuric acid spray, alcohol, toluene, ethyl acetate, etc.

Powder microscopy: glycerine, Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera.

PROCEDURE

HPTLC⁴: One gram of *Darvyadi Loha Choorna* content was dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 4 µl, 8 µl and 12µl of the above samples of were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. Rf, colour of the spots and densitometric scan were recorded.

Powder microscopy⁵: Pinch of *Darvyadi Loha Choorna* previously sieved is put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

RESULTS

HPTLC: The Rf value of sample of *Darvyadi Loha* obtained after HPTLC is depicted in Table 1. The HPTLC Photo documentation of sample of *Darvyadi Loha Choorna* is depicted in Fig 1 The densitometric scan report of *Darvyadi Loha* at UV 254nm and UV 366 nm is depicted in Fig 2a and Fig 2b respectively.

Powder microscopy: Result of Powder microscopy is given in Figure 3.

DISCUSSION

The given sample of *Darvyadi Loha Choorna* has been standardized as per standard testing protocol. The results of standardization parameters are represented in table 1. HPTLC results, Rf values, densitograms are represented in respective tables and figures.

On analysing the HPTLC reports, it was observed that at 254nm(Fig.2a), three peaks were observed at Rf value of 0.12(Dark green) and 0.24 (Dark green) and 0.30 (Dark green) for *Darvyadi Loha* .At 366nm(Fig.2b) , six peaks were observed at Rf value 0.12(Fluorescent blue), 0.19 (Flourescent yellow),

0.28(Fluorescent blue), 0.46 (Fluorescent aqua blue), 0.73 (Fluorescent blue), 0.86(Fluorescent blue) .At 620nm, six peaks were observed at Rf value of 0.12 (Purple) ,0.24(Purple) ,0.32(Purple) ,0.48(Purple) ,0.73 (Purple) and 0.93 (Purple) for *Darvyadi Loha*.

Powder microscopy: The observations recorded from powder microscopic studies are shown in figure 3. The powdered drug observed under microscope showed Parenchymal cells in surface, Parenchymal cells with starch, group of sclereids, brown matter, starch grains and vessels.

CONCLUSION

Genuineness of the drug needs to be standardised using approved parameters for every formulation. The result of Powder microscopy can be considered for standardisation of *Darvyadi Loha*. The peaks observed in HPTLC can be considered for fingerprint profile analysis in order to get standard markers of the sample drug. To get a clear picture of the composition, there should be standard markers and test should be conducted with different solvent system which is out of the limit of the study. It is difficult to compare and interpret the results and it requires much detailed study with trial and error method.

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5. D.R.Lohar, Protocol for testing, 1st Ed. Ghaziabad: Pharmacopeial Laboratory for Indian Medicines, Government of India, Ministry of Health and Family Welfare, 42: 200.

TABLE

1. Table 1: Rf values of sample of *Darvyadi Loha capsule*

Short UV	Long UV	Post derivatisation
0.12 (D. green)	0.12 (F. blue)	0.12 (Purple)
-	0.19 (F. yellow)	-
0.24 (D. green)	-	0.24 (Purple)
-	0.28 (F. blue)	-
0.30 (D. green)	-	-
-	-	0.32 (Purple)
-	0.46 (F. aqua blue)	-
-	-	0.48 (Purple)
-	0.73 (F. blue)	0.73 (Purple)
-	0.86 (F. blue)	-
-	-	0.93 (Purple)

FIGURES

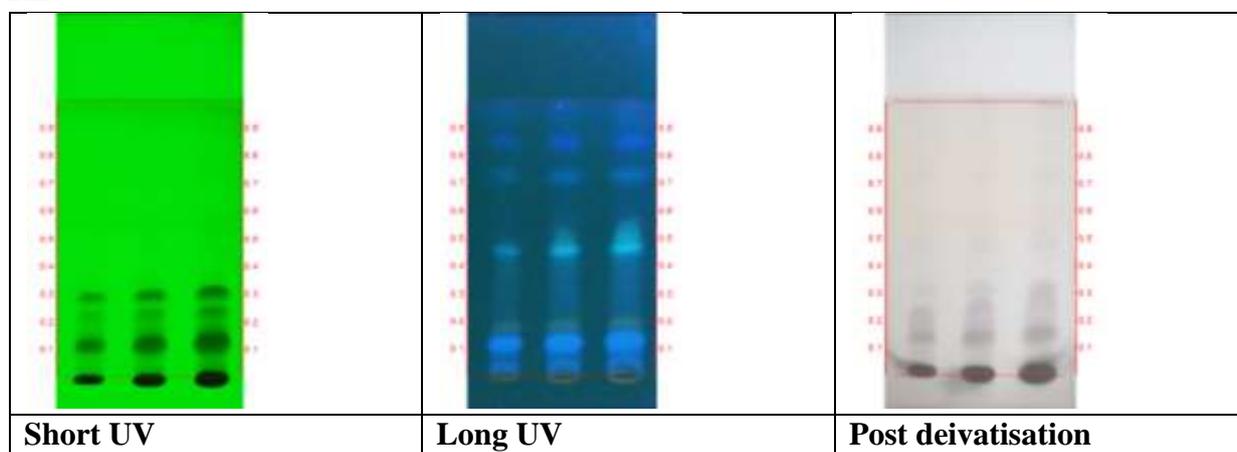


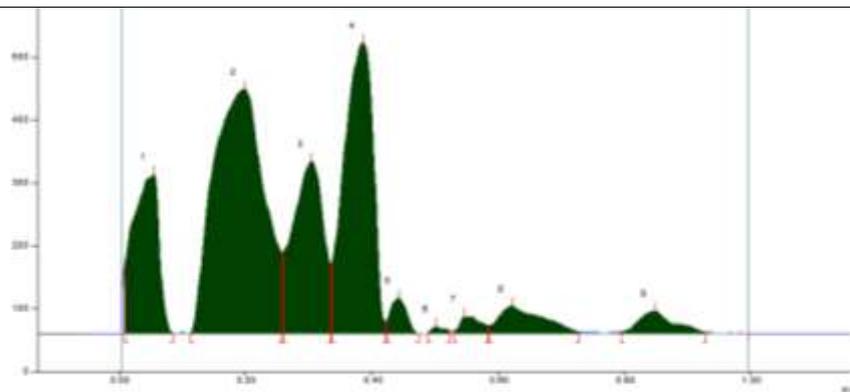
Figure 1: HPTLC Photo documentation of sample of *Darvyadi loha capsule*

Track 1: *Darvyadi loha capsule* - 4µl

Track 2: *Darvyadi loha capsule* - 8µl

Track 3: *Darvyadi loha capsule* - 12µl

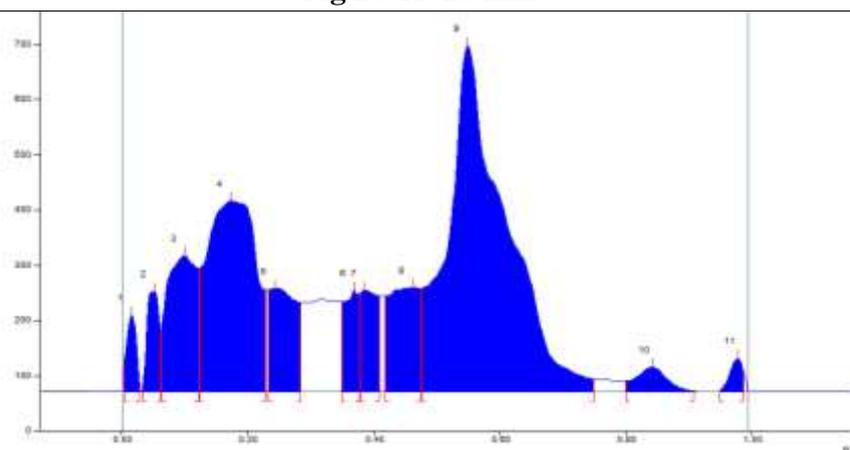
Solvent system: Toluene: Ethyl acetate (9:1)



Track 3, ID: Darvyadi loha capsule

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	110.0 AU	0.05 Rf	252.6 AU	16.31 %	0.09 Rf	0.2 AU	7633.2 AU	12.64 %
2	0.11 Rf	0.6 AU	0.20 Rf	388.0 AU	25.05 %	0.26 Rf	29.0 AU	22586.8 AU	37.40 %
3	0.26 Rf	129.4 AU	0.30 Rf	273.2 AU	17.65 %	0.34 Rf	09.6 AU	9556.5 AU	15.82 %
4	0.34 Rf	110.9 AU	0.39 Rf	461.6 AU	29.81 %	0.42 Rf	18.4 AU	15254.3 AU	25.26 %
5	0.42 Rf	19.9 AU	0.44 Rf	56.2 AU	3.63 %	0.48 Rf	0.1 AU	1024.9 AU	1.70 %
6	0.49 Rf	0.4 AU	0.50 Rf	11.0 AU	0.71 %	0.53 Rf	3.7 AU	158.1 AU	0.26 %
7	0.53 Rf	4.7 AU	0.55 Rf	26.8 AU	1.73 %	0.59 Rf	12.3 AU	635.1 AU	1.05 %
8	0.59 Rf	12.5 AU	0.62 Rf	43.6 AU	2.81 %	0.73 Rf	2.7 AU	2132.2 AU	3.53 %
9	0.80 Rf	2.8 AU	0.85 Rf	35.5 AU	2.29 %	0.93 Rf	1.8 AU	1407.7 AU	2.33 %

Fig 2a. At 254nm



Track 3, ID: Darvyadi loha capsule

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	55.3 AU	0.02 Rf	138.3 AU	5.80 %	0.03 Rf	8.9 AU	1485.8 AU	1.58 %
2	0.03 Rf	5.4 AU	0.05 Rf	181.0 AU	7.59 %	0.06 Rf	09.2 AU	2551.0 AU	2.72 %
3	0.06 Rf	116.7 AU	0.10 Rf	245.8 AU	10.31 %	0.12 Rf	23.5 AU	8124.4 AU	8.65 %
4	0.12 Rf	223.6 AU	0.17 Rf	345.3 AU	14.47 %	0.23 Rf	84.7 AU	19269.3 AU	20.53 %
5	0.23 Rf	184.7 AU	0.24 Rf	187.0 AU	7.84 %	0.28 Rf	61.6 AU	5675.3 AU	6.05 %
6	0.35 Rf	162.8 AU	0.37 Rf	183.3 AU	7.68 %	0.38 Rf	77.3 AU	3076.7 AU	3.28 %
7	0.38 Rf	178.7 AU	0.39 Rf	184.3 AU	7.72 %	0.41 Rf	73.1 AU	3584.4 AU	3.82 %
8	0.42 Rf	173.3 AU	0.46 Rf	188.3 AU	7.89 %	0.48 Rf	86.8 AU	6615.1 AU	7.05 %
9	0.48 Rf	187.1 AU	0.55 Rf	627.0 AU	26.28 %	0.75 Rf	22.5 AU	41057.4 AU	43.74 %
10	0.80 Rf	19.3 AU	0.84 Rf	44.9 AU	1.88 %	0.91 Rf	0.9 AU	1571.7 AU	1.67 %
11	0.95 Rf	0.8 AU	0.98 Rf	60.3 AU	2.53 %	0.99 Rf	36.9 AU	858.1 AU	0.91 %

Fig 2b. At 366nm

Figure 2: Densitometric scan of the sample of *Darvyadi Loha*

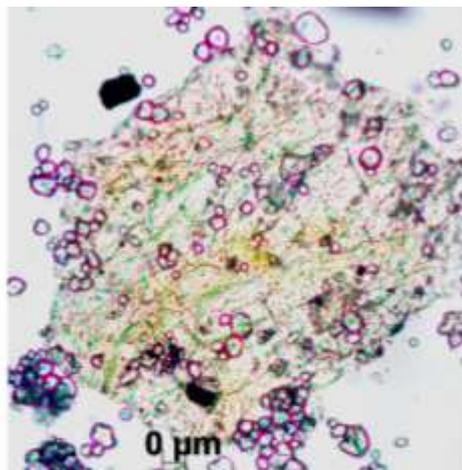


Fig 3.1 Parenchyma cells in surface

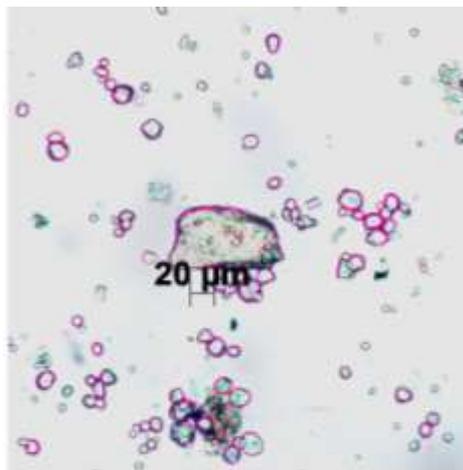


Fig 3.2 Parenchyma cells with

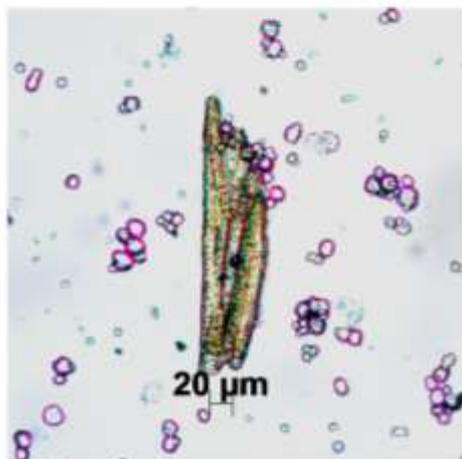


Fig 3.3 Sclereids



Fig 3.4 Group of sclereids

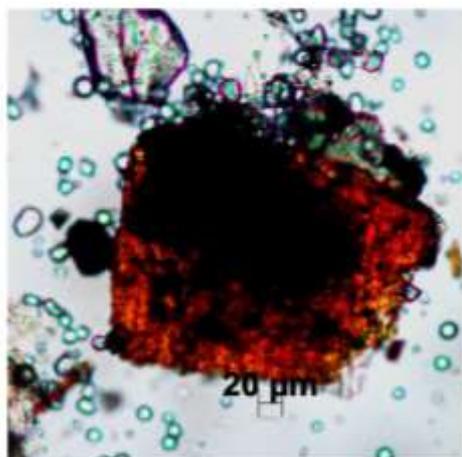


Fig 3.5 Brown matter

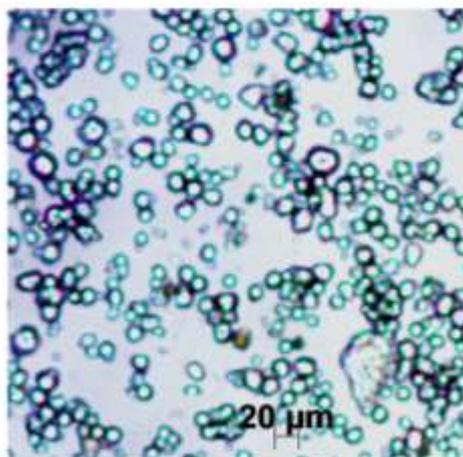


Fig 3.6 Starch grains

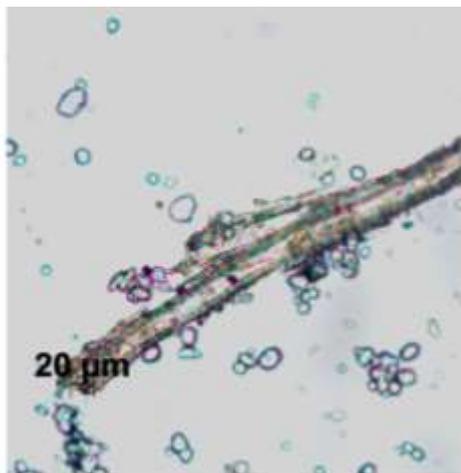


Fig 3.7 Vessels

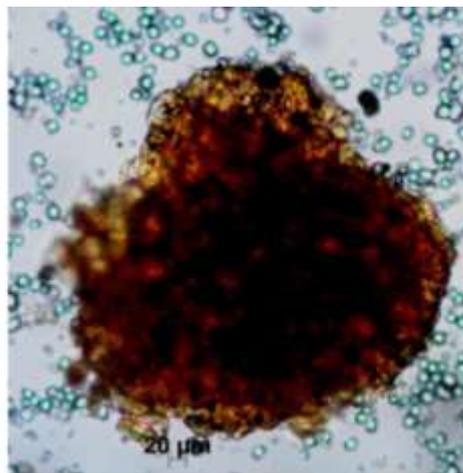


Fig 3.8 Parenchyma cells

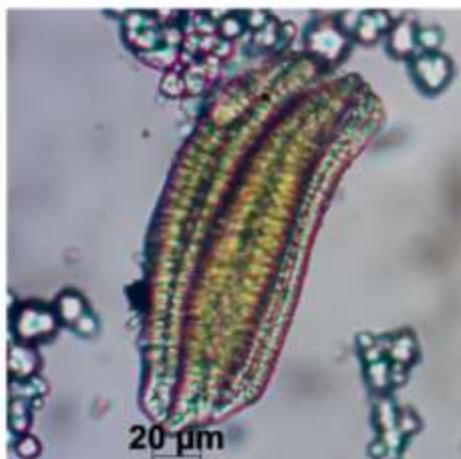


Fig 3.9 Sclereids



Fig 3.10 Parenchyma cells with thick

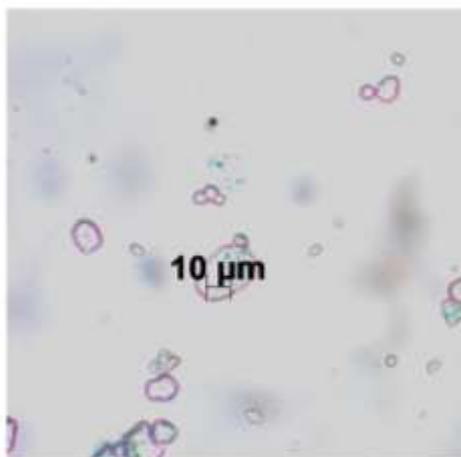


Fig 3.11 Starch grain

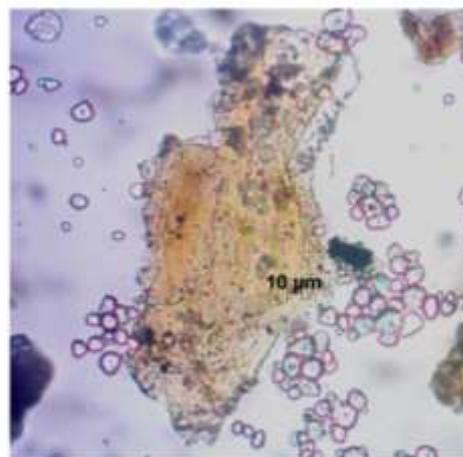


Fig 3.12 Parenchyma and starch

Figure 3: Powder microscopy of Darvyadi Loha