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DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF GALLIC ACID IN CHITRAK HARITAKI– AN AYURVEDIC FORMULATION

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Gallic acid in Ayurvedic formulations of Chitrak Haritaki of different manufactures. The alcoholic extract of Chitrak Haritaki, Haritaki fruit and Amalaki fruit samples were applied on TLC Aluminium plate pre coated with Silicagel60 GF254 and developed using Toluene : Ethyl acetate: Formic acid (5:4:1) v/v as a mobile phase. The plate was sprayed (derivatized) with 0.1% Ferric Chloride solution and detection and quantification were carried out densitometrically using anUV detector at wavelength of 254 nm. Content of marker compound in the samples were found similar.

Keywords: Chitrak Haritaki, Haritaki fruit, Amalaki fruit, Gallic acid, Standardisation, HPTLC.

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INTRODUCTION

Chitrak haritaki is a very famous Ayurvedic medicine used in treating chronic respiratory conditions. It is in herbal jam form. It is also known as Chitrak haritaki Avaleha, Chitraka Haritaki etc.Avaleha suggests that it is a herbal jam. Chitraka and haritaki are two herbs, which are the main ingredients of this product.

Chitrak Haritaki Uses:

It is used in the treatment of chronic respiratory conditions, Asthma, bronchitis, rhinitis and tuberculosis. It is also used to improve digestion power and to treat bloating and intestinal worm.

Chitrak Haritaki Dose:

3 – 6 grams once or two times a day after food with milk. This medicine is quite hot in nature. Hence it is advised to be taken along with milk, which is a coolant and has a calming effect over stomach.

Chitrak Haritaki Ingredients: A 4.8 liters of decoction is prepared with each of - Chitraka – Plumbago zeylanica, Amalaki-Embellica officinallis, Guduchi – Tinospora cordifoliasand Dashamoola. It is added with 4.8 kg of jaggery and 3.072 kg of Haritaki – Terminalia chebula.This mixture is heated till semi solid consistency.It is added with **Trikatu** – pepper, long pepper and ginger – 96 g each, Cinnamon – 96 g, Tejpatra – Cinnamomum tamala – 96 g, Yavakshara – 24 gand 384 grams of honey.

Plumbago zeylanica Linn Syn. *Plumbago rosea* Linn (Family-Plumbaginaceae) known vernacularly as Chitrak, Chitra, Chitraka, Chitrakmul, Agni, Pathi, Ushana, Chita, Chitramulam, Ceylong Leadwort or white Leadwort is one of the main ingredient of this formulations and is found wild in the tropics, subtropics and throughout India including West Bengal, Bihar and peninsular India. The roots have a short fracture an acrid and biting taste and disagreeable odour.The root and root bark are bitter, stomachic carminative, astringent to bowels, anthelmintic, piles bronchitis, itching, diseases of lever, consumption and ascetics. It acts as a powerful sudorific. Leaves are caustic, versicant aphrodisiac and good for scabies. Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanolone. Elliptinone, isozeylinone, catechol tannin, isoshinanolone, dihydrosterone and β- sitosterol also islated from plant.. Plumbagin shows as anticancer and antitumor activity. Aspartic acid, tryptophan, tyrosine, threonine, alanine, histidine, glycine, methionine, hydroxyproline, were isolated from the aerial parts. Lupeol and lupenyl acetate have been isolated from the root.

Literature survey reveals that the TLC, HPLC and HPTLC methods are reported but no method as yet is reported for the determination of Gallic acid in Haritaki fruit and Amalaki fruit. A simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of Gallic acid in in Haritaki fruit and Amalaki fruit and its compound formulations (1-8).

EXPERIMENTAL

Material and Method:

1)The Chitrak Haritaki of three different manufactures was procured from the Local Market Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded for further study.

(i) CH1DB (ii) CH2BY (iii) CH3ZB

2)The Haritaki fruit and Amalaki fruit were procured from the Local Market, Ghaziabad and also identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded as SD1 and SD2 respectively for study.

H.P.T.L.C. (High Performance Thin Layer Chromatography):

Equipment

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm^2) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

Chemical & Reagents

Analytical grade; Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Alcohol, Ferric chloride and n-Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (20x10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard Gallic acid procured from Sigma S L 0777, B. No. #037K0068.

Sample & Standard preparation

Sample preparation: 1g of coarsely powdered crude drugs and Citrak Haritaki samples were extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 10 ml in a volumetric flask and used for H.P.T.L.C.

Standard Preparation: 5mg of standard Gallic acid dissolved in 5ml of absolute alcohol and made up to 5ml in standard volumetric flask.

Chromatography:

Procedure

TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethyl acetate: Formic acid (5:4:1) v/v as mobile phase. absolute alcohol extract of samples and Gallic acid standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20x10 cm^2) with SS lid was used for development of TLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with 0.1Ferric Chloride solution and HPTLC finger print profile was snapped by Cammag Reprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization (Fig. 1). The plate was scanned before derivatization using Camag TLC Scanner III at wavelength 270nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Table No-1 HPTLC details of alcoholic extract of Citrak Haritaki

Sr. No.	Detection/ Visualization	Citrak Haritaki (Track T1, T2 & T3)		Standard- Gallic acid (Track S1, S2 & S3)		Haritaki Fruit (Track SD1)		Amalaki Fruit (Track SD2)	
		R_f. values	Colour of band	R_f. Values	Colour of band	R_f. Values	Colour of band	R_f. Values	Colour of band
1.	Under UV 254 nm	0.42 0.50 0.63 0.68 0.80	dark grey grey grey grey grey	0.42	dark grey	0.31 0.42 0.50 0.58 0.63	grey dark grey grey grey grey	0.10 0.42 0.58 0.63 0.68 0.97	grey Dark grey grey grey grey
2.	Under UV 366 nm	0.42 0.58 0.63 0.68 0.84 0.89 0.95	dark grey sky blue sky blue green green red red	0.42	dark grey	0.10 0.42 0.84 0.95	sky blue dark grey green sky blue	0.10 0.42 0.58 0.84 0.89 0.97	sky blue dark grey sky blue green sky blue sky blue
3.	After derivatization	0.42 0.50	dark blackish grey dark blackish grey	0.42	dark blackishgrey	0.38 0.42 0.58 0.66	dark blackish grey dark blackish grey blackish grey blackish grey	0.42 0.50 0.58 0.66	dark blackish grey dark blackish grey blackish grey blackish grey

Linearity of Detector Response and Assay:

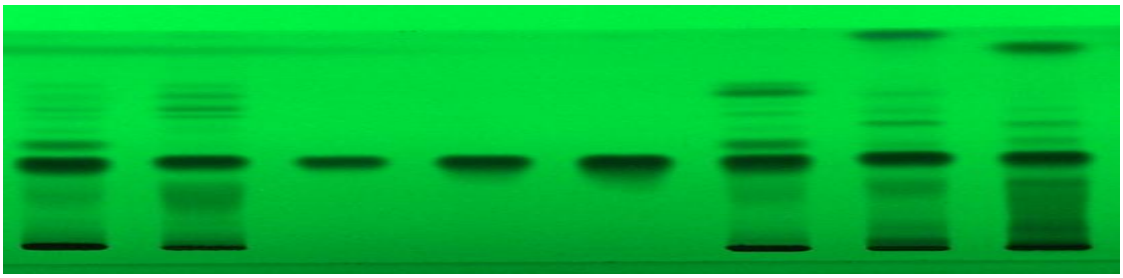
In order to establish linearity, standard solution of Gallic acid (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20X10 cm^2; 0.2 mm thick), 2µl, 4µl, 6µl on Track No. S1, S2 & S3 respectively and for assay, 9µl of alcoholic extract of samples applied on Track No. T1, T2 & T3 and 3µl on Track No. SD1 and SD2 on the same plate. TLC plate was developed to 8 cm distance above the position of the sample application and removed from the chamber and air dried at room temperature. This HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (Fig-1). The plate was scanned immediately before derivatization using Camag TLC Scanner III at wavelength 270nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that

Gallic acid appeared at R_f. 0.42 (dark grey colour). The peaks, graph and spectra obtained were given in Fig-2 and 3 and R_f. values, colour of bands (Table No-1), quantity of Gallic acid, linearity, standard deviation & regression coefficient found via graph (Table No-2) and calculated quantity of Gallic acid were given in Table No-3.

Table No- 2 Quantity applied on plate and values found via graph

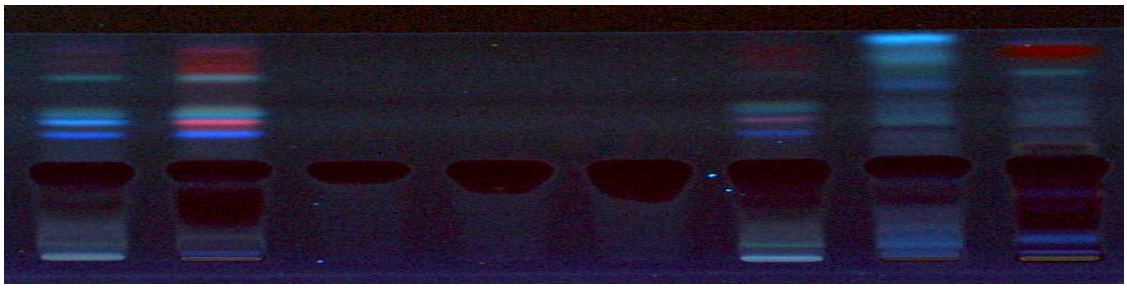
S. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of Gallic acid via graph	Linearity & Regression Coefficient and Standard deviation via graph
1.	T1	9µl	900µg	3.807µg	Y = 16281.461 + 6951.001 * X r = 0.99935 sdv = 1.60%
2.	T2	9µl	900µg	4.364µg	
3.	S1	2µl	2µg	2.000µg	
4.	S2	4µl	4µg	4.000µg	
5.	S3	6µl	6µg	6.000µg	
6.	T3	9µl	900µg	3.972µg	
7.	SD1	3µl	300µg	5.417µg	
8.	SD2	3µl	300µg	5.812µg	

T1 - Alcoholic extract of CH1DB
T2 - Alcoholic extract of CH2BY
S1 - Gallic acid Std. alcoholic solution (1mg/ml)
S2 - Gallic acid Std. alcoholic solution (1mg/ml)
S3 - Gallic acid Std. alcoholic solution (1mg/ml)
T3 - Alcoholic extract of CH3ZB
SD1 - Alcoholic extract of Haritaki Fruit
SD2 - Alcoholic extract of Amalaki Fruit



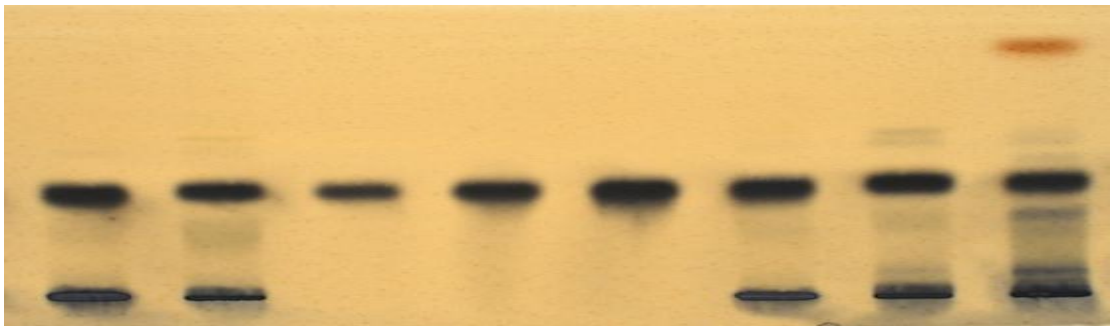
T1 T2 S1 S2 S3 T3 SD1 SD2

UV-254 nm



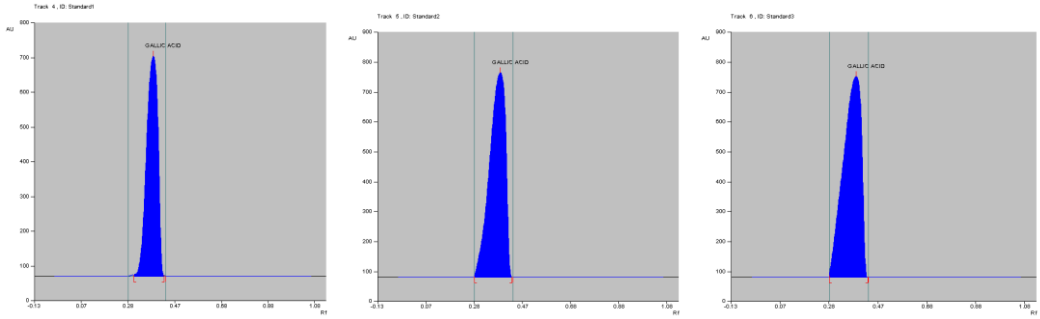
T1 T2 S1 S2 S3 T3 SD1 SD2

UV-366 nm



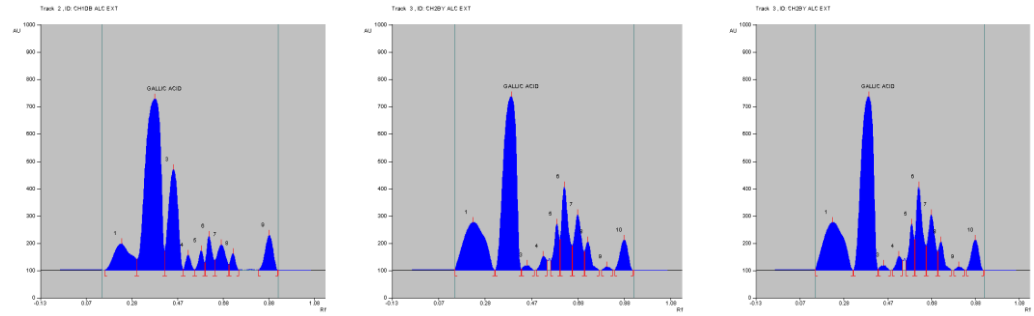
After derivatization

Fig- 1: H.P.T.L.C. Finger print of Citrak Haritaki

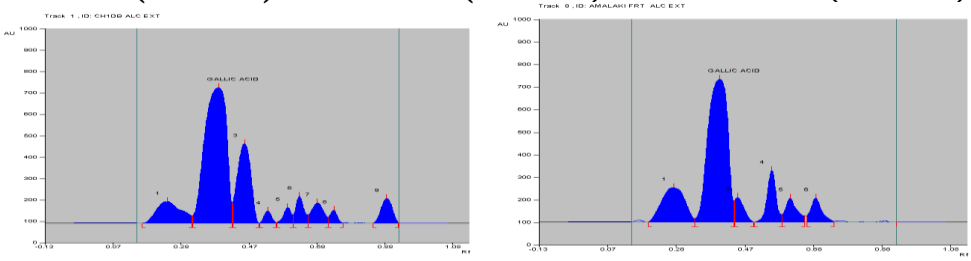


(Track S1) **(Track S2)** **(Track S3)**

Peaks of Gallic acid @ 254nm



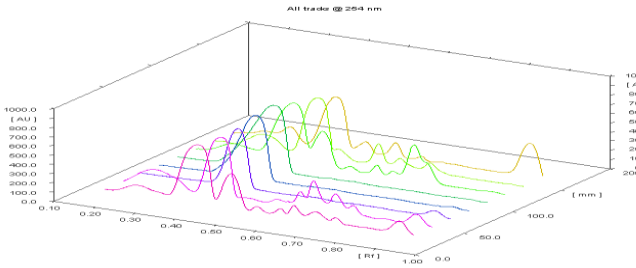
(Track T1) **(Track T2)** **(Track T3)**



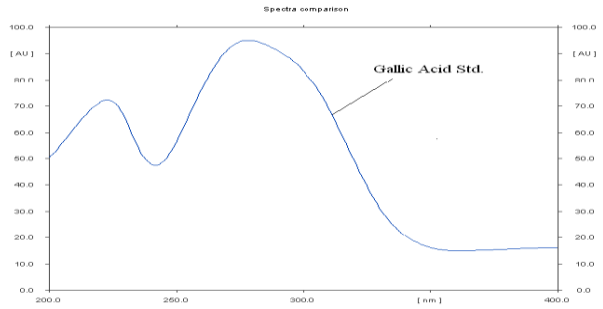
(Track SD1) **(Track SD2)**

Peaks of Citrak Haritaki, Haritaki and Amalaki Fruit @ 270nm

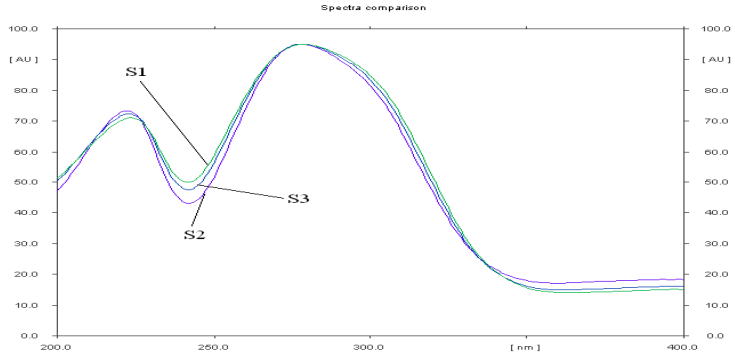
Fig-2: Peaks of Citrak Haritaki in all Tracks



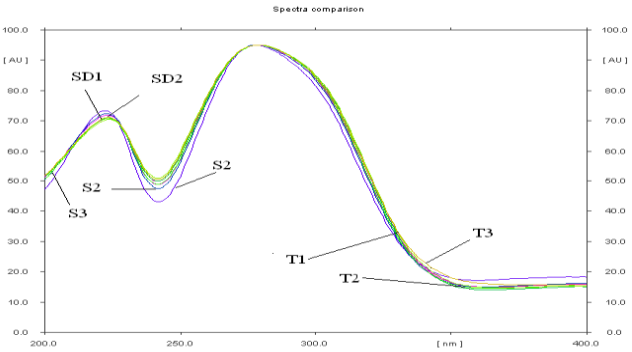
3D representation of Gallic acid



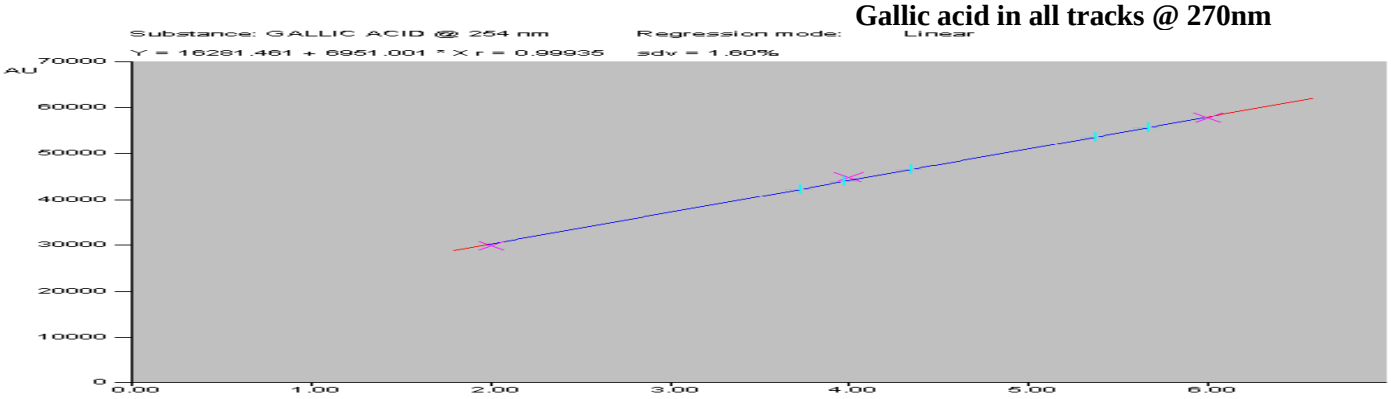
Spectra of Gallic acid @ 254nm



Spectra of Gallic acid in all Std.@ 270nm



Super Imposable UV Spectra of Gallic acid in all tracks @ 270nm



Conc. in µg

Graph Area vs AU

Fig-3: 3D representation, Spectrra and Graph of Citrak Haritaki

Table No. 3: Summary of results

Sr. No. ↓	**Sample from →**	CH1DB	CH2BY	CH3ZB	**Haritaki Fruit**	**Amalaki Fruit**
1.	**Quantity of Gallic acid in 1g**	4.230mg	4.849mg	4.413mg	18.057mg	19.373mg
2.	**% Gallic acid**	0.4230%w/w	0.4849%w/w	0.4413%w/w	1.8057%w/w	1.9373%w/w

RESULT AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate: Formic acid (5:4:1) v/v and the active principle Gallic acid resolved as a dark grey colour band at R_f. 0.42 very efficiently from the other components in alcoholic extract of samples (Fig. 1). Sharp peaks of Gallic acid (Standard and samples) were obtained when the plate was scanned at wavelength 254nm (Fig. 2). Quantity of Gallic acid found in samples were obtained automatically (Table No. 2) via graph (Fig.3) and % Gallic acid found in samples was calculated (Table No.3). Quantity of Gallic acid found in CH1DB is 4.230mg in 1g drug sample (0.4230% w/w), in CH2BY is 4.849 mg in 1g drug sample (0.4849%w/w), in CH3ZB is 4.413 mg in 1g drug sample (0.4413%w/w) and Quantity of Gallic acid found in Haritaki fruit is 18.057 mg in 1g drug sample (1.8057%w/w) and in Amalaki fruit is 19.373mg in 1g drug sample (1.9373%w/w).

The robustness of the method was studied, during method development, by determining the effect of small variation, of mobile phase composition (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of R_f. or response to Gallic acid was observed, indicating the robustness of the method.

CONCLUSION:

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Citrak haritaki* powder and quantitative determination of Gallic acid in fruit powder of Haritaki and Amalaki.

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