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PHARMACEUTICAL AND HPTLC ANALYSIS OF *YASHTIMADHU GHRITA* – AN AYURVEDIC MEDICATED GHEE

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ABSTRACT

Snehakalpana (preparations with fats), a commonly used Ayurvedic dosage form, allows active ingredients to be absorbed into fatty media. To address the lack of data for fat-based formulations, *Yashtimadhu Ghrita* (YG) was formulated and analyzed using HPTLC to estimate glycyrrhizin content. Various tests were conducted on raw drugs and prepared ghee. The pH, refractive index, specific gravity, acid value, saponification value, and iodine value of YG were determined as 6-7, 1.457, 0.912, 1.792, 145.66, and 41.467 respectively. Phytochemical analysis showed the presence of carbohydrates, cardiac glycosides, saponins, and tannins in chloroform and hexane extracts of YG. After developing an HPTLC quantitative analysis method using the chloroform extract after trial and errors, glycyrrhizin was estimated at 1.506 mg in 50 mg of YG's chloroform extract, accounting for over 50% incorporation from the drug (514.2 mcg in 10 mg of GG's alcoholic extract) into the media. The R_f value of the medicated ghee obtained at 254 nm with glycyrrhizin as the standard was 0.331 ± 0.021. The coefficient of variation was 41.07%. The study acknowledges the importance of HPTLC as a precise analytical tool for determining the active constituents of drugs. The formulation and analysis of YG provide valuable insights into the quantitative estimation of glycyrrhizin in a fat-based formulation, contributing to the knowledge base for such Ayurvedic formulations.

Keywords: Chromatography, *glycyrrhizin,* quantification, *Yashtimadhu Ghrita*

INTRODUCTION

Snehakalpana, a unique method in Ayurveda, involves preparing medications with fatty media such as ghee or oil. It utilizes a 1: 4: 16 ratio of *kalka* (paste), *sneha* (fat), and *kashaya* (decoction) to incorporate the active pharmaceutical ingredients of the herb **[1]**. The property of *Ghrita* (ghee) to assimilate the properties of processed drugs (*samskarasyanuvartana*) enhances the therapeutic benefits of medicines prepared with ghee as the base **[2]**. This method of extracting active ingredients into the fat media through boiling is a novel drug delivery system in Ayurvedic pharmaceutics.

Glycyrrhiza glabra Linn., commonly known as *Yashitmadhu* (YM), is a readily available herb with multiple actions on various general and systemic ailments. It is extensively used in Indian systems of medicine for treating gastric ulcers, cough, UV-induced skin irritation, stomatitis, inflammatory nervous diseases, unhealed wounds, chronic hepatitis, dry eye disease, and more **[3]**. Additionally, Ayurveda attributes properties such as *keshya* (beneficial to hair), *kantya* (promoting throat health), *chakshushya* (promoting eye health), *rasayana* (improving health and longevity), and *medhya* (beneficial for intellect) to *Yashtimadhu* **[4]**. The root part of this herb is primarily used in Ayurvedic formulations and contains flavonoids, phenolic compound, volatile compounds, and potent saponins like glycyrrhizin and glycyrrhetinic acid which are considered to be the characteristic phytoconstituent of the drug **[5]**.

Chromatography, a crucial analytical technique in chemistry, is employed to separate and analyze constituents of a drug at different levels of concentration. High-Performance Thin-Layer Chromatography (HPTLC) is a type of chromatography known for its ability to achieve complex separations. It utilizes optimized adsorbents and uniform layers, resulting in highly efficient analysis. HPTLC also enables detection in the nanogram and picogram ranges using UV absorption and fluorimetric detection, respectively, in a short time **[6]**.

Yashtimadhu Ghrita (YG) is a ghee-based formulation processed with *Glycyrrhiza glabra* and cow's ghee. It is commonly used in the management of conditions such as fissure-in-ano, stomatitis, GERD, and dry eye disease **[7 – 10]**. Ayurvedic Pharmacopoeia of India holds standards for various other Ayurvedic formulations, yet there is a lack of basic physicochemical and chromatographic analytical data for important and widely used formulations like YG. Hence selecting and analyzing such formulations like YG

based on specific physicochemical parameters and conducting HPTLC quantitative analysis for glycyrrhizin estimation with respect to its concentration in the raw drug is crucial to contribute towards the analytical data study of Ayurvedic formulations and that is the main core of the study.

MATERIALS AND METHODS

I. Pharmaceutical part:

The preparation of YG took place at the Rasashastra and Bhaishajyakalpana laboratory of KAHER's Shri BMK Ayurveda Mahavidyalaya in Belagavi. The raw drugs for YG, including coarse and fine powder of the root of YM (*Yashtimadhu*), were obtained from the GMP certified KLE Ayurveda Pharmacy in Belagavi (voucher no. 08417), while the cow ghee (*Go Ghrita*) was sourced from the local market. The authenticity of the YM root and ghee was verified by the Drug Testing Laboratory of the same institution.

To prepare the *kashaya* for YM, one part of coarse powder (300 g) was boiled with eight times the amount of water (2400 ml) until it reduced to one-fourth (600 ml) in an open vessel. The temperature was maintained at 103 degrees Celsius during the desired reduction. The contents were then strained through an unbleached cloth to obtain the *kashaya*.

The *Ghrita* preparation followed the standard *Snehakalpana* ratio of 1:4:16 for *kalka* (paste), *sneha* (fat), and *drava* (liquid), respectively **[1]**. The *kalka* of YM was prepared by grinding the fine powder (37.5 g) with a small quantity of water until it formed a proper bolus. In a vessel, the cow ghee (150 g) was melted completely over heat, and the previously prepared YM *kashaya* was added and mixed thoroughly. The prepared *kalka* bolus was then added to the vessel and mixed well. The entire mixture, along with the ghee, was allowed to boil on low heat until the signs of complete processing of ghee (*sneha siddhi lakshanas*) were observed. The temperature during the *snehapaka* (boiling of fat) was maintained at 100°C, and regular temperature recordings were made. The *snehapaka* process spanned two days. Finally, the fire was extinguished, and the medicated ghee was strained through a clean dry cloth. It was then stored in an airtight container after self-cooling. The entire preparation of YM *kashaya* and YG was carried out using a standard LPG cylinder setup.

II. Analytical part:

The entire physico chemical analysis of the raw drugs and YG was carried out in Central Research Facility of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi. All the tests mentioned in table 01 for raw drugs and YG were done using the SOP mentioned in the Ayurvedic Pharmacopoeia of India (API) **[11]**.

Image 01: Raw ingredients for preparation of YG

Image 02: Prepared YG

Table 01: List of analytical tests performed for raw drugs and final product

Sl. No	Name of the test	YM	*Ghrita*	YG
1.	Organoleptic character test	+	+	+
2.	Loss on drying (moisture content)	+	+	+
3.	Total ash value	+	-	-
4.	Acid insoluble ash	+	-	-
5.	Water soluble ash	+	-	-
6.	Water soluble extractive value	+	-	-
7.	Alcohol soluble extractive value	+	-	-
8.	Microbial limit test	+	+	-
9.	Preliminary phytochemical analysis	+	-	+
10.	Refractive index	-	+	+
11.	Specific gravity	-	+	+
12.	Acid value	-	+	+
13.	Saponification value	-	+	+
14.	Iodine value	-	+	+
15.	pH	-	+	+
16.	TLC	+	-	+
17.	HPTLC	+	-	+

III. Method development for TLC:

The TLC development of YG was conducted through a trial and error method due to the absence of a standardized protocol. A thorough literature review of articles and research on TLC and HPTLC analysis of Ayurvedic ghee formulations was performed **[12, 13]**. Chloroform and hexane were selected as the solvents for YG extraction. The extracts were prepared by adding 5g of YG to separate conical flasks with 50 ml of each solvent, followed by orbital shaking at 30˚ C for nine hours. After filtration and evaporation, different combinations of toluene, ethyl acetate, and benzene were used as TLC media based on previous studies. Three trials were conducted using TLC plates coated with TLC silica gel 60 F254, with chloroform and hexane extracts loaded separately as mentioned in table 02. The plates were visualized under UV light at 254 nm and 366 nm. Trials showing visible separation of phytoconstituents at both wavelengths were subjected to quantitative analysis using HPTLC for glycyrrhizin estimation. HPLTC for raw drug YM was done as per the SOP **[14]**.

Table 2: Trial details of TLC for YG

Sl. No	Trial number	Mobile phase	Ratio of mobile phase
1.	Trial 1	Toluene: ethyl acetate	9: 1
2.	Trial 2	Toluene: ethyl acetate	7: 3
3.	Trial 3	Benzene: ethyl acetate	9: 1

IV. HPTLC Analysis:

The entire HPTLC analysis was carried out in Dr. Prabhakar Kore's Basic Science Research Centre, Belagavi. The specification regarding the materials and equipments used in the analysis are given in **Table 3**.

Table 3: Equipment and material specification for HPTLC

Instrument	CAMAG HPTLC system, consisting of Linomat 5 spotting device and TLC scanner 4 with vision CATS
Development mode	Ascending
Chamber type	CAMAG, twin trough chamber 20 × 20 cms
Absorbent	Merck, HPTLC Silica gel $60F_{254}$ (20cm X 10cm), thickness –8mm, no.of tracks-15, band length- 8mm
Solvent system	Butanol: deionised water: glacial acetic acid (7 : 2 : 1) for raw drug YM and Toulene: ethylacetate (7 : 3) for YG
Solvent running distance	70 mm
Scanning wavelength	254 nm
Lamp used	Deuterium and tungsten
Slit	5 × 0.2 mm, micro
Scanning speed	20 mm/s
Scanner type	Single λ, multiple λ and spectrum
Measurement mode	Absorbance
Standard	Glycyrrihzin (100% pure from Yucca Enterprises, Wadala, Mumbai)
Standard preparation	1 mg of glycyrrhizin standard in l ml of ethanol was prepared for the analysis of raw drug YM and YG
Experimental conditions	Temperature 26 ± 2˚C, relative humidity 40%

The method of estimation was done in two different plates as both extract of the raw drug YM and the medicated YG had different solvents. The specifications for both the analysis is furnished in **Table 4**. Both the samples' glycyrrhizin content was estimated using the standard glycyrrhizin in the specified concentration.

Table 4 : Specifications of test samples and their mobile phases

S. No	Drug Tested	Extraction media	Test Solution	Mobile Phase
1	*Yashtimadhu* – raw drug	Ethanol	10 mg in 1 ml of ethanol	Butanol: deionised water: glacial acetic acid (7:2: 1)
2	*Yashtimadhu Ghrita*	Chloroform	50 mg in 1 ml of chloroform	Toluene : ethylacetate (7 : 3)

Table 5 : Volume of samples and standards loaded on the TLC plate

Track number	Volume (µl)		Sample type
	YM (ethanol extract)	YG (chloroform extract)	
1	0.5	0.5	Sample
2	1	1	Sample
3	1.5	1.5	Sample
4	2	2	Sample
5	2.5	2.5	Sample
6	3	3	Sample
7	3.5	3.5	Sample
8	4	4	Sample
9	4.5	4.5	Sample
10	5	5	Sample
11	0.5	0.5	Standard
12	1	1	Standard
13	1.5	1.5	Standard
14	2	2	Standard
15	2.5	2.5	Standard

RESULTS:

I. Pharmaceutical part:

The temperature of the *snehapaka* gradually decreased during the preparation, as shown in **Table 6**. The total processing time for the *ghrita* was one hour and 20 minutes (one hour on the first day and 20 minutes on the second day). From the 150 ml of *ghrita* added, the final yield of YG obtained was 109.8 gm, and the weight of the *kalka* after processing was 147 gm.

II. Analytical part:

Results of all preliminary physico chemical analysis of YM, plain ghee and YG are enlisted in **Tables 07 to 10**. **Table 11** shows the results of preliminary TLC done for YM and YG. R_f obtained from HPTLC for both the samples are enlisted in **Table 12**.

Table 6: Temperature of *snehapaka* at different time intervals

Sl. No	Day	Time of *paka*	Temperature observed (in Celsius)
1	1	7 pm	91
2	1	7.1 5 pm	89
3	1	7. 30 pm	85
4	1	8 pm	83
5	2	12. 05 pm	83
6	2	12. 25 pm	83

Image 03: *Kalka* after preparation of YG

Table 7: Oraganoleptic characters

Sl. No.	Character	YM		*Ghrita*		YG
		API standard	Observed	API standard	Observed	
1	Form / Part	Root	Root	Semisolid with granular texture	Semisolid with granular texture	Ghee
2	Colour	Yellowish brown / dark brown	Yellowish brown	White to yellow	Yellowish	Yellowish brown
3	Odour	Faint and characteristic	Faint and characteristic	Rich and characteristic	Rich and characteristic	Faint and characteristic
4	Taste	Sweetish	Sweetish	Pleasant	Pleasant	Slightly sweet
5	Touch	---	---	Oily	Oily	---

Table 08: Physico chemical analysis of YM

Sl. No.	Name of test	API standards	Observed results
1.	Foreign matter	Nil	Nil
2.	Loss on drying	NA	1.287%
3.	Total ash value	≤10%	8.227%
4.	Acid insoluble ash	≤2.5%	1.265%
5.	Water soluble ash	NA	1.119%
6.	Water soluble extractive value	≥20%	34.977%
7.	Alcohol soluble extractive value	≥10%	14.348%
8.	Microbial limit test	NA	less than limits

Table 09: Physico chemical analysis of *Ghrita* and YG

Sl. No.	Name of test	*Ghrita*		YG
		API standards	Observed results	Observed results
1.	Moisture content	not more than 0.5 %	0.246%	1.345%
2.	Refractive index	NA	1.455	1.457
3.	Specificgravity @ 25° C	NA	0.921	0.912
4.	Acid value	NA	1.009	1.792
5.	Saponification value	<225	154.275	145.66
6.	Iodine value	<35	33.42	41.467
7.	Microbial limit test	NA	trace amounts of microbial colonies found	not done
8.	pH	NA	5.5 – 6	6 - 7

Note: API standards for YG is not available

Table 10: Phytocehmical analysis of YM and YG

Sl. No.	Test for phytochemicals	YM		YG	
		ASE	WSE	CSE	HSE
1	Carbohydrates	+	+	+	+
2	Reducingsugar	+	-	-	+
3	Monosaccharides	-	-	-	-
4	Pentose sugar	+	-	-	-
5	Hexose sugar	-	-	-	-
6	Non - reducing polysaccharides	-	-	-	-
7	Proteins	+	+	-	+
8	Aminoacids	-	-	-	-
9	Steroids	+	-	+	-
10	Cardiac glycosides	-	+	+	+
11	Saponins	+	+	+	+
12	Flavonoids	-	+	-	-
13	Alkaloids	+	+	-	+ (Drgendroff's test)
14	Tannins	+	+	+ (lead acetate test)	+ (lead acetate test)
15	Anthraquinone glycosides	+	-	+	-

ASE – Alcohol soluble extract, WSE – Water soluble extract, CSE – Chloroform soluble extract, HSE – Hexane soluble extract

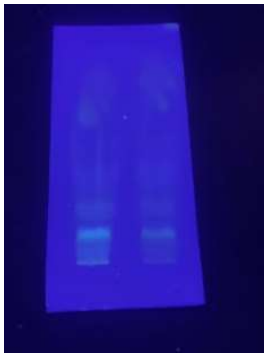

Image 04: TLC trial 1 - 366 nm with chloroform extract (left) and hexane extract (right) of YG

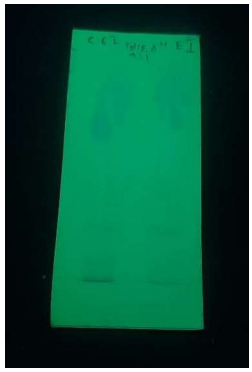

Image 05: TLC trial 1 - 254 nm with chloroform extract (left) and hexane extract (right) of YG

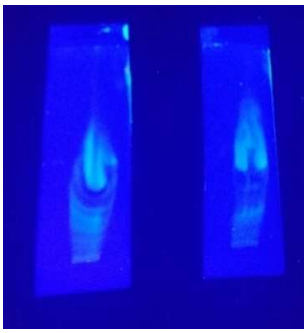

Image 06: TLC trial 2 - 366 nm with chloroform extract (left) and hexane extract (right) of YG

Table 11: TLC results of YM and YG

Sample	Mobile phase	R_f values
YM alcoholic extract (API standards)	n butanol: deionised water: glacial acetic acid (7:2: 1)	0.18, 0.21, 0.25, 0.57, 0.75
YM alcoholic extract	n butanol: deionised water: glacial acetic acid (7:2: 1)	Long wave – 0.098, 0.2, 0.34, 0.95, 0.98 Short wave – 0.07, 0.25, 0.31
YG chloroform extract	Toluene: ethyl acetate (7: 3)	Long wave – 0.04, 0.08, 0.13, 0.16, 0.2 Short wave – 0.09

Table 12: R_f values of YM and YG obtained from HPTLC

Substance: YM ethanolic extract and YG chloroform extract						
Track	R_f		X axis (mm)		Y axis (mm)	
	YEE (0.331±0.038)	YGCE (0.331±0.021)	YEE	YGCE	YEE	YGCE
1	0.329	0.315	20	20	28.4	27.5
2	0.326	0.337	31.4	31.4	28.2	28.9
3	0.326	0.339	42.8	42.8	28.2	29
4	0.323	0.311	54.2	54.2	28	27.3
5	0.326	0.329	65.6	65.6	28.2	28.4
6	0.327	0.321	77	77	28.3	27.9
7	0.324	0.313	88.4	88.4	28.1	27.4
8	0.326	0.323	99.8	99.8	28.2	28
9	0.324	0.313	111.2	111.2	28.1	27.4
10	0.326	0.313	122.6	122.6	28.2	27.4
11	0.329	0.313	134	134	28.4	27.4
12	0.331	0.344	145.4	145.4	28.5	29.3
13	0.332	0.315	156.8	156.8	28.6	27.5
14	0.335	0.313	168.2	168.2	28.8	27.4
15	0.339	0.322	179.6	179.6	29	28.6

YM ethanolic extract (YEE); YG chloroform extract (YGCE)

Image 07: HPTLC of YEE in 254 nm

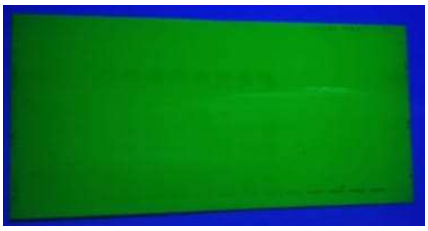

Image 08: HPTLC of YGCE in 254 nm

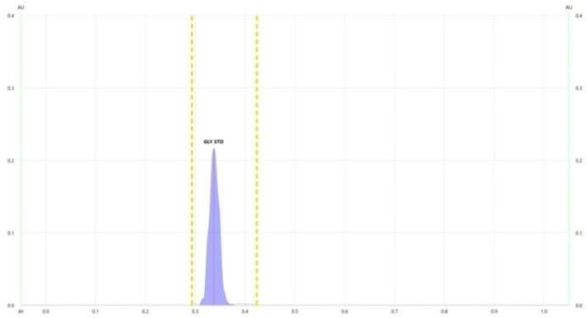

Image 09: Peak of standard glycyrrhizin

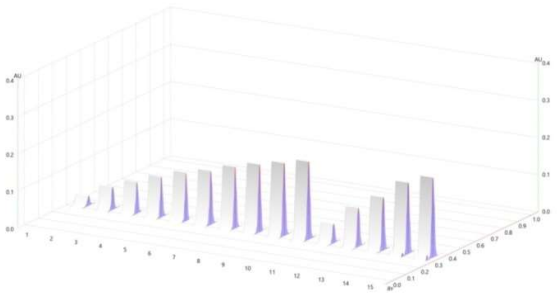

Image 10: Fingerprinting analysis of YGCE

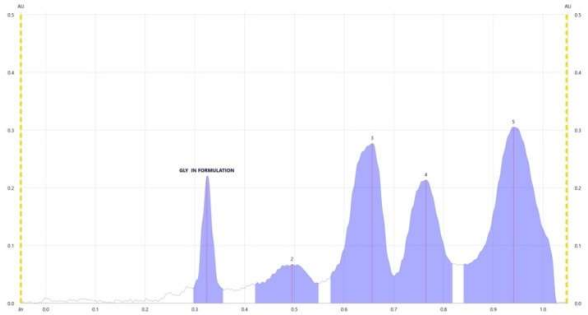

Image 11: Peak of glycyrrhizin in YGCE

DISCUSSION :

The main objective of the present study was to perform basic physico-chemical and HPTLC of commonly practiced drugs like YM and YG. By analyzing the analytical data of such important drugs and formulations, we can gain extensive knowledge about their quality, potency, and properties, which may undergo changes during various pharmaceutical steps and procedures. This information can be gathered and interpreted to better understand the drug's characteristics and potential benefits in treating various conditions.

YG was prepared according to the standard *Snehakalpana* ratio in Ayurveda, using YM *kashaya* as the *dravadravya*. The temperature pattern in Table 05 indicates that reaching *sneha siddhi lakshana* signifies complete evaporation of YM *kashaya*, retaining only the active constituents. The increase in *kalka* weight can be attributed to the solid part of YM *kashaya* after evaporation.

Results of physico-chemical analysis of YM and ghee are in accordance with pharmacopoeial standards indicating the genuineness of drugs used. The odor of YG resembled that of the raw drug YM, indicating successful processing of the drug into the fatty medium during the pharmaceutical procedure. The moisture content analysis of YM and YG revealed that the former method measures both water and volatile content, while the latter specifically indicates the percentage of moisture **[15]**. Physico-chemical analysis confirmed that both YM and plain cow ghee met pharmacopoeial standards, ensuring sample purity for the pharmaceutical preparation. The increase in moisture content in YG compared to plain cow ghee can be attributed to the addition of YM *kashaya* during the ghee preparation. The saponification value of YG differed from plain ghee, suggesting molecular weight changes in short and long chain fatty acids due to interactions between GG's phytoconstituents and lipid molecules during processing. Previous studies have reported varying iodine values for YG, influenced by the type of *drava dravya* used **[12, 16]**. It may be due to this reason that classically it is advised to add only water when no specificatino is laid regarding the type of drava dravya to be used **[1]**. The pH range of YG (6 to 7) makes it safe and suitable for external ophthalmic procedures and other external treatments in Ayurveda for various diseases.

Phytochemical screening of YM revealed the presence of carbohydrates, proteins, saponins, alkaloids, and tannins in both alcoholic and water extracts. YG's chloroform and hexane extracts contained carbohydrates, cardiac glycosides, saponins,

and tannins, indicating the incorporation of active constituents during *Snehakalpana*. Qualitative or quantitative detection of saponins like 20α-rhaoglycyrrhizin, 11-deoxorhaoglycyrrhizin, galacturonoylglycyrrhizin, and others confirms their presence in YG, as isolated from GG roots **[17]**.

Quantification analysis for YG has not been conducted in chromatographic studies to date. This study aimed to estimate glycyrrhizin in YG, using chloroform and hexane (nonpolar solvnent) for extraction due to its ghee base (nonpolar solute) using toluene and ethyl acetate (7:3) as the mobile phases as resulted from the trials. Centrifuging was done in order to make the solute particles get evenly distributed in their respective solvents. Chromatogram development with chloroform extract took 13 minutes, showing a retention factor (R_f) of 0.331±0.021, matching the R_f of the standard glycyrrhizin peak. Fingerprinting comparison (Image 10) confirmed glycyrrhizin presence. Calibration yielded a concentration of 1.506 mg in 50 mg of chloroform extract of YG and 514.2 mcg in 10 mg of alcoholic extract of raw YM, indicating the successful incorporation of over 50% of glycyrrhizin from YM into the ghee medium during the specific boiling procedure.

CONCLUSION:

This study explores the unique process of *Snehakalpana* in Ayurvedic pharmaceutics, demonstrating its ability to incorporate over 50% of a drug's active concentration into fat media like ghee. While the precise mechanism remains unknown, formulations like YG offer enhanced therapeutic benefits compared to raw drugs. The use of HPTLC allows for quantifiable conclusions regarding YG, based on standard phytoconstituents found in *Glycyrrhiza glabra*.

LIMITATIONS AND SCOPE FOR FURTHER STUDY:

This study quantitatively analyzed glycyrrhizin concentration in ghee using chloroform as a nonpolar solvent unlike other studies, yielding specific and reliable results with shorter development time for the chromatogram. Furthermore there is a need to verify precision and accuracy for more conclusive data.

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