ORIGINAL ARTICLE

Quantitation of Vitamin C from Marketed Chyawanprash Using UV Spectrophotometer

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ABSTRACT Chyawanprash (CP) is an antioxidant paste created by blending around 50 herbs and spices in a synergistic fashion. It contains Vitamin C as a key ingredient. All of the substances are mixed in precise proportions and exposed to unique pharmaceutical techniques to develop products with the greatest health benefit. In the pharmaceutical sector, however, noncompliance with normal manufacturing processes is a typical blunder. It made it essential to test the products' quality before releasing them for sale. The content of a product's primary ingredient, i.e., Vitamin C ensures quality of Chyawanprash. In this study, a rapid and simple UV spectrophotometric method was developed for quantitation of Vitamin C from a few marketed Chyawanprash. Buffer and sodium oxalate solution were employed to keep the pH acidic and prevent Vitamin C oxidation in aqueous media. The absorption was measured at 266 nm. The response was found to be linear over 2.5-12 µg/mL with r^2 value 0. 998. The proposed method was also found to be specific, precise, accurate, and linear, and it was effectively used to estimate Vitamin C from commercially available Chyawanprash.

Keywords: Chyawanprash, Vitamin C, Noncompliance, Sodium oxalate, UV spectrophotometer

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INTRODUCTION

According to Ayurvedic Pharmacopeial Index (API), Chyawanprash is a traditional Indian polyherbal formulation with a semisolid and sticky in nature.[1] Chyawanprash is classified as Rasayana in Ayurvedic texts, and its main purpose is to maintain the body's integrity in order to delay the ageing process, improve longevity, and improve digestion.[2] It is the primary source of health care for respiratory tract disorders such as bronchial spasms, cough, asthmatic breathing, and tuberculosis. It can also be used as an immunomodulator and memory booster.[3] Formulation comprises of more than 50 medicinal plants ingredients such as AmLaki (*Emblica officinalis*), Bilva (*Aegle marmelos*), Agnimantha (*Premna Integrifolia*), Syonak (*Oroxylum indicum*), Kasmari (*Gmelina arborea*), Patala (*Stereospermum suaveolens*), Bala (*Sida cordifolia*), Salaparni (*Desmodium gangeticum*), Prsniparni (*Uraria picta*), Mudgaparni (*Phaseolus trilobus*), Mashparni (*Teramnus labialis*), Pippali (*Piper longum*), Goksura (*Tribulus terrestris*), Brhati (*Solanum indicum*), kantakari (*Solanum surattense*), Srngi (*Pistacia integerrima*), Bhumyamalaki (*Phyllanthus amarus*), Draksha (*Vitis vinifera*), Jeevanti (*Leptadenia reticulate*), Puskaramul (*Inula racemosa*), Agaru (*Aquilaria agallocha*), Haritaki (*Terminalia chebula*), Guduchi (*Tinospora cordifolia*), Rddhi (*Habenaria intermedia*), Jivaka (*Malaxis acuminate*), Rsabhaka (*Malaxis muscifera*), Sati (*Hedychium spicatum*), Mustak (*Cyperus rotundus*), Punarnava (*Boerhaavia diffusa*), Meda (*Polygonatum cirrhifolium*), Ela (*Elettaria cardamomum*), Candan (*Santalum album*), Utpala (*Nymphaea stellata*), Vidari (*Pueraria tuberose*), Vrsamula (*Adhatoda vasica*), Kakoli (*Lilium polyphyllum*) and Kakanasika (*Marytynia annua*) in various amounts.[4]

One of the main active ingredients (35%) of Chyawanprash is Amla (*Emblica officinalis*) a richest source of Vitamin C a

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powerful antioxidant, and polyphenols including flavonoids.[5]

Vitamin C aids in the recovery of energy wasted by the body.[6] Vitamin C is necessary for the formation of collagen, a protein that provides bones, muscles, and blood vessels shape. L-ascorbic acid refers to the reduced form of Vitamin C, while dehydroascorbic acid refers to the oxidized version. Both kinds are biologically active in humans. The sum of both kinds of Vitamin C activity equals total Vitamin C activity. It dissolves in water and produces somewhat acidic solutions. Vitamin C is the common name for ascorbic acid. Ascorbic acid is a kind of Vitamin C known as just a "vitamer." Vitamin C IUPAC name is 2, 3-dihydro-L-threohexo-1, 4-lactone. Ascorbic acid, in the form of Vitamin C, has a number of important biological functions, include organism maintenance, control of Vitamin C deficit (scurvy), stimulation of collagen formation, inhibition of melanogenesis, and antioxidation.[7-10]

Various characteristics, including as morphological, phytochemical, and physicochemical properties, have been specified for Chyawanprash to date. To the best of knowledge, no scientific evidence has even been reported in regards to the proper analytical testing of Chyawanprash for the content determination of Vitamin C in formulation. As a consequence, the study intends to standardized the traditional polyherbal formulation Chyawanprash by Vitamin C. For the estimation of Vitamin C, a number of analytical methods are proposed, including titrimetric[11], fluorimetry[12], Spectrophotometry[13-15], and high-performance liquid chromatography (HPLC)[16-18], each with its own set of benefits and costs.

In aqueous solutions, vitamin C is unstable. In addition, the reversible transition of L-ascorbic acid to dehydroascorbic acid, followed by the irreversible reaction of 2, 3-diketo-L-gulonic acid, demonstrates its instability. These problems can be avoided by using stabilizer.

The goal of this research was to develop a simple and fast UV spectrophotometric method to quantify Vitamin C in Chyawanprash using sodium oxalate as a stabilizer.

METHODS AND MATERIALS

Reagents

All reagents used were of analytical reagent grade, sodium oxalate, disodium hydrogen phosphate, sodium dihydrogen phosphate, Hydrochloric acid, ascorbic acid.

Instruments

UV-Visible spectrophotometer (Shimadzu 1601), Single pan analytical balance (Dhona 200 D).

Method

Buffer solution (pH = 5.8)

A mixture of sodium dihydrogen phosphate (0.09 M) and disodium hydrogen phosphate (0.005) was prepared by dissolving 13.15 g of $NaH_2PO_4.H_2O$ and 1.30 g of $Na_2HPO_4 \cdot 7H_2O$ in 800 mL of distilled water.

Stabilizer Solution

Prepared by dissolving 0.67 g of sodium oxalate in 1000 mL of the buffer solution.

Linearity Study

Preparation of Standard Solution

Accurately weighed 100 mg of Vitamin C was diluted up to 100 mL of stabilizer solution. Adequate aliquots of above solution were diluted to 25 mL to obtain concentration in the range 2.5-12.5 µg/mL.

Calibration Curve

All the standard solution scanned in between 200 nm-400 nm and absorbance recorded at 266 nm. Graph of concentration (as x-value) versus absorbance (as y-value) was plotted (Figure 2). The correlation coefficient, y-intercept and slope of the regression were calculated and mentioned below in Table 1.

Formula

Concentration of test solutions were calculated by using slope and intercept equation obtained from the calibration curve of Desloratadine.

Slope and intercept equation: $y = m x \pm c$

where, y - y axis value i.e. Absorbance of test solution

x - x axis value i.e., Concentration of test solution

m - slope of linear curve

c - y intercept at zero concentration

Quantitation of Vitamin C from Chyawanprash

Three marketed Chyawanprash i.e., Dabur Chyawanprash

Table 1: Beers Law Study of Vitamin C

S. No.	Conc. µg/mL	Abs. at 266 nm
1	2.5	0.191
2	5	0.369
3	7.5	0.517
4	9.5	0.696
5	12	0.879

Figure 1: Overlay UV Spectra of Standard Ascorbic Acid Solution

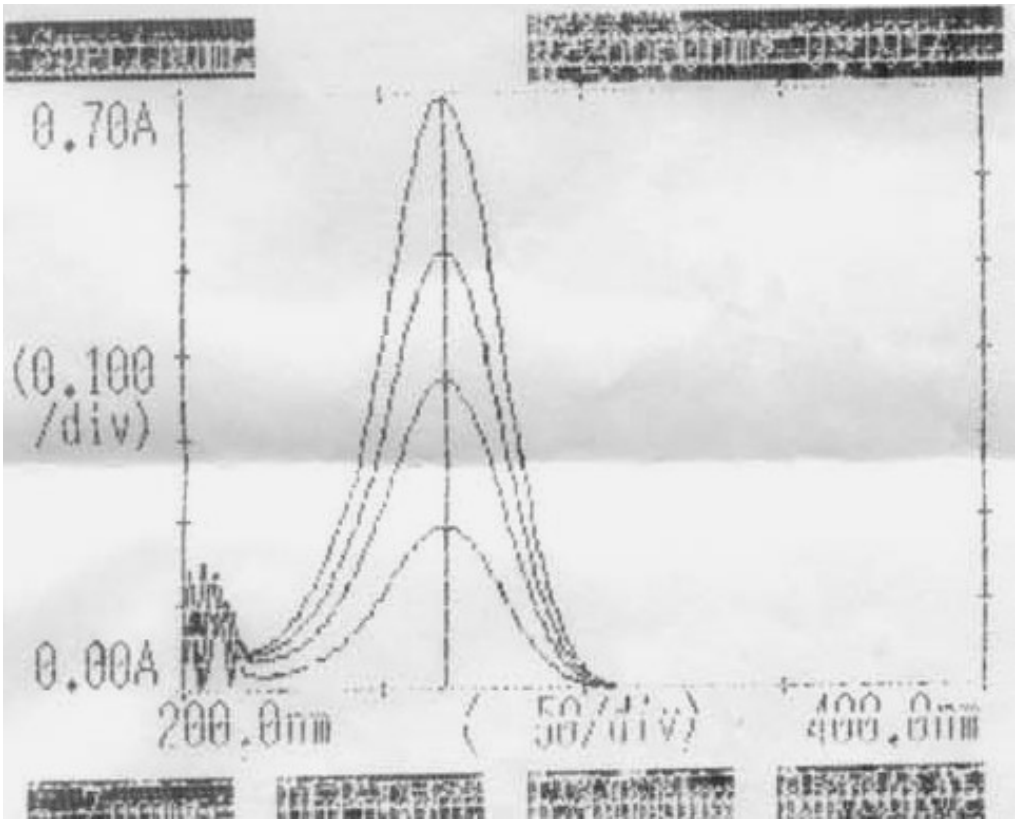
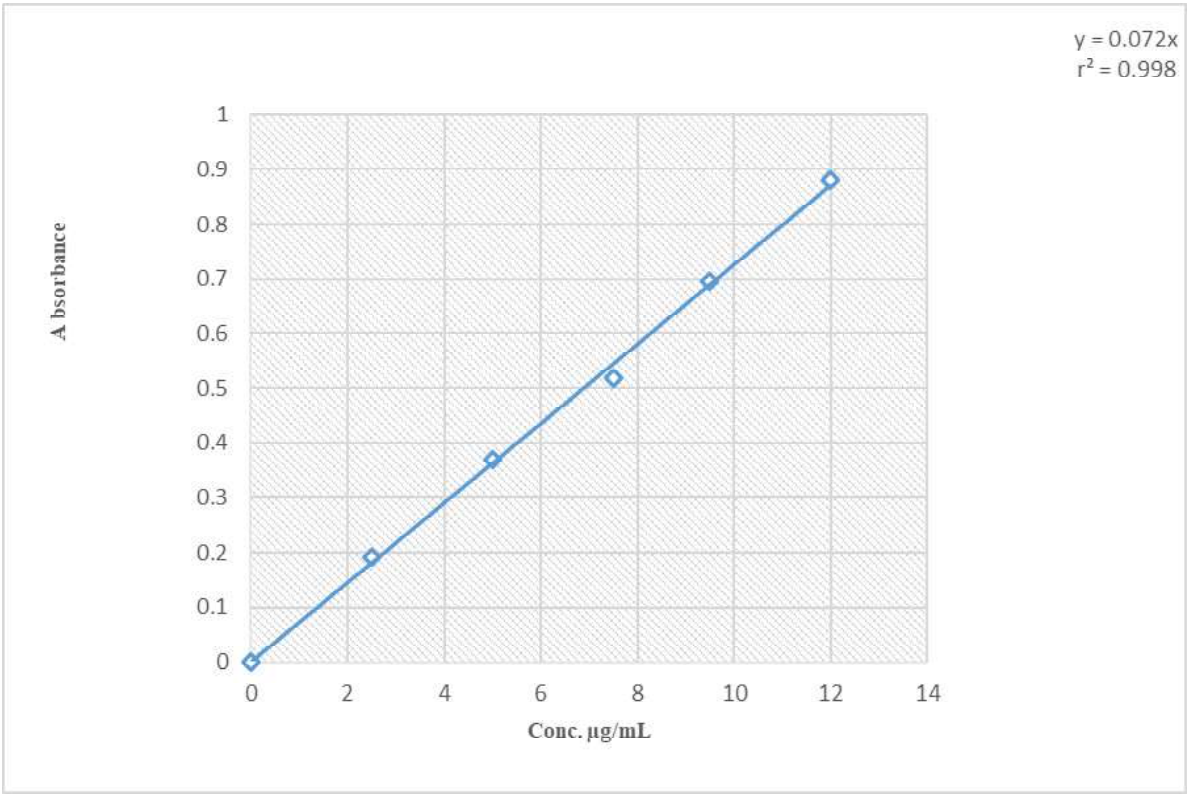


Figure 2: Linear Curve of Vitamin C



(DBC), Amrit Prash (AMP) and Sona Chandi Chyawanprash (SCC) were selected for the study. The Vitamin C content of marketed products was determined by using slope and intercept equation.

Linearity Study of Marketed Formulation

Accurately weighed 100 mg of Chyawanprash was transferred to 100 mL volumetric flask and diluted to mark in stabilizer solution. The above solution was filtered and aliquots of filtrate were transferred into 25 mL volumetric flask. Final volume was adjusted to mark with stabilizer solution to prepare series of test solution (200-1000 µg/mL).

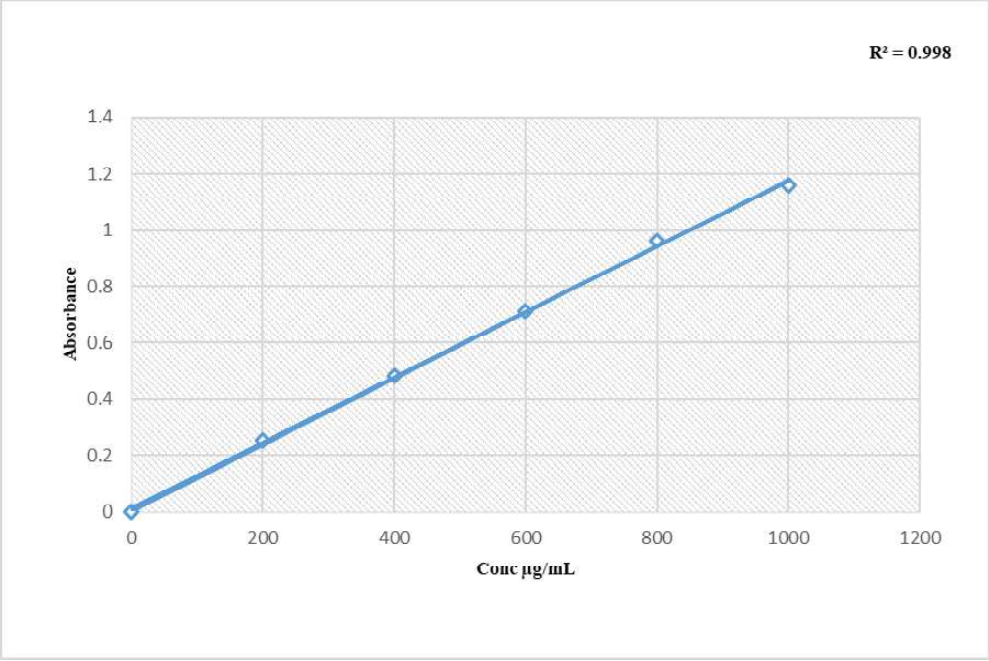
Table 2: Observation of Linearity Study of Marketed Formulation

S. No.	Conc. µg/mL	Absorbance at 266 nm		
		DBC	AMP	SCC
1	200	0.253	0.161	0.24
2	400	0.484	0.307	0.467
3	600	0.713	0.474	0.821
4	800	0.96	0.625	0.963
5	1000	1.159	0.808	1.315

Table 3: Summarized Table of Vitamin C Content in Marketed Formulation

Formulation	Test No.	ABS at 266 nm	Conc. (µg/mL)	Vit. C content per 100 gm	Mean
DBC	Test 1	0.271	3.7276	1.86	1.55
	Test 2	0.471	6.4833	1.62	
	Test 3	0.68	9.3668	1.17	
AMP	Test 1	0.17	2.3438	1.66	1.56
	Test 2	0.323	4.4471	1.61	
	Test 3	0.471	6.4849	1.42	
SCC	Test 1	0.24	3.3287	1.17	1.03
	Test 2	0.467	6.4771	1.11	
	Test 3	0.821	11.386	0.81	

Figure 3: Linear Curve of DBC



Absorbance of each solution was measured at 266 nm shown in Table 2. Graph was plotted in between absorbance and test concentration to assure linear response (Figures 3, 4 and 5).

From above, three test concentrations 200, 400 and 600 µg/mL were studied for the content of Vitamin C. Content per 100 gm was determine and reported in Table 2. It was performed in triplicate to assure repeatability Table 3.

Figure 4: Linear Curve of AMP

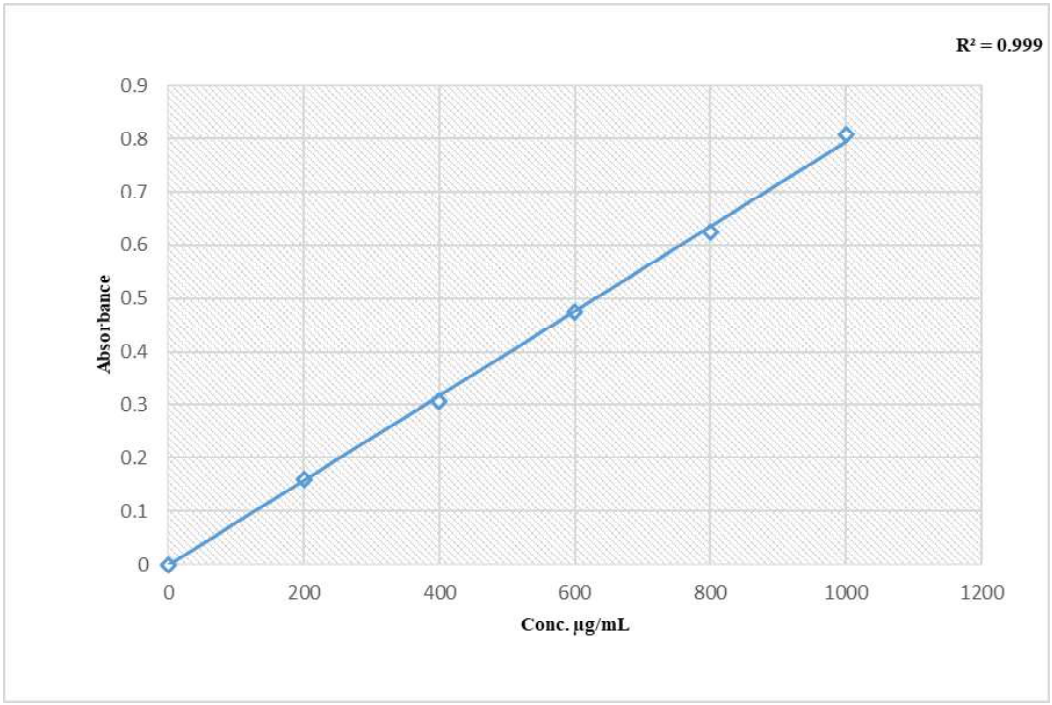


Figure 5: Linear Curve of SSC

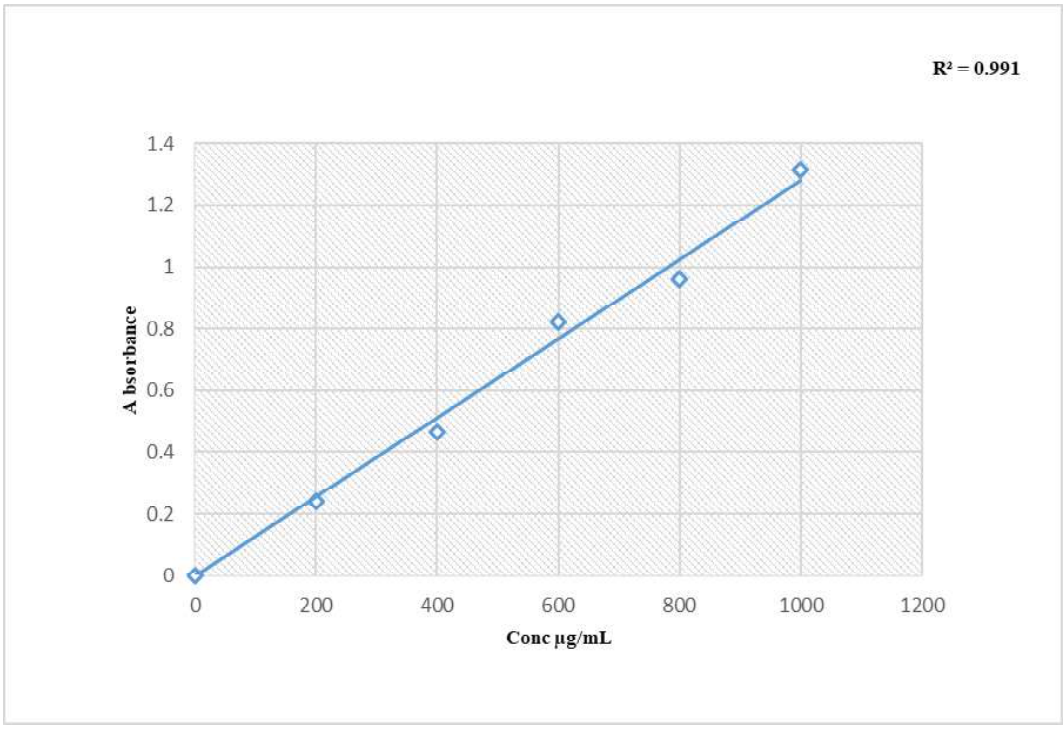


Table 4: Results of Repeatability Study

Sample	Vitamin C content per 100 gm		
No.	DBC	AMP	SCC
I	1.64	1.09	1.61
II	1.66	1.09	1.62
III	1.57	1.1	1.63
Mean	1.62	1.09	1.62
Standard deviation	0.047	0.07	0.01
% RSD	0.029	0.0642	0.0061

RESULTS AND DISCUSSION

Absorption of Vitamin C solutions is dependent upon the pH of the aqueous media. Above pH 5.0, Vitamin C exists predominantly as the monoanion and has maximal absorption at 266 nm. Because of this, Phosphate buffer containing Sodium dihydrogen phosphate – disodium hydrogen phosphate (pH = 5.8) was used throughout this work.

Vitamin C is readily and reversibly oxidized to dehydroascorbic acid, which is present in aqueous media as a hydrated hemiketal. The biological activity is lost when the dehydroascorbic acid lactone ring is irreversibly opened, giving rise to 2,3-diketogulonic acid. In the present work, sodium oxalate in the buffer solution was used to stabilize Vitamin C in the aqueous media.

A linearity investigation was carried out to ensure that the proposed method's response was linear. The UV response (y) of Vitamin C standard solution was found to be linear over a concentration (x) range of 2.5-12 g/mL, with a linear regression equation y = 0.072x and r^2 value 0.998.

The concentration of test solution of commercial formulations Dabur Chyawanprash (DBC), Amrit Prash (AMP), and Sona Chandi Chyawanprash (SCC) was determined using the aforementioned linear regression equation. Vitamin C concentration per 100 g of each formulation was later determined and found 1.55 g (DBC), 1.56 g (AMP), and 1.03 g, respectively (SCC). The analytical procedure's repeatability was also assessed, and it was determined to be exact, with a standard deviation value in the range of 0.01-0.07.

CONCLUSION

A variety of methods for analyzing vitamin C have been published in the literature. Using Phosphate buffer and Sodium oxalate as a stabilizer, a UV-Spectrophotometric method was developed and successfully employed to quantify Vitamin C from three commercially available Chyawanprash.

The proposed method used a simple sample preparation method and produced a linear, accurate analytical result in a short amount of time at a reasonable cost. As a result, the proposed method should be used to estimate Vitamin C in commercial pharmaceutical, nutraceutical, food, and cosmetic goods.

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