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Estimation of Gallic Acid in Different Commercial Samples of Amruthotharam Kashayam by Using HPLC

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Abstract

Amruthotharam Kashayam (AK) is used by a practitioners of Indian system of medicine (Ayurveda) for ailments like chronic fever. Commercial AK samples were collected from the local market in Coimbatore, India and subjected to HPLC conditions using Gallic acid as a marker. Mean value of Gallic acid in commercial samples was found to be 2.93% while reference range was 2.80-4.44%.

1 Introduction

Nowadays high performance liquid chromatography (HPLC) is gaining more popularity than conventional TLC (thin layer chromatography) for herbal drug analysis. HPLC is more speed, accuracy, sensitivity, reproducibility and automation than any other chromatography analysis. So it is more useful in herbal drug analysis than TLC and HPTLC (High performance thin layer chromatography). HPLC is having flexibility of detectors like Ultra Violet and Refractive index.

By taking these facts under consideration OSADHI (Office for Standardization of Ayurvedic Drugs & Herbs Initiative), a drug department of Arya Vaidya Pharmacy Research foundation (formerly AVTAR <AVT Institute for Advanced Research>) a research division of Arya Vaidya Pharmacy Ltd Coimbatore has started work on HPLC analysis of both raw as well as finished products codified in classical Ayurvedic texts .

OSADHI is working out the reference range for markers in classical formulations as well as trying to develop reference range library of selected formulations by using HPLC technique.

Amruthotharam Kashayam is one of the leading polyherbal formulation used by Kerala vaidyas traditionally for management of chronic fevers as well as rheumatoid arthritis It contain Amruta (*Tinospora cordifolia*), Haritaki (*Terminalia chebula*) and Sunthi (*Zingiber officinale*)[1] as key ingredients. Amruta contains Tinosporaside, Tinsosporic acid, caridioside etc[2]. Haritaki contains tannis, gallic acid, chebulagic acid, ellagic acid [3] while sunthi contains volatile oil 1%, 6% gingerol, 8% gingerol,10% gingerol and shagol etc[4].

The study focused on one major chemical component namely Gallic acid from Haritaki which is one of the major ingredient of Amruthotharam Kashayam. Gallic acid (3,4,5-trihydroxybenzoic acid) possesses important medicinal properties (Fig 1). It blocks histamine release and pro-inflammatory cytokine production in mast cells. Gallic acid has anti-fungal and anti-viral properties.

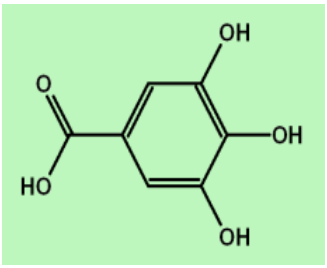

Fig 1: Chemical Structure of Gallic acid

Gallic acid acts as a antioxidant and helps to protect our cells against oxidative damage, cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used a remote

astringent in cases of internal haemorrhage also used to treat albuminuria and diabetes[5-8].

2 Materials and Methods

The Amruthotharam Kashayam manufactured in different batches by Arya Vaidya Pharmacy Ltd were collected from its different outlets in Coimbatore. All these samples were kept under room temperature.

2.1 Chemicals and Instruments

Solvents used were HPLC grade water, acetonitrile and phosphoric acid (Qualigens).

Standard gallic acid from Sigma Aldrich Bangalore was used as a marker. The mobile phase was passed through bacterial filter 0.2 microns and sonnicated before use. All analyses were run in triplicate and averaged. The Schimadzu HPLC Model 7725 (2004) LC-10 system consisted AT VP, SPD -10 A-VP, UV detector,SCL-10 A system controller, Class 10-VP 6.12 SP5 integrations software and Rheodyne. Micro syringe loading sample injector fitted with 20 micro liter injection loop was used for analysis. Base line resolution of gallic acid was obtained at 27+/- using Phenomenax Luna C18 Column.

2.2 HPLC conditions

2.2.1 Solvent system

Acetonitrile /phosphoric acid (35:65 V/V) at flow rate 1ml/ min using detector Ultra violet visible at 260 nm. Column temp was 27 °C and volume of injection of sample was 20 micro liter.

2.2.2 Sample preparation

10 ml of Amruthotharam Kashayam were taken and centrifuged at 2000 rpm for 10 minutes and sediments were discarded. 1ml of clear supernatant solution was separated and dilute to 10 ml with HPLC grade water. After that 0.1 ml of stock solution was added into 10ml standard flask and make 10 ml of mobile phase. The prepared sample was then passed through 0.2 micron sepak filter. This method employed for all other Amruthotharam Kashayam samples.

2.2.3 Estimations

20 micro liter of sample of Amruthotharam Kashayam were injected in HPLC column and eluted with ACN (Acetonitrile): Phosphoric acid (35:65V/V) solvent system by using the binary gradient system. Peaks were obtained at 260 nm and average retention time was found to be 10.5 minute. The HPLC chromatogram was compared with standard gallic acid. From area of peak of gallic acid in the standard and those present in Amruthotharam Kashayam were calculated (% by W/W) (Table 1 & 2).

Table 1: Estimation of gallic acid in different commercial samples of Amruthotharam Kashayam by using HPLC

Name of the sample	Gallic acid	Amruthotharam Kashayam	Amruthotharam Kashayam	Amruthotharam Kashayam	Amruthotharam Kashayam	Amruthotharam Kashayam
Source	Sigma aldrich Bangalore	AVP factory Kanjikode	AVP factory Kanjikode	AVP factory Kanjikode	AVP factory Kanjikode	AVP factory Kanjikode
Date manufactured	12 -01- 2007	July 2007	Oct 2007	Jan 2008	Feb 2008	Apr 2008
Date received on	10.03.2007	8-11-2008	8-11-2008	8-11-2008	8-11-2008	8-11-2008
Date tested on	01-11-2008	24-11-2008	24-11-2008	24-11-2008	25-11-2008	25-11-2008
Quantity received	100gm	200ml	200ML	200ML	200ML	200ML
Description	White colour powder	Light brown liquid	Light black Liquid	Light brown liquid	Brown liquid	Light brown liquid
Value obtained	98 % (W/W)	4.44 %(W/W)	4.14 %(W/W)	2.53%(W/W)	2.40 %(W/W)	1.81%(W/W)
Reference range	100 %	2.80-4.44 %	2.80-4.44%	2.80-4.44 %	2.80-4.44 %	2.80-4.44 %

Same method was applied for the rest of the Amruthotharam Kashayam samples.

3 Results and Discussions

Estimation of reference range and mean of Gallic acid by using HPLC technique in Amruthotharam Kashayam samples manufactured by Arya Vaidya Pharmacy in different batches was the primary aim of this study. Satisfactory retention time and resolution were obtained using C18 column.

An average retention time was found to be 11 min for gallic acid in the samples of Amruthotharam Kashayam.

All chromatograms including standard are shown in the Fig 2-10. The concentration of gallic acid was found between range 2.80-4.44% and mean value is 2.9%.

Table 2: Estimation of gallic acid in different commercial samples of Amruthotharam Kashayam by using HPLC

Name of the sample	Amruthotharam Kashayam	Amruthotharam Kashayam	Amruthotharam Kashayam
Source	AVP factory Kanjikode	AVP factory Kanjikode	AVP factory Kanjikode
Date manufactured	June 2008	Aug 2008	Feb 2007
Date received on	8-11 -2008	9-11-2008	10-10-2008
Date tested on	25-11-2008	26-11-2008	28-10-2008
Quantity received	200 ml	200 ml	200 ml
Description	Light brown liquid	Light brown liquid	Light brown liquid
Value obtained	3.03 %(W/W)	3.39%(W/W)	1.76%(W/W)
Reference range	2.80-4.44 %	2.80-4.44 %	2.80-4.44 %

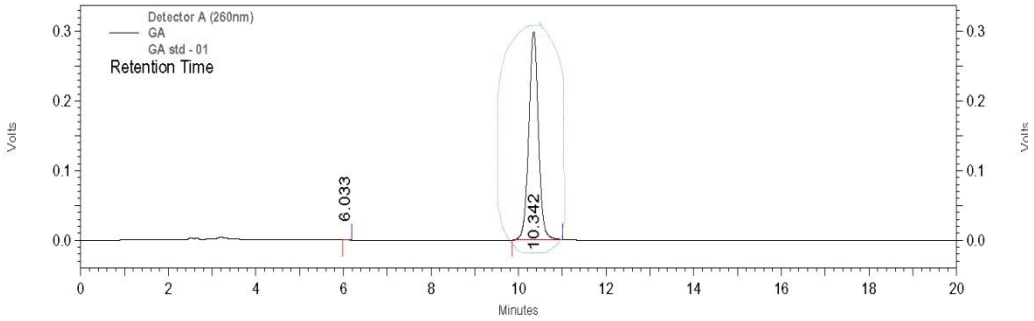

Fig 2: Standard Gallic acid

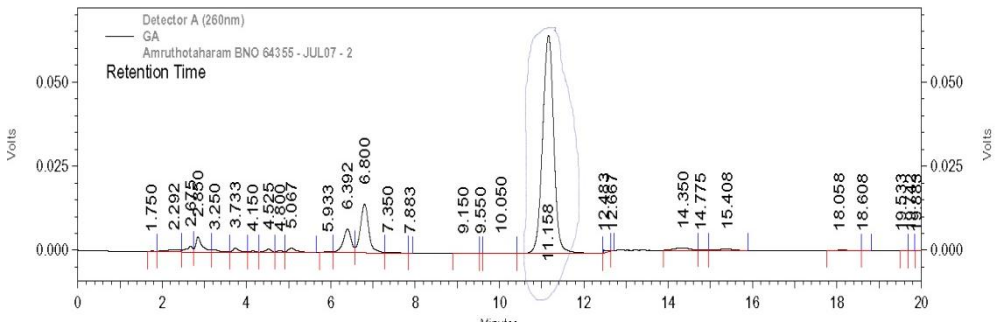

Fig 3: HPLC chromatogram of Amruthotharam Kashayam July 2007

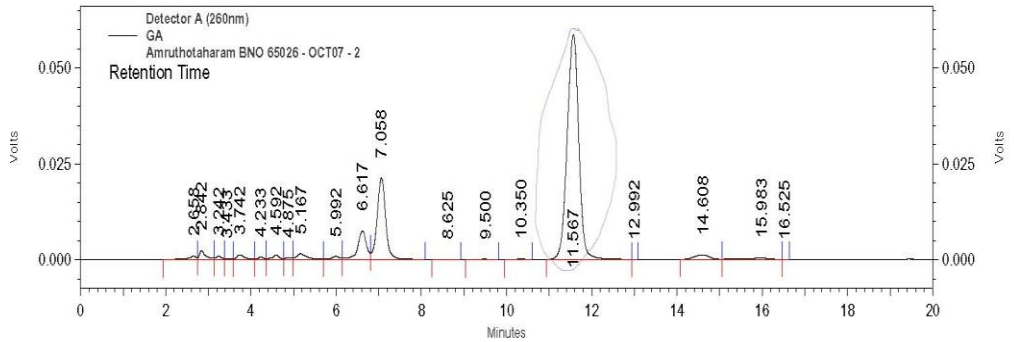

Fig 4: HPLC chromatogram of Amruthotharam Kashayam Oct 2007

Samples manufactured in different manufacturing dates are showing variation. Further study by using advance techniques like Mass Spectrometry needed to focus more on the data generated.

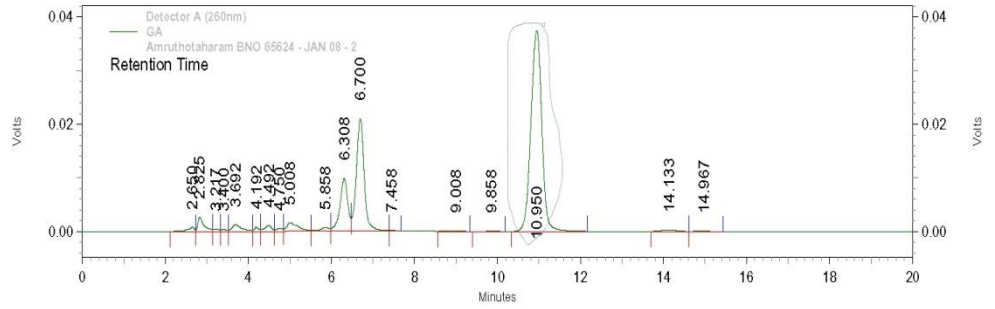

Fig 5: HPLC chromatogram of Amruthotharam Kashayam Jan 2008

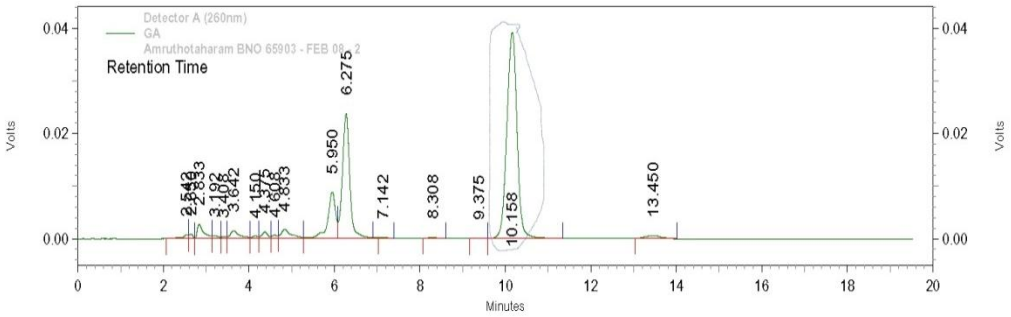

Fig 6: HPLC chromatogram of Amruthotharam Kashayam Feb 2008

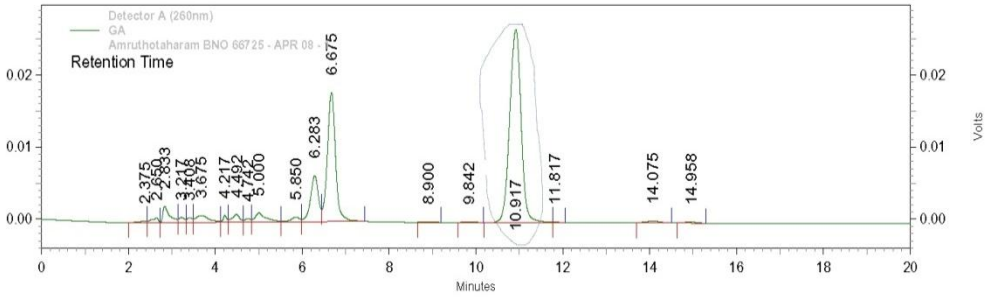

Fig 7: HPLC chromatogram of Amruthotharam Kashayam Apr 2008

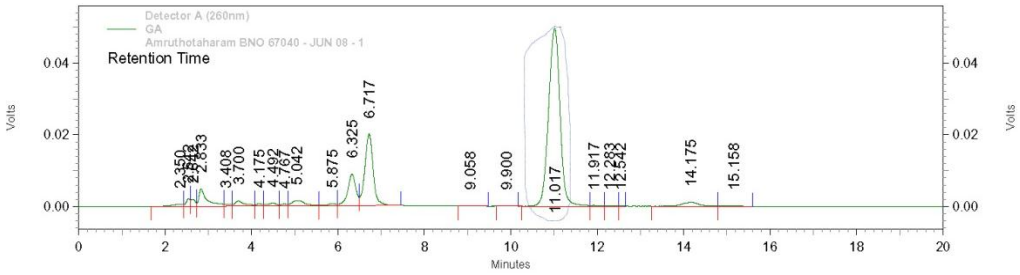

Fig 8: HPLC chromatogram of Amruthotharam Kashayam June 2008

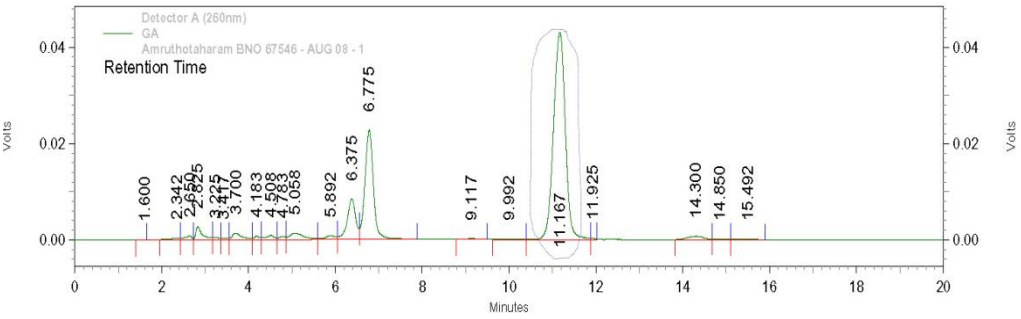

Fig 9: HPLC chromatogram of Amruthotharam Kashayam Aug 2008

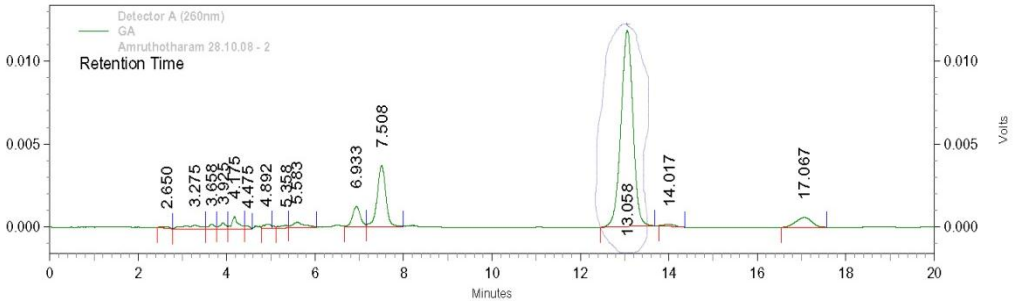

Fig 10: HPLC chromatogram of Amruthotharam Kashayam Feb 2007

4 Conclusion

Till today the estimation of gallic acid in Amruthotharam Kashayam not carried out by any ayurvedic pharmacy. So this study was an attempt to produce the reference range of gallic acid in the samples of Amruthotharam Kashayam by using HPLC technique. The reference range found to be 2.80-4.44% and mean value was 2.93%. The study should get carried out on the more commercial samples of Amruthotharam Kashayam prepared by other pharmacies in states like Kerala, Tamil Nadu, Karnataka and rest of India.

5 Acknowledgement

We would like to acknowledge Shree P R Krishnakumarji, Managing Director Arya vaidya Pharmacy Coimbatore for providing samples on request of Amruthotharam Kashayam for this work and giving guidance from time to time. We would also like to thank colleagues Anita Mahapatra and Reshmi Sarin for providing references and technical inputs for this work.

6 Competing interests

The author and co-author has no competing interest about this article.

7 Authors contributions

PB prepared the main script of this article. MP assisted in preparing the script. PB procured the samples from factory. PB identified both marker plus method to run the samples.BS has run the samples on HPLC.

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