

ABSTRACT

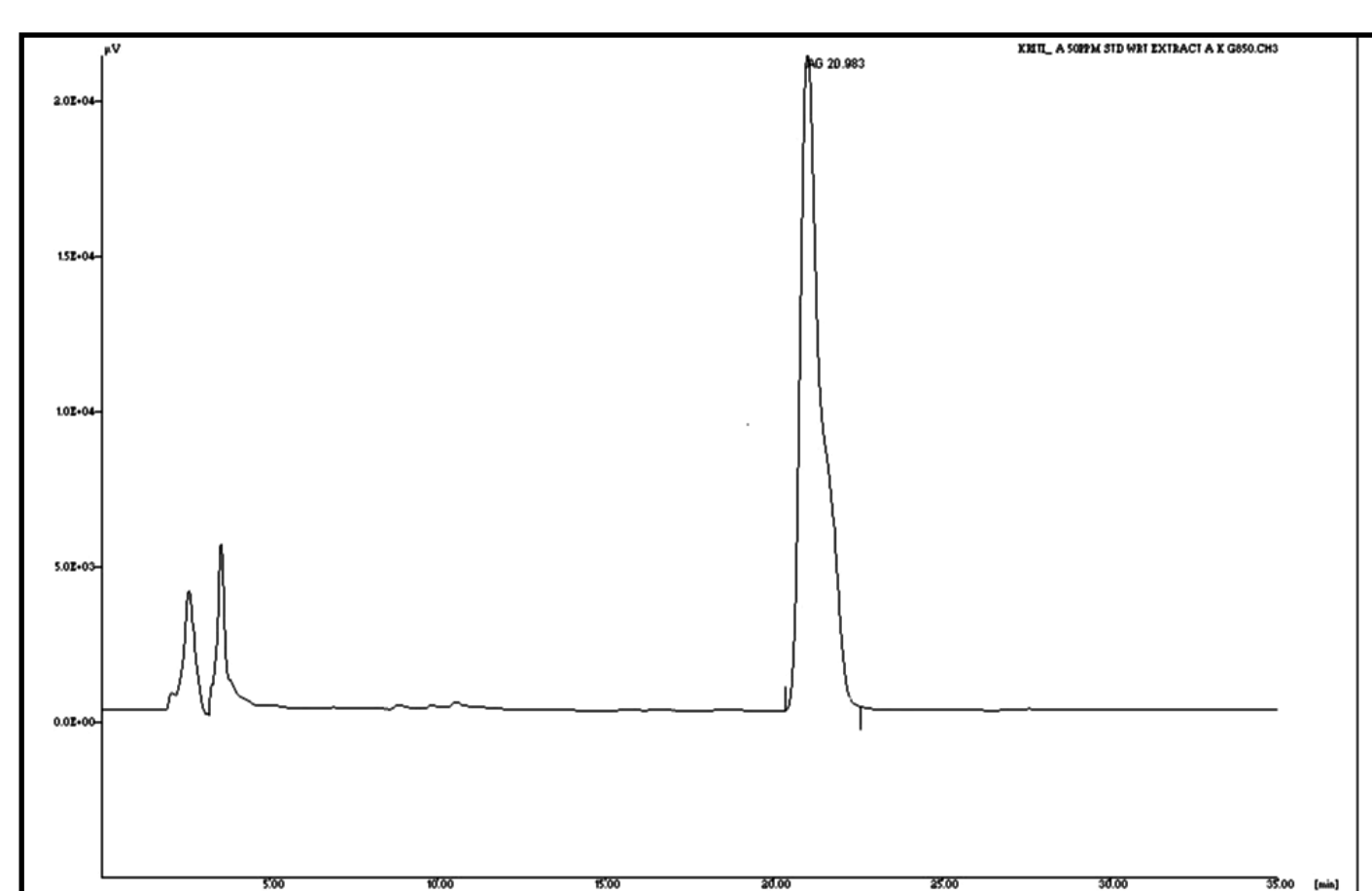
Objective: To develop a simple, accurate, sensitive, precise and reproducible RP-HPLC method using a poly-herbal formulation, HEPASAVE syrup (Cadila Pharmaceutical Ltd.).
Materials and Methods: Hepasave syrup, an Ayurvedic Proprietary Medicine which is used particularly as a powerful hepato-protective, an antioxidant, and a bitter tonic. The ingredients of the formulation include Amalaki, Haritaki, Bibhitaki, Vasa, Bhunimba, Katuka, Nimba, Amruta, Galo, Sarpankha and also a flavored syrup base. Present investigation includes estimation of three biomarkers namely Andrographolide (AG), Gallic acid (GA) and Kutkin (KT) using RP-HPLC method. Ethyl acetate extracts has been prepared to check for presence of active phytoconstituents using the mobile phase water : ACN (75:25), pH 3.45 maintained by 0.1% solution of o- phosphoric acid, with 1.0 mL/min flow rate using Column C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 µm particle size, an injection volume of 20 µL and a run time of 35 min. **Results:** Desired resolution of andrographolide (R_t: 21.55 min), gallic acid (R_t: 3.46 min) and kutkin (R_t: 5.69 min) with symmetrical and reproducible peaks was achieved for the working standards and the with the formulation extract. The amount of andrographolide, gallic acid and kutkin in the formulation were found to be 0.56 %w/v, 0.554 %w/v and 0.44 %w/v; while in the ethyl acetate extract, it was found to be 3.354 %w/v, 3.28 %w/v and 1.8 %w/v, respectively. **Conclusion:** The calculated amounts of biomarkers in the formulation by RP-HPLC method provided an information about the reliability of the method developed for the simultaneous estimation of AG, GA and KT in the formulation.

INTRODUCTION

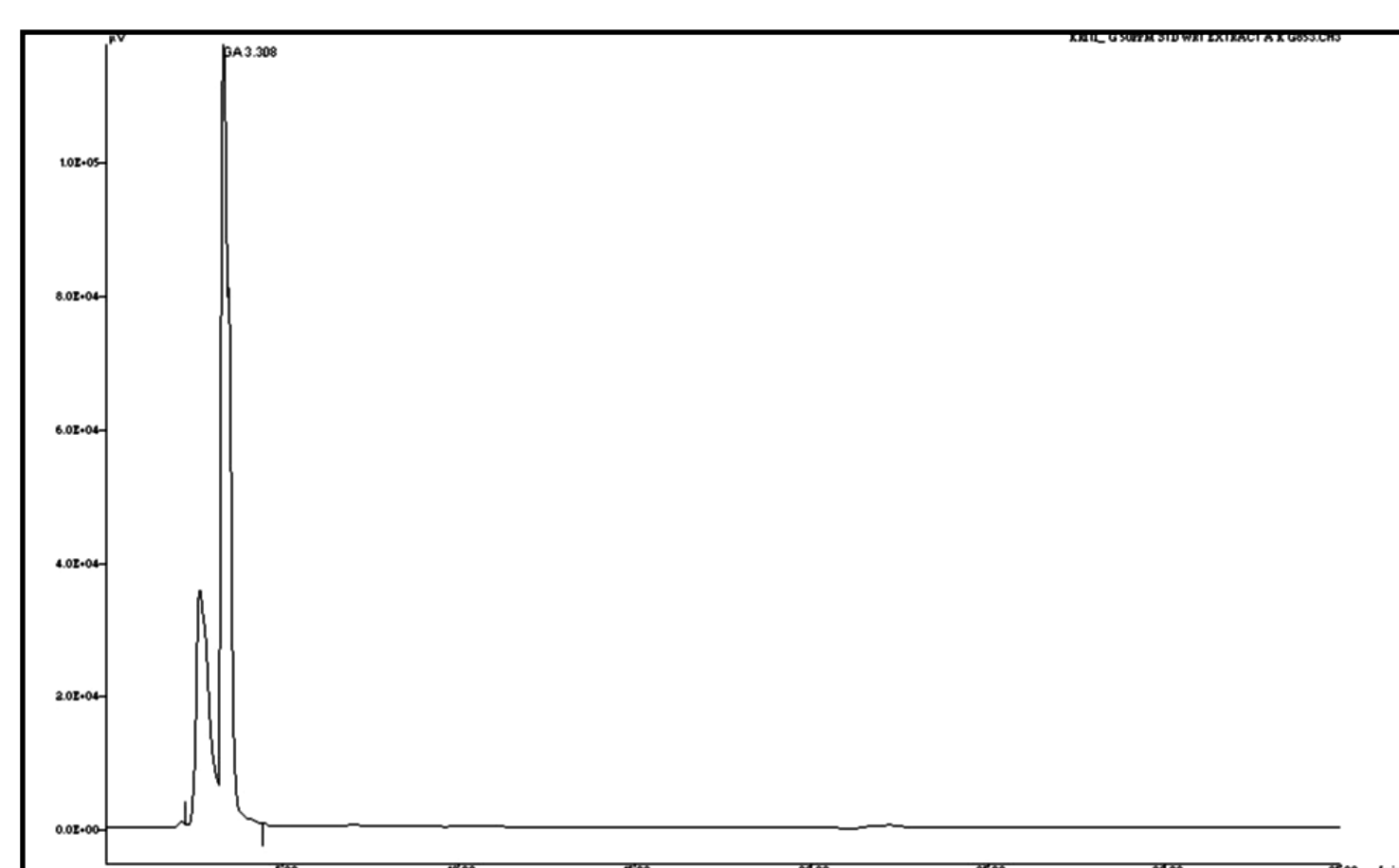
- * The Polyherbal formulation, Hepasave syrup, an Ayurvedic Proprietary Medicine was analyzed under the study. It is used particularly as a Powerful Hepato-protective, an Antioxidant, and a Bitter tonic.
- * The herbal constituents of the formulation include *Amalaki fruit i.e. Phyllanthus emblica Ab.*, *Haritaki fruit i.e., Terminalia chebula Ab.*, *Bibhitaki fruit i.e., Terminalia bellerica Ab.*, *Vasa leaf i.e., Adhatoda vasica Ab.*, *Bhunimba herb i.e., Andrographis paniculata*, *Katuka root i.e., Picrorrhiza kurroa*, *Nimba stem bark i.e., Azadirachta indica Ab*, *Amruta stem i.e., Tinospora cordifolia*, *Sarpankh herb i.e., Tephrosia purpurea Ab.*, and also a Flavored syrup base.
- * The major active phyto-constituents are Gallic acid (*Amalaki fruit i.e. Phyllanthus emblica Ab.*), Gallic acid (*Haritaki fruit i.e., Terminalia chebula Ab.*), Gallic acid (*Bibhitaki fruit i.e., Terminalia bellerica Ab.*), Andrographolide (*Bhunimba herb i.e., Andrographis paniculata*), Kutkin (*Katuka root i.e., Picrorrhiza kurroa*) and Vasicine (*Vasa leaf i.e., Adhatoda vasica Ab.*)

CHROMATOGRAPHIC CONDITIONS

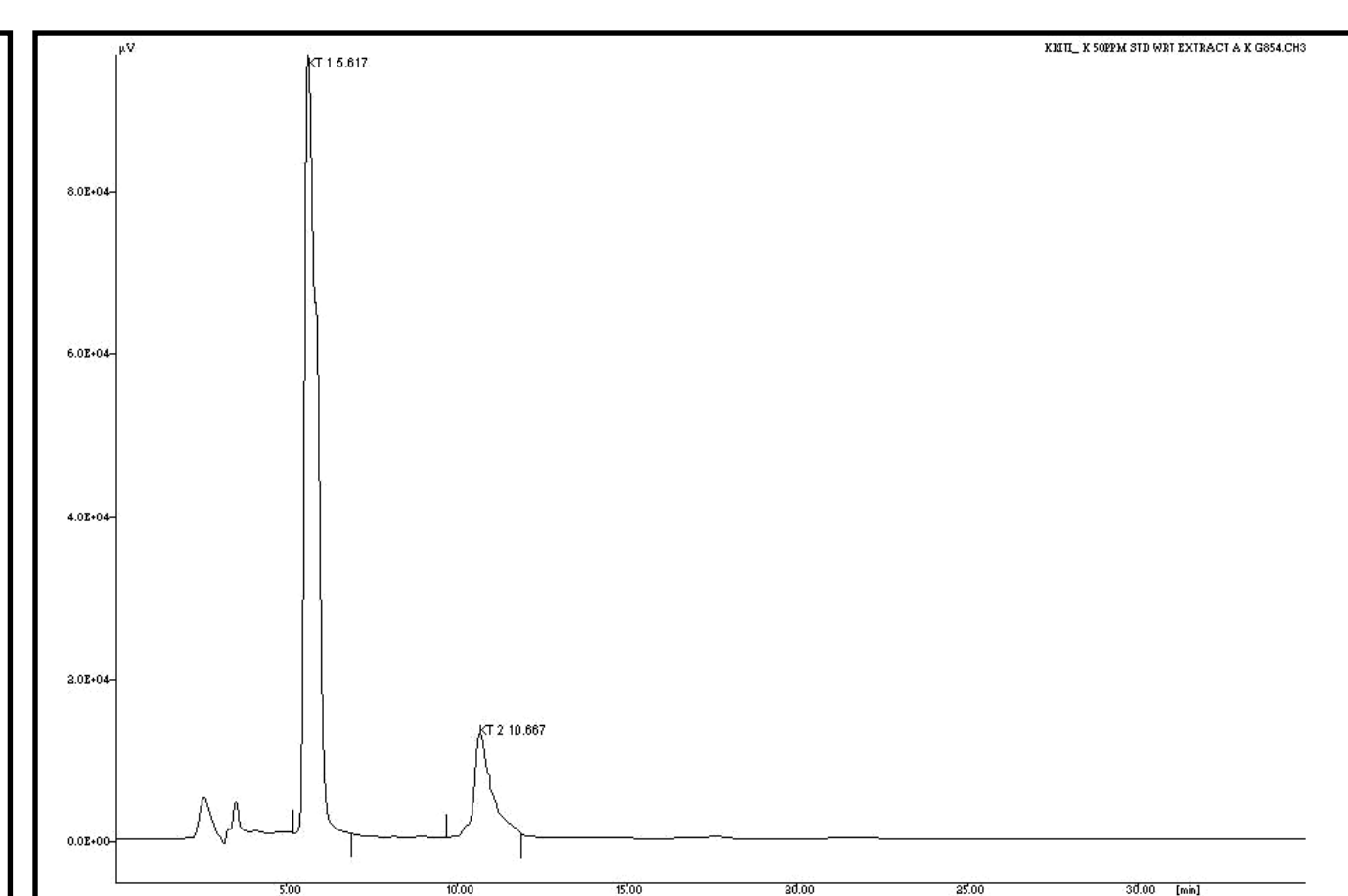
Sr. no.	Chromatographic conditions	Value of Chromatographic condition
1.	Diluent	ACN: Water (25: 75); pH- 3.45 (0.1% Ortho Phosphoric acid)
2.	Column	C18 PUROSPHERE STAR Hyber 250 × 4.5 mm i.d., with 5 µm particle size
3.	Column temperature	25°C
4.	Sample temperature	25°C
5.	Mobile phase	ACN: Water (25: 75); pH- 3.45 (0.1% Ortho Phosphoric acid)
6.	Flow rate	1.0 mL/min
7.	Detection wavelength	254 nm
8.	Injection volume	20 µL
9.	Blank	Methanol



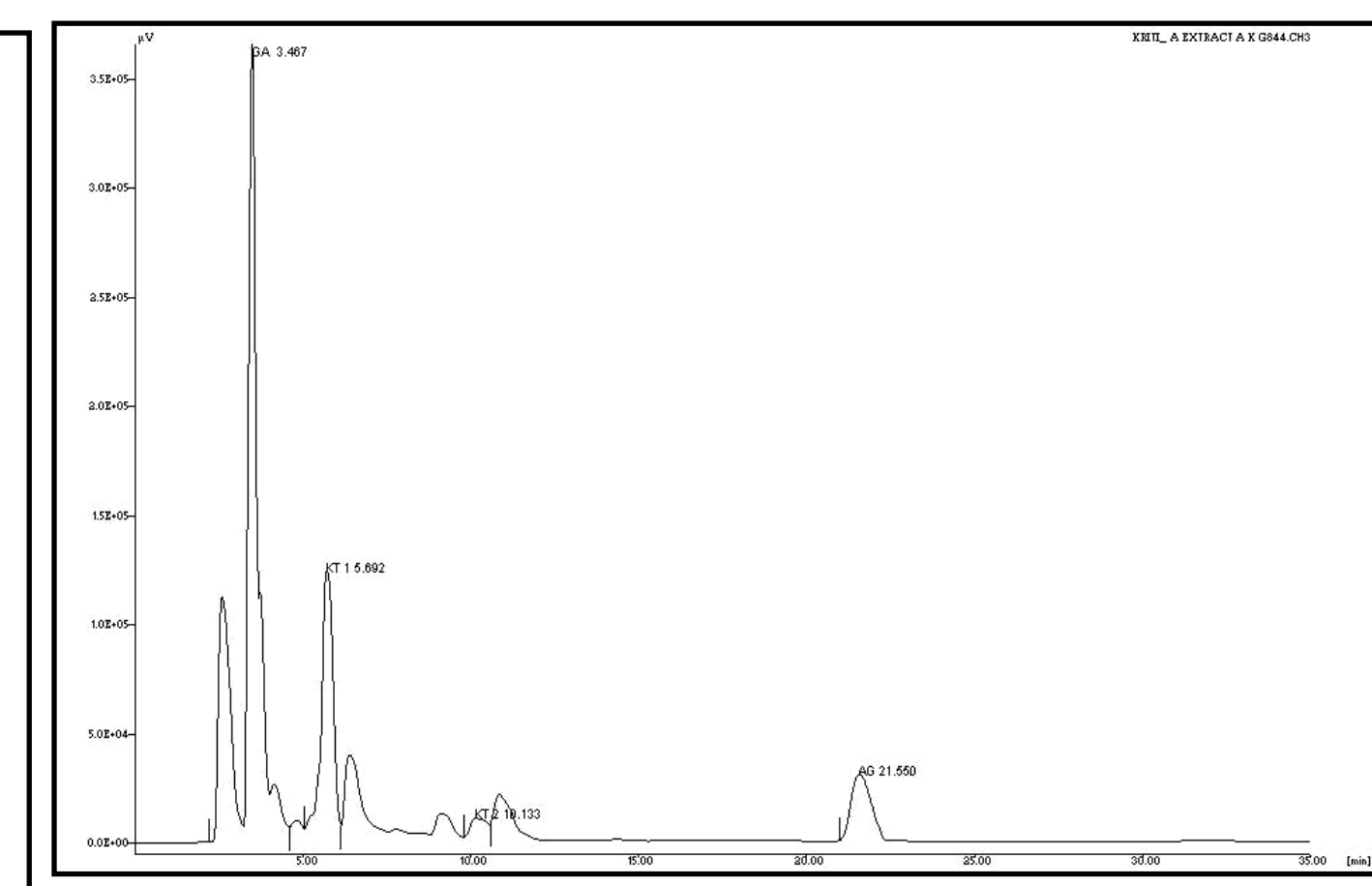
HPLC Chromatogram of Andrographolide (Rt:20.98 min)



HPLC Chromatogram of Gallic acid (Rt: 3.30min)



HPLC Chromatogram of Kutkin (Rt: 5.61min)



Chr. of Ethyl acetate extract of Formulation [Andrographolide (Rt: 21.55 min), Gallic acid (Rt: 3.46 min), and Kutkin (Rt: 5.69 min)]

AG, GA and KT obtained from the RP- HPLC Chromatogram of the Formulation Extract

Sr. No.	Name	Rt (min)	Area (µV.sec)	Theoretical Plates	Assymetry
1.	Andrographolide	21.550	1735538	1462	1.67
2.	Gallic acid	3.467	8664849	1780	1.24
3.	Kutkin	5.692	2461420	1906	1.07

Quantity of AG, GA and KT in the Ethyl acetate extract and in the formulation, HEPASAVE, estimated by HPLC

Sr. no.	Name of Biomarker	Amount estimated in the Ethyl acetate extract (% w/v)	Amount estimated in the Formulation (%w/v)
1.	Andrographolide	3.354	0.56
2.	Gallic acid	3.28	0.554
3.	Kutkin K1	1.8	0.44

CONCLUSION-HEPASAVE contains various Biomarkers as its constituents, including Andrographolide, Gallic acid and Kutkin, which have been estimated for their amount in the Polyherbal formulation. The amount of Andrographolide in the sample was found to be greater than that of Gallic acid and Kutkin, which supports the fact that HEPASAVE is a powerful hepatoprotective as AG has been reported in many studies for its hepatoprotective activity, followed by Gallic acid, which is a chemical constituent of almost all the renowned and reported antioxidant medicinal plants, which helps HEPASAVE to protect the liver against toxicity and inflammation. The Future scope to the study includes the HPLC method employed in the current study can be validated as per Official guidelines, i.e ICH guidelines or U.S. Pharmacopoeia, for the estimation of Andrographolide, Gallic acid and Kutkin in Hepasave Tonic, or a new method can also be validated for the same. The current HPLC methods can be further modified to develop a simultaneous estimation method for the estimation of Andrographolide, Gallic acid, Kutkin as well as Vasicine, which can be applied to other formulations containing Andrographolide, Gallic acid, Kutkin and Vasicine.

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