Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

STANDARDIZATION OF AN AYURVEDIC FORMULATION "JATYADI GHRITA" BY RP-HPLC

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Received: 09 Mar 2013, Revised and Accepted: 15 Apr 2013

ABSTRACT

In traditional Indian medicinal treatise there are several Ayurvedic formulations mentioned which have been claimed as potential wound healing agents like Madhu Ghrita and Jatyadi ghrita. Jatyadi ghrita (JG) is a medicated ghee formulation popularly used in the treatment of various topical wounds. It is a polyherbal preparation containing eleven ingredients. Though JG has its composition recorded in ancient Ayurvedic texts, there have been minimal attempts to standardize its. In this research paper an attempt has been made to develop standardization method for some of the ingredients of Jatyadi Ghrita.Quantitative estimation was done by reported RP-HPLC methods using markers glycyrrhetinic acid, ursolic acid, karanjin, curcumine, berberine and kutkin which are major constituents of formulation. A standard laboratory reference sample of Jatyadi Ghrita and four marketed samples were evaluated as per methods. Data has been provided to demonstrate applicability of the methods to standardization of Jatyadi Ghrita.

Keywords: Jatyadi Ghrita, RP-HPLC, Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine, Kutkin.

INTRODUCTION

Herbal medicines are generally available as a mixture of more than one plant constituents. It is important to quantify the maximum possible numbers of markers in such herbal formulations through which the formulation may be assessed.Jatyadi Ghrita, a poly herbal preparation containing eleven ingredients, was found in authentic books like Ashtanga Hrudaya Uttarasthana 25/67 and used as a topical product prescribed for the treatment of all kind of wounds of the body. [1]As it is difficult to estimate each and every ingredient for its chemical constituents, few of the main ingredients of Jatyadi Ghrita have been identified and standardized. The formulation describes the presence of *Glycyrrhiza glabra, Jasminum grandiflorum, Pongamia pinnata, Curcuma longa, Berberis aristata* and *Picrorhiza kurroa* the principal constituents of which are Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumine, Berberine and Kutkin respectively[2-7]. Separate HPLC methods were available for analysis of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumine, Berberine and Kutkin[8-11].

MATERIALS AND METHODS

Selection and authentication of medicinal plants

All the plants, viz. *Jasmine grandiflorum* Linn.(leaf), *Azadirachta indica* A.Juss.(leaf), *Berberis aristata* D.C.(stem), *Curcuma longa* Linn.(Rhizome), *Picrorhiza kurroa* Royle.(Root), *Trichosanthes dioica* Roxb.(leaf), *Pongamia pinnata* Linn.(seed), *Hemidesmus indicus* Linn.(Root), *Glycyrrhiza glabra* Linn.(Root), *Rubia cordifolia* Linn.(Root)and *Vetiveria zizanioids* Linn. (Root) were collected from Mankarnika Aushadhlay, Pune. All these plants were authenticated by Botany group, Plant Sciences Division, Agharkar Research Institute, (No.3/187/2010/Adm. 367), Pune.

Preparation of Jatyadi ghrita

In laboratory formulation of Jatyadi ghrita was prepared as per Ayurvedic Formulary of India[1]. All the plant parts, viz. *Jasminum grandiflorum, Azadirachta indica, Berberis aristata, Curcuma longa, Picrorrhiza kurroa, Trichosanthes dioica, Pongamia pinnata, Hemidesmus indicus,Glycyrrhiza glabra, Rubia cordifolia,* and *Vetiveria zizanioids* were dried in oven at 45^0 c until they were free from moisture. They were then powdered and sieved through.60# and stored in air tight container for further use. All the drugs were taken in the equal amount that is 1.47 gm for 100gm. Decoction of each drug and 1.47 gm of copper sulphate in 10 ml water were mixed together and passed through muslin cloth. Bees wax 1.47 gm and 76.8 gm Cow's ghee was heated on water bath at 100^0C. The decoction and copper sulphate solution was mixed in it in melted condition with propeller mixer at 100 RPM for 15 min. A reference sample of Jatyadi Ghrita (JGL) prepared in the laboratory and four marketed formulations JGAV, JGA, JGR and JGN were chosen for the standardization.

HPLC analysis:

HPLC analysis was performed by using Waters (2487) with Empowers-3 software with different chromatographic conditions for different constituents as mentioned in Table 1.

Table 1: Chromatographic Conditions

Chromato-graphic Parameter → Constituents ↓	Column	Mobile Phase	Flow Rate (ml/min)	Wavelength (nm)	Run Time (min)
Glycyrrhetinic acid	Hypercil BDS C_{18},250x4.6 mm,5µ	A] 1% Acetic acid in Water. B] 1% Acetic acid in Acetonitrile.	1.0	249	60
Ursolic acid	Hypercil BDS C_{18},250x4.6 mm,5µ	Methanol: Phosphate buffer (pH-3.0) (90:10v/v)	0.8	203	20
Karanjin	Microbondapack 300x3.9mm,10µ	Methanol: Water (80:20v/v)	1.0	217	15
Curcumin	Hypercil BDS C_{18},250x4.6 mm,5µ	Acetonitrile: Water (10:90v/v)	1.0	423	8
Berberine	Hypercil BDS C_{18},250x4.6 mm,5µ	Acetonitrile: Water (10:90v/v)	0.6	265	20
Kutkin	Inertsil ODS-3, C_{18},250x4.6 mm,5µ	0.1%Orthophosphoric acid in Water: Acetonitrile (75:25v/v	1.0	265	15

Preparation of standard solutions

Stock solutions of all markers prepared separately in methanol. Final concentration was made up by further diluting with methanol which was mentioned in Table 2.

Preparation of sample solutions

Accurately weighed 5 gm formulations were extracted with equal amount of hexane and methanol (20 ml each) by means of separating funnel. It was shaken vigorously and allowed to stand for 5 min for separating the two layers. Methanolic layer was again treated with 10 ml hexane till it was free from fat. Hexane layers were discarded. The volume was made with methanol up to 25 ml by using volumetric flask and filtered through 0.22 micron filterpaper.This preparation was used as stock solution. Final concentration was made up by further diluting with methanol which was mentioned in Table 2.

Comparison was done between reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN.

Table 2: Concentration of Standard and Sample solutions

Analysed Constituents	Glycyrrhetinic acid	Ursolic acid	Karanjin	Curcumin	Berberine	Kutkin
Standard solution	150 (µg/ml)	60 (µg/ml)	60 (µg/ml)	600 (µg/ml)	600 (µg/ml)	60 (µg/ml)
Test solution	150 (µg/ml)	60 (µg/ml)	60 (µg/ml)	600 (µg/ml)	600 (µg/ml)	60 (µg/ml)

Inject the prepared standard and sample solutions and quantitative analysis was carried out by comparison with respective peak areas.

RESULTS AND DISCUSSIONS

HPLC finger printing profile:

HPLC study of methanolic extracts of the in-house formulation and marketed formulations were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. Inject the prepared standard and sample solutions and quantitative analysis was carried out by comparison with respective peak areas. The HPLC fingerprint profiles of the formulations are presented in Fig 1-5.

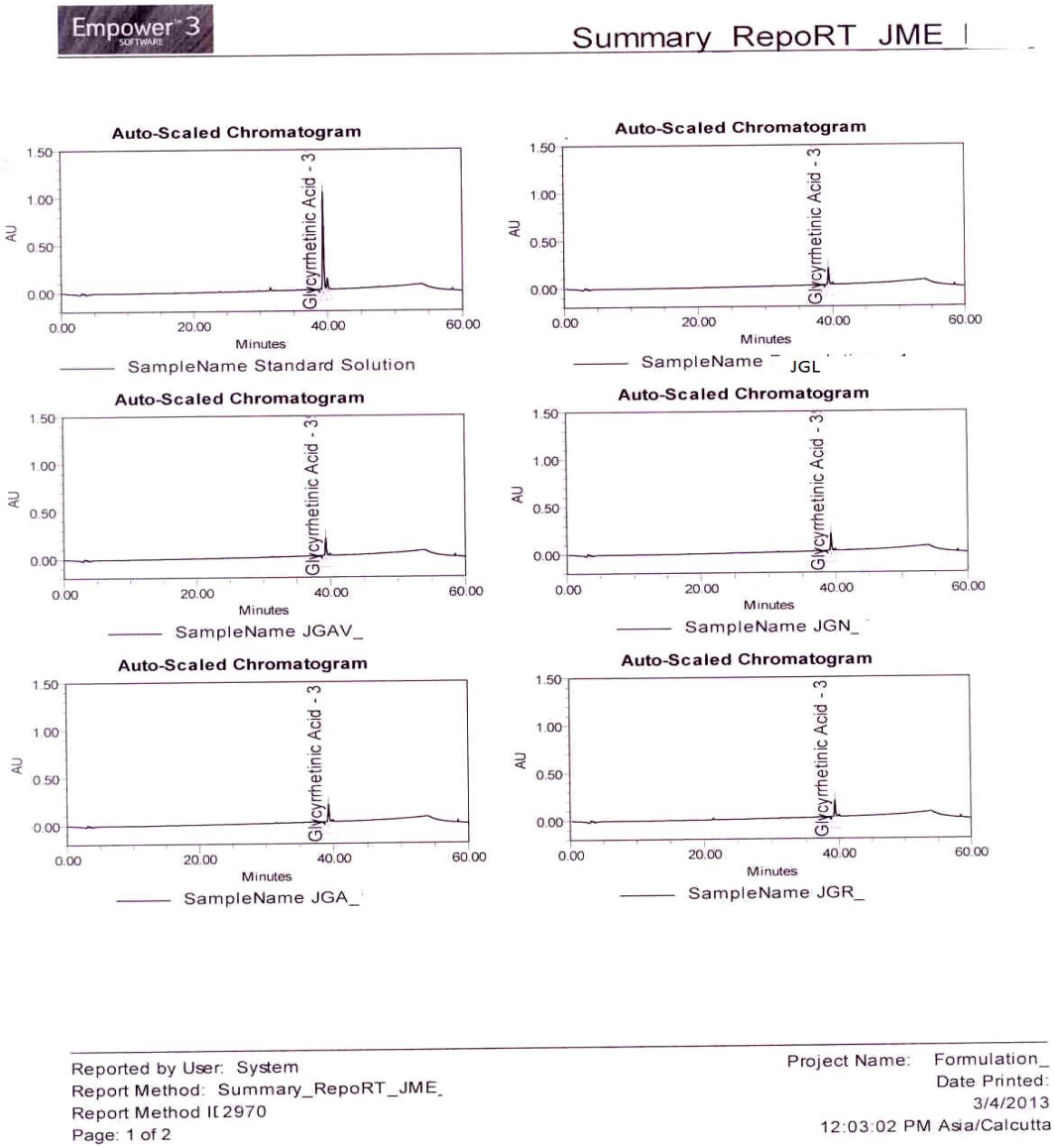

Fig. 1: HPLC Fingerprinting of Standard solutions and Sample solution of Glycyrrhetinic acid.

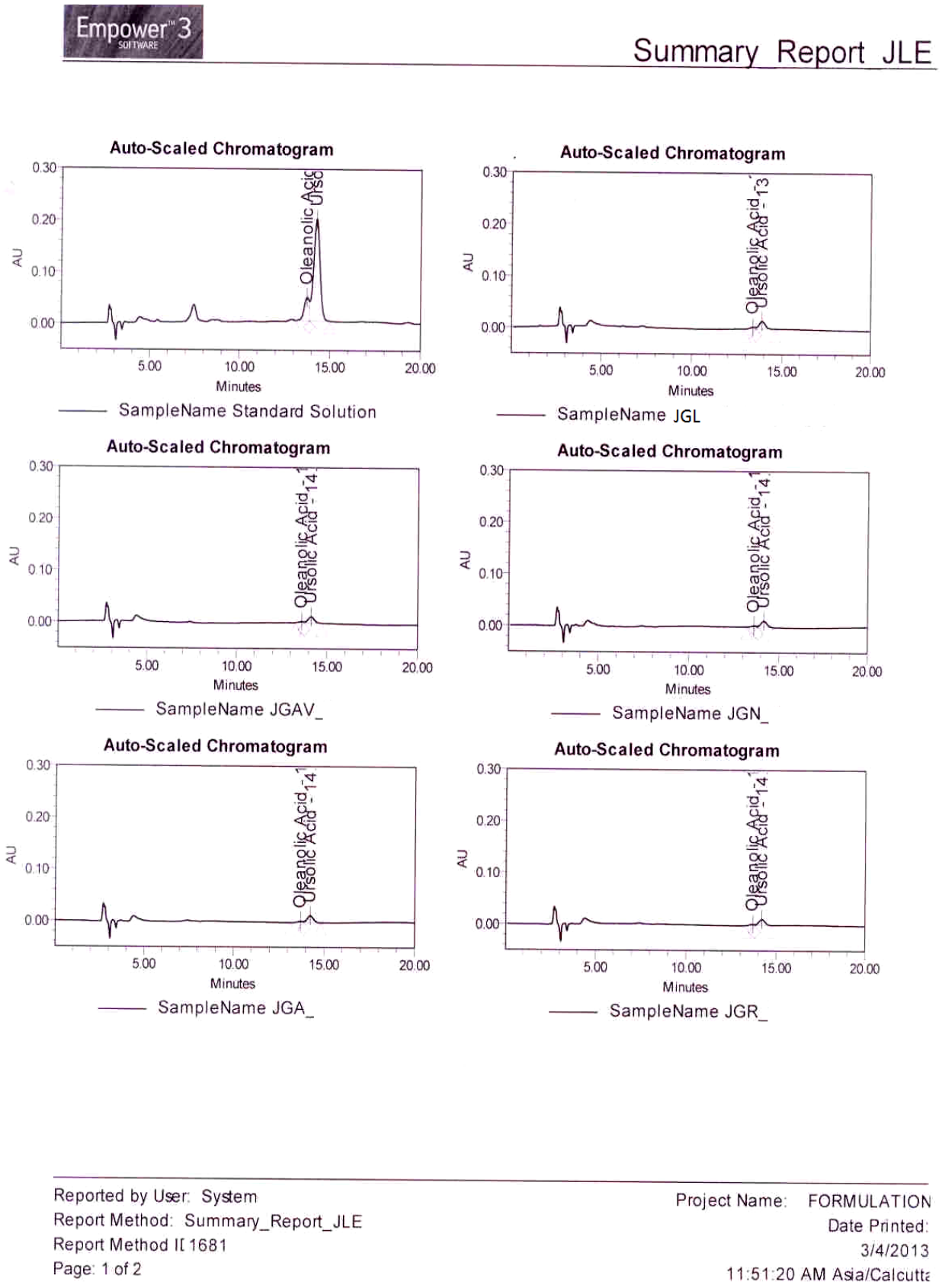

Fig. 2: HPLC Fingerprinting of Standard solutions and Sample solution of Ursolic acid.

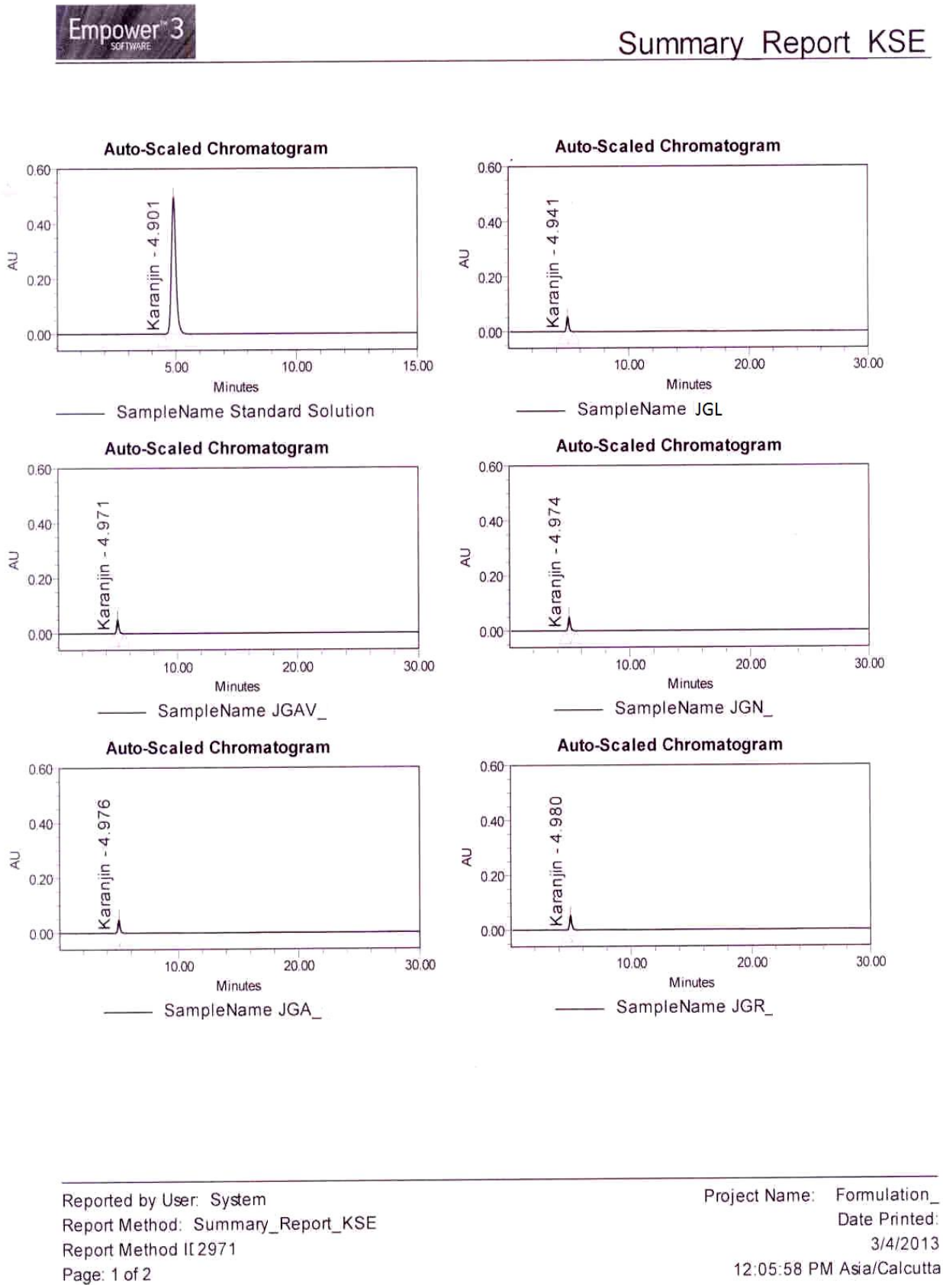

Fig. 3: HPLC Fingerprinting of Standard solutions and Sample solution of Karanjin.

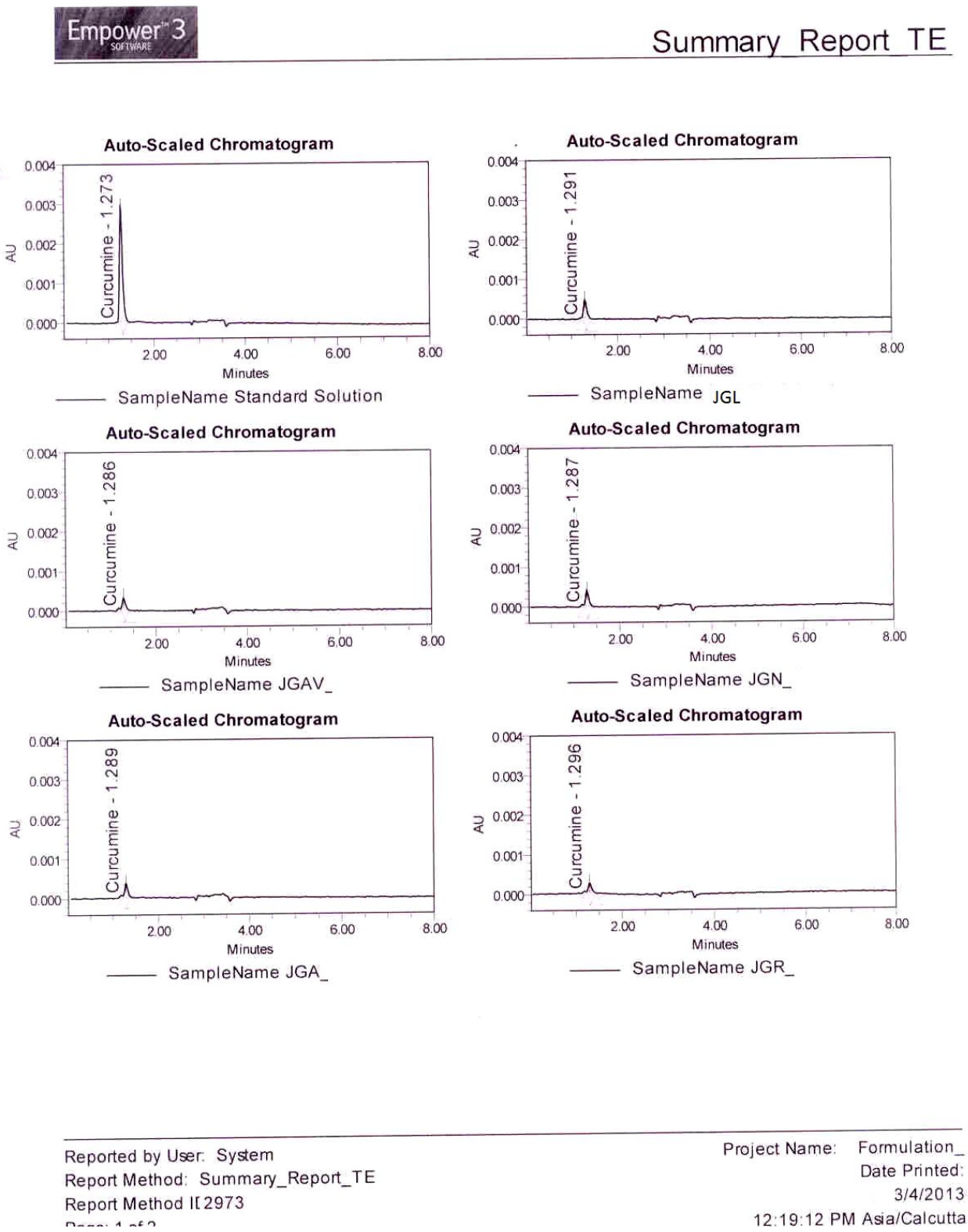

Fig. 4: HPLC Fingerprinting of Standard solutions and Sample solution of Curcumin.

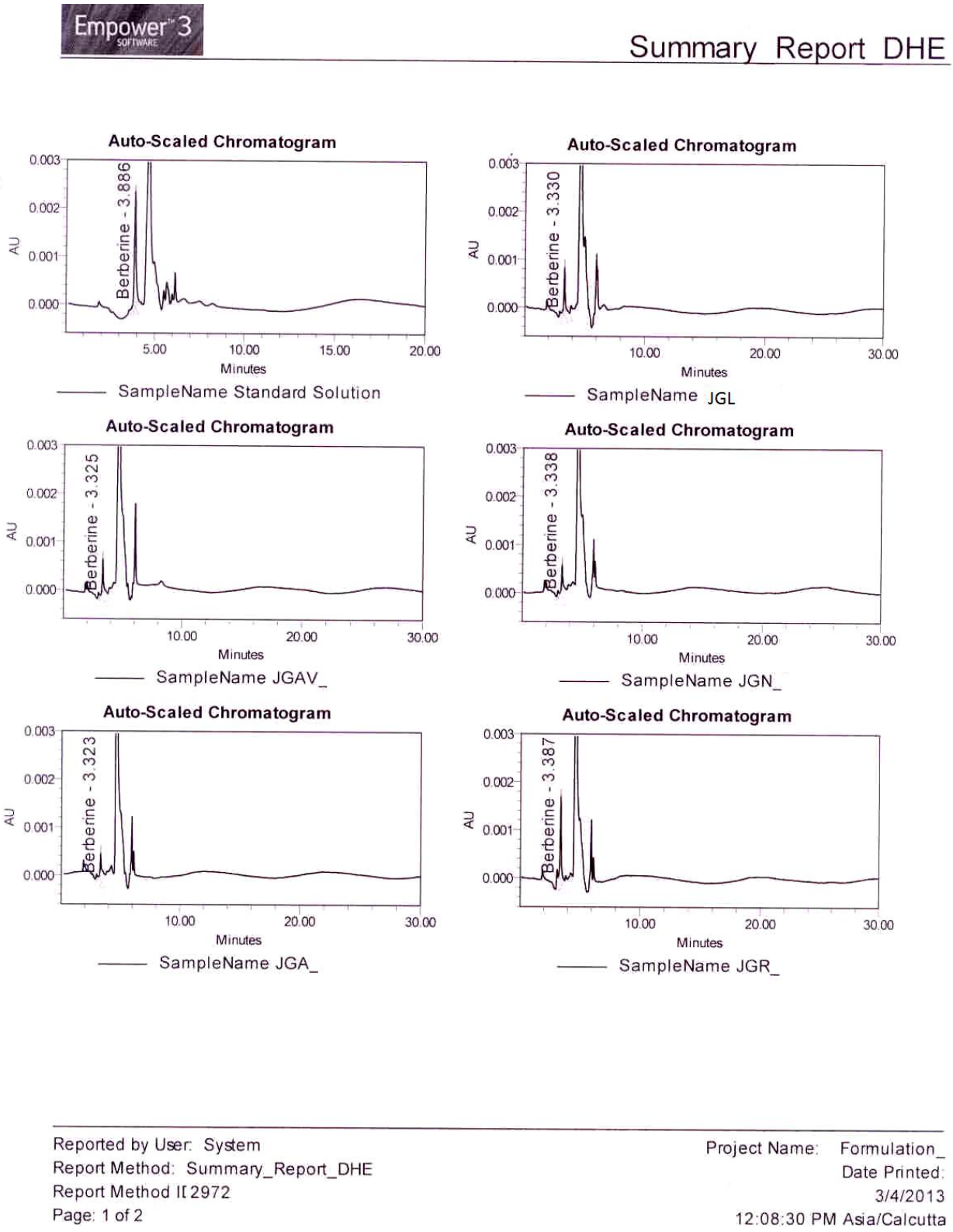

Fig. 5: HPLC Fingerprinting of Standard solutions and Sample solution of Berberine.

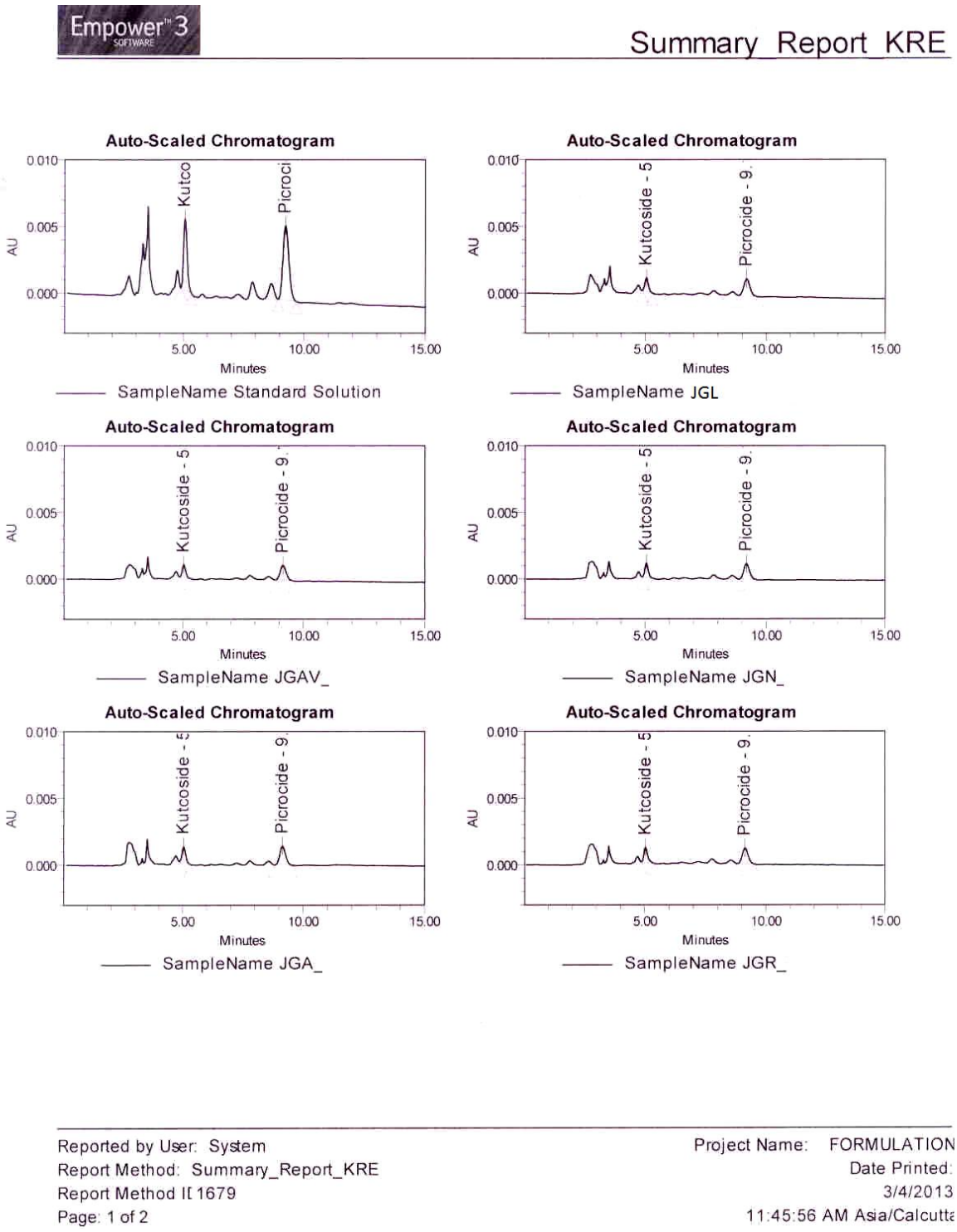

Fig. 6: HPLC Fingerprinting of Standard solutions and Sample solution of Kutkin.

The percentage of concentration for each constituents were found in the reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN mentioned in Table 3.

Table 3: Concentration of Constituents

S. No.	Formulations → Constituents	Percentage Concentration				
		JGL	JGAV	JGA	JGR	JGN
1	Glycyrrhetinic acid	1.75	1.38	1.3	1.46	1.33
2	Ursolic acid	1.46	1.42	1.38	1.32	1.3
3	Karanjin	1.23	1.22	1.15	1.17	1.16
4	Curcumin	1	1.03	1.02	0.93	0.96
5	Berberine	1.01	1.1	1.06	0.9	1.07
6	kutkin	0.89	0.82	0.45	0.50	0.54

There was no more difference in the concentration of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumine, Berberine and Kutkin in laboratory and marketed formulations.

HPLC study of formulations was calculated the presence ingredient. The marker compound Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin were estimated by HPLC in Jatyadi ghrita samples. The chromatogram shows the presence of these components in the formulation. HPLC fingerprint profile of the Jatyadi ghrita formulations are depicted in figure 1-5 indicates the presence of all the ingredients in proportional quantity in the formulations.

CONCLUSION

This study was focused on quantitative estimation of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumine, Berberine and Kutkin which are principal constituents of Jatyadi Ghrita by using modern methods of analysis. From this research paper it can be concluded that there was no more difference in the concentration of Glycyrrhetinic acid, ursolic acid, karanjin, curcumin, berberine and kutkin in laboratory reference and marketed formulations, which will go a long way for prescribing a dependable standard to these preparations.

REFERENCES

1. Anonyms. The ayurvedic formulary of India. 2nd ed. Ministry of health and family welfare. Dept. of Indian systems of medicine and homeopathy:New delhi; Part-I: p.84.
2. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy.43rd ed. Nirali prakashan; 2009. p.52-56.
3. Sadhu SK, Khan MS, Ohtsuki T, et al. Secoiridoid components from Jasminum grandiflorum. Phytochemistry 2007; 68(13):1718-21.
4. Tanaka T, Iinuma M, et al. Flavonoids in root bark of Pongamia pinnata. Phytochemistry. 1992; 31:993-8.
5. Lal B, Kapoor AK, Asthana OP. Efficacy of curcumin in the management of chronic anterior uveitis. Phytotherapy Research 1999; 13:318-322.
6. Lect EJ, Elango V, Hussain FS, et al. Secobisbenzlisoquinoline or simple isoquinoline dimmer. Heterocycle 1983; 20(3):p.425-9.
7. Weinges K, Kloss P, Henkels WD. Natural products from medicinal plants. XVII. picroside-II, a new 6-vanilloylcatapol from *Picrorhiza kuroa* Royle and Benth. Justus Liebigs Ann Chem. 1972; 759:173-182.
8. Willard HH, Merritt LL. Instrumental methods of analysis. 7th ed. CBS publishers and distributors Pvt. Ltd. 1986; p.159, 287,614.
9. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Himalaya publishing house. 2007; p.2.172, 2.78, 2.635-2.638.
10. Skoog DA, Holler FJ. Instrumental analysis. Cengage Learning.2009; p.411, 477,893.
11. Sethi PD. Quantitive analysis of drugs in pharmaceutical formulations. 3rd ed, CBS publishers and distributors pvt.Ltd.1997; p.51.