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Development of Analytical Method for Quality Control of Polyherbal Formulation

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Abstract: *The objective of these research work was to develop a simple, rapid and reliable HPTLC method for standardization for antidiabetic formulation. Development of method carried out by using Gymnemic acid and Berberine as bioactive marker reported to have an antidiabetic activity. Chromatographic analysis was performed using silica gel 60 F254 TLC plate, CAMAG Linomat 5 applicator and solvent system consisting of toluene: ethyle acetate: methanol: formic acid (6:2:1.5:0.5) and Ccl4: methanol: acetic acid (4:1:0.5). Densitometry scanning performed under reflectance absorbance mode at 360 nm to identify the spot. The Rf value of gymnemic acid and Berberine was found to be 0.03 and 0.11 respectively. No analytical method has been reported so far associated with polyherbal formulation associated with polyherbal formulation (Mahantak vati) containing Gymnemic acid and Berberine on antidiabetic activity.*

Keywords: Polyherbal formulation, marker compounds, HPTLC method development.

I. INTRODUCTION

India is known for its traditional medicinal systems— Ayurveda, Siddha, and Unani. Medical systems are found mentioned even in the ancient Vedas and other scriptures. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is "science of life," because ancient Indian system of health care focused on views of man and his illness. It has been pointed out that the positive health means metabolically well balanced human beings. Ayurveda is also called the "science of longevity" because it offers a complete system to live a long healthy life. It offers programs to rejuvenate the body through diet and nutrition. It offers treatment methods to cure many common diseases such as food allergies, which have few modern treatments. However, one should be aware that Ayurvedic nutrition is not a "magic bullet" system but requires the full participation of the patient to succeed. It is an interactive system that is user-friendly and educational. It teaches the patient to become responsible and self-empowered. Ayurveda is not a nutritional system for those seeking an escape or excuse to further abuse their body or mind. It is a system for empowerment, a system of freedom, and long life. Hence, the modern food habits are affecting the balanced nutrition.

1.1 Advantages of Herbal Medicine

- They have large amount of use.
- They have better patient tolerance as well as acceptance.
- The medicinal plants have renewable source of cheaper medicines.
- Improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology.
- Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
- They are cheap in cost.
- They are not harmful.
- They are more effective than any synthetic drug.
- Throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form as well as a pure chemical upon which modern medicines are constructed.

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II. MATERIAL USED FOR THE STUDY

Formulation chosen for the study

2.1 Mahantak Vati:

Mahantak vati composed of eighteen crude drugs Gymnema sylvestre, Syzygium cumini, Pterocorpous marsupium, Barberies aristate, Trigonella foenum graecum,Triphala triturated in Gomutra , Pongamia glabra, Asphaltum, Azadirachta indica,Tribulus terrestries ,Tinospora cardifolia, Andrographis Paniculata ,calcined zinc,Abhrak bhasma, Akik Ashti, Pottasium sorbate,Sodium benzoate. Mainly prescribed in Ayurvedic system of medicine as diabeties .

TABEL: Ingredients of Mahantak Vati

SR NO	CRUDE DRUG	BOTANICAL NAME	QUANTITY
1	Mesha Shringi	Gymnema sylvestre	120 mg
2	Jambu beeja	Syzygium cumini	120 mg
3	Bijaka	Pterocorpus marsupium	100 mg
4	Daruharidra	Berberies Aristata	100 mg
5	Methi	Trigonella foenum gracium	80 mg
6	Gomutra Bhavita		q.s
7	Triphala		80 mg
8	Karanja	Pongamia glabra	60 mg
9	Shilajeet	Asphaltum	60 mg
10	Nimba	Azadirachta indica	40 mg
11	Gokshura	Tribullus Terrestries	40 mg
12	Bhunimba	Andrographis peniculata	40 mg
13	Yasad Bhasma	Calcined zinc	40 mg
14	Abhrak Bhasma	Calcined mica	40 mg
15	Akik Pishti		40 mg
16	Pottasium sorbate		2.4 mg
17	Sodium benzoate		1.2 mg

III. EXPERIMENTAL WORK

3.1 Organoleptic Evalution

- Color
- Odour
- Taste
- Texture
- Width
- Length
- Hardness

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3.2 Extractive Values

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.

- **Water Soluble Extractive Value:** 5 gm of powder macerated with 100 ml water for 24 hrs and shake after 6 hrs and stand for 18 hrs after that filtered that and after 25 ml filtrate evaporated and dry in petri dish at 105°c after that weighed it and calculated.
- **Alcohole Soluble Extractive Value:** 5 gm powder macerated with 100 ml 70% ethanol for 24 hrs and shake after 6 hrs and stand for 18 hrs after that filtered that.25 ml filtrate evaporated and dry in petri dish for 105°c after that weighed it and calculated.
- **Ether Soluble Extractive Value:** 5 gm of powder extracted with ethyl ether in Soxhlet extract for 20 hrs evaporate it at 105°c.Weighed it and calculated.

3.3 Physico-Chemical Properties

A. Moisture Content

Moisture content affects the physical, chemical and microbiological properties of pharmaceutical finished dosage forms. In direct compression process, high and extra low moisture content could be it affects the hardness of tablet. Grind the tablet and make the powder. These powder pass through 40 no mesh and after 120 no mesh. 10 gm powder put into evaporating dish and powder dried at 105 °c for 5 hrs and weigh.

B. Bulk Density

Bulk density of tablet is ratio of a tablets (Mass) to its bulk volume (volume) Measurement with graduated cylinder.

C. Tapped Density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample.

D. Carr's Index

The Carr's index is frequently used in pharmaceutics as an indication of the compresiblity of a powder. In a free-flowing powder, the bulk density and tapped density would be close in value, therefore, the Carr index would be small. On the other hand, in a poor-flowing powder where there are greater interparticle interactions, the difference between the bulk and tapped density observed would be greater, therefore, the Carr index would be larger.

E. Hausner's Ratio

The ratio of tapped density W/V_{50} to fluffy density (W/V_0 g/ml) is known as the Hausner ratio. A good flow is indicated by a Hausner ratio greater than 1.25, and a poor flow may have a value of 1.5

Hausner ratio	Flow character
1.00-1.11	Excellent
1.12-1.18	Good
1.19-1.25	Fair
1.26-1.34	Passable
1.35-1.45	Poor
1.46-1.59	Very poor
>1.60	Very, very poor

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F. Determination of PH of Drug

pH is a critical factor for all those medications prepared as aqueous liquid forms, because it has an impact on the solubility of the molecule, determining the stability of medications, the biological tolerability of the formulation, and the activity of the molecule.

G. Ash Value

Ash values are helpful to determine the quality as well as purity of a crude drug, especially when the drug is present in powdered form. The object of ashing crude drugs is to remove the traces of organic matter which may be interferes in an analytical determination.

H. Total Ash

2 gm of drug put in that crucible heat that at 450°c while it not become white color. After cool that and weigh .and calculate.

I. Acid Insoluble Ash

Take a ash and boil them and put with 2 M HCL (25 ML) for 5 min after filter that and wash with hot water. after cool that and weigh and calculate.

J. Water Soluble Ash

Take a Ash and boil that with 25 ml water for 5 min and after filter that and wash with hot water. Cool. weigh that on weighing balance and calculated.

3.4 HPTLC (High Performance Thin Layer Chromatography)

Stationary Phase

A simple and precise HPTLC method were developed for simultaneous estimation of two antidiabetic herbal drug i.e Gymenic acid and barberine. The method was tailored to analyse both drugs in commercial dosage form (tablet). Chromatographic separation was performed over silica gel precoated TLC plates (60 F254,10cm X 10 cm, E.MERCK KGaA) via linear ascending technique using toluene : ethyle acetate :methanol: formic acid (6.2:1.5:0.5) for Gymnemic acid . and Ccl4: methanol: acetic acid (4:1:0.5) for Barberine .

Mobile Phase

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are based on their diverse selectivity properties. Mobile phase for Gymnemic acid is Toulene : Ethyle acetate : methanol : formic acid
(6:2 :1.5:0.5) and for Barberine Ccl4 : methanol : acetic acid (4:1:0.5)

Prewashing

Plates are handled at the upper edge to avoid contamination. Plates are used without pretreatment unless chromatography produces impurity fronts due to contamination of the plate. For reproducibility and quantitative analysis, layers are often prewashed using 20 ml methanol. The methanol is used as a prewashing solvent, a mixture of methanol and even mobile phase is used, per trough in a 20 × 10 cm twintrough chamber (TTC). four 10 × 10 cm plates can be developed back-to-back in each trough of the TTC. Remove the plate and dry it for 20 min in a clean drying oven at 120˚C. Equilibrate plate with laboratory atmosphere (temperature, relative humidity) in a suitable container providing protection from dust and fumes.

Preparation of Plate

TLC plates can be made with suitable apparatus. such layers do not adhere well to the glass support. Precoated plates use small quantities of very high molecular weight polymer as binder overcomes most limitations of a homemade layer.Precoated layers are reasonably abrasion resistant, very uniform in layer thickness, reproducible, preactivated, and ready to use. They are available with glass or aluminum or polyester support. Aluminum foil plates are less expensive to buy, cheaper, can be cut, and therefore easy to carry around or transport or mail. Glass plates are the best for highest quality of results. Most often, layers containing a fluorescent indicator F 254 are used. This enables the visualization of

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samples in a UV cabinet very simply, instantly, and in a nondestructive manner. used size of plates in TLC is 10 × 10 cm is widespread.

Steps Involved in HPTLC

1. Sample Application
2. Chromatogram Development
3. Derivatization
4. Evaluation: Detection
5. Evaluation: Documentation

Sample Application

The sample application plays an important role and its techniques are spot application and Spraying – on samples. Sample application is the first step in chromatography and it affects the quality of the result at the end of the process. The choice of the application technique and the device depend on the requirements. Spot wise sample application using a fixed volume capillary is the simplest way. Sample volumes of 0.5 to 5 µL can be applied as spots onto conventional layers without drying, on HPTLC layers it is up to 1 µL per spot. Spraying-on samples as narrow bands of larger volumes is the best resolution that can be achieved with the chromatographic system selected. Large sample volumes or samples with high matrix content can be sprayed-on in the form of rectangles and focused into narrow bands.

Chromatogram Development

In this technique in addition to stationary and mobile phases, a gas phase is present. This gas phase can significantly influence the result of the separation.

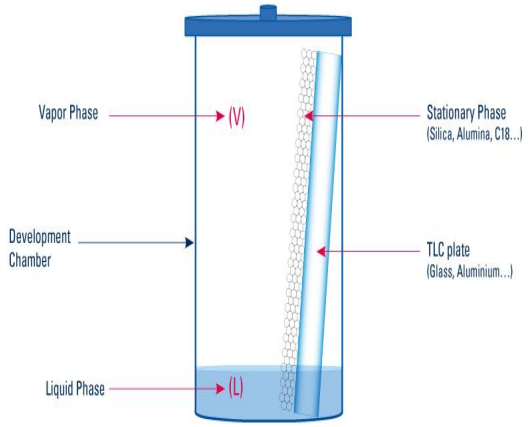

Diagram: Chromatographic Process

The lower end of the plate should be immersed and act by capillary action the developing solvent moves up the layer until the desired distance is reached and chromatography is stopped.

Derivatizer

The Derivatizer presents an automated spraying device which sets a new standard of reproducibility in the reagent transfer onto TLC plates by employing a unique "micro droplet" spraying technology (patent pending). The Derivatizer ensures homogeneous and reproducible application of all common reagents. To meet the diverging physicochemical properties of the different reagents, e.g. acidity or viscosity, four different color-coded spray nozzles are employed, and the user can select from six spraying level .

Detection

The chromatogram is evaluated under white or ultraviolet light. Options range from visual inspection of electronic images to quantitative determinations using video or densitometry.

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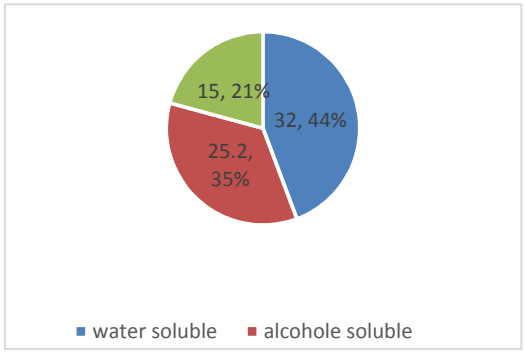
IV. RESULT AND DISCUSSIO

APPEARANCE

1. **Color:** Black green
2. **Odor:** Musty
3. **Taste:** Slightly bitter
4. **Texture:** Oval and crystaline
5. **Width:** 96 mm
6. **Length:** 48 mm
7. **Hardness:** 4 kg

EXTRACTIVE VALUES



MOISTURE CONTENT: 8 % W/W

BULK DENSITY, TAPPED DENSITY, HAUSNER RATIO:

1. **BULK DENSITY:** 0.5
2. **TAPPED ENSITY:** 0.6
3. **HAUSNER RATIO:** 1.3

Ph OF DRUG:

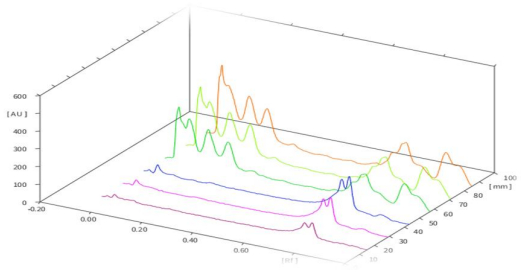
1. **Ph OF 1% w/v:** 5.3
2. **Ph OF 10 % w/v:** 5

ASH VALUE:

1. **TOTAL ASH:** 9 %w/w
2. **WATER SOLUBLE ASH:** 2.8 %w/w
3. **ACID INSOLUBLE ASH:** 1.8 %w/w

HPTLC

GYMNEMIC ACID:



All tracks at wavelength (GYMNEMIC ACID)

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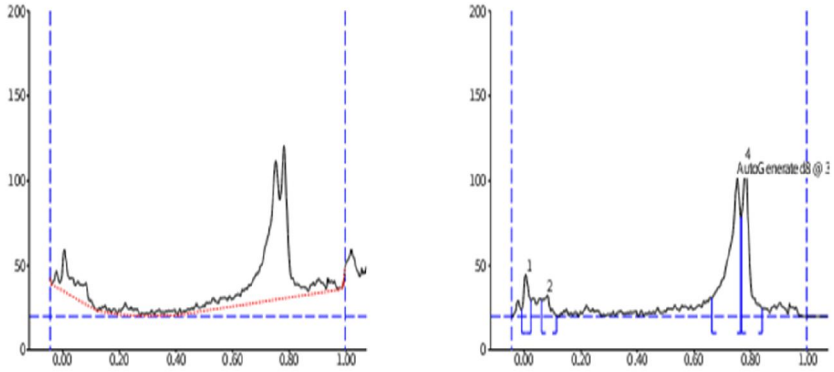

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2	0.06	9.5	0.08	12.9	6.15	0.11	0.4	252.7	5.16	unknown
3	0.66	10.9	0.75	81,8	39.01	0.77	58.5	2635.4	53.79	Auto gen
4	0.77	59.0	0.78	90.2	43.03	0.84	4.7	1678.2	34.25	unknown

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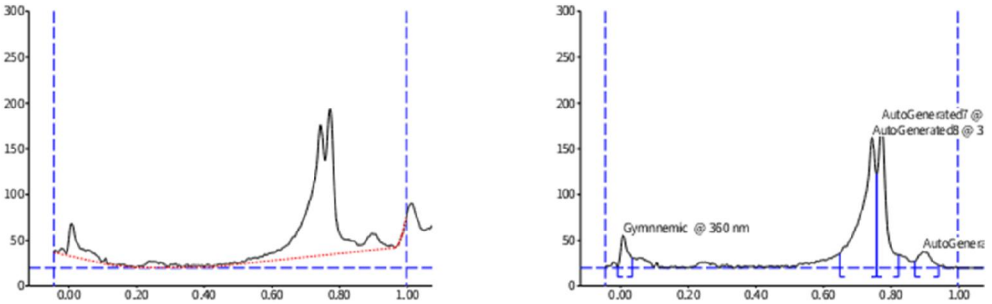

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1	-0.01	1.4	0.01	35.6	10.01	0.03	10.5	577.9	6.64	GYMNEMIC
2	0.65	15.5	0.75	142.8	40.13	0.76	103.2	4577.4	52.57	AUTO
3	0.76	103.4	0.77	159.1	44.71	0.82	13.9	3000.0	34.45	AUTO
4	0.87	7.5	0.90	18.3	5.15	0.94	2.9	552.4	6.34	AUTO

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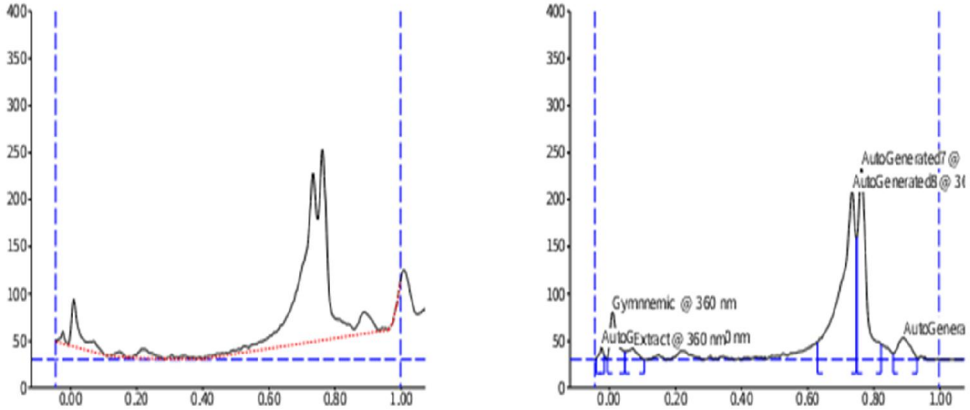

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1	-0.04	1.4	-0.02	13.5	2.80	-0.01	4.4	122.3	1.02	
2	-0.01	1.6	0.01	50.5	10.48	0.05	8.5	797.1	6.65	Gymnemic
3	0.05	8.5	0.07	12.1	2.51	0.11	0.2	289.7	2.42	extract
4	0.63	18.7	0.74	179.0	37.19	0.75	129.1	6082.6	50.77	
5	0.75	130.3	0.76	202.9	42.16	0.82	16.6	3984.8	33.26	
6	0.86	6.6	0.89	23.4	4.86	0.93	2.1	703.2	5.87	

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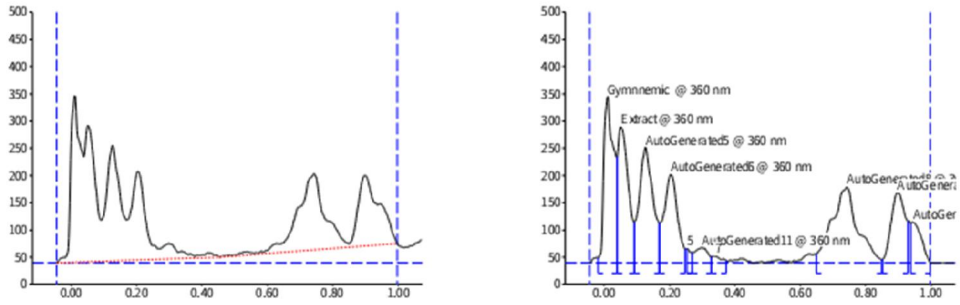

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1	-0.02	9.7	0.01	305.5	22.72	0.04	193.4	7196.6	17.18	Gymnemic
2	0.04	196.6	0.05	250.7	18.64	0.09	75.5	6316	15.08	Extract
3	0.09	75.5	0.13	212.7	15.82	0.17	73.9	6952.8	16.60	
4	0.17	74.0	0.20	162.9	12.12	0.25	25.9	5287.6	12.63	
5	0.25	26.3	0.25	26.3	1.95	0.27	19.5	262.7	0.64	
6	0.27	19.1	0.30	28.5	2.12	0.33	11.2	872.9	2.08	
7	0.33	11.4	0.34	13.3	0.99	0.37	5.2	289.3	0.69	

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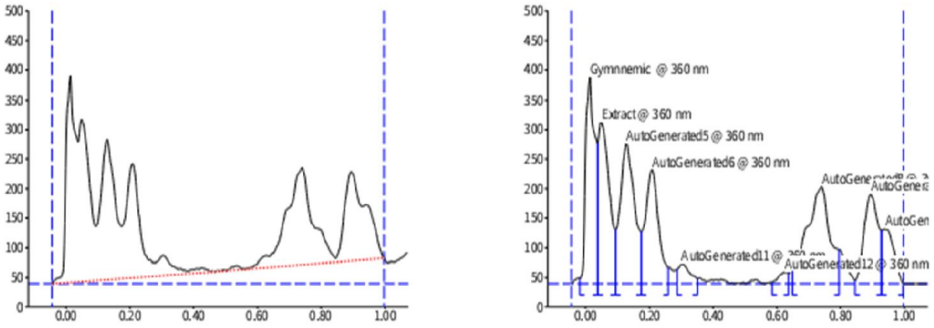

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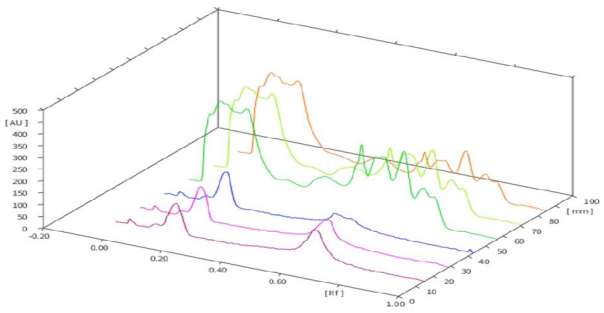
2	0.04	238.8	0.05	274.4	18.11	0.09	91.3	7430.3	15.33	Extract
3	0.10	91.8	0.13	237.6	15.68	0.17	88.3	8401.9	17.34	
4	0.18	88.6	0.21	192.8	12.73	0.26	27.8	6280.4	12.96	
5	0.29	24.5	0.30	33.6	2.22	0.35	8.9	985.4	2.03	
6	0.59	2.5	0.63	20.1	1.33	0.64	18.3	479.5	0.99	
7	0.65	22.5	0.74	164.1	10.83	0.80	57.0	9089.9	18.76	
8	0.85	6.4	0.90	150.1	9.96	0.93	90.1	5145.4	10.62	
9	0.93	90.1	0.94	92.7	6.12	1.00	1.5	2692.2	5.57	

TLC profile of Gymnemic acid under 360 nm

BERBERINE



All tracks at wavelength

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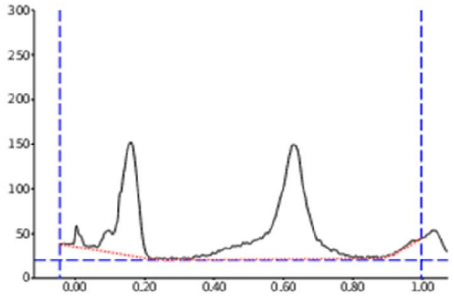

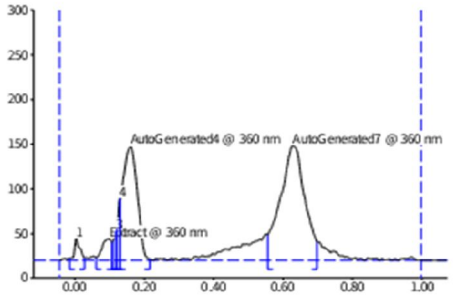

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1	-0.01	2.5	0.00	24.4	6.01	0.03	2.7	306.1	2.44	
2	0.06	3.3	0.10	24.4	6.01	0.11	20.8	518.8	4.14	Extract
3	0.11	21.0	0.12	32.5	7.99	0.12	32.5	206.1	1.64	
4	0.12	35.1	0.13	69.1	17.01	0.13	69.1	370.8	2.96	
5	0.13	68.8	0.16	127.5	31.38	0.22	1.4	4083.8	32.55	
6	0.56	28.7	0.63	128.3	31.60	0.70	20.6	7061.6	56.28	

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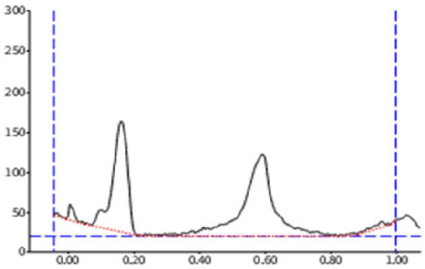

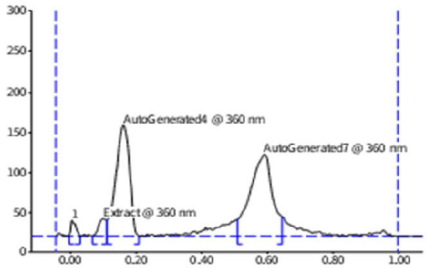

PEAK	START Rf	START Ht	MAX Rf	MAX Ht	MAX %	END Rf	END Ht	AREA	AREA %	ASSIGNED SUB
1	-0.00	2.3	0.00	20.4	7.15	0.03	0.3	240.5	2.28	
2	0.07	0.6	0.10	22.2	7.80	0.11	20.9	399.3	3.78	Extract
3	0.11	20.6	0.16	139.0	48.84	0.21	0.1	4533.7	42.94	
4	0.51	21.7	0.59	103.1	36.21	0.65	24.3	5383.6	50.99	

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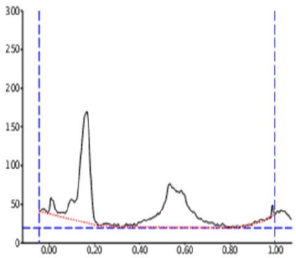

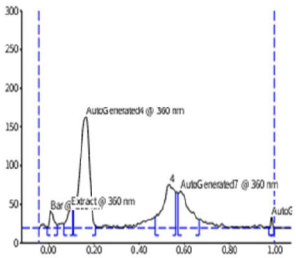

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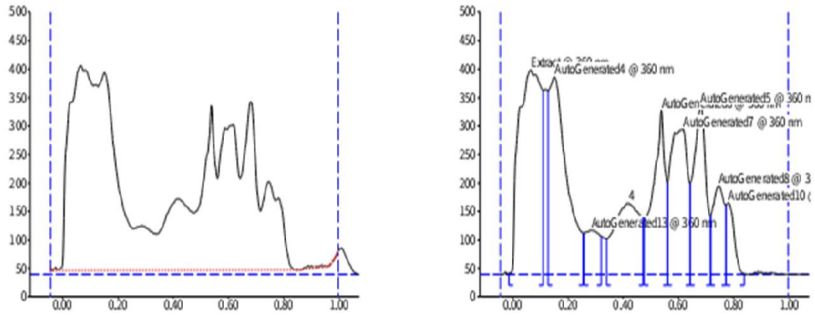
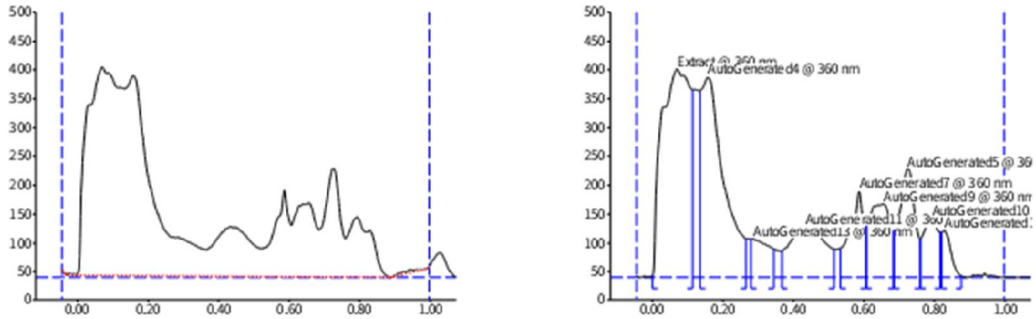
PEAK	START Rf	START Ht	MAX Rf	MAX Ht	MAX %	END Rf	END Ht	AREA	AREA %	ASSIGNED SUB
1	-0.01	0.3	0.01	21.6	7.02	0.04	3.4	318.4	3.33	Bar
2	0.07	5.1	0.10	25.9	8.44	0.11	23.2	473.8	4.96	extarct
3	0.11	0.11	0.17	143.1	46.55	0.21	3.4	4684.5	49.03	
4	0.47	14.3	0.54	55.5	18.06	0.56	45.0	2250.5	23.55	
5	0.57	44.3	0.59	47.3	15.39	0.67	10.7	1757.6	18.40	
6	0.98	1.1	0.99	13.9	4.53	1.00	0.6	69.6	0.73	

TRACK 4, ID



PEAK	START Rf	START Ht	MAX Rf	MAX Ht	MAX %	END Rf	END Ht	AREA	AREA %	ASSIGNED SUB
1	-0.01	1.9	0.07	359.5	17.70	0.11	323.7	22549	24.25	Extract
2	0.13	322.8	0.15	347.3	17.10	0.26	72.8	17655	18.99	
3	0.26	73.4	0.29	77.5	3.82	0.32	67.5	3124.3	3.36	
4	0.34	63.1	0.42	125.0	6.16	0.47	100.5	9232.7	9.93	
5	0.48	100.4	0.54	288.8	14.22	0.56	160.8	9901.6	10.65	
6	0.56	161.3	0.62	255.5	12.58	0.64	160.8	12327.6	13.26	
7	0.64	161.2	0.68	295.6	14.56	0.72	103.7	10374.6	11.16	
8	0.72	104.2	0.75	155.2	7.64	0.77	122.0	5000	5.38	
9	0.77	122.3	0.78	126.1	6.21	0.84	1.3	2814	3.03	

TRACK 5, ID



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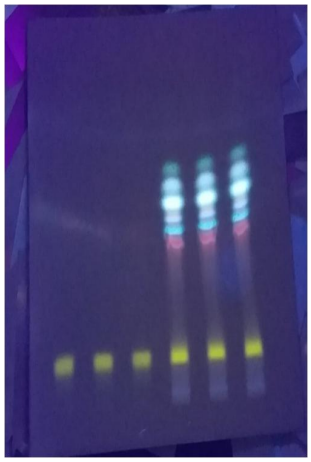
PEAK	START Rf	START Ht	MAX Rf	MAX Ht	MAX %	END Rf	END Ht	AREA	AREA %	ASSIGNED SUB
1	0.00	3.6	0.07	362.2	23.79	0.11	326.6	22719.2	31.44	Extract
2	0.14	324.6	0.16	348.3	22.87	0.27	68.2	17626.0	24.39	
3	0.28	67.5	0.29	68.2	4.48	0.34	50.2	2664.1	3.69	
4	0.37	46.8	0.43	87.4	5.74	0.52	49.0	6977.5	9.66	
5	0.53	51.2	0.59	150.6	9.89	0.61	90.3	4753.1	6.58	
6	0.61	90.9	0.66	128.9	8.47	0.69	82.9	5911.7	8.18	
7	0.69	83.0	0.73	188.9	12.41	0.76	67.9	6517.8	9.02	
8	0.76	68.3	0.79	105.0	6.90	0.82	79.3	3400.0	4.71	
9	0.82	79.7	0.83	83.0	5.45	0.88	2.8	1684.1	2.33	



TLC profile of Berberine extract under 360 nm

V. SUMMERY

Traditional systems of medicine have been in vogue for centuries and use of plantbased medicine has been increasing all over the world especially for conditions like cancer, high blood pressure, allergies, and for general wellbeing. Commercialization and manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine. Hence it is necessary to come up with a systematic approach to develop well-designed methodologies for the quality control of polyherbal formulations. By considering these facts, the aim of the present research work was to develop high standard quality parameters for some polyherbal formulations which are frequently used in Ayurveda system of medicine. The work has given emphasis on the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles along with the conventional parameters followed for the standardization of polyherbal Ayurveda herbal formulations. The one formulation have been selected for the development of modern quality control standards with conventional parameters. The common and conventional quality control parameters such as organoleptic evaluations like colour, odour, taste and consistency; physico-chemical evaluation like total ash, acid soluble ash water soluble ash, ph 1 % solution, ph of 10% solution, extractive values, water soluble matter, alcohole soluble matter.

HPTLC (High performance thin layer chromatography) was performed to develop fingerprint profiles of the formulations. HPTLC fingerprinting of Gymnemic acid extract and Berberine extract Mahantak vati shows the six, six peaks in chromatogram. For Gymnemic acid Toulene:ethyle acetate :methanol: formic acid (6:2:1.5:0.5) and for Berberine Ccl4:methanol:acetic acid (4:1:0.5) mobile phase are used .

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VI. CONCLUSION

In conclusion, HPTLC new multiple marker based methods has been developed, validated and applied for live analysis of 2 markers in one traditional compound multi component Ayurveda formulation. The multiple marker based modern quality control approaches used in present investigations are the first attempt using traditional Ayurveda formulations and should be applied for all other existing traditional formulations for evaluation of their quality and indirectly their bio efficacy.

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