Asian Journal of Chemistry Vol. 20, No. 1 (2008), 509-513

Application of HPTLC in Standardization of Homoeopathic Mother Tincture *Andrographis paniculata* and Its Comparison with Market Products

D. SHANBHAG and SUNITA JAYARAMAN*†
Department of Chemistry, D.G. Ruparel College, Mahim, Mumbai-400 016, India
E-mail: sunita_75in@yahoo.com

A simple and accurate HPTLC method has been developed for the quantification of andrographolide and fingerprinting of the in-house mother tincture considered here to be a standard with that of different marketed samples available from manufacturers of homoeopathic medicines in India. This HPTLC method was quantitatively evaluated in terms of stability, repeatability, accuracy and calibration providing the utility in the analysis of the mother tincture.

Key Words: HPTLC, Mother tincture andrographolide, Fingerprint.

INTRODUCTION

Andrographis paniculata is therapeutically used as an antipyretic, anti-inflammatory, antidiarrhoeal and in skin diseases. About 0.5 to 0.9 % of the active principle andrographolide which is a diterpene lactone is present in the plant[1-3]. Homoeopathy is a holistic system of therapy which works at reinforcing the body's own natural capacity to heal and achieve a gentle and lasting cure. Mother tinctures (MQ) are defined as the original tincture prepared with the aid of alcohol, directly from the crude drug. They are the precursors of the corresponding potencies of the respective drug and the starting point for the production of most homoeopathic medicines. The in-house standard mother tincture was strictly prepared as per the procedure laid down in the Homoeopathic Pharmacopoeia of India (HPI). The objective of this work is to make an in-house standard mother tincture and compare it with different marketed samples using its fingerprint characteristics and to further quantify them with specific active principle of the known fraction[4].

†Present Address: Anchrom Testlab Pvt. Ltd., A-101, Shree Aniket Apts., Navghar Road, Mulund (E), Mumbai-400 081, India.

EXPERIMENTAL

Authentic plant of *Andrographis paniculata* (Bafco, India) was used to prepare the mother tincture. Andrographolide ($C_{20}H_{30}O_5$ m.p. 228 °C, purity 99 % by HPLC) was purchased from Natural Remedies, Bangalore. The solvents 99.9 % absolute ethanol, HPLC water, chloroform, methanol were of Analytical Grade purity (Merck Ltd.)

Preparation of standard mother tincture: The dried plant was coarsely powdered, 10 g of this powder was used and the requisite amount of alcohol and water was added as specified in HPI and the standard mother tincture was prepared by the percolation method[5]. This tincture was transferred to suitable glass container and stored for further study.

Preparation of standard andrographolide: Weigh 10 mg andrographolide in a 10 mL volumetric flask. To this 10 mL ethanol was added (1 µg/µL). 1 mL of this standard solution was taken in another volumetric flask, this was further diluted to 10 mL with ethanol (0.1 µg/µL) and was used for present studies.

Standardization of standard other tincture: Camag HPTLC system[6] comprising of Linomat 5 as sample applicator and TLC Scanner3 controlled by winCATS software version 1.3.4 was used for quantitative evaluation. Stationary phase used was Merck precoated TLC aluminium foil silica gel $60F_{254}$ and the mobile phase used was chloroform:methanol (7:1) v/v. Samples and standard were applied as 8 mm bands with 6 mm distance between the tracks. Tank saturation was given with filter paper for 15 min. Ascending development for a distance of 80 mm in a twin trough chamber was completed in *ca.* 15 min. Volume of standard MQ was first optimized at 6µl for fingerprinting. The λ_{max} of andrographolide was found to be 232 nm after taking the spectra of the standard of andrographolide. Quantitative measurement in the absorbance mode was done at 225 nm using a slit dimension of 6.00 × 0.45 mm.

Linearity response: 4 µL volume of the standard mother tincture that was applied along with the standard andrographolide gave higher area hence could not be quantified. The standard mother tincture was diluted. Standard mother tincture was diluted with ethanol and was used for further studies. The volume of the standard mother tincture was optimized to 4 µL for quantification. It was then simultaneously applied with different concentration of standard andrographolide. The method was found to be linear with a regression of 0.99898 and a standard deviation of 2.48 % and the amount of andrographolide was calculated in the mother tincture.

Standardization of the standard mother tincture by fingerprint method: Standardization of the mother tincture was done by evaluating its fingerprint characteristics, using HPTLC method[7,8]. Standard mother tincture was chromatographed simultaneously along with six other mother

tinctures available in market at 4 µL on the same plate for comparison. Multi wavelength (MWL) scan was done for finding the optimum wavelength for scanning. The optimum wavelength was found to be 225 nm. The entire plate was further scanned at this wavelength for quantitation and spectral match. Many fractions of standard mother tincture were matched with the help of its characteristic spectra with that of other marketed samples. Individual λ_{max} of each fraction was also found with the help of spectral scanning and then the plate was scanned with these selected wavelengths in MWL mode. The pattern of the peaks was compared for the standard mother tincture and marketed samples (Table-1).

It was observed that the response for various concentrations of standard andrographolide was linear in the range of 200 to 1000 ng with a coefficient of variation of 0.99916 and a standard deviation of 2.32 %. Andrographolide was quantified and the amount was calculated in individual mother tinctures. With this method we compared all available mother tinctures and the active principle was also quantified. Thus the method can be said to be standardized.

Quantification of andrographolide in market samples and standard mother tincture: The amount of andrographolide was calculated in standard mother tincture (B) and market samples (B1 to B6) and the results are tabulated in Table-2.

RESULTS AND DISCUSSION

The decomposition of the analyte during application or development was confirmed by two-dimensional chromatography. The chromatogram did not show any extra fractions. Repeatability of the method was checked by scanning 15 tracks of 2 µL volume standard mother tincture. The co-efficient of variation (CV) was found to be 0.368 %.

Accuracy: The percentage recovery of andrographolide was calculated using the above method. The average recovery values obtained were 85.79 to 100.58 %, which confirms that the method is validated.

Conclusion

The HPTLC fingerprinting characteristics of *Andrographis paniculata* mother tinctures obtained from manufacturer (B1 to B6) and the in-house standard mother tinctures (B) had been scanned at 225 nm wavelength. From the results obtained after densitometric scanning, it was observed that the standard mother tinctures (B) of *Andrographis paniculata* shows 9 peaks. The marketed samples B1 shows 9 peaks, B2 shows 8 peaks, B3 shows 9 peaks, B4 shows 8 peaks, B5 shows 7 peaks and B6 shows 9 peaks. Value of the six marketed tinctures (B1 to B6) was found to show

TABLE-1
ANALYSIS OF DIFFEENT ANDROGRAPHIS PANICULATE MOTHER TINCTURES AT SCANNING WAVELENGTH 225 nm

Peak	B			B1			B2			B3		
	Ref.	Max. Ht.	Area (%)	Ref.	Max. Ht.	Area (%)	Ref.	Max. Ht.	Area (%)	Ref.	Max. Ht.	Area (%)
1	0.06	23.5	2.70	0.05	83.8	6.12	0.04	0.17	1.51	0.05	29.8	2.08
2	0.18	10.2	1.76	0.18	26.3	1.42	0.18	14.1	1.21	0.19	70.1	10.29
3	0.22	14.7	2.63	0.21	52.1	4.95	0.22	33.2	6.34	0.27	41.3	7.06
4	0.28	12.7	2.34	0.36	365.1	39.59	0.27	24.0	2.92	0.36	150.3	24.98
5	0.37	217.2	47.95	0.42	50.2	4.81	0.36	343.9	50.57	0.44	38.3	9.13
6	0.45	27.6	7.50	0.44	40.6	2.63	0.48	96.3	15.81	0.49	47.5	8.54
7	0.50	42.5	10.13	0.49	94.4	11.31	0.57	33.0	4.81	0.52	31.7	5.44
8	0.53	27.9	5.32	0.52	48.9	6.09	0.78	56.2	17.46	0.59	11.8	2.68
9	0.79	39.7	19.66	0.77	115.0	23.08	–	–	–	0.77	91.9	29.79

Peak	B4			B5			B6		
	Ref.	Max. Ht.	Area (%)	Ref.	Max. Ht.	Area (%)	Ref.	Max. Ht.	Area (%)
1	0.05	27.6	1.61	0.05	12.3	1.44	0.06	42.0	2.91
2	0.18	26.1	2.43	0.22	40.0	6.67	0.22	79.2	5.69
3	0.28	37.7	4.95	0.28	60.4	17.17	0.27	66.0	9.03
4	0.37	428.7	59.36	.037	124.3	34.94	0.37	73.9	12.03
5	0.44	38.2	6.45	0.50	56.2	21.06	0.45	81.0	12.58
6	0.49	82.1	13.13	0.61	14.8	3.53	0.50	107.4	13.49
7	0.61	14.6	2.22	0.79	29.6	15.18	0.53	77.9	8.82
8	0.79	35.4	9.85	–	–	–	0.61	42.7	5.92
9	–	–	–	–	–	–	0.77	156.6	29.52

TABLE-2
AMOUNT OF ANDROGRAPHOLIDE IN *Andrographis paniculata* MOTHER TINCTURES

Sample	Wt. of Andrographolide (mg) in 100 mL sample
B	21.95
B1	42.26
B2	40.36
B3	13.97
B4	54.62
B5	11.82
B6	4.89

minimum 7 different peaks with R_f values similar to standard mother tinctures (B) and they are similar within themselves. So from this study, it was confirmed that *Andrographis paniculata* tincture contains different components with R_f values (0.04-0.06, 0.18-0.19, 0.21-0.22, 0.27-0.28, 0.36-0.37, 0.44-0.45, 0.48-0.50, 0.51-0.53, 0.61, 0.77-0.79). These components must be considered to determine quality of any further sample of the same. The spectral analysis also indicates that spectra with particular R_f values of various components (0.05, 0.18, 0.22, 0.28, 0.36, 0.44, 0.49, 0.52, 0.79) have similar pattern within themselves. It may be concluded that samples procured from the market that are showing lesser peaks may not be up to the standard level.

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(*Received*: 22 November 2006; *Accepted*: 14 September 2007) AJC-5852