Development of High Performance Thin Layer Chromatography Finger Print Parameters on Maka Vallati Ilekiyam – Part-I

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ABSTRACT : High performance thin layer chromatographic finger print parameters had been developed for the siddha medicine ‘Maka Vallati Ilekiyam’ to fix standards.

INTRODUCTION

In indigenous systems of medicine, several parameters are used as standards. Thin layer chromatography is employed to get tlc profiles of various extracts and can be used in standardizing the raw drugs as well as the herbal medicines. Ordinary tlc plates do not give better resolution particularly when the medicine involves the use of several herbs and quantification of makers by conventional solvent extraction followed by colorimetry is laborious and expensive. HPTLC offers better resolution and estimation of active principles can be done with reasonable accuracy in a shorter time.

‘Maka Vallati Ilekiyam’ is a siddha herbal medicine containing thirty four ingredients. The medicine is prescribed for various ailments particularly for venereal diseases and is claimed to possess anti-AIDS potential and tlc studies on maka vallati ilekiyam1. The medicine is also analysed by HPTIC. Herein we report HPTLC studies on the various extracts of maka vallati ilekiyam and the integration spectrum which will be useful as standard parameters.

MATERIALS AND METHODS

The medicine ‘maka vallati ilekiyam’ was procured from IMPCOPS, Madras and extracted in n-Hexane, benzene and CHCl₃ by soxhlet apparatus the HPTLC was performed on 20 X 20(cm) aluminum sheets procoated with silica gel 60 f254 of 0.2 mm thickness. CAMAG HPTLC linear thin trough (20 X 20 cm) was saturated with the developing solvent: toluene: EtOAc: diethyl amine (5:4:1) for 2 hrs at 20 – 250c. The sample 2 or 5 µl was applied in a 5mm width band through LINOMAT IV in a space of 5 mm.

The CAMAG TLC scanner II with computer and modified TLC software were used for scanning the chromatogram. The extracts were scanned at wavelengths 366, 270, 254 mm².

RESULTS AND DISCUSSION

The HPTLC integration spectrum of hexane, benzene and CHCl₃ extracts are shown in Fig.1-6. In the spectrum of
hexane extract are shown in Fig.1-6. In the spectrum of hexane extract at $\lambda_{\text{max}}$ 366nm, about 30.82% of the compound remained at the point of application and the remaining compounds remained at the point of application and the remaining compounds were resolved into four spots of which the spot of Rf.0.12 showed the presence of 25.66%. The scanning at $\lambda_{\text{max}}$ 270 nm resolved into four spots with a major compound (17%) at Rf.0.46 while the percentage of other spots were ranging from 7.21 to 14.55. Thus the hexane extract at $\lambda_{\text{max}}$ 366 and 270 nm showed four and seven spots respectively, out of which two spots of R.f.0.78 and 0.91 were common, making the total compounds to nine (Fig. 1&2, Table-1).

Benzene extract $\lambda_{\text{max}}$ 366 nm resolved into two spots of Rf. Value 0.13 and solvent front (1.00). The constituent at solvent front (1.00) being the major with a concentration of 28.58% and the other spot of Rf.0.13 with 5.55% while 65.9% of compounds remained at the point of application. The scanning at $\lambda_{\text{max}}$ 270nm, showed the presence of seven spots having the percentage from 5.9 to 42.72. The major spot at Rf.0.95 had the concentration of 42.72%. Thus benzene extract at $\lambda_{\text{max}}$ 366 and 270 nm resolved into two and seven spots respectively, which makes the total number of spots as nine (Fig. 3 & 4, Table-1).

CHCL$_3$ extract on scanning at $\lambda_{\text{max}}$ 270nm, revealed the presence three spots with Rf. 0.47, 0.67 and solvent front (1.00). The spot at solvent front was the major one having 60.78% and that with Rf. 0.67 was found to be the minor with a concentration of 8.78% only (Fig.6). Thus the CHCL$_3$ extract at $\lambda_{\text{max}}$ 254 and 270 nm resolved into three spots at each wavelength total number common (Table – 1).

In the hexane and benzene extracts the compounds with Rf. Values 0.46, 0.50, 0.56 were found to be common. At shorter wavelength i.e $\lambda_{\text{max}}$ 270nm scanning, resolution was better for hexane and benzene extracts. This $\lambda_{\text{max}}$ 270 nm can be taken for obtaining optimum HPTLC results for this medicine. Though further work to characterize the other chemical constituent and quantitative estimation with marker compounds are also necessary, these data can also be used to lay down the standards for ‘Maka vallati ilekiyam’ along with the previously reported data$^1$.

**CONCLUSION**

HPTLC studies have shown that it is more versatile than the ordinary TLC methods, as the spots were well resolved. The quantification of different markers can be carried out in a single run by comparison with authentic chemicals. The data presented above can also be considered along with the other values for fixing standards to this medicine ‘Maka vallati ilekiyam’.

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REFERENCES

1. Saraswathy, A. Girija Rani Mohan, and Veluchamy, G. Chemical analysis of Maka Vallati Ilekiyam. Journal of Research in Ayurveda and Siddha – Communicated.

2. Sethi, P.D., High Performance thin layer Chromatography Quantitative Analysis of Pharmaceutical Formulations – First Edition. CBS Publishers and Distributors 4596 I.A. 11-Darya Ganji, New Delhi – 12, 1996.

TABLE -1
HPTLC FINGER PRINT PARAMETERS OF MAKA VALLATI ILEKIYAM

| Developing System | Extract  | Wave length $\lambda_{\text{max}}$ nm | Rf. Value | No. of spots |
|-------------------|----------|---------------------------------------|-----------|-------------|
| Toluene: EtoAc: Diethyl amine (5:4:1) | n-Hexane | 366 | 0.12 | 0.78 | 0.91 | 1.00 | Nine |
|                   |          | 270 | 0.46 | 0.50 | 0.56 | 0.63 |
|                   |          |    | 0.78 | 0.82 | 0.91 | |
|                   | Benzene  | 366 | 0.13 | 0.79 | 0.67 | 1.00 | Four |
|                   |          |    | 0.46 | 0.50 | 0.56 | |
|                   |          |    | 0.79 | 0.83 | 0.95 | |
|                   | CHCL3    | 254 | 0.43 | 0.67 | 1.00 | |
|                   |          | 270 | 0.47 | 0.67 | 1.00 | |
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Fig. 3

**Opt-Cara Vallati Zeletan**

**Hexane extract (2μL)**

Toluene : EtOAc : Diethylamine (5 : 4 : 1)

| RF  | max | AIO  |
|-----|-----|------|
| 0.01| 44.9| 65.67|
| 0.13| 3.8 | 5.55 |
| 1.00| 19.5| 28.58|

Total height = 68.7

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**Opt-Cara Vallati Zeletan**

**Hexane extract (3μL)**

Toluene : EtOAc : Diethylamine (5 : 4 : 1)

| RF  | max | AIO  |
|-----|-----|------|
| 0.46| 7.3 | 13.90|
| 0.50| 1.1 | 5.91 |
| 0.56| 4.8 | 9.15 |
| 0.65| 5.1 | 9.82 |
| 0.79| 4.1 | 7.78 |
| 0.90| 5.6 | 10.63|
| 0.95| 22.4| 42.72|

Total height = 52.5
Fig. 2

HPTLC-MASA VALLATI ILEKIVAM

Chloroform extract (5µl)

Toluene : EtOAc : Diethylamine
(5 : 4 : 1)

Wavelength 250 nm

| Rf  | Max | A10 |
|-----|-----|-----|
| 0.44| 3.7 | 15.37|
| 0.67| 3.3 | 13.82|
| 1.00| 17.1| 76.01|

Wavelength 270 nm

| Rf  | Max | A10 |
|-----|-----|-----|
| 0.47| 8.7 | 30.44|
| 0.67| 2.5 | 8.78 |
| 1.00| 17.5| 60.78|

Total length=28.7