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QUALITATIVE ANALYSIS OF *EUPATORIUM PERFOLIATUM* MOTHER TINCTURE USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

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ABSTRACT

Introduction- Chromatography is used for both qualitative and quantitative drug examination, especially for mother tinctures and to check the purity and identify any adulterants that may be present.

Objective - To evaluate the quality of the Homoeopathic Pharmaceutical Company's *Eupatorium perfoliatum* Mother Tincture utilising its fingerprinting properties.

Methodology- The qualitative analysis of *Eupatorium perfoliatum* Mother Tincture Using High Performance Thin Layer Chromatography (HPTLC) has been performed to assess the quercetin content.

Results- Analysis using High Performance Thin Layer Chromatography (HPTLC) revealed that the Rf value of quercetin was about 0.5 in all the three samples. The regression equation was found

to be Y1 (sample A) = 0.0009x+ 0.0016, Y2(sample B) =0.0009x+0.0021, Y3(sample C) = 0.0008+0.0029 with respective to concentration. Additionally, a correlation coefficient of 1.0 suggests that the relationship between concentration and area is linear.

Conclusion- The quercetin content was found in all the three samples of mother tinctures, but sample C was considered to be standard (R2 = 0.9979).

Keyword: HPTLC, Qualitative analysis, *Eupatorium perfoliatum*, Mother tincture, Quercetin-3-glucoside

INTRODUCTION

The qualitative and quantitative investigation of pharmaceuticals, particularly mother tinctures, can benefit from chromatography. H.P.I. [Homoeopathic Pharmacopoeia of India] often uses paper chromatography and thin layer chromatography (TLC and PC For the evaluation of botanical materials, High Performance Thin Layer Chromatography (HPTLC) is a priceless quality assessment technique. This examination can be viewed with many light wavelengths, giving a more comprehensive profile of the drug than is generally seen with more focused methods of analysis. In 1901, Russian botanist Mikhail Tsevet developed chromatography. Its meaning is "Colour Writing". Since its inception, this approach of standardizing plant compounds has advanced greatly, with numerous improvements made along the way. It is one of the most crucial methods for homoeopathic mother tincture standardization and is based on the concepts of analysis, identification, purification, and quantification of mother tincture components. In order to check the purity of mother tinctures and identify any adulterants that may be present, it can be used for both qualitative and quantitative examination. The foundation of this procedure is the adsorption of substances between the mobile phase and stationary phase. As the name implies, the stationary phase is fixed while the mobile phase flows over it and the components are separated by adsorption **[1-3]**. Native to North America, boneset (*Eupatorium perfoliatum* L.) has a long history of use as an antipyretic and cold cure by Native Americans and early European settlers. Traditional doctors frequently employed boneset as a laxative, diaphoretic, and remedy for cough, discomfort, and the flu. In the past, boneset was frequently used for illnesses like "break bone fever," "ague" (malarial fever, dengue fever) **[4-9]**.

MATERIALS AND METHODS

1. **Test Samples** - *Eupatorium perfoliatum* Mother Tincture from 3 different pharmaceutical companies.
2. **Chemicals**
    1. Quercetin-3-glucoside
    2. Methanol
    3. Ethyl acetate
    4. Toulene
    5. Glacial acetic acid
3. **Chromatographic condition**

Instrument: CAMAG HPTLC system, consisting of Linomat 5 spoting device and TLC scanner 4 with vision CATS software from KAHER's Basic Science and Research Centre Belagavi, Karnataka - 500010.

Absorbent: Merck, HPTLC Silica gel $60F_{254}$ (20cm X 10cm), thickness –8mm, no. of tracks-15, band length- 8mm

Solvent system: Toluene: Ethyl acetate: Glacial acetic acid [7:2:1]

Solvent run up to: 70 mm.

Scanning wavelength: 254 nm and 366 nm

Standard preparation: A 1 mg/ml solution of quercetin standard was prepared in methanol.

Measurement mode: Absorbance

Procedure:

Using a Linomat V sample applicator l, 2, 4, 6, and 8 of pharmaceutical mother tincture of *Eupatorium perfoliatum* were put as bands on silica gel 60 F 254 plates (Merck) alongside real samples of standard quercetin. The applicator's speed was kept at 150 nl per second. The band's width was maintained at 8 mm. The chromatogram was generated in a saturated chamber up to a 70 mm depth. After the plate had dried, it was scanned in absorbance mode at 254 and 366 nm. The calibration curve between concentration and area of the standard quercetin was used to calculate the quantity of quercetin.

OBSERVATIONS AND RESULTS

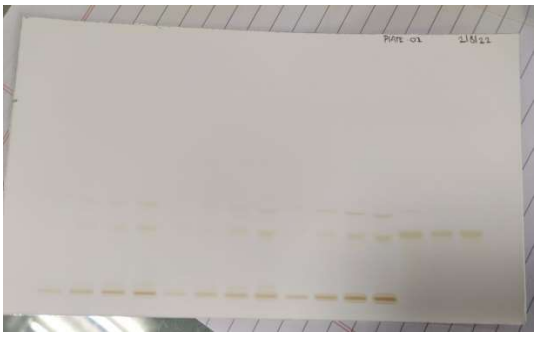

Figure 1: HPTLC Photograph of Samples and Various Concentrations of quercetin under Normal light

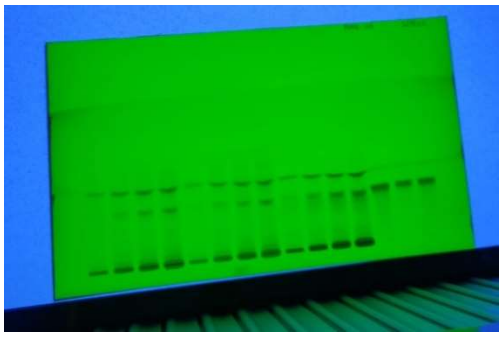

Figure 2: HPTLC Photograph of Samples and Various Concentrations of quercetin Under 254 nm of UV Light

Figure 3: HPTLC Photograph of Samples and various concentrations of quercetin under 366nm of UV Light

Sample A

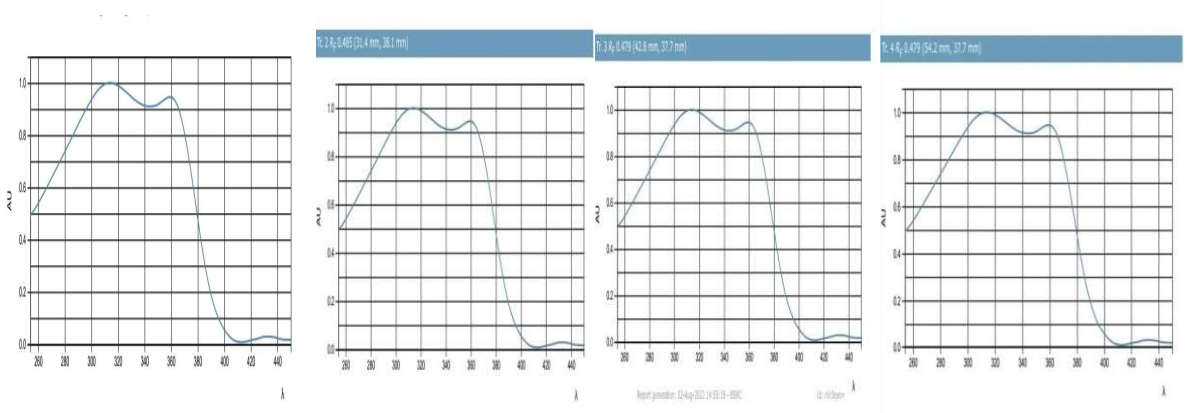

Figure 4a,4b,4c,4d: Peaks of Test Sample A

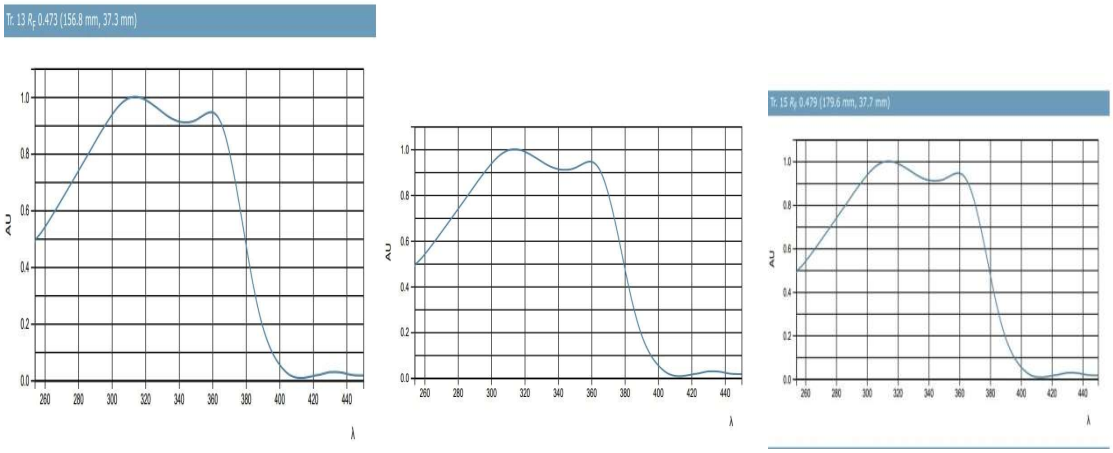

Figure 7a,7b,7c: Peaks of Standard quercetin

Table 1 A: Rf values of sample A

Substance EP A (R_F 0.483 +/- 0.021):			
Track	R_F	X (mm)	Y (mm)
1	0.485	20.0	38.1
2	0.485	31.4	38.1
3	0.479	42.8	37.7
4	0.479	54.2	37.7
13	0.473	156.8	37.3
14	0.481	168.2	37.8
15	0.479	179.6	37.7

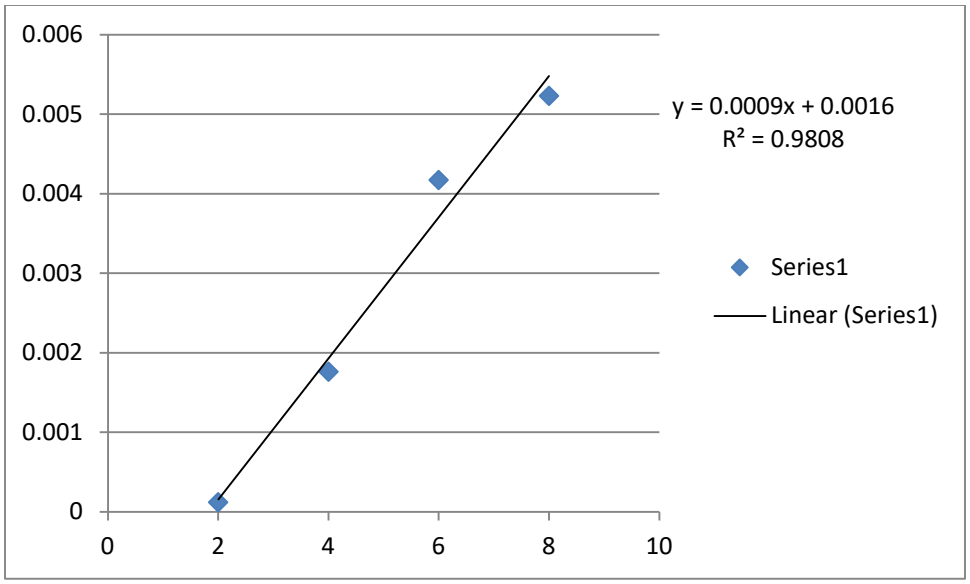

Figure 8A: Calibration curve of Sample A

SLOPE	**0.000887**
SE	**0.000481**
SD	**0.000963**
LOD	**3.580995**
LOQ	**10.8515**

Table 2A: Correlation table of Sample A

Substance name	Track	*R*	r (s,m)	r(e,m)	Ref.spectrum	Correlation
EPA	1	0.485	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPA	1.000000
EPA	2	0.485	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPA	1.000000
EPA	3	0.479	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPA	1.000000
EPA	4	0.479	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPA	1.000000
EPA	13	0.473	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPA	1.000000
EPA	14	0.481	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPA	1.000000
EPA	15	0.479	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPA	1.000000

Sample B

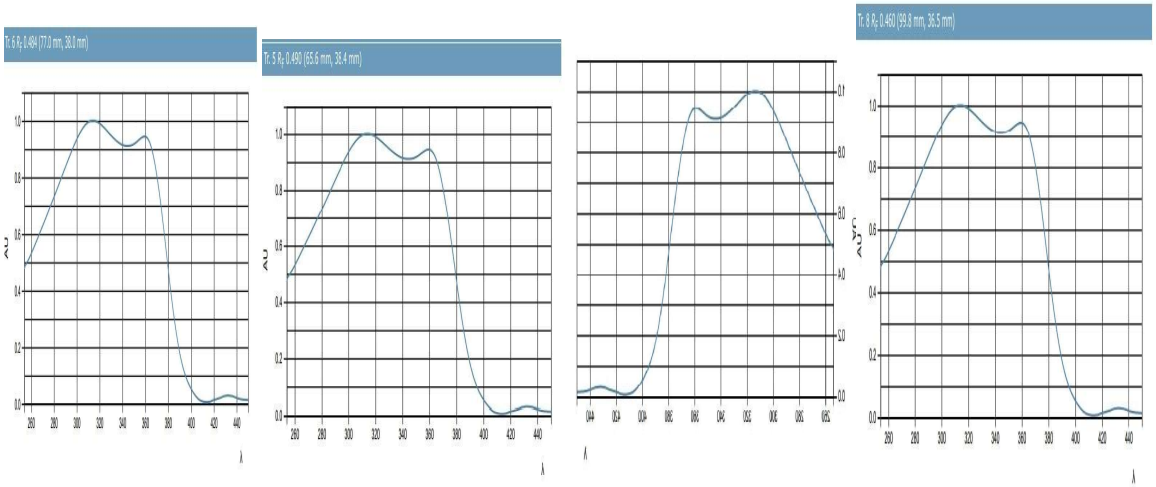

Figure 5a, 5b,5c,5d: Peaks of Test Sample

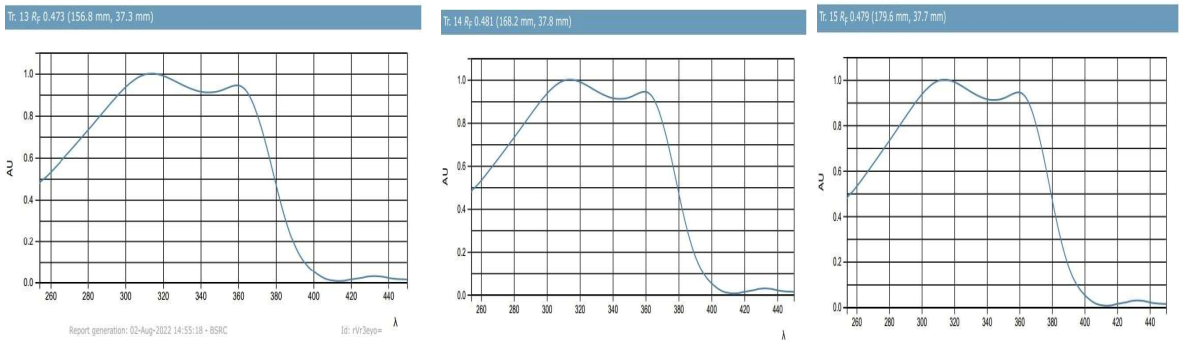

Figure :7a,7b,7c: Peaks of Standard quercetin

Table 1B: Rf values of sample B

Substance EP C (R_F 0.465 +/- 0.035):			
Track	R_F	X (mm)	Y (mm)
5	0.490	65.6	38.4
6	0.484	77.0	38.0
7	0.471	88.4	37.2
8	0.460	99.8	36.5
13	0.473	156.8	37.3
14	0.481	168.2	37.8
15	0.479	179.6	37.7

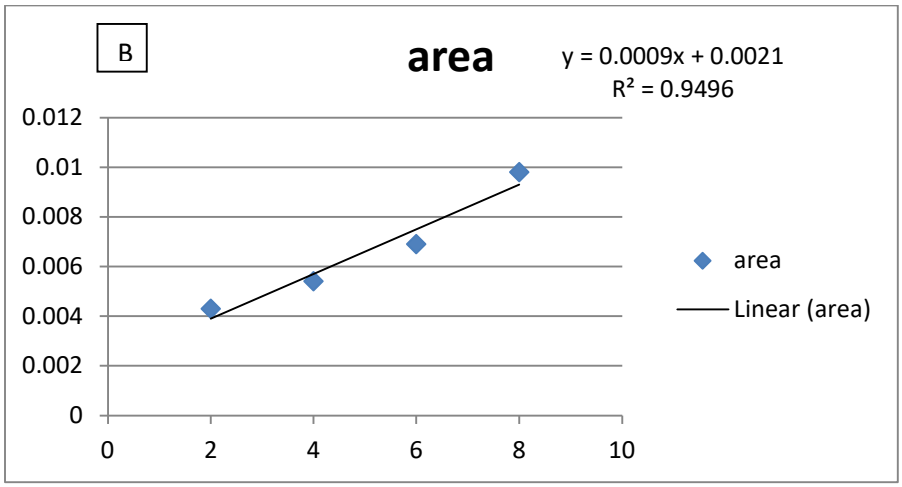

Figure 8B: Calibration curve of Sample B

SE	**0.000803**
SD	**0.001606**
LOD	**5.889539**
LOQ	**17.84709**

Table 2B:Correlation table of Sample B

Substancename	Track	R_F	r(s,m)	r(e,m)	Ref.spectrum	Correlation
EPB	5	0.490	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPB	6	0.484	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPB	7	0.471	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPB	8	0.460	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPB	13	0.473	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPC	1.000000
EPB	14	0.481	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPB	15	0.479	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPC	1.000000

Sample C

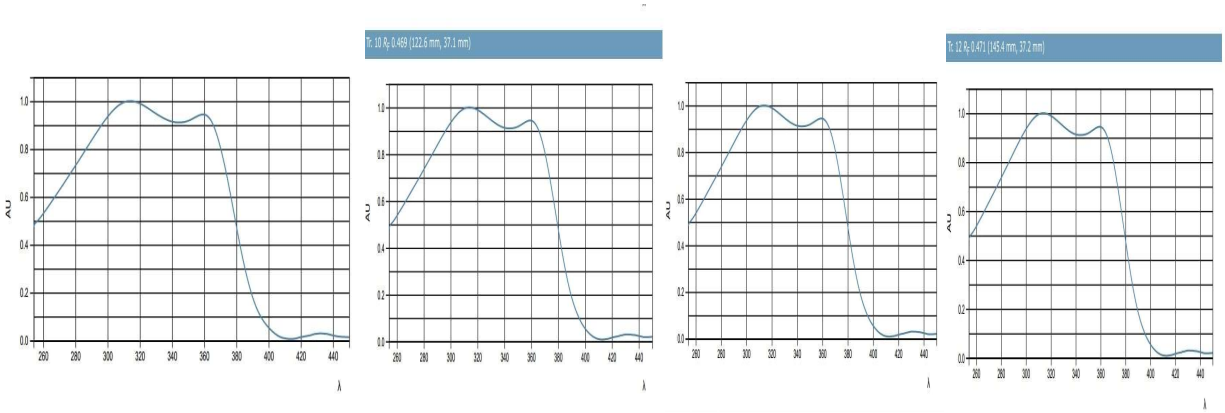

Figure 6a, 6b, 6c, 6d: Peaks of Test Sample C

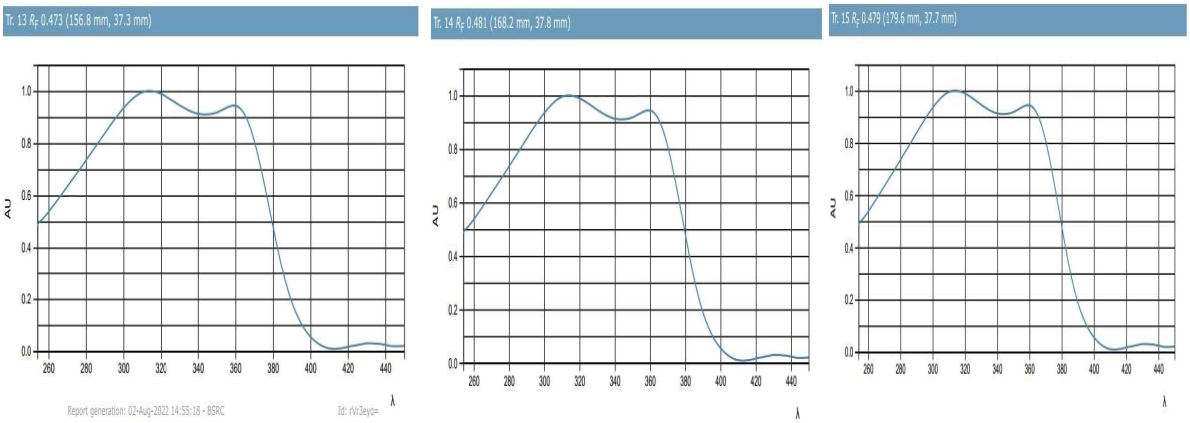

Figure 7a,7b,7c: Peaks of Standard Quercetin

Table 1C: Rf values of sample C

Substance EP C (R_F 0.472 +/- 0.035):			
Track	R_F	X (mm)	Y (mm)
9	0.473	111.2	37.3
10	0.469	122.6	37.1
11	0.469	134.0	37.1
12	0.471	145.4	37.2
13	0.473	156.8	37.3
14	0.481	168.2	37.8
15	0.479	179.6	37.7

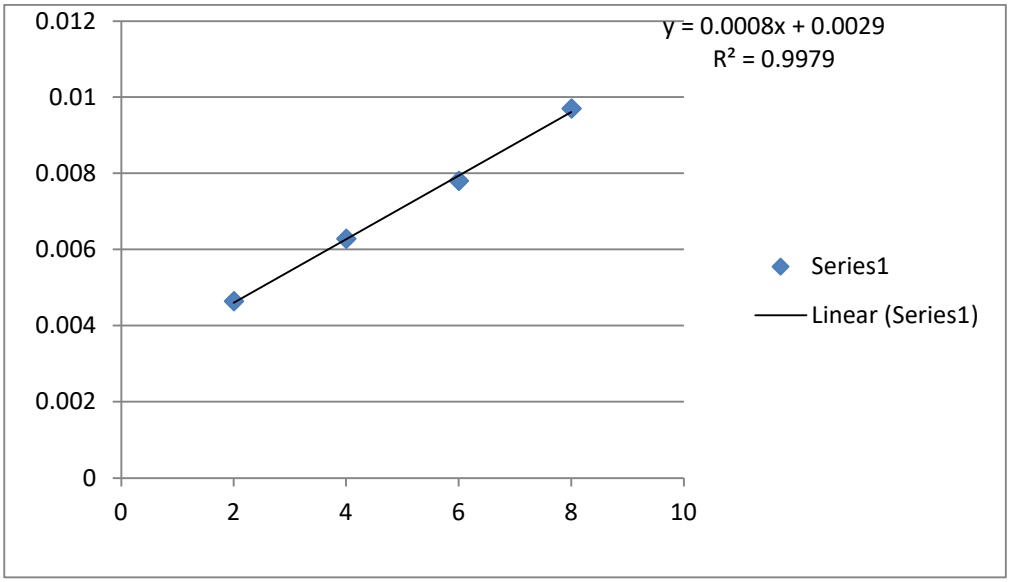

Figure 8C: Calibration curve of Sample C

SLOPE	**0.000835**
SE	**0.000148492**
SD	**0.000296985**
LOD	**1.173712573**
LOQ	**3.556704768**

Table 2C: Correlation table of Sample C

Substance name	Track	R_F	r(s,m)	r(e,m)	Ref.spectrum	Correlation
EPC	9	0.473	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPC	10	0.469	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPC	11	0.469	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPC	12	0.471	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPC	13	0.473	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPC	1.000000
EPC	14	0.481	0.000000	0.000000	Tr.13,Rf0.473,Sub.EPC	1.000000
EPC	15	0.479	0.000000	0.000000	Tr.14,Rf0.481,Sub.EPC	1.000000

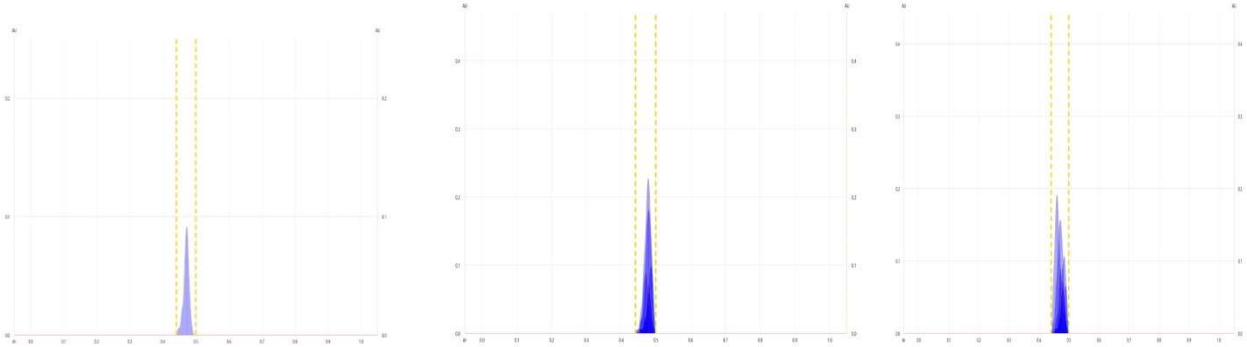

Figure 9: Quercetin Std Peak **Figure 9a: Quercetin and Sample A peak** **Figure 9b: Quercetin and Sample B Peak**

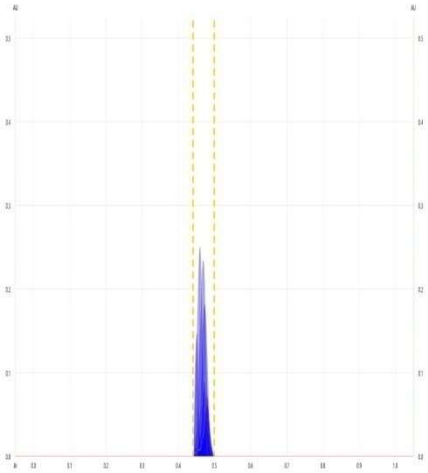

Figure 9c: Standard and Sample C peak

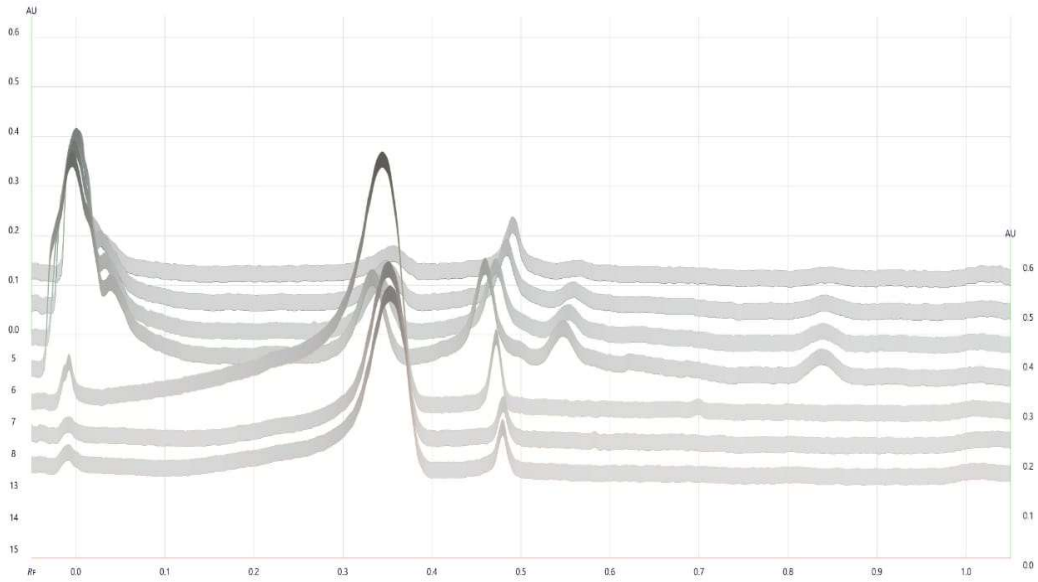

Figure 10a: Standard and EPA peaks

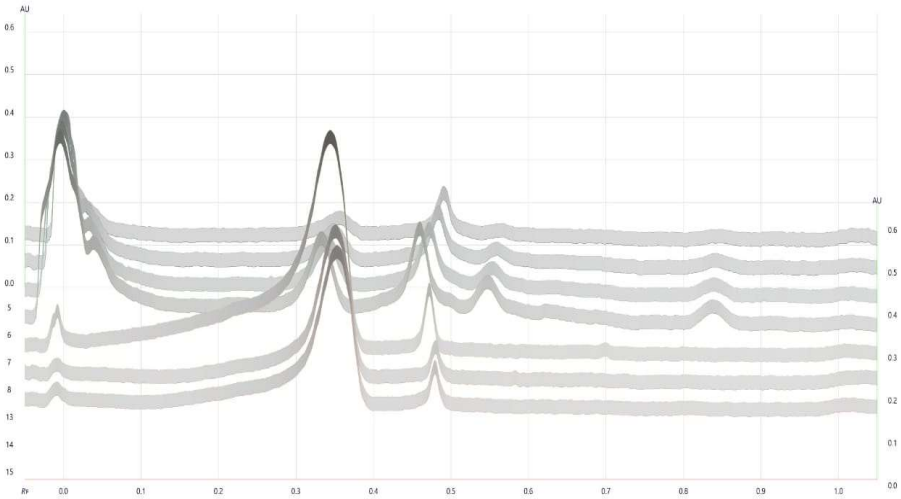

Figure 10b: Standard and EPB peaks

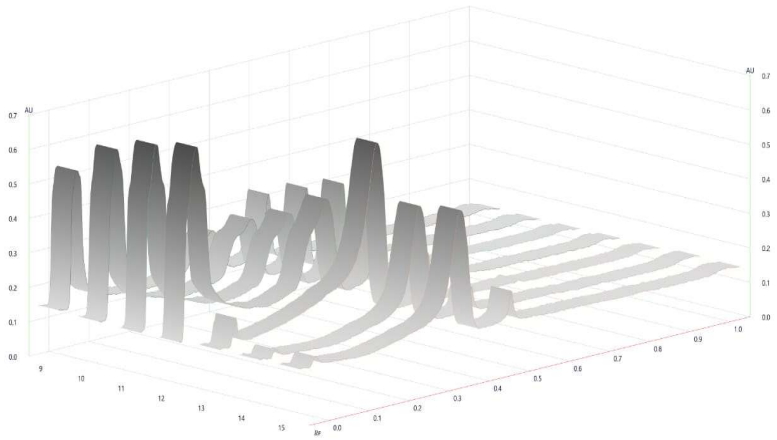

Figure 10c: Standard and EPC peaks

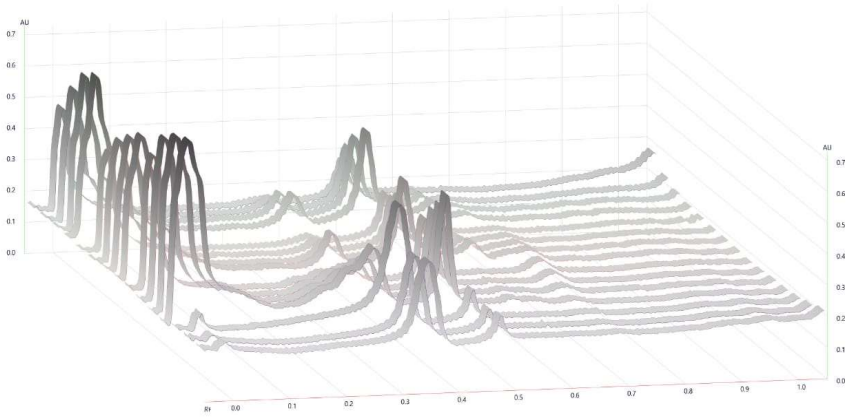

Figure 10d: Dimetric peaks of standard and all three samples

DISCUSSION

The two crucial processes in designing the analytical procedure—sample preparation and creation of an appropriate mobile phase of the solvent system—become more significant for herbal drugs due to the complexity of chemical components and their affinity for different solvents. In the current study, the quercetin concentration of 3 different Mother Tincture *Eupatorium perfoliatum* samples (A, B, and C) from 3 different pharmaceutical companies is analysed. Using Toulene: Ethyl acetate: Glacial acetic acid (7: 2: 1) and drying in air and scanning, the necessary resolution of quercetin with symmetrical and reproducible peaks was obtained after experimenting with several mobile phase compositions **(Figures. 1, 2, and 3)**. For quercetin, the calibration curve was linear from 2 to 8 g. Quercetin may be accurately quantified using this method, which also offers good resolution and separation of quercetin from other components of *Eupatorium perfoliatum*. The reflectance spectra of the test samples were compared with a standard quercetin to determine the

purity of the peaks **(Figures 4, 5, 6, 7)**. Additionally, the standard and sample's spectra show identical peaks and valleys at precisely the same wavelengths **(Figure 9, 10)**. The wavelengths and Rf values of standard quercetin match those of the sample, demonstrating that the sample and standard *Eupatorium perfoliatum* mother tinctures both contain the same active ingredient (quercetin). So, quercetin's Rf value was roughly 0.5 **(Table 1 A, B, C)**. The regression equation was found to be of Y1 (sample A) = 0.0009x+0.0016, Y2(sample B) =0.0009x+0.0021, Y3(sample C) = 0.0008+0.0029 with respective to concentration (**Figure 8A, B, C)**. Further a correlation coefficient of 1.0 indicates good linearity between concentration and area **(Table 2 A, B, C)**.

CONCLUSION

A practical, sensitive, and trustworthy qualitative HPTLC approach has been created. For qualitative quercetin monitoring in *Eupatorium perfoliatum* , the proposed HPTLC approach is quick, easy to use, and accurate. It can also be utilised for standard quality checks. The qualitative assessment of quercetin was examined using the HPTLC fingerprinting technique in three separate samples of mother tinctures from three different pharmaceutical companies. All three of the mother tincture samples tested positive for quercetin, however only sample C was deemed to be standard (R2 = 0.9979). Because the leaves contain a significant amount of quercetin, they can be utilised to treat a variety of maladies.

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Nil.

Conflict of Interest

Nil

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