

Mixed stationary phase for TLC: separation of components from Manjistha root extract

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Thin layer chromatography owes its popularity to several advantages, being faster, easy to perform and ability to use a variety of mobile phase compositions because detection is post mobile phase removal. Silica gel is the most commonly employed stationary phase. This poses certain limitations in terms of selectivity, hence suitable modifications are attempted. Selectivity can be improved using modified silica but the process of modification can be tedious and non reproducible. We report here use of mixed bed stationary phase for separation of components in extract of roots of *Rubia cordifolia* L. (family Rubiaceae), a common medicinal plant used in the preparation of various formulations in Ayurveda. Microcrystalline cellulose (MCC) was prepared and used as a stationary phase with silica in the ratio 10 : 90 and 5 : 95 w/w respectively. Mobile phase composition was optimized and an additional component was separated, which was not observed on silica TLC plate. Moreover, characterization studies of prepared MCC were performed. The FTIR spectra obtained resembled the commercial MCC spectra with all the characteristic peaks obtained well in the prepared MCC. The dynamic light scattering data of the prepared MCC showed the mean particle diameter of 0.715 μm . The TGA-DTA data showed no deviations in the plot, even when the analysis was carried out in the presence of air instead of nitrogen.

Keywords: Microcrystalline cellulose, mixed stationary phase, *Rubia cordifolia*, thin layer chromatography, silica gel.

Thin layer chromatography (TLC) remains one of the undisputed chromatographic techniques due to several advantages, being faster, easier, economic, free from carryover since fresh stationary phase used every time and ability to use a variety of mobile phase compositions because detection is post mobile phase removal. Though several other materials were studied as TLC stationary phases during its evolution and development¹, silica gel is the most commonly employed stationary phase, as of today. This poses certain limitations in terms of selectivity, hence suitable modifications are attempted^{1,2}. Thus, a comparative study is reported for the analysis of amino acids on silica gel (SIG) and polyaniline modified SIG as stationary phases using 1% ethanolic solution of boric

acid as mobile phase³. There is a report of simple and efficient TLC method for separation of gold ($\text{Au}_{25}\text{L}_{38}$) metal clusters⁴. Newer developments in the area continue, for instance, micro-TLC approach comprising one and two dimensional separation to generate fingerprints for fast screening of environmental samples originated from sewage and ecosystem waters⁵. Cellulose in native form and as microcrystalline form has been used as stationary phase for several separations^{6,7}. Cellulose has unique advantages of being relatively mild stationary phase, compared to silica gel and also, shows good separation of chiral compounds¹. For moderately polar compounds, however, it does not yield good separations. For a mixture of two compounds of varying polarity, a mixture of silica gel and micro crystalline cellulose (MCC) has been reported to be useful⁸. Such mixed stationary phases (silica gel containing 25% MCC) are commercially available as precoated plates. As phytochemicals are typically a mixture of varying polarity and tend to contain some chiral compounds as well, we considered the option of using such *mixed* stationary phase for improving separation of extract of manjishta roots. This approach has shown promising results and we demonstrate here improved separation of components of Manjistha root extract, prepared in methanol and chloroform.

Rubia cordifolia L. (Rubiaceae), also known as Manjistha, has been used as a traditional herbal medicine in Ayurveda for centuries for many healthcare disorders⁹. It is an important medicinal plant used for treatment of various diseases such as tumours¹⁰⁻¹², inflammations, urinary disorders and as antimicrobial¹³, hepatoprotective, hypoglycemic¹⁴ and antipsychotic¹⁵. The roots of *R. cordifolia* are listed officially in *Chinese Pharmacopeia* for treatment of arthritis, chronic bronchitis, uterine hemorrhage and uteritis¹⁶.

TLC fingerprinting studies have been reported on *R. cordifolia* root extracts and its commercial samples, the components of Manjistha on silica TLC plates were separated in a ternary solvent system of toluene : diethyl ether : acetic acid in the ratio of 1 : 1 : 1 for the methanolic extract of Manjistha. Also, TLC studies were undertaken for extract prepared in different solvents. Another combinations of solvents used for resolving the components of Manjistha were benzene, ethyl acetate for diethyl ether extract¹⁷.

We have attempted to improve the separations selectivity and separation efficiency by making use of silica gel mixed with microcrystalline silica (MCC) as stationary phase (mixed stationary phase) and succeeded in separating an additional component. MCC was chosen because there is possibility of chiral interactions between MCC and components of Manjistha, which may also have chiral centre. Silica does not have this advantage.

For chromatography, TLC grade silica (Acme's Laboratory Chemicals Silica Gel G, particle size: max. 76 μm), Column grade silica gel (Acme's Laboratory, 60–120

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