INSTRUCTION MANUAL FOR
2L-ChiralTLC (IA, IC, ID, IE, IF)

Please read this instruction sheet completely before using these plates

TLC Description

Plate Specification :

Immobilized-type CSPs (Chiral stationary phases) and silica-gel with a fluorescence indicator (254nm) in the form of a bilayer on an aluminum plate.

Base Material : Aluminum

Chiral selectors of CSP layer :

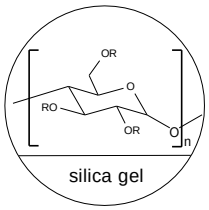
IA : Amylose tris(3,5-dimethylphenylcarbamate) IC

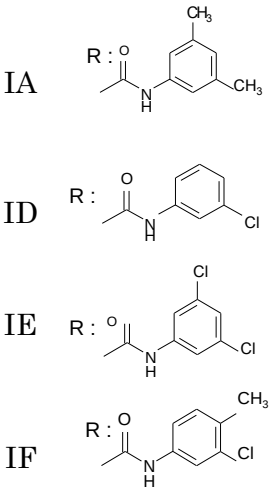
: Cellulose tris(3,5-dichlorophenylcarbamate)

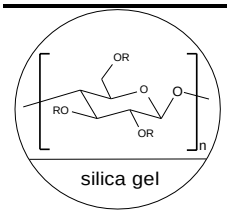
ID : Amylose tris(3-chlorophenylcarbamate)

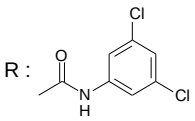
IE : Amylose tris(3,5-dichlorophenylcarbamate)

IF : Amylose tris(3-chloro-4-methylphenylcarbamate)









Operating Instructions

Size	200 mm width X 100 mm length
Layer Thickness	approximately 270 μm (CSP layer + silica-gel layer)
Particle size	CSP : 20μm
Recommended sample amount	1 ～ 5 μL
Detection	Silica-gel layer includes a fluorescence indicator (UV254nm). Samples which have ultraviolet absorption can be observed as spots (shadow). Staining reagent can also be used.

Important reminder

- 2L-ChiralTLC are intended only for research and experimental proposes.
- Intended for use with organic solvents only. Use with aqueous solvents can cause coating to wash off.
- An impact shock or stress should be avoided as, the base material is aluminium. TLC plates that are cut may have sharp edges. To prevent from injuring fingers, please use proper protective equipment.
- Do not press and rub the surface of TLC plates. Silica-gel layer may lift off. When you cut a TLC plate, protection of the surface of the TLC plate is recommended.
- UV lamps should be handled in accordance with their instruction manuals.
- Once the vacuum-sealed package is opened, unused plates should be stored in a desiccator with a desiccant.

How to use 2L-ChiralTLC

1) Preparation of TLC plates

Open a vacuum-sealed package and remove a 2L-ChiralTLC plate. Confirm the position of "sample application zone" and the direction of developing by reference to Fig. 1. "Sample application zone" has only CSP layer (no silica-gel layer). The type of CSP is printed on the top of TLC plate.

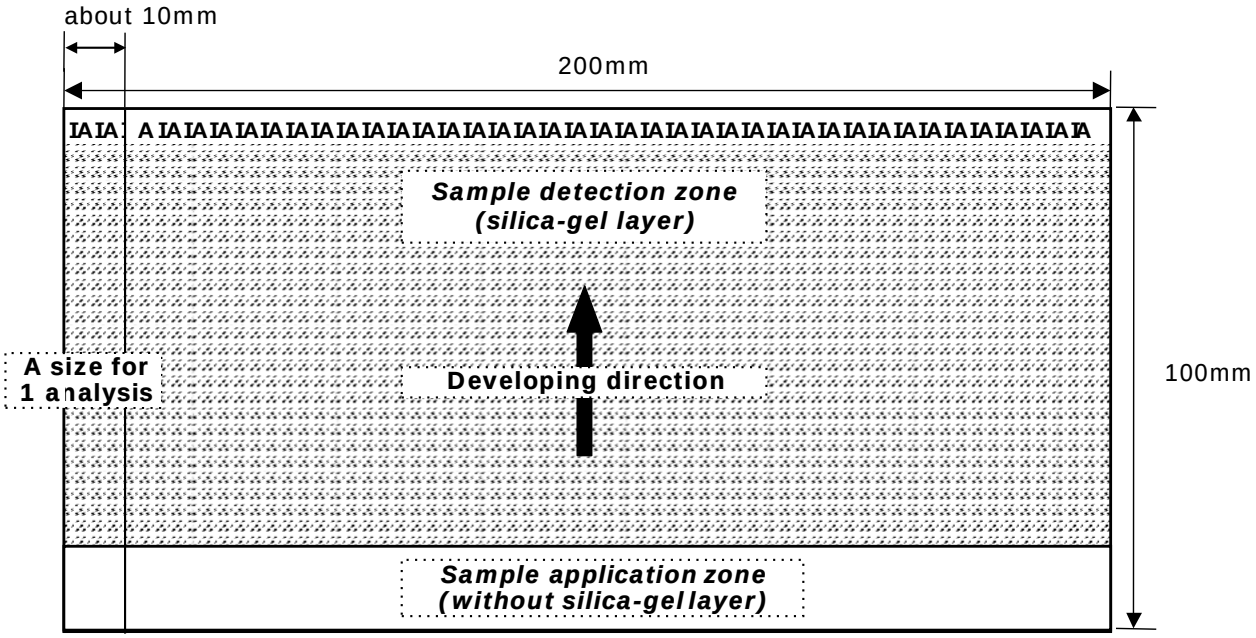


Fig. 1. An example of 2L-ChiralTLC and the size for 1 analysis (20 analyses per 1 plate)

Cut 2L-ChiralTLC into a proper size with scissors. 10mm in width per one analysis is recommended. Do not press the surface of the TLC plate with fingers to prevent from peeling the Silica-gel coat. When you cut a TLC plate, it is recommended to protect the surface to avoid contamination with your fingers. In addition, to prevent from injuring fingers, please use proper protective equipment.

2) Sample spotting

Spot the sample on "sample application zone". ("Sample application zone" is the bottom end of the TLC plate where only the CSP is coated) The recommended spotting position is within approximately 10 mm of the bottom end of the TLC. (Fig. 2). Best spotting results are obtained by dipping a microcapillary pipet into a sample solution, removing the pipet, and gently touching the end of the pipet to the surface of the TLC plate until a spot of no more than 2 mm in diameter is obtained. Allow spot to dry, and repeat spotting procedure if necessary, to increase the amount of sample on the plate. All spots should be dry before proceeding to developing step.

Before spotting, it is recommended to verify the UV detection of the sample by spotting on the top of the silica-gel layer zone. (Fig. 3)

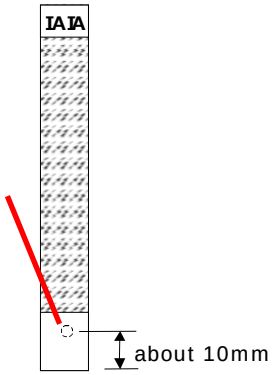


Fig. 2. The position of Sample spotting

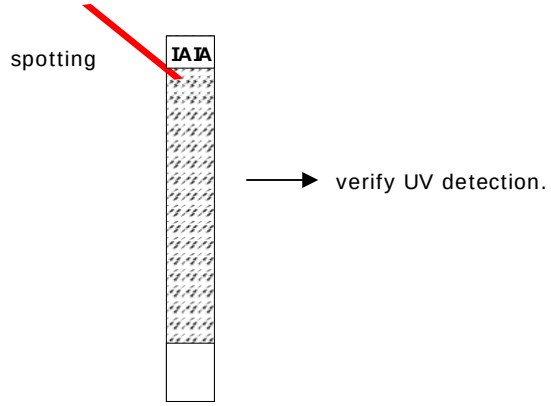


Fig. 3. The verification of UV detection of the Sample

3) Developing (Sample separation)

Put a filter paper into the developing chamber and fill with a saturated vapor of the solvent. Then, put the TLC plate in a developing chamber. Confirm that the solvent level is lower than the position of sample spot (Fig. 4). After the TLC has been developed, dry the TLC plate.

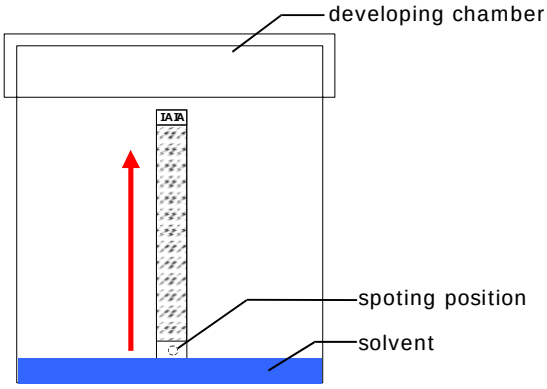


Fig. 4. Developing

4) UV detection

Irradiate UV light to the TLC plate and observe the sample spot (shadow) in the sample detection zone. Measure the distance of the sample spots from the sample spotted position (A and B in Fig. 5) and the solvent front from the sample spotted position (C in Fig. 5). Calculate Rf (Relative to Front) value. (Fig. 5)

If sample spots can't be observed, spraying and drying an organic solvent (e.g. Ethanol) on the surface of TLC plate may help the detection. An accompanying atomizer is for this purpose.

In the case of detection of samples which don't have enough UV absorption, use staining reagent to detect the spot. (staining reagent : phosphomolybdic acid-ethanol, iodine stain, p-anisaldehyde, or ninhydrin) If you need to use staining reagent, it is recommended to verify the coloring and the detection of the sample by spotting the sample on a TLC plate before using 2L-ChiralTLC® (an achiral standard silica-gel TLC plate is recommended).

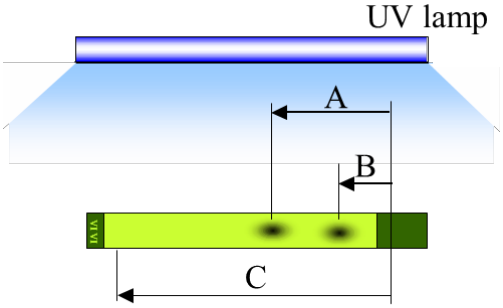


Fig. 5. UV detection

$$Rf_1 = A / C$$
$$Rf_2 = B / C$$

Recommended Method

A - Solvents

2L-ChiralTLC can be used with a wide range of organic miscible solvents, such as mixtures of alkanes/alcohol, pure alcohol or acetonitrile (CH_3CN)) to developing solvents containing methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc) among others.

We recommend that the conditions shown in Table 1 are used as the basis for initial method development for 2L-ChiralTLC. After the initial evaluation the most promising methods can be optimised using the suggested ranges below. MtBE and chlorinated solvents may also be used in their pure form as developing solvents. Moreover, in the case of solvents with strong elution intensity, such as THF and ethyl acetate, it is advised to mix them with a hydrocarbon solvent (e.g. hexane or heptane) to modulate retention and selectivity.

Table 1. Recommended organic miscible solvents

	Alkane❶/ Alcohol❷	Alkane❶/ EtOAc	Alkane❶/ $CHCl_3$	Alkane❶/ THF	MTBE / EtOH
Typical starting conditions	90:10	90:10	70:30	90:10	100:0
Advised optimization range	95:5 ~ 0:100❸	95:5 ~ 0:100	95:5 ~ 0:100	95:5 ~ 0:100	100:0 ~ 40:60

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ Methanol, ethanol, and 2-propanol are raised as typical alcohol.
Moreover, as alcohol other than the above, 1-propanol, 1-butanol, 2-butanol, etc. can be used.
Depending on a sample, separation may change greatly with kinds of alcohol.
❸ As for the mixed solvent of alcohol, viscosity may become high with composition. Assuming to use CHIRALFLASH, please adjust the composition of solvent mixture not to exceed the maximum working pressure range of CHIRALFLASH. Usually, higher composition of alcohol gives shorter retention time and higher Rf value.
Due to limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH or the other alcohol listed above together with MeOH in order to obtain homogenous solvent mixtures. A maximum of 5% MeOH in n-Hexane only may be used without adding other alcohol.

For initial and routine method development, it is recommended to use pure alcohol, EtOAc, THF, MTBE, $CHCl_3$ and mixtures of alkanes. For higher productivity on CHIRALFLASH, it is effective to use a solvent where the solubility of the sample is comparatively high as an initial examination solvent.

B – Additives

For basic samples or acidic samples, it is necessary to add an additive into the developing solvent in order to get appropriate spots, otherwise broad and/or tailing spot form may be observed.

1. Add 0.1 volume percent of acid (for acidic samples) or base (for basic samples) to developing solvent.
2. Before developing, soak TLC plate for about 1 minute in above solvent and dry it. This will ensure that the entire plate has been conditioned with the chosen additive.
3. Once the above steps are taken, the TLC plate is ready to be used.

Depending on samples, EDA or AE is often more effective than DEA. If you use these additives, 2 volume percent of alcohol can be necessary to be completely mixed, because of their low miscibility to low polar solvents.

Basic Samples require Basic additives	Acidic Samples require Acidic additives
Diethylamine (DEA) Ethylenediamine(EDA) 2-Aminoethanol (AE) Butyl amine (BA)	Acetic acid
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

⇨ ***STRONGLY BASIC solvent additives or sample solutions <u>MUST BE AVOIDED</u>, as they are likely to damage the silica gel used in this TLC.***

Method Development for CHIRALFLASH

The CSP used for 2L-ChiralTLC and CHIRALFLASH is the same (i.e. 20µm CSP). Therefore, the eluent used on 2L-ChiralTLC is directly applicable to CHIRALFLASH, if the sample is separated on 2L-ChiralTLC. The protocol of method development for CHIRALFLASH using 2L-ChiralTLC is described below.

1. Spot sample solutions and develop with each 2L-ChiralTLC (IA, IC, ID, IE and IF) and each solvent (Refer to the Table 1). [Screening]

2. If good separation is achieved on TLC, the mobile phase can be directly transfered to the separation on the corresponding CHIRALFLASH. If a good separation on CHIRALFLASH is confirmed, move to the optimization of separation conditions (injection amount or mobile phase etc.). If the method is being transferred to CHIRALFLASH, it may be desirable to optimize the separation to obtain higher Rf values, since this will speed up the separation and use less solvent

- The example of separation of Flavanone(FLV)

1. Sample solutions are spotted on 2L-ChiralTLC IA, IC and developed with several solvents. (Fig. 6) (In case of no separation, try other combinations of CSP types and mobile phases.)

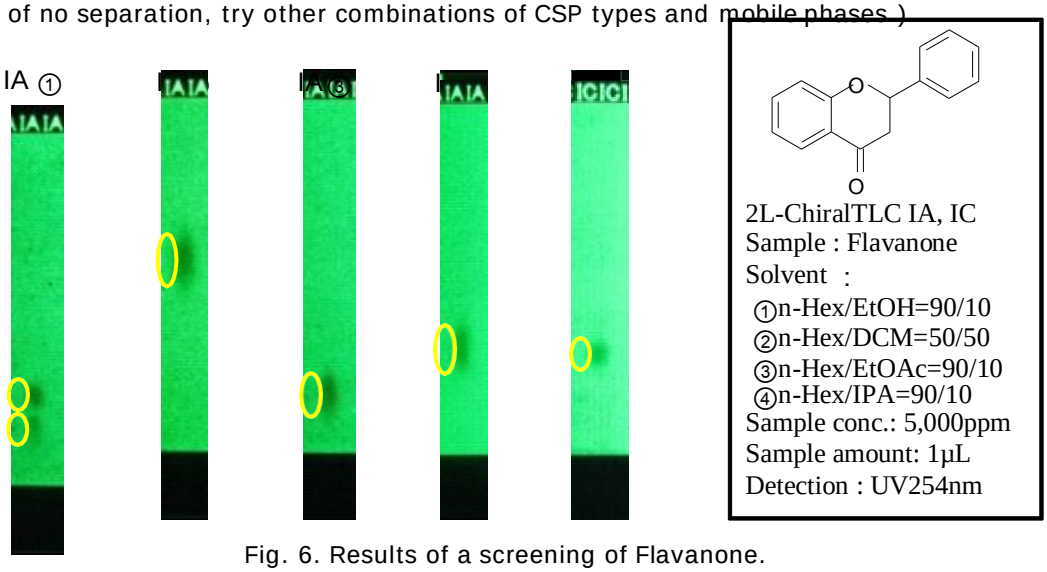


Fig. 6. Results of a screening of Flavanone.
(n-Hex=n-Hexane, EtOH=Ethanol, DCM=Dichloromethane, EtOAc=Ethyl acetate, IPA=2-Propanol)

2. Inject a certain amount of sample on CHIRALFLASH with the same mobile phase with TLC. If a good separation on CHIRALFLASH is confirmed, increase the sample loading amount and fix the separation conditions. (Fig. 7)

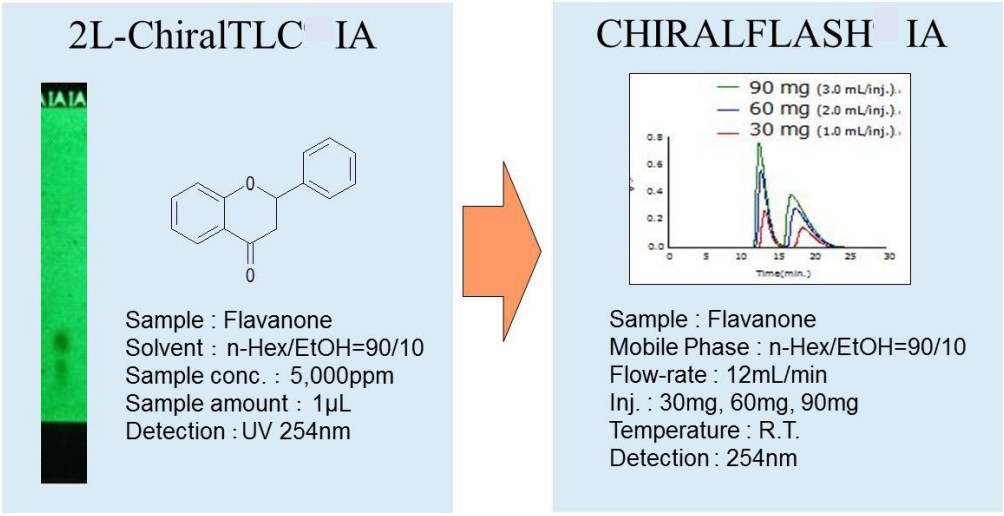
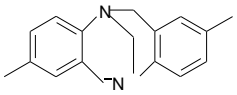


Fig 7. The optimization of separation conditions on CHIRALFLASH IA

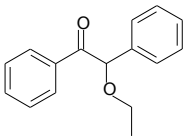
Applications of 2L-ChiralTLC

Applications of 2L-ChiralTLC IA

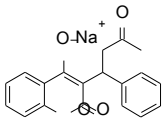
1. Tröger's base (TB)
Solvent : n-Hex/EtOH=90/10 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL
Detection : : UV254nm



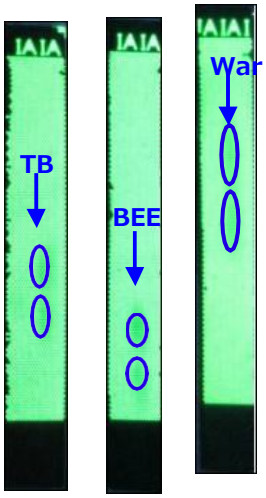
2. Benzoin Ethyl Ether (BEE)
Solvent : n-Hex/EtOAc=90/10 (v/v)
Sample conc. : 10mg/mL
Sample amount : 1μL
Detection : : UV254nm



3. Warfarin Sodium (War)
Solvent : n-Hex/IPA/TFA=50/50/0.1 (v/v/v)
Sample conc. : 25mg/mL
Sample amount : 1μL
Detection : : UV254nm

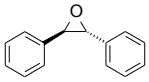


1. TB : Rf_1=0.49, Rf_2=0.35
2. BEE : Rf_1=0.33, Rf_2=0.19
3. War : Rf_1=0.78, Rf_2=0.60

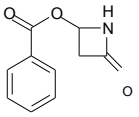


Applications of 2L-ChiralTLC IC

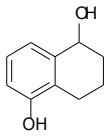
1. trans-Stilbene oxide (TSO)
Solvent : n-Hex/IPA=90/10 (v/v)
Sample conc. : 50mg/mL
Sample amount : 1μL Detection
: : UV254nm



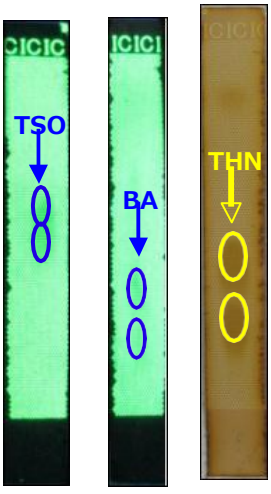
2. 4-Benzoyloxy-2-azetidinone (BA)
Solvent : n-Hex/IPA=50/50 (v/v)
Sample conc. : 100mg/mL
Sample amount : 1μL
Detection : : UV254nm



3. 1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene(THN)
Solvent : DCM/MeOH/AcOH=100/0.1/0.1(v/v/v)
(twice developing)
Sample conc. : 50mg/mL
Sample amount : 1μL
Detection : : Iodine stain



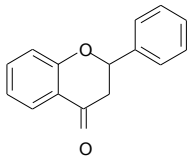
1. TSO : Rf_1=0.61, Rf_2=0.54
2. BA : Rf_1=0.41, Rf_2=0.28
3. THN : Rf_1=0.46, Rf_2=0.26



Applications of 2L-ChiralTLC ID

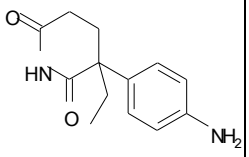
1. Flavanone (FLV)
Solvent : MeOH=100
Sample conc. : 5mg/mL
Sample amount : 1μL

Detection : : UV 254nm



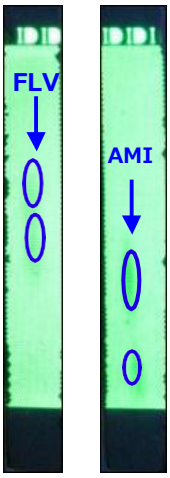
2. Aminoglutethimide (AMI)
Solvent : EtOAc/DEA=100/0.1 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL

Detection : : UV254nm



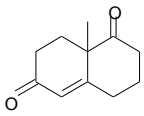
1. FLV : Rf_1=0.64, Rf_2=0.49

2. AMI : Rf_1=0.44, Rf_2=0.16



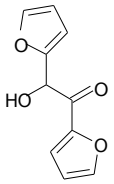
Applications of 2L-ChiralTLC IE

1. Wieland Miescher ketone (WMK)
Solvent : n-Hex/EtOH=60/40 (v/v)
(twice developing)
Sample conc. : 10mg/mL
Sample amount : 1μL
Detection : : UV 254nm



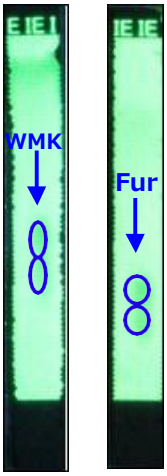
2. Furoin (Fur)
Solvent : n-Hex/EtOH=70/30 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL

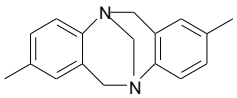
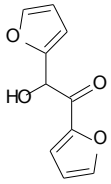
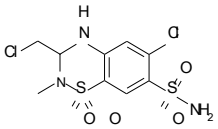
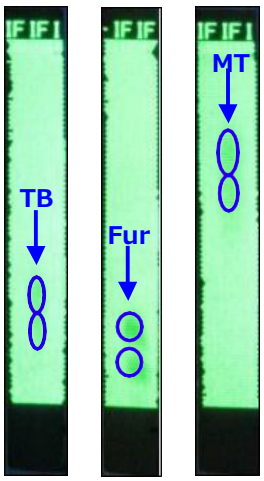
Detection : : UV254nm



1. WMK : Rf_1=0.51, Rf_2=0.41

2. Fur : Rf_1=0.37, Rf_2=0.28



Applications of 2L-ChiralTLC IF	
1. Tröger's base (TB) Solvent : MeOH=100 Sample conc. : 20mg/mL Sample amount : 1μL Detection : : UV 254nm  2. Furoin (Fur) Solvent : n-Hex/EtOH/MeOH=75/15/15 (v/v/v) Sample conc. : 50mg/mL Sample amount : 1μL Detection : : UV254nm  3. Methyclothiazide (MT) Solvent : n-Hex/EtOH/DEA=50/50/0.1 (v/v/v) (twice developing) Sample conc. : 10mg/mL Sample amount : 2μL Detection : : UV254nm 	1. TB : Rf_1=0.38, Rf_2=0.28 2. Fur : Rf_1=0.28, Rf_2=0.16 3. MT : Rf_1=0.76, Rf_2=0.65 

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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