biostep®

Ready-to-use

Application Notes from the lab for the lab

Handling of the TLC Quicktest Set of biostep GmbH

Introduction:

Thin-Layer Chromatography (TLC) and HPTLC (High-performance-TLC) are widely used methods for the separation and identification of substances of a mixture. There is a mobile phase that is a composition of different solvents and a stationary phase which is a solid layer on glass plates. In most cases this layer is made of silica gel. The separating principle is that the components of the mixture have different affinities to the mobile and the stationary phase. The mobile phase migrates by capillary forces through the stationary phase and transports the sample components. Due to adsorption and desorption processes at the stationary phase, the different substances will be separated from one another.

Material (Fig. 1):

H-separating chamber 50 x 50 mm, application template 50 x 50 mm, 1 frit rod 50 mm, micro capillaries 1 µl with holder, lipophilic and hydrophilic test solution, HPTLC ready-to-use glass plates 50 x 50 mm silica gel K60F254, Concise Practical Book of TLC
Solvents (not included):
for the hydrophilic test solution: n-butanol, Aqua dest., ethanol, glacial acetic acid (60 + 20 + 10 + 0.5 v/v)
for the lipophilic test solution: xylene or toluene

Workflow (Fig. 2 - 10):

Prepare samples according to the instructions given in the Practical Book or use the test solutions.
Insert a HPTLC glass plate with the white layer upwards into the application template so that it touches the right stopping point of the transparent toothing (Fig. 2). Push a capillary in the holder (Fig. 3) and dip it in one of the test solutions or your sample (Fig. 4). When the capillary is filled, apply it on the surface of the white layer by gentle dipping without damaging (Fig. 5). Use the indentations between the teeth. Let the solution flow out with interruptions for drying (e.g. with a hair-dryer) to ensure that the application spot is as little as possible. Apply e.g. 1 µl at position 1; 2 x 1 µl at position 3 and so on up to 5 x 1 µl at position 9. Let the sample dry before the separation (Fig. 6). During drying, mix the appropriate mobile phase according to the instruction given here or in the Practical Book if necessary. Put the frit rod in the H-chamber in the cavity (Fig. 7). If you need a chamber saturation, insert a piece of filtering paper on the bottom of the separating chamber. Wet this paper with reagent or solvent.
Lay the HPTLC glass plate with the white adsorbent layer downwards into the H-chamber over the frit rod. Be sure that the frit rod touches the smaller part of the plate below the sample dots (Fig. 8). Do not touch the adsorbent surface of the HPTLC plate! After the correct positioning of the plate, apply 1.0 ml of the mobile phase in the cavity of the frit rod (Fig. 9). The solvent will be soaked immediately by the frit rod and transported to the stationary phase. Cover the chamber using the glass lid. The migration and separation process can be monitored through the glass plates. As soon as the solvent front is some millimeters away from the end of the plate it can be removed to stop the separation (Fig. 10). The solvent front should be marked by scratching the surface. Let the glass plate dry. For drying a hair-dryer or a Thermoplate S_{plus} (BS121.845) in a fume hood is recommended. After drying the results can be recorded. Wash the frit rod three times with acetone and let them dry before using it further.

by P. Altenhofer

The HPTLC plate should now be investigated under UV-light (UV-Box BS131.210) to detect substances invisible in daylight. Document the results. For many substances it is also necessary to derivatize them with special reagents (see the instructions in the Practical Book). The sprayer SG e1 (BS130.605) is recommended for derivatisation. It works without CFC and the air pressure is generated by a silent pump powered by accumulators. Always spray in a spray box (BS124.105) or fume hood. After spraying the HPTLC plate, it is often necessary to heat the plate for finishing reaction. This can easily be done with the newly-developed Thermoplate S_{plus} (BS121.845). After cooling the TLC plate, document it again under daylight and UV-light.

If the work must comply to GxP, we also have semi-automated and fully-automated systems. For a professional sample application, we offer the HPTLC applicator AS 30 incl. software (BS130.500, BS130.532). Using the professional automatic sprayer ChromaJet DS 20 (BS130.700), the derivatisation is much more comfortable, reproducible and accurate. Regarding documentation, we offer the system ProViDoc DD 70 with a high-resolution 18 Mpixels reflex camera (BS140.061). Images under white and UV-light can be taken and analyzed by the Maxim TLC software (BG04-T0305). For accurate quantification, the HPTLC densitometer CD 60 is recommended (BS131.800).

**** Technical Changes reserved ****

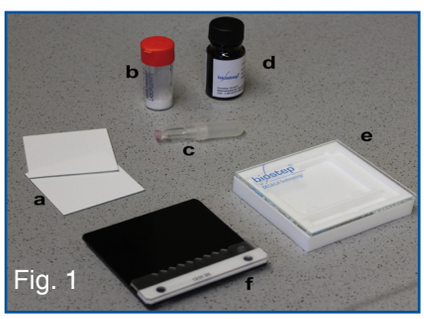


Fig. 1

Fig. 1
Materials of the TLC-Quick Set without Practical Book
a: HPTLC plates 50 x 50mm
b: Glass capillaries in container
c: Capillary holder
d: Test solution (hydrophilic or lipophilic)
e: H-separating chamber incl. frit rod
f: Application template

Fig. 2-10
Workflow in TLC/HPTLC. For descriptions see text.


Fig. 2

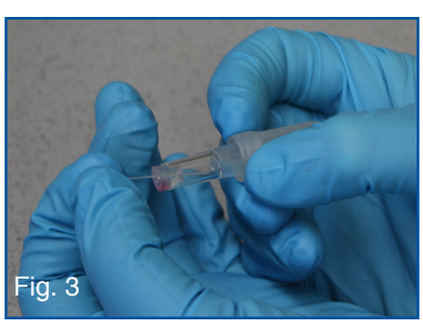


Fig. 3


Fig. 4

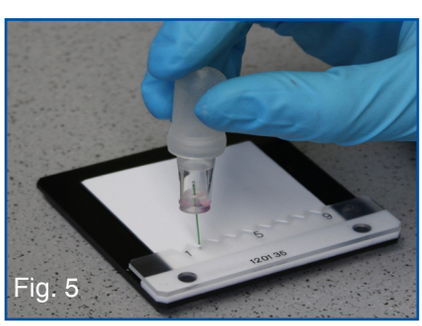


Fig. 5


Fig. 6

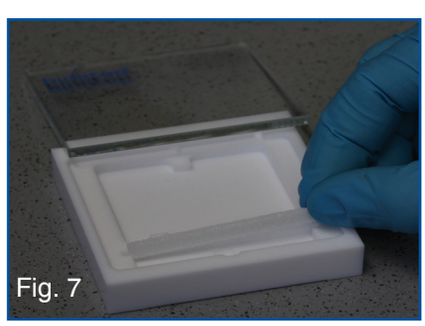
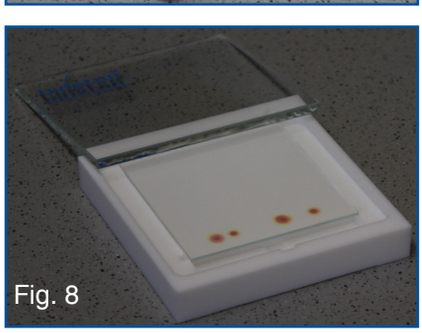


Fig. 7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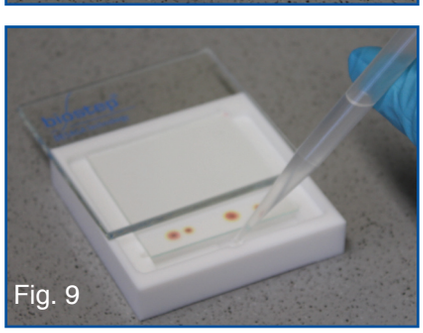
Fig. 9

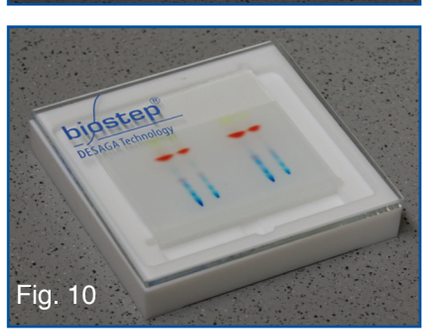

Fig. 10