

# Derivatization

The possibility of straight forward derivatization is a special feature of TLC/HPTLC

## Pre- and Postchromatographic Derivatization



It is an inherent advantage of TLC/HPTLC that all fractions remain stored on the plate and can be readily derivatized after chromatography. Substances that do not respond to visible or UV light can be rendered detectable. In many cases, substances or classes of substances can be identified by specific reagents, enabling their selective detection.

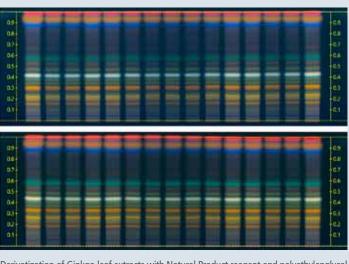
Pre-chromatographic derivatization is possible by overspraying the sample application zones with the Linomat 5 or the Automatic TLC Sampler ATS 4.

For the transfer of liquid reagents for postchromatographic derivatization, one can choose between spraying or dipping. Dipping and automated spraying are the preferred techniques, particularly when a quantitative evaluation is intended. Usually reagent transfer by spraying can not be circumvented when two reagent solutions have to be applied in sequence without intermediate drying, for instance diazotation followed by coupling.

Whenever reagents are transferred by spraying, an efficient reagent mist removing device should be used to protect laboratory personnel against poisonous or irritating sprays or solvent vapors.

In most cases the derivatization reaction needs to be completed by heat treatment. Heating the plate at the desired temperature with a plate heater specifically designed for this purpose is highly recommended. An oven used for this purpose will become permanently contaminated.

## Comparison of reagent transfer by automated spraying and dipping



Derivatization of Ginkgo leaf extracts with Natural Product reagent and polyethylenglycol solution by spraying with 2 mL (above) and dipping in 200 mL (below)



## CAMAG Chromatogram Immersion Device

For proper execution of the dipping technique, the plate must be immersed and withdrawn at a controlled uniform speed; otherwise tide marks may be left which interfere with densitometric evaluation. By maintaining a well defined vertical speed and immersion time, derivatization conditions can be standardized. The immersion device can also be used for the pre-washing of plates.

#### **Key features**

- Uniform vertical speed, freely selectable between 30 mm/s and 50 mm/s
- Immersion time selectable between 1 and 8 seconds and indefinitely (upward movement at another touch of the button)
- The device can be set to accommodate 10 cm and 20 cm plate height.
- · Battery operated

## **CAMAG TLC Sprayer**

The function is electro-pneumatic. Reagents are atomized into a fine aerosol spray with particles in the range of 0.3 to 10  $\mu m.$  This ensures a fairly homogeneous distribution over the layer at a low reagent consumption.

The TLC Sprayer consists of the charger and a pump unit with two kinds of spray heads, type A for spray solutions of normal viscosity (organic solvents), and type B for liquids of higher viscosity (*e.g.* sulfuric acid containing reagents).

## **Glass Reagent Sprayer**

This all glass reagent sprayer is a low cost alternative to the TLC/HPTLC Sprayer. It comes with a rubber pump but may also be operated from a compressed air or nitrogen supply. The Erlenmeyer flask may be closed with a standard glass stopper.

#### **Ordering information**

**022.6606 CAMAG® Chromatogram Immersion Device 3** for TLC

and HPTLC plates up to 20  $\times$  20 cm, without dip tank

022.6627 Dip tank for plates  $20 \times 20$  cm, with lid

022.6628 Dip tank for plates  $20 \times 10$  cm, with lid

022.6619 Bench top rack for three dip tanks

**022.6530 TLC Sprayer,** complete with spray head type A and B, reagent bottle 100 mL, reagent bottle 50 mL

022.6535 Pack of 5 spray heads type A and 1 type B

022.6538 Pack of 6 spray heads type B

022.6536 Reagent bottle 100 mL with cap, pack of 6

022.6537 Reagent bottle 50 mL with cap, pack of 6

022.6539 Service kit for TLC Sprayer

022.6100 Glass reagent spray with 100 mL Erlenmeyer flask

### **CAMAG Derivatizer**

Derivatization means another step in the TLC/HPTLC process, consequently causing an increase in the variance. If an experienced technician performs reagent transfer by manual spraying, the relative standard deviation of the measured values significantly rises up to 12.0%. In contrast with the Derivatizer, the standard deviation increases only slightly to between 2.5 and 4.5%

The Derivatizer is an automated spraying device which sets a new standard of reproducibility in the reagent transfer onto TLC/HPTLC plates by employing a unique "micro droplet" spraying technology. The Derivatizer ensures homogeneous and reproducible application of the most common reagents. To meet the diverging physicochemical properties of the different reagents, e.g. acidity or viscosity, four different color-coded spray nozzles are employed, and the user can select from six spraying levels. The Derivatizer is available for two different plate formats  $(20 \times 20 \text{ and } 20 \times 10 \text{ cm})$ .



Color-coded spray nozzles

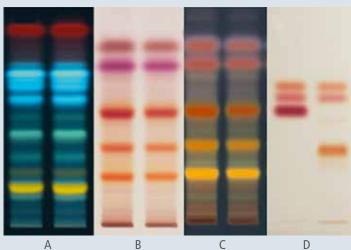


#### **Key features**

- Unique micro droplet spraying technology
- Reproducible and user-independent results
- Highly homogeneous reagent transfer
- Reagent consumption (2-4 mL)
- Environmentally friendly and safe handling through a closed system
- Easy cleaning



high homogeneity.



HPTLC chromatograms derivatized with Natural Product reagent/Polyethylene glycol solution under UV 366 nm (A), Anisaldehyde reagent under white light (B) and UV 366 nm (C), and Fast blue salt B reagent under white light (D)

The nozzle generates an extremely fine reagent mist, which evenly distributes in the chamber and gradually condenses on the TLC/HPTLC plate.

The following most common reagents have been tested and validated by the CAMAG laboratory for use with the Derivatizer:

- Sulfuric acid reagent (10% in methanol)
- Anisaldehyde reagent
- · Natural product reagent
- Polyethylene glycol solution
- Iodine solution (0.5% in ethanol)
- Dragendorff reagent
- Fast blue salt B reagent
- · Ehrlich's reagent
- Phosphomolybdic acid reagent
- Ninhydrin reagent
- Copper (II) sulfate reagent
- Aniline-diphenylamine-phosphoric acid reagent
- Vanillin reagent
- Potassium hydroxide solution (5% in methanol)
- Aqueous solutions (enzymatic solutions, etc.)

#### **Ordering information**

022.6000 CAMAG® Derivatizer with hoods for 20 x 10 and 20 x 20 cm plates

022.6010 CAMAG® Derivatizer with hood for 20 × 10 cm plates 022.6020 CAMAG® Derivatizer with hood for 20 × 20 cm plates

Detailed ordering information: www.camag.com/derivatizer



## **CAMAG TLC Plate Heater 3**

The TLC Plate Heater is designed for heating a TLC/HPTLC plate to a selected temperature after a staining reagent has been applied.

The Plate Heater has a CERAN® heating surface which is resistant to all common reagents and is easily cleaned. The  $20 \times 20$  cm heating surface has a grid to facilitate correct positioning of the TLC/HPTLC plate.

Programmed and actual temperature are digitally displayed. The temperature is selectable between 25 and 200  $^{\circ}$ C. The plate heater is protected from overheating.

## **CAMAG TLC Spray Cabinet 2**

The TLC Spray Cabinet is designed for the complete removal of excessive spray mist while spraying a TLC/HPTLC plate with reagent.

There is no deflection of the spray jet before it reaches the plate, an effect often encountered in a normal laboratory fume hood. Particles rebounding from the plate are completely removed. The Spray Cabinet is also useful for drying plates after development, with or without the assistance of a hair dryer.

The cabinet is made of PVC. The blower, a radial fan driven by a motor outside of the fume duct, produces an airflow of 130 cubic feet (3.7 cubic meter) per minute. The bottom of the spray cabinet has a built in tray, which is removable for easy cleaning.

#### **Ordering information**

022.3306 CAMAG® TLC Plate Heater 3

Stainless steel housing, flat ceramic top, for TLC/HPTLC plates up to  $20\times20$  cm, digital temperature display, temperature range 25–200 °C.

**022.6230 CAMAG® TLC Spray Cabinet 2** with blower and flexible exhaust hose 1.5 m

**022.6232 CAMAG® TLC Spray Cabinet 2** without blower, for connection to existing forced flow conduit, with 1.5 m flexible exhaust hose 127 mm diameter

022.6226 Exhaust hose extension 1.5 m with adapter

Further information can be found at www.camag.com/derivatization

