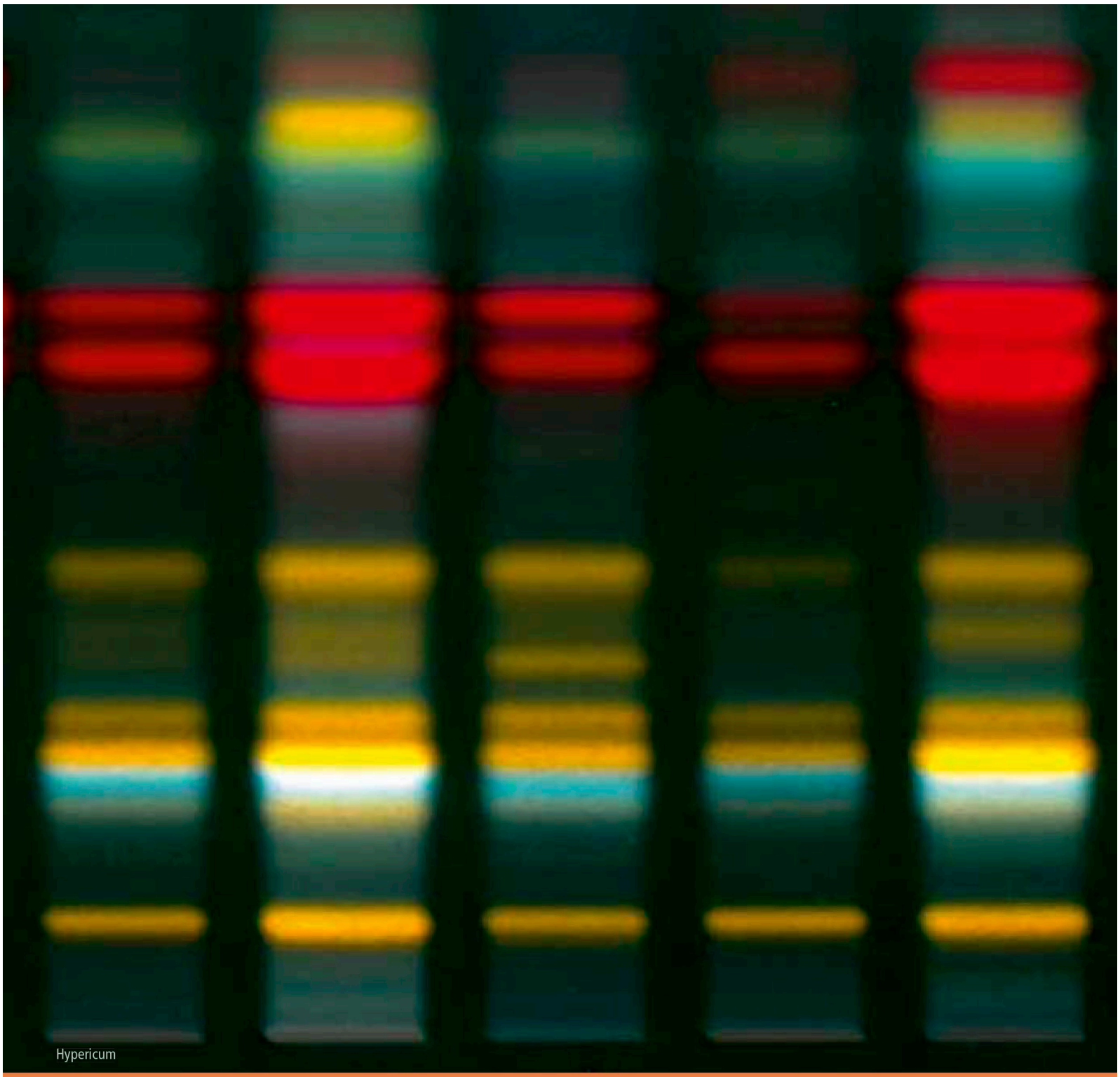


Hypericum

Chromatogram Development

Chromatogram development under reproducible standardized conditions is a key to the quality of the result

Chromatogram Development

TLC/HPTLC differs from all other chromatographic techniques in the fact that in addition to stationary and mobile phases, a gas phase is present, which can significantly influence the result of the separation.

The following considerations primarily concern silica gel as stationary phase and a process usually described as adsorption chromatography.

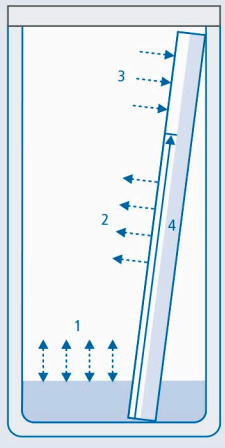
In the developing chamber four partially competing processes occur:

1 Between the components of the developing solvent and its vapor, an equilibrium will be established gradually. This process is called chamber saturation. Depending on the vapor pressure of the solvent components the composition of the gas phase can differ from that of the developing solvent.

2 The part of the layer which is already wetted with mobile phase contributes to the formation of the equilibrium.

3 While still dry, the stationary phase adsorbs molecules from the gas phase. Thereby polar components will be preferentially withdrawn from the gas phase and loaded onto the surface of the stationary phase. Allowing the plate to interact with the gas phase prior to starting chromatographic development is called layer preconditioning, which is not possible with all types of developing chambers. Lining the chamber with filter paper soaked with developing solvent supports this process. In case that preconditioning is not desired, a counter glass plate arranged a few mm apart suppresses it. This is called sandwich configuration.

4 During solvent migration, the components of the mobile phase may be separated by the stationary phase under certain conditions, causing the formation of secondary fronts, which is usually not desired.



Influence of relative humidity ("activity of the layer") with the same solvent migration distance

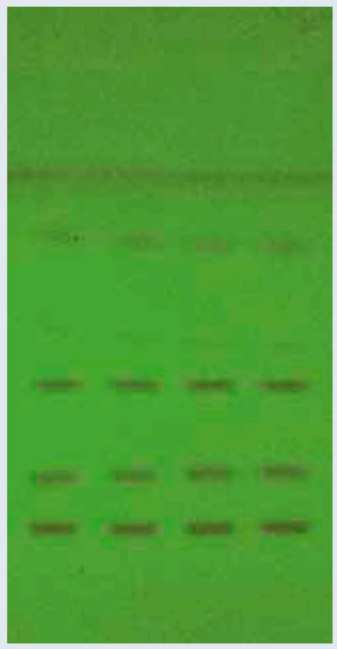
Choosing the type of developing chamber

Selection of the "appropriate" chamber is made during method development, depending on what parameters such as chamber saturation, preconditioning the layer, relative humidity, etc. influence the result. Often "practical" considerations are followed such as which chamber is available, which one must be used due to an SOP, or which one has been used in the past if a results comparison is to be made. Economical aspects like solvent consumption, optimal use of layer space, etc. are also considerations. Preconditioning of the layer with solvent vapor is possible with all type chambers described except the flat bottom chamber.

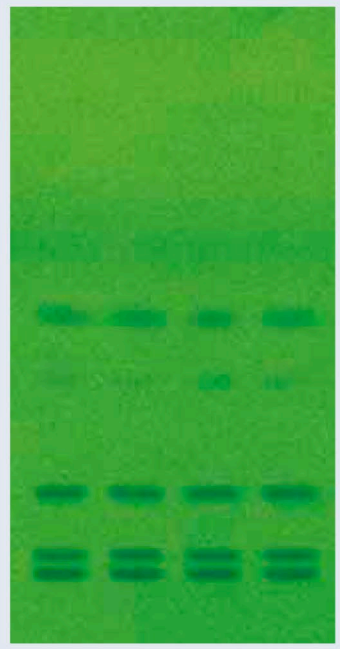
Efficient preconditioning at a controlled relative humidity is most conveniently effected with the ADC 2. Also the Horizontal Developing Chamber (HDC) provides this feature and – with limitations – the Twin Trough Chambers.

Sandwich configuration can be selected with the HDC.

Considered also should be the HPTLC Vario System (p. 18) which offers the time saving optimization of development conditions.



Development without preconditioning

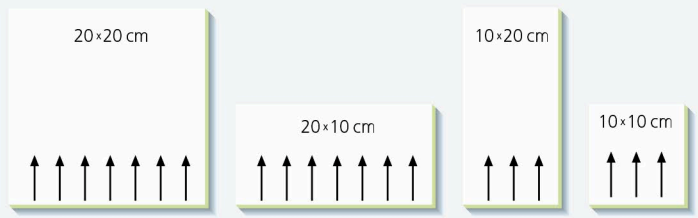


Development with preconditioning

Definition of plate and chamber formats

These format definitions are used in this catalog as well as in all CAMAG literature.

Note: certain plates can be developed in one direction only, e.g. plates with a concentration zone, GLP coded plates.



CAMAG Flat Bottom Chamber

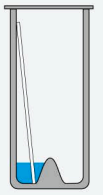
This is the classical developing tank for thin-layer chromatography. It permits the plate to be developed under conditions of partial or complete saturation of the tank atmosphere with solvent vapors. The degree of layer preconditioning can not be controlled unless additional accessories are used.

CAMAG Twin Trough Chamber

The CAMAG Twin Trough Chamber offer several ways to specifically influence chromatogram development in order to improve it.

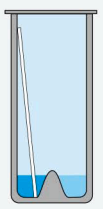
Twin Trough Chamber:
Low solvent consumption

20 mL of solvent are sufficient for a 20x20 cm chamber, 10 mL for the 20x10 cm chamber and 5 mL for a 10x10 cm chamber. This reduces not only solvent consumption but also disposal problems.



Reproducible preconditioning of the layer with solvent vapor

Developing solvent is placed in the trough opposite to the plate. Preconditioning can be performed with any solvent and for any duration. Development is started when developing solvent is placed into the trough with the plate.



Ordering information
CAMAG Flat Bottom Chamber

022.5259 Flat Bottom Chamber for plates 20 x 20 cm, with stainless steel lid
022.5250 Flat Bottom Chamber for plates 20 x 20 cm, with glass lid
022.5257 Flat Bottom Chamber for plates 20 x 20 cm, without lid

022.5150 Flat Bottom Chamber for plates 10 x 10 cm, with stainless steel lid
022.5151 Flat Bottom Chamber for plates 10 x 10 cm, without lid

022.5275 Flat Bottom Chamber light-weight for plates 20 x 20 cm, with glass lid
022.5270 Flat Bottom Chamber light-weight for plates 20 x 10 cm, with glass lid

CAMAG Twin Trough Chamber

022.5256 Twin Trough Chamber for plates 20 x 20 cm, with stainless steel lid
022.5255 Twin Trough Chamber for plates 20 x 20 cm, with glass lid
022.5258 Twin Trough Chamber for plates 20 x 20 cm, without lid

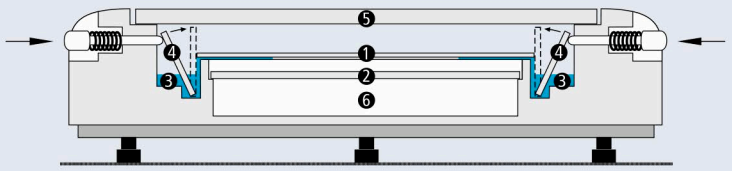
022.5254 Twin Trough Chamber for plates 20 x 10 cm, with stainless steel lid
022.5253 Twin Trough Chamber for plates 20 x 10 cm, with glass lid
022.5261 Twin Trough Chamber for plates 20 x 10 cm, without lid

022.5155 Twin Trough Chamber for plates 10 x 10 cm, with stainless steel lid
022.5156 Twin Trough Chamber for plates 10 x 10 cm, without lid

CAMAG Horizontal Developing Chamber

In the Horizontal Developing Chamber the HPTLC plate is developed from both opposing sides towards the middle. This permits the number of samples to be doubled as compared with development in a tank, provided the separation distance of 45 mm, i.e. 50 mm minus 5 mm distance from the edge, is sufficient. In case a longer separation distance is desired, the HDC can be used for development from one side.

In the Horizontal Developing Chamber, a plate can be developed in the sandwich as well as in the tank configuration.



1 HPTLC plate (layer facing down)
2 Glass plate inserted to establish sandwich configuration
3 Reservoir for developing solvent
4 Glass strip for solvent transfer by capillary action
5 Cover plate
6 Conditioning tray

CAMAG smartAlert solvent front monitor

smartAlert serves for dependable monitoring the development of a plate in a glass developing chamber.

- Gives acoustic and visual notice when the mobile phase has reached the desired developing distance.
- Replaces a timer or stop watch.
- Works with glass chambers for plate sizes 20 x 20, 20 x 10 and 10 x 10 cm.
- Battery operated

CAMAG smartCut plate cutter

Convenient and precise cutting of TLC/HPTLC plates

- Cuts plates with a glass thickness up to 3 mm
- Makes smooth cuts on sensitive layers
- Desired size can be read directly from a scale
- Easy handling

Ordering information

022.8535 CAMAG Horizontal Developing Chamber for plates 20 x 10 cm
022.8530 CAMAG Horizontal Developing Chamber for plates 10 x 10 cm

022.5300 CAMAG smartAlert solvent front monitor
022.4300 CAMAG smartCut plate cutter

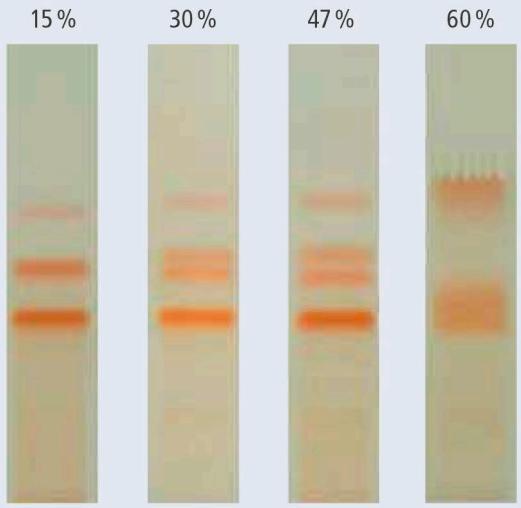
CAMAG Automatic Developing Chamber ADC 2

The Automatic Developing Chamber ADC 2 offers convenience, safety and reproducibility for the isocratic development of HPTLC plates and foils with the format 20 × 10 cm.

The Automatic Developing Chamber ADC 2 is the heart of a state-of-the-art HPTLC system. It performs the development step fully automatically, reproducibly, and independent of environmental effects. The activity and preconditioning of the layer, chamber saturation, developing distance and final drying can be pre-set and are automatically monitored by the ADC 2. Two modes of operation are possible: stand-alone with input of parameters via keypad, or remote operation by software with process monitoring, documentation of operating parameters, and reporting.

Key features

- Fully automatic development of 20 × 10 cm TLC/HPTLC plates
- A conventional 20 × 10 cm Twin Trough Chamber is used for development.
- This way, chromatographic conditions of already existing analytical procedures can be retained, but environmental and operational effects are excluded.
- Operation in stand-alone mode or software controlled
- The user is freed of all process monitoring responsibilities, operation is fully traceable.
- The option "Humidity Control" allows reproducible chromatography at defined activity of the layer. This feature is essential in method development when the influence of relative humidity shall be investigated.



Effect of relative humidity on separation of polyphenols in green tea

Mobile phase: toluene – acetone – formic acid 9:9:2

Note

The Automatic Developing Chamber ADC 2 meets all the requirements of GMP/GLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the option 21 CFR Part 11 "compliance ready" is required for each winCATS workstation.

Ordering information

022.8350 CAMAG Automatic Developing Chamber ADC 2, complete with standard Accessories and Equilink, without software ➔ *visionCATS*

022.5261 Twin Trough Chamber for ADC 2

Further information can be found in the special brochure "Automatic Developing Chamber ADC 2" and under www.camag.com/adc

The CAMAG AMD 2 System
Automated Multiple Development

The CAMAG AMD procedure allows thin-layer chromatography to be utilized for tasks that could not be performed by TLC in the past.

Only the AMD procedure can be successfully employed for reproducible gradient development with silica gel as the stationary phase. In column liquid chromatography, gradient elution is common, but on reversed phases only, because a normal phase column would be irreversibly degraded, which is not acceptable in a technique depending on multiple use of the stationary phase.

The principle of the CAMAG AMD procedure

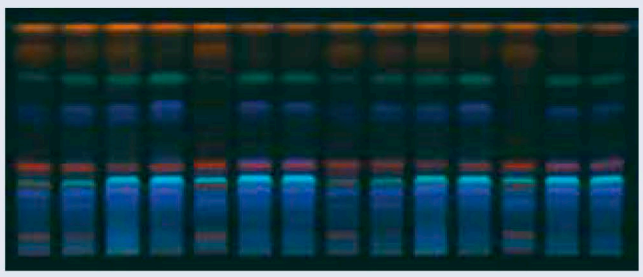
- The HPTLC plate is developed repeatedly in the same direction.
- Each successive run extends over a longer solvent migration distance than the one before.
- Between runs, the solvent is completely removed from the developing chamber and the layer is dried under vacuum.
- Each successive run uses a solvent of lower elution strength than that of the one used before. In this way, a stepwise elution gradient is formed.
- The combination of focusing effect and gradient elution results in extremely narrow bands. Their typical peak width is about 1 mm. This means that, within the available separation distance of 80 mm, up to 40 components can be completely resolved, i.e. with base line separation.

AMD 2 under winCATS

The AMD 2, like other computer controlled CAMAG instruments, communicates with winCATS. The gradient, made from up to 5 solvent bottles, is defined by input into a table in winCATS. Gradient and developing distance for each run are graphically displayed for verification. All individual runs of the developing program are performed fully automatic and monitored by winCATS.

Key features

- Multiple development using a solvent strength gradient
- Separation power improved over regular HPTLC development by about factor 3
- Data input and monitoring through winCATS
- Utilizing time outside working hours if required



Separation of various rhubarb samples by AMD

Detection: UV 366 nm

Gradient in 10 steps: Methanol – dichloromethane from 40:60 to 10:90 in 9 steps over 40 mm developing distance followed by one step methanol – dichloromethane 10:90 over 70 mm

Note

The AMD 2 with winCATS meets all the requirements of GMP/GLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the option 21 CFR Part 11 "compliance ready" is required for each winCATS workstation.

Ordering information

022.8860 **CAMAG AMD 2 System** comprised of chromatogram developing module, standard accessories and Equilink, without software

Further information can be found in the special brochure "AMD 2 System" and under WWW.camag.com/amd2

CAMAG HPTLC Vario System

Key features

- Development with six different solvents can be tested side by side.
- Sandwich as well as tank configuration can be simulated side by side, making results directly comparable.
- Six different conditions of pre-equilibration, including relative humidity, can be tested simultaneously.
- These variations of developing conditions can be freely combined.

Time saving optimization of separation conditions using the HPTLC Vario System

Application examples, schematic: F_1 … = developing solvents, C_1 … = conditioning liquids

Optimization of the developing solvent

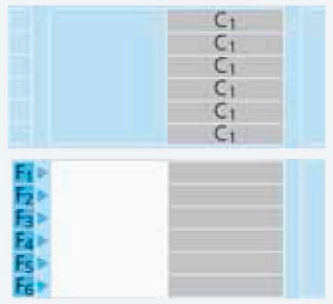
Development with 6 different solvents side by side, without preconditioning = development in sandwich configuration.

Optimization of the development solvent

Development with 6 different solvents side by side whereby the conditioning troughs contain the same six solvents = simulated tank development

Optimization of the development solvent after uniform layer preconditioning

First step: pre-equilibration of all six tracks with the same conditioning liquid; then development with six different solvents (in sandwich configuration).



Optimization of preconditioning

Pre-equilibration with six different conditioning liquids; then development of all tracks with the same solvent.

Ordering information

022.8550 HPTLC VARIO System complete, comprising
022.8555 HPTLC VARIO Chamber for 10×10 cm plates and
022.8556 HPTLC Scoring unit for the preparation of TLC/HPTLC plates