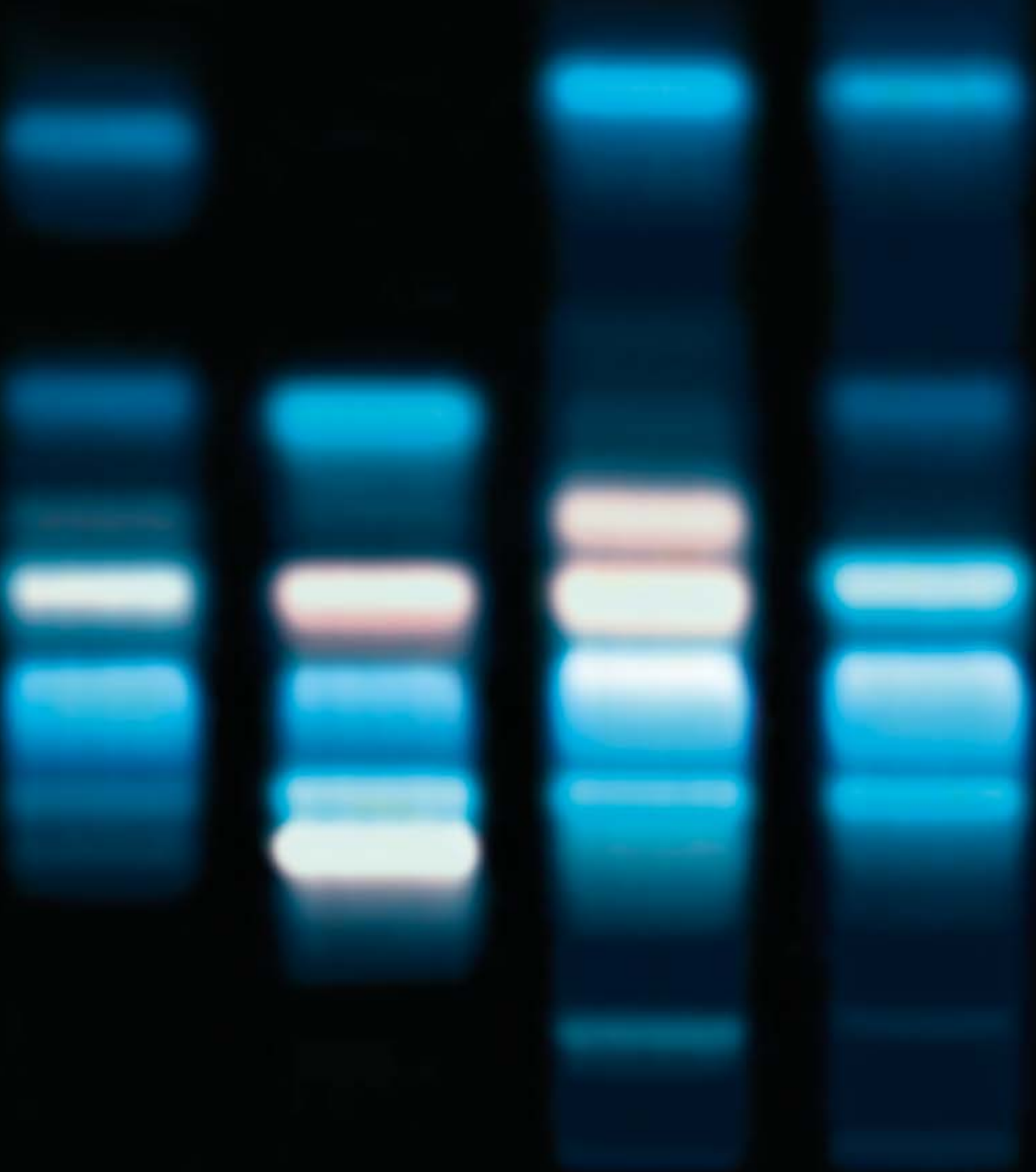


# INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY



**CAMAG**<sup>®</sup>

WORLD LEADER IN PLANAR CHROMATOGRAPHY





## CAMAG – Your partner in all fields of Planar Chromatography

CAMAG has the tradition of serving planar chromatography since 1961. We develop and manufacture sophisticated instruments and associated software for the state-of-the-art analytical technique. Our products are marketed directly in Switzerland, through daughter companies in Germany and in the USA, and through carefully selected distributors worldwide. We see ourselves as a flexible, customer friendly, science based company that has made its mark as a reliable partner in all branches of planar chromatography. We provide competent customer support and technical service for our products as well as education and training for our customers' lab personnel.

CAMAG has everything your lab may need for planar chromatography. This catalog will help you to quickly find information about the products suitable for your tasks. The catalog may also serve as a short guide through all steps of the planar chromatography procedure. Methodological explanations are set apart from specific product information.

Please visit our homepage [www.camag.com](http://www.camag.com) for additional information and the latest news.

Our team is always available for individual questions and support.

### Terminology used in this catalog

In order to emphasize that the state-of-the-art method is something different from the simple Thin-Layer Chromatography of yesterday, we are predominantly using the term Planar Chromatography but occasionally also TLC and HPTLC (High-Performance Thin-Layer Chromatography).

# Overview

| Steps of the TLC/HPTLC procedure | What is important?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample Application               | The samples are applied onto the plate either as spots (TLC) or as bands (HPTLC). Precision of the applied volume, exact positioning and compactness of application zone determine the quality of the final result.                                                                                                                                                                                                                                                                                                                                                                                                                |
| Chromatogram Development         | The mobile phase is drawn through the stationary phase by capillary action. Samples are separated into their components which remain in their position on the layer after the mobile phase has been evaporated. Chromatography is affected by the gas phase in the chromatographic chamber.                                                                                                                                                                                                                                                                                                                                        |
| Derivatization                   | Substances without chromophores or fluorophores can be visualized or made detectable through derivatization. The required reagents are transferred onto the chromatogram by spraying or immersion.                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Chromatogram Evaluation          | TLC/HPTLC chromatograms are captured images of the plates under white or ultraviolet light. Densitograms are profiles of each sample track obtained by scanning densitometry. Evaluation options range from visual inspection of electronic images to quantitative determinations using scanning densitometry or image profiles. For documentation, electronic images are easy to capture and to archive. They can be reproduced on screen without changes over time and thus compared with current images. Coupling HPTLC with Mass Spectrometry or bioluminescence detection (bioautography) expand the capability of TLC/HPTLC. |
| Software                         | <i>visionCATS</i> software organizes the workflow of HPTLC, controls the involved CAMAG instruments, manages data, and generates analysis reports in conformity to cGMP/cGLP.                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Complete Systems<br>Consumables  | With a complete system a lab can efficiently start working with HPTLC.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Services                         | CAMAG offers support with HPTLC feasibility studies, training, instrument qualification, literature services and more.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

| What is offered by CAMAG?                                                                                                                                |                                                             | Where can I find?                     |
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## High-Performance Thin-Layer Chromatography

The high performance version of planar chromatography distinguishes the technique. HPTLC comprises the use of chromatographic layers of utmost separation efficiency and the employment of state-of-the-art instrumentation for all steps in the procedure, precise sample application, standardized reproducible chromatogram development and software controlled evaluation. Of course, conventional TLC, manually performed and using inexpensive equipment still has its place in almost all laboratories as a convenient tool for simple and rapid separations.

HPTLC as opposed to column chromatography (GC, HPLC) utilizes a flat (planar) stationary phase and an open system. The basic steps sample application, chromatogram development and evaluation as well as any optional ones are performed relatively independent in time and location, which, on the one hand offers unsurpassed flexibility but on the other hand makes total automation difficult. Since many samples can be processed in parallel, HPTLC is rapid and cost effective.

### Remarkable features of planar chromatography:

In addition to chromatogram detection/evaluation under visible or ultra-violet light, pre- and postchromatographic derivatization is readily available, for which a practically unlimited variety of reagents can be used, since it is performed in the absence of the mobile phase.

Unlike with column chromatography, the user has a complete overview of the chromatogram as all fractions remain stored on the plate and any substances remaining in the start position are detectable. It is another advantage of planar chromatography that sample preparation can often be simplified, due to the one-time use of the stationary phase.

Standardized HPTLC analysis methods – qualitative or quantitative – can be validated. For customers regulated by cGMP/cGLP, we offer Installation Qualification (IQ) and Operating Qualification (OQ) for our instruments.

CAMAG's modern software concept ensures the reliable operation of our HPTLC systems and the documentation/storage of operating parameters and results as required by the customer.

HPTLC-MS online coupling, the comparatively new hyphenation of HPTLC and mass spectrometry, has the potential to become an indispensable technique for many analytical laboratories.

## Application Fields



### Pharmaceutical applications

- Quality control
- Content Uniformity Test (CUT)
- Identity- and purity checks
- Stability tests, etc.



### Herbals

- Identification
- Stability tests
- Detection of adulteration
- Assay of marker compounds, etc.



### Clinical applications

- Lipids
- Metabolism studies
- Drug screening
- Doping control, etc.



### Food and feed stuff

- Quality control
- Additives (e. g. vitamins)
- Pesticides
- Stability tests (expiration), etc.



### Cosmetics

- Identity of raw material
- Preservatives, coloring materials, etc.
- Screening for illegal ingredients, etc.



### Industrial applications

- Process development and optimization
- Process monitoring
- Cleaning validation, etc.



### Environment

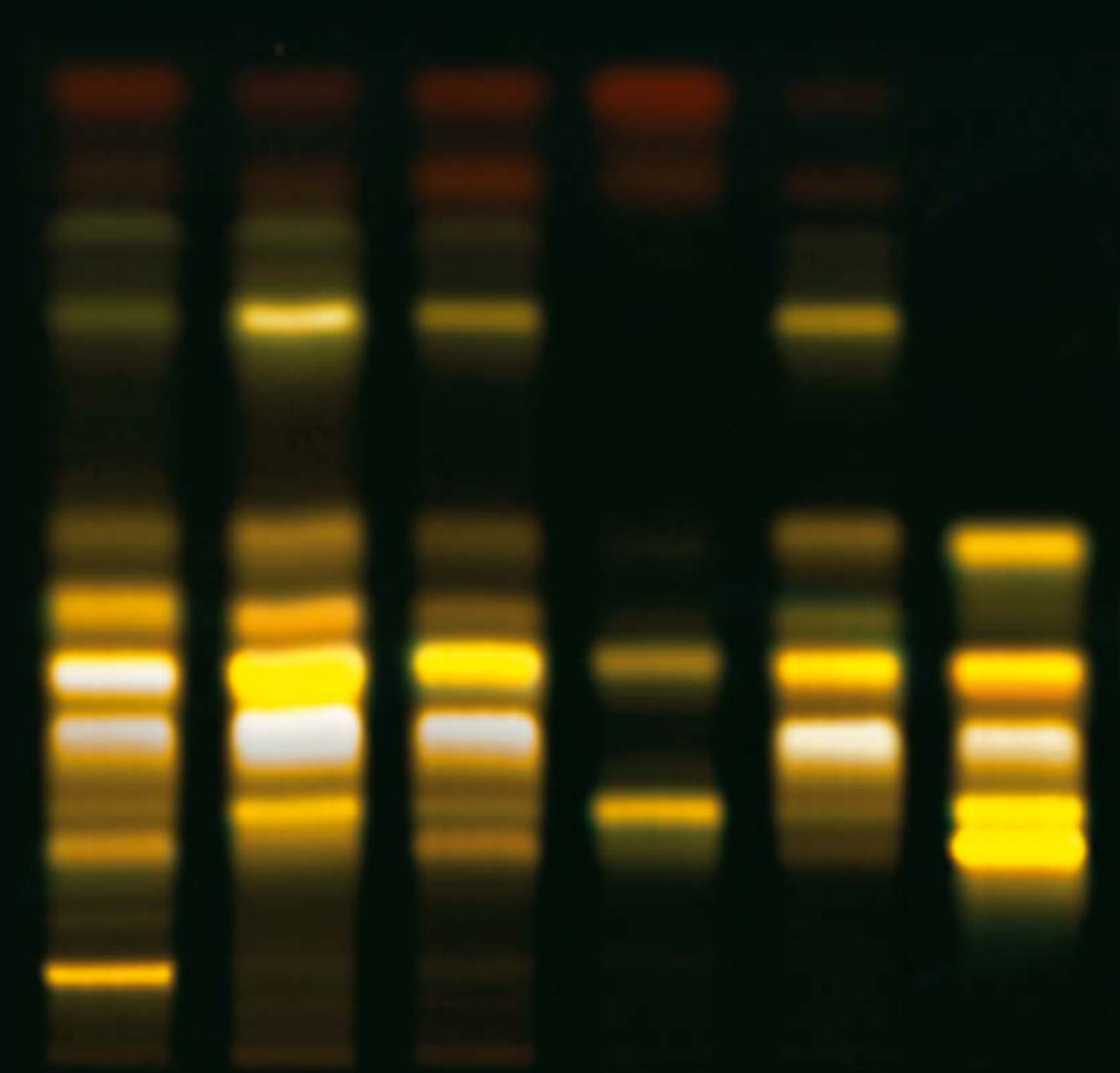
- Water
- Soil
- Residue analysis, etc.



### Forensics

- Detection of document forgery
- Investigation of poisoning
- Dyestuff analyses, etc.





# Sample Application

Sample application determines  
quality and reproducibility of the analysis

# Sample Application – Variations

Application of samples, reference standards and/or reference materials is the first step in the workflow of TLC/HPTLC and it affects significantly the quality of the result at the end of the process.

The choice of the application technique and the device depend on the requirements of precision, sample volumes, number of analyses and the desired grade of automation.

Spotwise sample application using a fixed volume capillary is the simplest way. Sample volumes of 0.5 to 5  $\mu\text{L}$  can be applied as spots onto conventional layers without intermediate drying, on HPTLC layers it is up to 1  $\mu\text{L}$  per spot.

Bandwise application by spray-on techniques allows the application of significantly larger volumes. Starting zones in the form of narrow bands ensure the best resolution that can be achieved with the chromatographic system selected.

## Effect of the solvent and the technique of sample application on the chromatogram

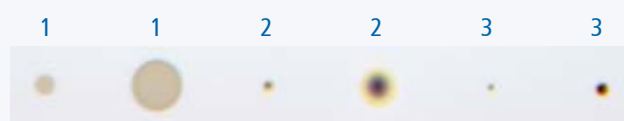
Mobile phase: toluene; detection: white light

Test dye mixture (0.5 and 5  $\mu\text{L}$ ) dissolved in

1: methanol

2: toluene

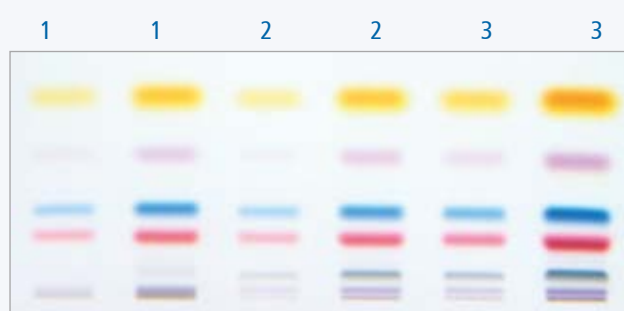
3: hexane



Contact application, prior to development



Developed plate after contact application of spots



Developed plate after spray-on application of bands





## CAMAG Nanomat 4 and Capillary Dispenser

The Nanomat 4 serves for easy application of samples in the form of spots onto TLC and HPTLC layers, precisely positioned and without damage to the layer. The actual sample dosage is performed with a disposable capillary pipette, which is precisely guided, thus ensuring that the chromatogram can be scanned automatically according to a programmed pattern.

### The Nanomat 4 is suitable for

- Conventional TLC plates including self-coated plates up to 20 × 20 cm
- HPTLC plates 10 × 10 cm and 20 × 10 cm
- TLC and HPTLC sheets up to 20 × 20 cm

### Capillary pipettes

The capillary pipettes are loaded into the dispenser in magazines. Capillaries of 0.5, 1.0, 2.0, and 5.0 µL volume are available. Each capillary size requires an appropriate dispenser magazine. With the Universal Capillary Holder capillary pipettes are taken from the dispenser, then filled with sample solution and placed against the applicator head of the Nanomat 4.

### Ordering information

#### 040.1500 CAMAG® Nanomat 4 Complete-Kit

022.4730 CAMAG® Nanomat 4,  
022.7655 Capillary Dispenser,  
022.7786 Universal Capillary Holder,  
022.7661 Dispenser Magazine for  
1 µL capillaries,  
022.7771 Disposable Capillary  
Pipettes 1 µL,  
pack of 5 × 100

022.7660 Dispenser Magazine for 0.5 µL  
capillaries, without capillaries  
022.7661 Dispenser Magazine for 1 µL  
capillaries, without capillaries  
022.7662 Dispenser Magazine for 2 µL  
capillaries, without capillaries  
022.7665 Dispenser Magazine for 5 µL  
capillaries, without capillaries

022.7770 Capillary Pipettes 0.5 µL  
pack of 5 × 100  
022.7771 Capillary Pipettes 1 µL  
pack of 5 × 100  
022.7772 Capillary Pipettes 2 µL  
pack of 5 × 100  
022.7775 Capillary Pipettes 5 µL  
pack of 5 × 100

Further information at [www.camag.com/nanomat](http://www.camag.com/nanomat)

## CAMAG Automatic TLC Sampler 4 (ATS 4)



Automatic sample application is a key factor for productivity of the HPTLC laboratory. The ATS 4 offers fully automatic sample application for qualitative and quantitative analyses as well as for preparative separations. It is suited for routine use and high sample throughput.

Samples are either applied as spots through contact transfer (0.1–5 µL) or as bands or rectangles (0.5 to > 50 µL) using the spray-on technique. Starting zones in the form of narrow bands offer the best separation attainable with a given chromatographic system. Application in the form of rectangles allows precise application of large volumes without damaging the layer. Prior to chromatography, these rectangles are focused into narrow bands with a solvent of high elution strength.

The ATS 4 allows “overspotting”, i.e. a sequential application from different vials onto the same position. This technique can be used e.g. in pre-chromatographic derivatization, spiking, etc.

### Key features

- Fully automatic sample application, suitable for routine use
- Application of spots, bands, or rectangles
- Data input and monitoring through *visionCATS* software
- Application of solutions onto TLC/HPTLC plates
- Application of sample volumes between 0.1 and 5 µL by contact transfer
- Spray-on application of sample volumes between 0.5 and > 50 µL
- Heated Spray Nozzle (option)



### Heated Spray Nozzle for ATS 4 (optional)

Heating at 60 °C cuts the time required for the application of aqueous solutions about in half. This is useful e.g. for trace analysis where comparatively large sample volumes have to be applied in order to reach a low detection limit.

### Ordering information

**022.7400 CAMAG® Automatic TLC Sampler 4 (ATS 4)**, incl. 25 µL Dosing syringe (695.0053), Spray-on needle for dosing syringe (695.0046), Contact transfer needle for dosing syringe (695.0047), without software

**022.7410 CAMAG® Automatic TLC Sampler 4 (ATS 4)**, with heated spray nozzle, incl. 25 µL Dosing syringe (695.0053), Spray-on needle for dosing syringe (695.0046), Contact transfer needle for dosing syringe (695.0047), without software

### Note

The Automatic TLC Sampler ATS 4 with *visionCATS* meets all the requirements of cGMP/cGLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the *visionCATS* option “21 CFR Part 11” is required.

Detailed ordering information: [www.camag.com/ats4](http://www.camag.com/ats4)

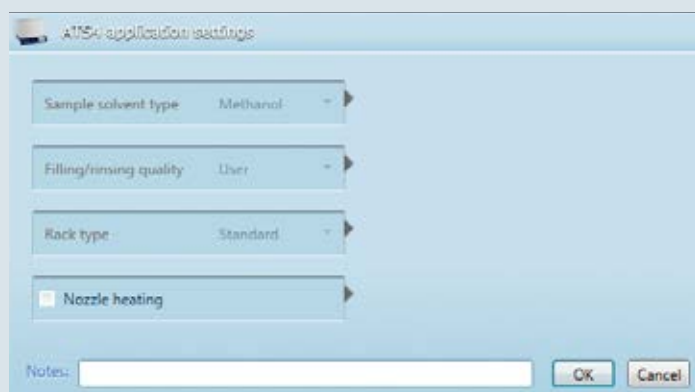


### Operation of the ATS 4 with *visionCATS*

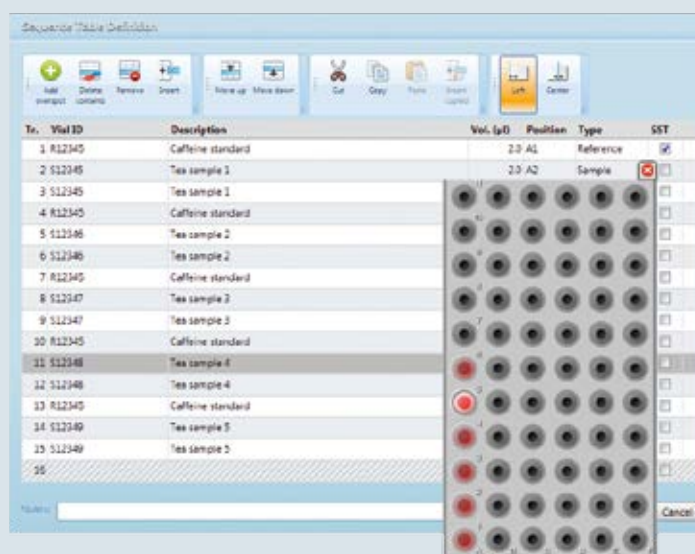
Precise sample application is a crucial factor for the quality of the HPTLC analysis and the results obtained. When using *visionCATS* HPTLC software with its easy to navigate user interface to control the ATS 4, a fully automated sample application for routine use and high sample throughput is supported.

The dialog box for instrument parameters offers user-friendly default combinations. For instance, the user can select the solvent type most similar to the solvent actually used. The software will then automatically adapt the instrument defaults to optimize its application regarding viscosity, volatility and surface tension. Another example of a pre-selected combination is the filling/rinsing quality which determines how often the syringe is rinsed, the filling process repeated, etc. All these pre-selections can be individually adjusted to a specific task.

The dialog for entering the sequence of samples is clearly arranged and easy to use. Tracks can be automatically arranged evenly spaced across the plate, sample designations can be inserted from a prepared list, etc. The program progress is displayed on screen as long as the instrument remains connected to the computer.



The dialog box for ATS 4 application settings offers user-friendly default combinations. All pre-defined parameters can be individually adjusted.



Easy sequence setup with *visionCATS*: the sequence table holds all information required to fully automated run a series of samples, e.g. sample location (rack position and application position), sample volume, sample name, etc.

## CAMAG Linomat 5



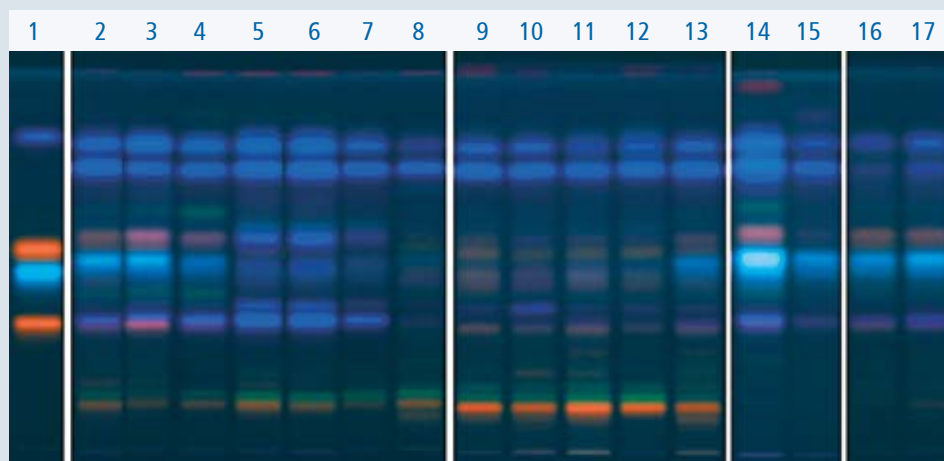
With the Linomat 5 samples are sprayed onto TLC/HPTLC plates in the form of bands with nitrogen or compressed air. Sample application is automatic, only changing the syringe (filling, inserting and rinsing) is manual. The Linomat is suitable for small sample throughput.

### Software-controlled operation with *visionCATS*

The Linomat 5 is controlled by *visionCATS*, the new generation of HPTLC software. Instrument handling is now easier and more convenient. Simply select a plate format, fill in the sequence table and start working. With *visionCATS*, the Linomat 5 can be IQ/OQ qualified and used in a cGMP environment.

### Operation in stand-alone mode

For those who use the Linomat 5 infrequently stand-alone mode is available. Up to ten methods can be entered either manually via the keypad or downloaded to the instrument from a computer running a licensed *visionCATS* program. In stand-alone mode the keypad is used to enter sample application parameters or to select a saved method.



### Sample application as bands

HPTLC fingerprint (flavonoids) of green and black tea samples representing different geographic origins.

#### Track assignment

1 reference substances with increasing  $R_f$ :  
rutin, chlorogenic acid, hyperoside, gallic acid

2–8 Samples from China  
9–13 Samples from Japan  
14–15 Samples from India

For comparison:

16–17 Black tea from Sri Lanka  
Tracks taken from different plates

### Ordering information

**022.7808 CAMAG® Linomat 5**, including one dosing syringe 100 µL, without software

695.0014 Dosing Syringe 100 µL for Linomat

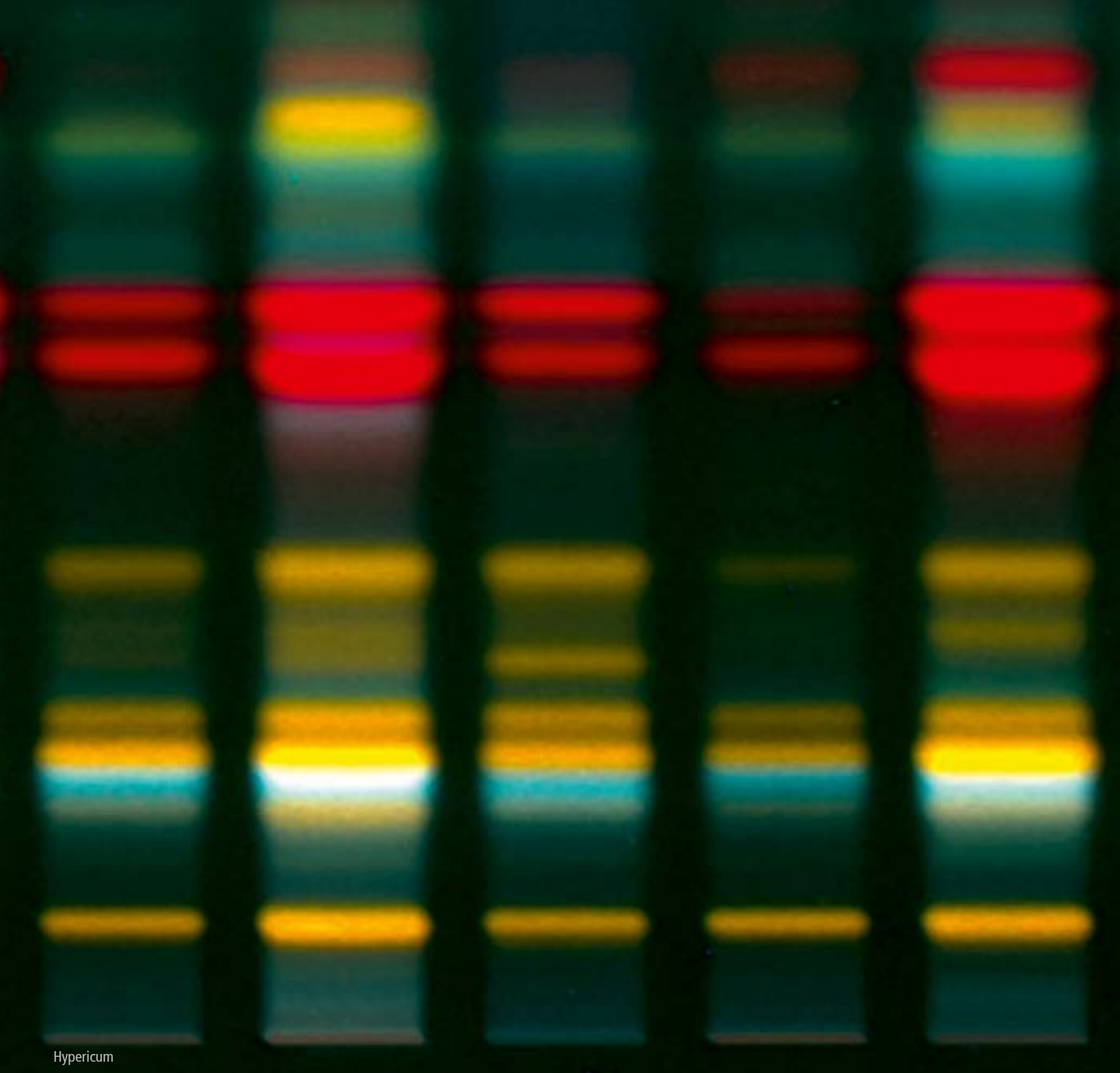
695.0015 Dosing Syringe 500 µL for Linomat

### Note

The Linomat 5 with *visionCATS* meets all the requirements of cGMP/cGLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the *visionCATS* option "21 CFR Part 11" is required.

Detailed ordering information: [www.camag.com/linomat5](http://www.camag.com/linomat5)





# Chromatogram Development

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

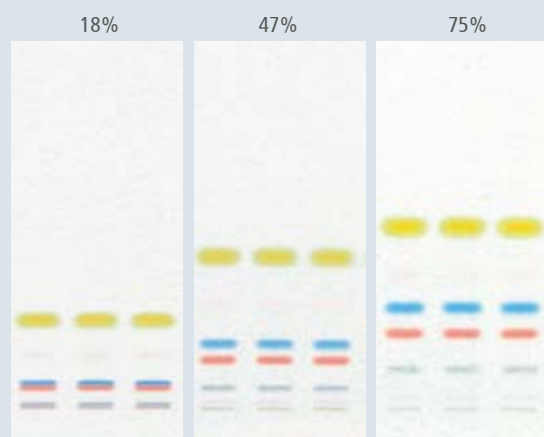
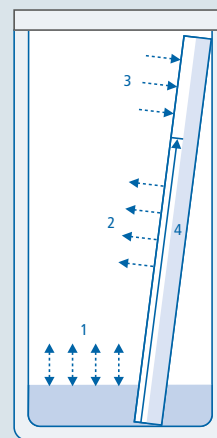
# Chromatogram Development

TLC and HPTLC differ from all other chromatographic techniques in the fact that in addition to stationary and mobile phases, a gas phase is present. This gas phase 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





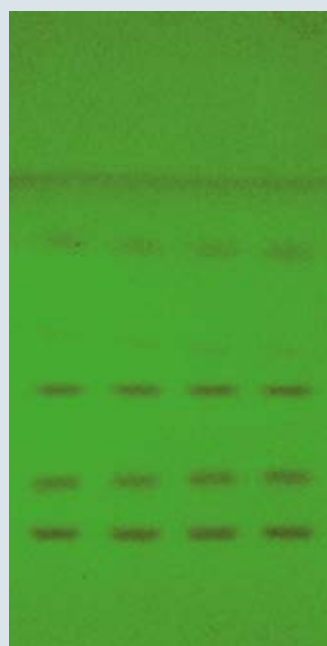
## Choosing a developing chamber

Selection of the “proper” chamber is done during method development and generally follows “practical” considerations such as which chamber is available, which one must be used due to an SOP or following a guideline, or which one has been used in the past if a results comparison is to be made. However, a focus should also be on economical aspects such as time requirement and solvent consumption and on reproducible conditions.

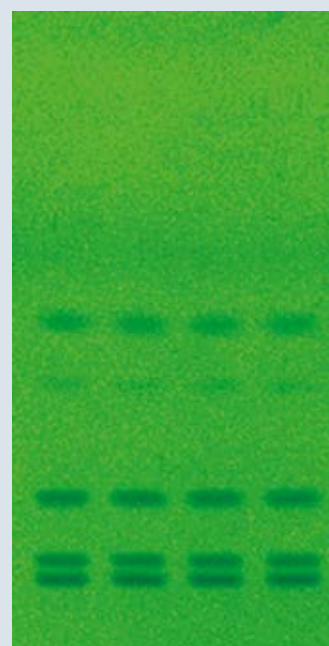
The **Horizontal Developing Chambers** have proven to be exceptionally economical, flexible and reproducible in operation. Although designed for applications where the plate is developed from two sides, they are also suitable for single-sided developments in unsaturated, saturated and sandwich configuration as well as for preconditioning of HPTLC plates. Please note that results are not comparable to vertical developing chambers.

The **Automatic Developing Chamber 2 (ADC 2)** is a software-controlled device for reproducible plate development. Unsurpassed for reproducibility and universal applicability in HPTLC. This instrument does not only eliminate any effects of the operator when introducing the plate into a saturated chamber, but also the activity of the layer prior to start of chromatography can be set and drying of the chromatographed developed plate is rapid, homogeneous, and complete. For development a conventional 20 × 10 cm Twin Trough Chamber is used. This way chamber geometry and chromatographic conditions of already existing analytical procedures can be retained, but environmental and operational effects are standardized.

In case the sample contains polar and non-polar components, which must be separated in the same analysis, the principle of **AMD 2 System Automated Multiple Development** can be employed. Development is performed on the basis of a solvent gradient from polar to non-polar over several steps with intermediate drying.



Development without preconditioning

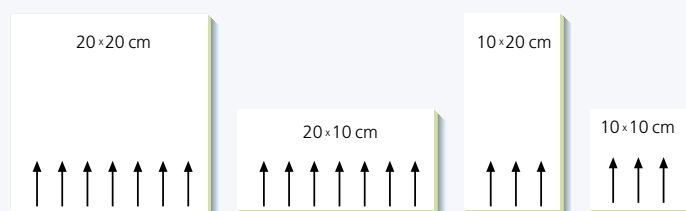


Development with preconditioning

## Definition of plate and chamber formats

These format definitions are used in all CAMAG literature.

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





## CAMAG Flat Bottom Chamber

This is the classical developing tank for TLC/HPTLC. 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 presaturation cannot be controlled unless additional accessories are used.



## CAMAG Twin Trough Chamber

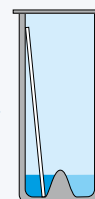
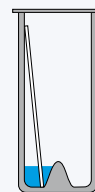
The Twin Trough Chambers offer several ways to specifically affect the TLC/HPTLC separation in order to improve it. Also it reduces the required volume of developing solvent compared to Flat Bottom Chambers.

### Twin Trough Chamber: Low solvent consumption

20 mL of solvent are sufficient for a 20 × 20 cm chamber, 10 mL for the 20 × 10 cm chamber and 5 mL for a 10 × 10 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 for plates 20 × 20 cm, with stainless steel lid
- 022.5250 for plates 20 × 20 cm, with glass lid
- 022.5257 for plates 20 × 20 cm, without lid
- 022.5150 for plates 10 × 10 cm, with stainless steel lid
- 022.5151 for plates 10 × 10 cm, without lid
- 022.5275 light-weight for plates 20 × 20 cm, with glass lid
- 022.5270 light-weight for plates 20 × 10 cm, with glass lid

#### CAMAG® Twin Trough Chamber

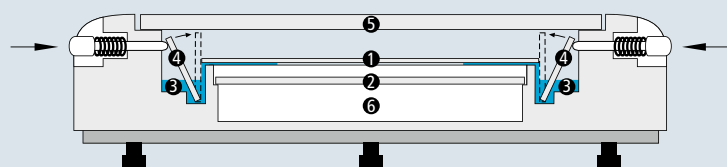
- 022.5256 for plates 20 × 20 cm, with stainless steel lid
- 022.5255 for plates 20 × 20 cm, with glass lid
- 022.5258 for plates 20 × 20 cm, without lid
- 022.5254 for plates 20 × 10 cm, with stainless steel lid
- 022.5253 for plates 20 × 10 cm, with glass lid
- 022.5251 for plates 20 × 10 cm, without lid
- 022.5155 for plates 10 × 10 cm, with stainless steel lid
- 022.5156 for plates 10 × 10 cm, without lid



## CAMAG Horizontal Developing Chambers

In the Horizontal Developing Chambers, the HPTLC plates can be 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. This chamber type is often used for screening purposes.

Plates can be developed in sandwich as well as in unsaturated and saturated chamber configuration. The chamber is suitable for all kinds of solvents.



- 1 HPTLC plate (layer facing down)
- 2 Glass plate for sandwich configuration
- 3 Reservoir for developing solvent
- 4 Glass strip
- 5 Cover plate
- 6 Conditioning tray

## CAMAG smartAlert solvent front monitor

The smartAlert serves for dependable monitoring the development of a glass 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 × 20, 20 × 10 and 10 × 10 cm
- Battery operated



## CAMAG smartCut plate cutter

Convenient and precise cutting of TLC/HPTLC plates

- Cuts glass plates with a 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 × 10 cm  
 022.8530 CAMAG® Horizontal Developing Chamber for plates 10 × 10 cm

- 022.5300 CAMAG® smartAlert solvent front monitor  
 022.4300 CAMAG® smartCut plate cutter

## CAMAG Automatic Developing Chamber 2 (ADC 2)



The ADC 2 offers convenience, safety and reproducibility for the isocratic development of HPTLC plates and foils with the format  $20 \times 10$  cm.

The ADC 2 is a device for reproducible plate development. It performs the development step fully automatically, and independent of environmental effects. The activity and pre-conditioning of the layer, chamber saturation, developing distance and final drying can be preset and automatically monitored by the ADC 2. Two modes of operation are possible: stand-alone with input of parameters via keypad, or remote operation from *visionCATS* with process monitoring, documentation of operating parameters, and reporting.

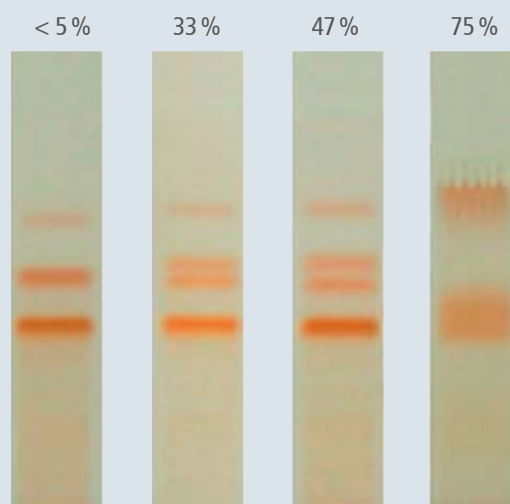
### Key features

- Fully automatic development of  $20 \times 10$  cm HPTLC plates
- A conventional  $20 \times 10$  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

Developing solvent: toluene, acetone, formic acid (9:9:2)

Derivatization: Fast Blue Salt B



Polyphenols in green tea

### Ordering information

**022.8380 CAMAG® Automatic Developing Chamber 2 (ADC 2),** including CAMAG® Twin Trough Chamber for ADC 2 (022.5261) for  $20 \times 10$  cm plates and Option Humidity Control for CAMAG ADC 2, without software

Detailed ordering information: [www.camag.com/adc2](http://www.camag.com/adc2)

### Note

The ADC 2 with *visionCATS* meets all the requirements of cGMP/cGLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the *visionCATS* option "21 CFR Part 11" is required.



## CAMAG AMD 2 System Automated Multiple Development



The AMD 2 is a software-controlled HPTLC chamber for gradient development. It is used for difficult analytical tasks that cannot be solved by isocratic HPTLC.

The separation of complex samples is a challenging task for every chromatographic system, particularly when they span a wide polarity range. The AMD procedure offers an excellent solution as it allows stepwise gradient elution over increasing separation distances. As a result acids, bases, neutral, hydrophilic and lipophilic substances can be separated in a single AMD run. This makes AMD suitable for a variety of fields of applications. The method is frequently used in lipid analysis and in routine analysis of drinking water. Pigment formulations with a complex composition, resins as well as additives of mineral oil products are other typical applications of AMD analysis.

### The principle of the CAMAG AMD procedure

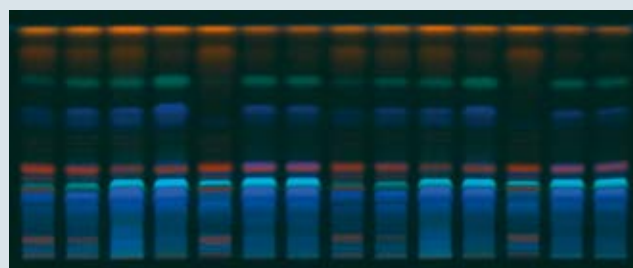
- The HPTLC plate is developed repeatedly in the same direction.
- Each successive run extends over a longer solvent migration distance than the one before.
- Between runs, the solvent is completely removed from the developing chamber and the layer is dried under vacuum.
- Each successive run uses a 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 baseline separation.

The AMD 2, like other software-controlled CAMAG instruments, communicates with *visionCATS*. The gradient, made from up to 5 solvent bottles, is defined by input into a table in *visionCATS*. Gradient and solvent migration distance for each run can be shown graphically for verification. Then all individual runs of the developing program are performed fully automatic and monitored by *visionCATS*.

### Key features

- Multiple development in the same direction with increasing solvent migration distances
- Enhanced separation capacity with baseline separation of up to 40 components
- Software-controlled with *visionCATS*
- Utilizing time also after working hours

### Separation of various rhubarb samples by AMD 2



HPTLC chromatogram of Rhubarb under UV 366 nm, derivatized with Natural Product reagent: AMD gradient in 10 steps: methanol – dichloromethane (40:60) to (10:90) in 9 steps, 40 mm solvent migration distance, then one isocratic step methanol – dichloromethane (10:90) over 70 mm solvent migration distance

### Ordering information

022.8860 CAMAG® AMD 2 System Automated Multiple Development, chromatogram developing module including standard accessories, without software

Detailed ordering information: [www.camag.com/amd2](http://www.camag.com/amd2)

### Note

The AMD 2 with *visionCATS* meets all the requirements of cGMP/cGLP 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.



# CAMAG HPTLC Vario System



## Key features

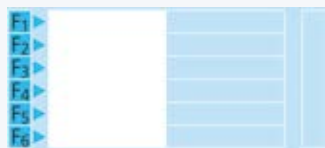
- Development with six different solvents can be tested side by side.
- Sandwich configuration as well as tank configuration can be simulated side by side, making results directly comparable.
- Six different conditions of pre-equilibration, including relative humidity, can be tested simultaneously.
-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

## Time saving optimization of separation conditions using the HPTLC Vario System

Application examples, schematic:  $F_1 \dots$  = developing solvents,  $C_1 \dots$  = conditioning liquids

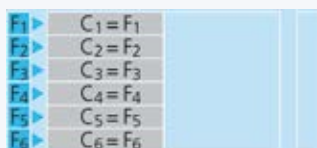
### Optimization of the developing solvent

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



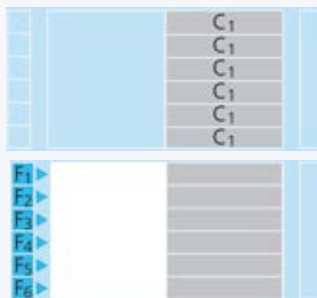
### Optimization of the development solvent

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



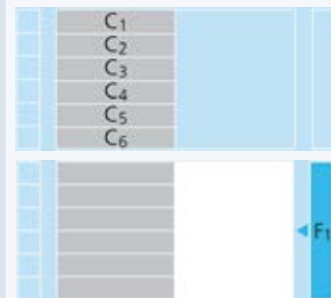
### Optimization of the development solvent after uniform layer preconditioning

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 CAMAG® HPTLC Vario System, consisting of  
022.8555 CAMAG® HPTLC Vario Chamber and  
022.8556 HPTLC Scoring Unit



# Derivatization

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

# Pre- and Postchromatographic Derivatization

020 °C

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.

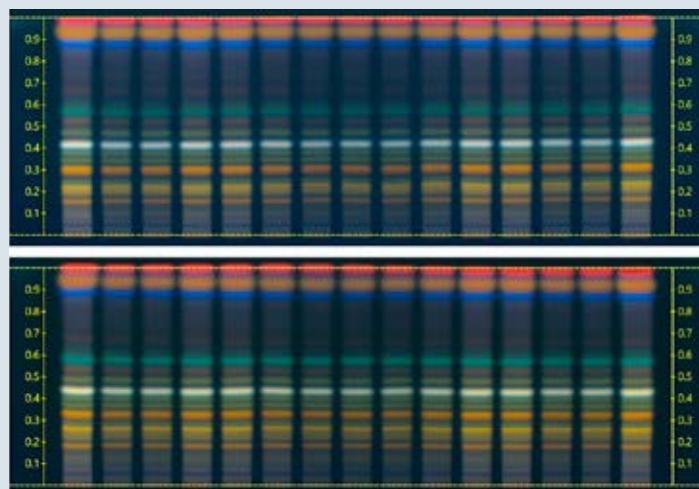
Pre-chromatographic derivatization is possible by overspraying the sample application zones with the Linomat 5 or the Automatic TLC Sampler ATS 4.

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.

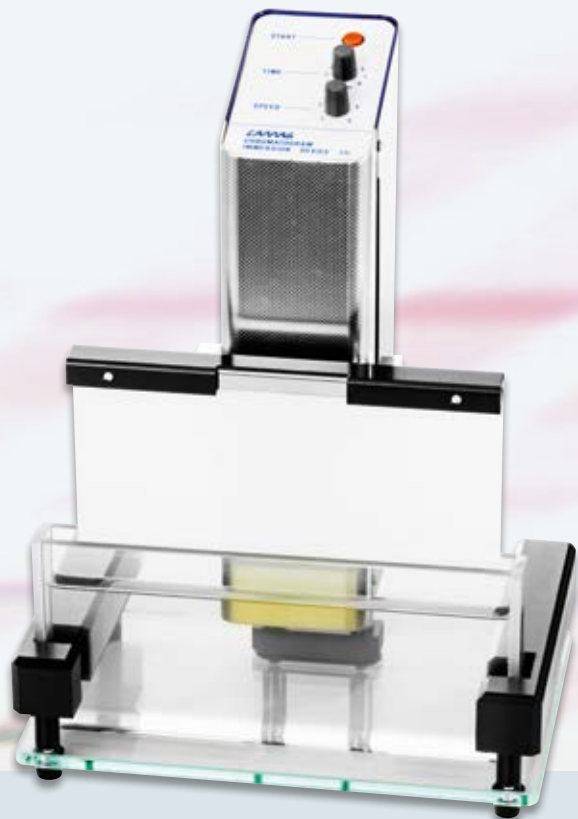
Whenever reagents are transferred by spraying, an efficient reagent mist removing device should be used to protect laboratory personnel against poisonous or irritating sprays or solvent vapors.

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 3

For proper reagent transfer, the chromatogram must be immersed and withdrawn at a controlled uniform speed. By maintaining a well defined vertical speed and immersion time, derivatization conditions can be standardized and "tide marks", which can interfere with densitometric evaluation, are avoided.

### Key features

- Uniform vertical speed, freely selectable between 2.5 cm/s and 4.5 cm/s
- Immersion time selectable between 0 and 8 seconds and indefinitely
- The device can be set to accommodate 10 cm and 20 cm plate height
- Battery operated, independent of power supply



## CAMAG TLC Sprayer

The TLC Sprayer consists of a charger and a pump unit with two kinds of spray heads. Spray head type A is for spray solutions of normal viscosity, *e.g.* lower alcohol solutions. Spray head type B is for liquids of higher viscosity, *e.g.* sulfuric acid reagents

### Key features

- Easy to use, with electro-pneumatic spray function
- Formation of fine aerosol with particles of 0.3 to 10  $\mu\text{m}$
- Low reagent consumption

## CAMAG Glass Reagent Sprayer

This all glass reagent sprayer is a low cost alternative to the TLC 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 × 20 cm, without dip tank
- 022.6627 Dip tank for plates 20 × 20 cm, with lid
- 022.6628 Dip tank for plates 20 × 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 Sprayer, all glass, with 100 mL Erlenmeyer flask

# CAMAG Derivatizer

Derivatization means another step in the process, consequently causing an increase in variance. Even if an experienced technician manually sprays a reagent, the relative standard deviation (RSD) of the measured values rises up to 12%. Using the Derivatizer, the RSD increases by less than 5%, thus providing very reproducible results, equal to those obtained with the Chromatogram Immersion Device, yet consuming considerably less reagent.

The Derivatizer sets a new standard of reproducibility and convenience in reagent transfer onto TLC/HPTLC plates by employing a unique "micro droplet" spraying technology (patent pending). The device is available for two different plate formats (20 x 20 and 20 x 10 cm) and is suitable for all common reagents.

In addition to the unsurpassed homogeneous reagent distribution, the Derivatizer offers other advantages as compared to manual spraying and immersion.



- Environmentally friendly and safe handling through a closed system
- Reproducible and user-independent results
- Low reagent consumption (2–4 mL) through efficient operation
- Intuitive handling and easy cleaning

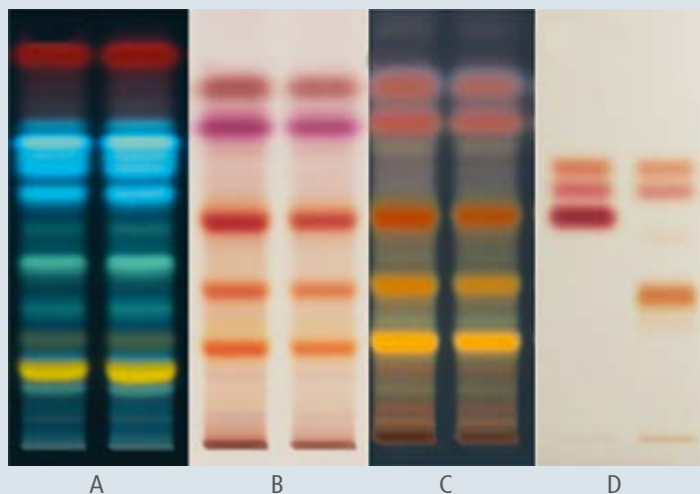


Color-coded spray nozzles



### Examples of derivatized chromatograms

Visual observation of results achieved with the Derivatizer reveals a very 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.*)

For alternative spraying reagents and reagents that are problematic to spray, please visit: [www.camag.com/derivatizer](http://www.camag.com/derivatizer)

### Ordering information

022.6000 CAMAG® Derivatizer with hoods for 20 × 10 and 20 × 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](http://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 × 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 °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

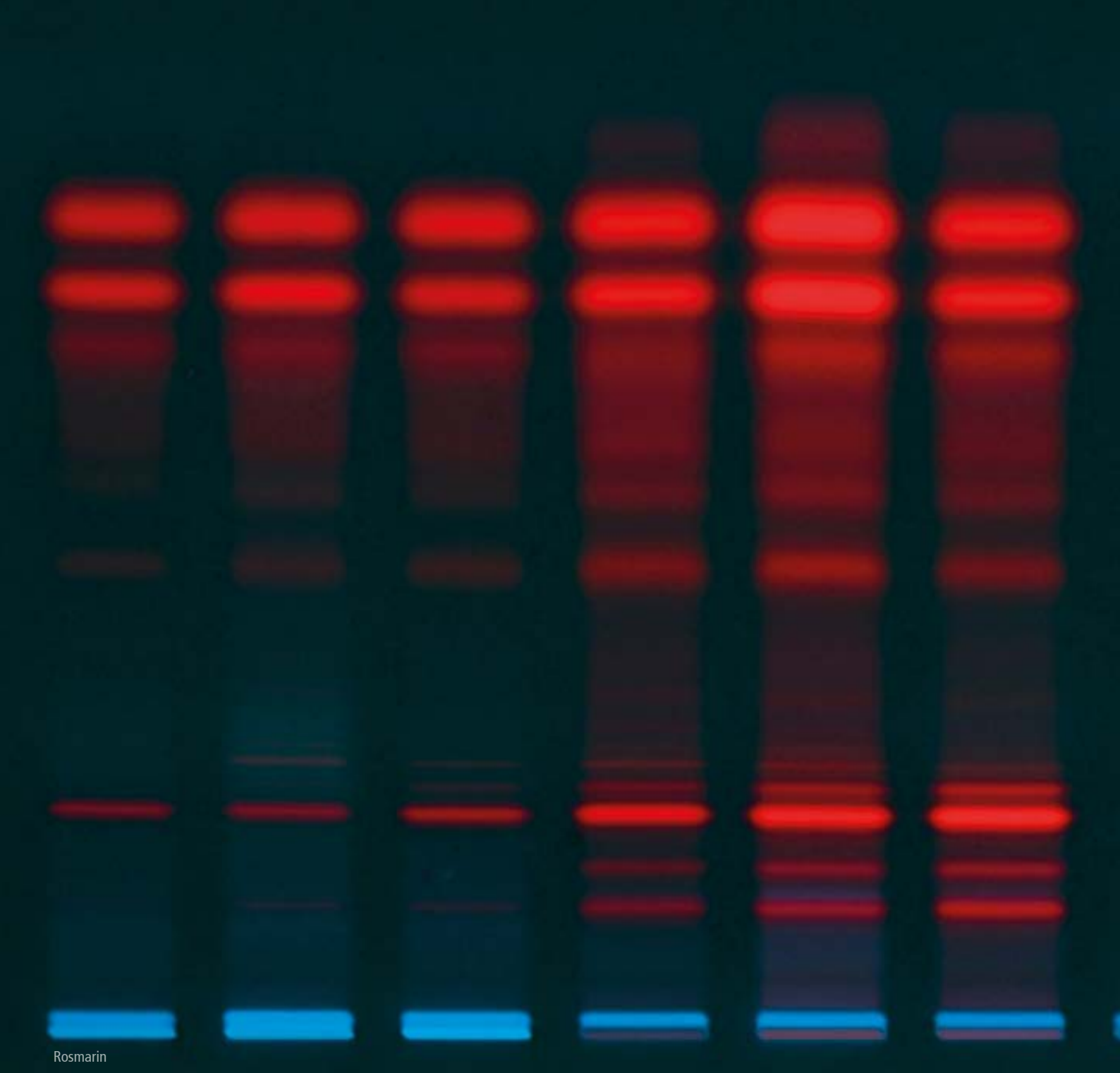
#### 022.3306 CAMAG® TLC Plate Heater 3

Stainless steel housing, flat ceramic top, for TLC/HPTLC plates up to 20 × 20 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



# Chromatogram Evaluation

Chromatogram inspection under UV light \_\_\_\_\_ UV Lamp 4  
Documentation, image acquisition \_\_\_\_\_ TLC Visualizer 2  
Bioactivity detection \_\_\_\_\_ BioLuminizer 2  
Scanning densitometry \_\_\_\_\_ TLC Scanner 4  
Hyphenation of TLC/HPTLC with MS \_\_\_\_\_ TLC-MS Interface 2

# Chromatogram Inspection under UV Light



## CAMAG UV Lamp 4

The UV Lamp 4 is designed primarily for use in a TLC/HPTLC laboratory. Users benefit from a convenient one-button operation for each UV tube. In order to reduce the user's risk of UV radiation exposure, the UV Lamp 4 is equipped with two safety features: in addition to the built-in timer (which automatically switches off the lamp after 10 minutes) a tilt sensor automatically turns off the lamp in case the lamp is tilted more than 30 degrees. Beyond optimized handling and improved safety features, the UV Lamp 4 comes with a more homogeneous illumination and higher UV light intensity.

### Key features

- Two UV tubes for illumination (1 × UV 254 nm, 1 × UV 366 nm, each 8 W)
- Convenient handling through one button operation for each UV tube
- Homogeneous illumination
- High level of user safety through tilt sensor and timer

**Two types of UV light are required for inspecting chromatograms:**

#### Long-wave UV light 366 nm

Under long-wave UV light fluorescent substances appear as bright, often differently colored zones, on a dark background. The sensitivity increases with the intensity of the UV light and also with the efficiency visible light is eliminated.

#### Short-wave UV light 254 nm

Under 254 nm UV light substances absorbing light of that wavelength appear as dark zones on a bright background, when the TLC/HPTLC layer contains a fluorescent indicator excited by UV 254 nm.

## CAMAG UV Cabinet 4

The UV Cabinet 4, a combination of the UV Lamp 4 and the Viewing Box 4, is specially designed for UV observation with minimal influence of ambient light. Thanks to a compact footprint, the UV Cabinet 4 requires only minimum space. The observation port has a built-in UV filter in the viewing window ensuring effective eye protection. The interior is accessible via a roller shutter on the front.

### Key features

- Chromatogram inspection with minimal influence of ambient light
- Eye protection through UV filter in the viewing window
- Minimum space requirements through compact footprint

### Ordering information

**040.2000 CAMAG® UV Cabinet 4**, incl. CAMAG® UV Lamp 4  
and CAMAG® Viewing Box 4

**022.9160 CAMAG® UV Lamp 4**, 254/366 nm, 2 x 8 W

**022.9060 CAMAG® Viewing Box 4**



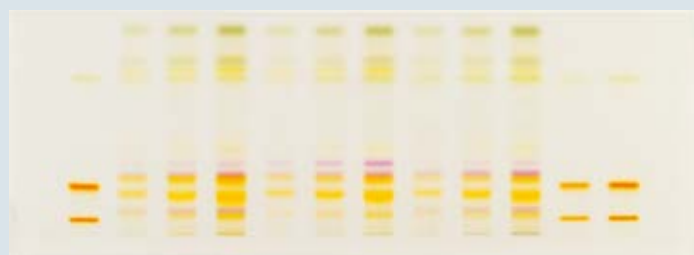
## Documentation/ Image Acquisition



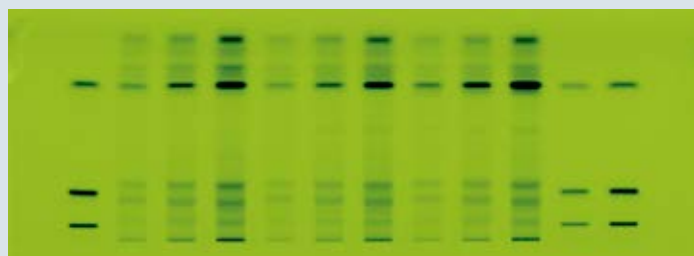
For electronic image acquisition the camera – like the human eye – captures polychromatic visible light. Under white light illumination it is the light reflected from the layer background. Under long-wavelength UV light (366 nm) it is the light emitted by fluorescent substances. When short-wavelength UV light (254 nm) is used, substances absorbing UV 254 nm appear as dark zones, provided the layer contains a fluorescence indicator (fluorescence quenching).

The TLC Visualizer 2 is CAMAG's imaging and documentation system operated with *visionCATS*. The software enables an image-based evaluation of chromatograms for quantitative evaluation.

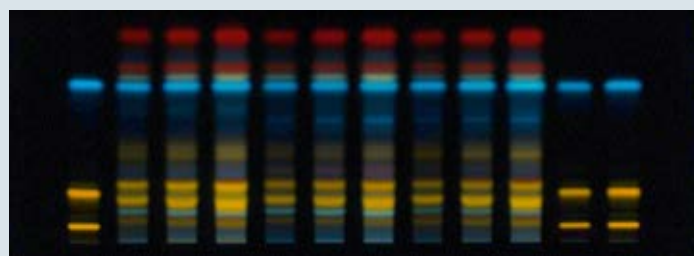
The strength of the electronic image acquisition is the overview of the complete chromatogram.



Chromatogram under white light



Chromatogram under UV 254 nm



Chromatogram under UV 366 nm



## CAMAG TLC Visualizer 2



Professional imaging and documentation system for TLC/HPTLC chromatograms and other planar objects with a state-of-the-art digital CCD camera, connected by USB 3.0

The visual presentation of the complete chromatogram showing all samples and standards side by side is one of the most convincing arguments for (High-Performance) Thin-Layer Chromatography. No other chromatographic technique can directly express the result as a color image and make it available for visual evaluation.

To reproducibly acquire and preserve best quality images of TLC/HPTLC chromatograms under different illuminations this high-end imaging and documentation system is available. With its digital CCD camera a maximum resolution of 82  $\mu\text{m}$  on the plate is obtained.

For electronic image acquisition the camera – like the human eye – captures polychromatic visible light. Under white light illumination it is the light reflected from the layer background. Under long-wavelength UV light (366 nm) it is the light emitted by fluorescent substances. When short-wavelength UV light (254 nm) is used, substances absorbing UV 254 appear as dark zones, provided the layer contains a fluorescence indicator (fluorescence quenching).

### Key features

- Reproducible high-quality images acquired under homogenous illumination with the selected light
- Easy and intuitive operation with *visionCATS*
- High-dynamic-range imaging (HDRI)
- Side by side comparison of tracks originating from the same or different plates and/or different illumination modes
- Various image enhancement tools, e.g. Spot Amplification ("Spot Amp"), Clean Plate Correction and Exposure Normalization
- Image-based profile generation from reference and sample tracks, and subsequent peak integration and calibration
- New digital CCD camera with a maximum resolution of 82  $\mu\text{m}$  on the plate
- USB 3.0 for easy PC connection
- Meets all requirements to be used in a cGMP/cGLP environment
- IQ/OQ qualification and 21 CFR Part 11 ready

### Ordering information

**022.9810 CAMAG® TLC Visualizer 2 documentation system** with 12 mm lens. Suited for object formats up to about 21 × 28 cm (20 × 20 cm TLC plates). *visionCATS* software is not included.

**022.9811 CAMAG® TLC Visualizer 2 documentation system** with 16 mm lens\*. Suited for object formats up to about 16 × 21 cm (20 × 10 and 10 × 10 cm TLC/HPTLC plates). *visionCATS* software is not included.

\*16 mm lens leads to an increased image resolution for HPTLC plates

Detailed ordering information: [www.camag.com/tlcvisualizer2](http://www.camag.com/tlcvisualizer2)

### Note

The TLC Visualizer 2 with *visionCATS* meets all the requirements of cGMP/cGLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the *visionCATS* option "21 CFR Part 11" is required.

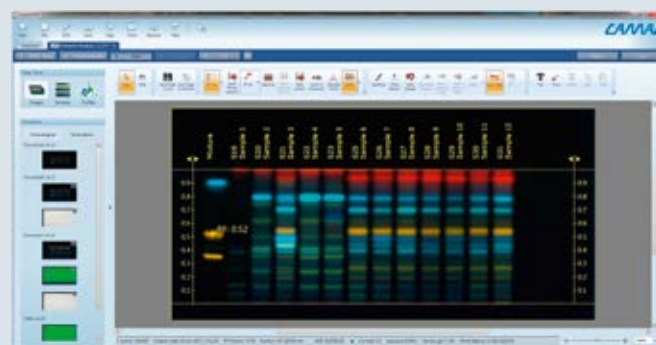
## TLC Visualizer 2 operated under *visionCATS*

*visionCATS* organizes the workflow of TLC/HPTLC, controls the involved CAMAG instruments, and manages data. Professional image acquisition and documentation of TLC/HPTLC chromatograms ensuring highest reproducibility is the major purpose of the TLC Visualizer 2.

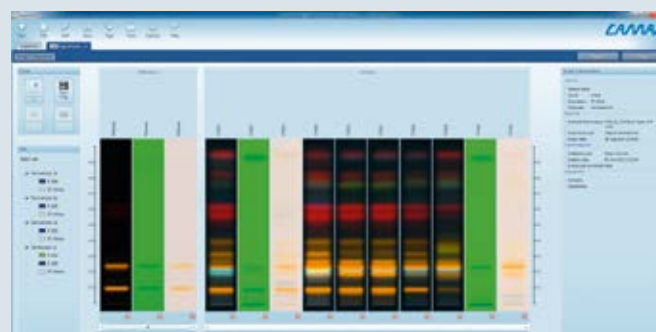
For the evaluation of acquired images sophisticated enhancement tools and also functions for annotation and determination of position ( $hR_f$ ) are required.

This is where *visionCATS* comes into play and virtually unleashes the power of TLC/HPTLC. *visionCATS* features a powerful database at its heart, enabling an all new sample-based approach. The state-of-the-art software supports low-noise, high-dynamic-range imaging (HDRI) and includes a comprehensive set of image enhancement tools.

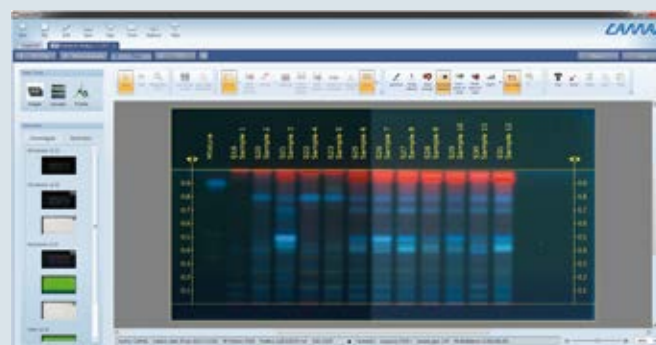
With the "Comparison Viewer", tracks originating from the same or different plates and/or different illumination modes can be compared on the same screen side by side which allows the creation of "virtual plates". Detailed on-screen instructions effectively guide the user through the image acquisition process.



"Data View" allows for visual evaluation of a plate in different illumination modes and offers a broad range of helpful tools, e.g. a  $R_f$  tool displays the  $R_f$  value of zones on the digital image or a rectangular zoom function.



"Comparison Viewer": Selected tracks of images taken from different plates under UV 254 nm, white light and UV 366 nm can be displayed side by side.



"Exposure Normalization" for visualization of weak zones (right) and original image (left)

# Selective Detection of Bioactive Compounds

## CAMAG BioLuminizer® 2



The BioLuminizer® 2 is a detection system specifically designed to detect bioluminescence on HPTLC plates.

The BioLuminizer® 2 system is consisting of a compartment excluding any extraneous light, climate controlled for extended stability of the plate, and a 16 bit CCD digital camera of high resolution and high quantum efficiency. It features ergonomic design and easy, intuitive handling in stand-alone mode using the special software.

With the BioLuminizer® 2 bioactivity can be detected and registered economically and with short response time. Special antibacterial protection measures are not necessary, as the bioluminescent bacteria *Allivibrio fischeri* are non-pathogenic.

Hyphenating TLC/HPTLC and bioassay is an excellent tool for identification of single toxic compounds in complex sample matrices.

The method is suitable for the detection of toxins in foodstuff, beverages, cosmetics, wastewater, drinking water, and for the detection of bioactivity in natural products. After chromatographic separation of the complex sample the plate is immersed in a suspension of bioluminescent bacteria *Allivibrio fischeri*. The reaction takes place within a very short time. All zones with inhibitory or toxic effects appear as dark zones on the luminescent plate background.

### Key features

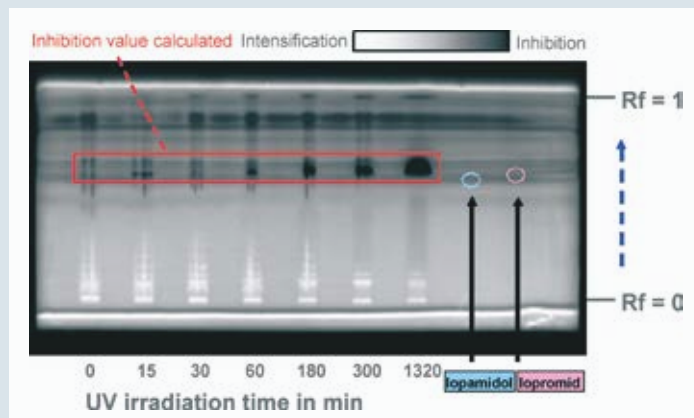
Stand-alone operation using BioLuminizer® 2 Software

- Cooled 16 bit CCD camera with high resolution and high quantum efficiency
- Optimized plate compartment, which preserves a favorable environment for luminescent bacteria (the bacteria are kept moist)
- Small footprint
- Easy to clean

### Ordering information

022.9750 CAMAG BioLuminizer® 2 for detection of bioluminescence patterns on HPTLC plates, including software

Detailed ordering information: [www.camag.com/bioluminizer](http://www.camag.com/bioluminizer)



### Example

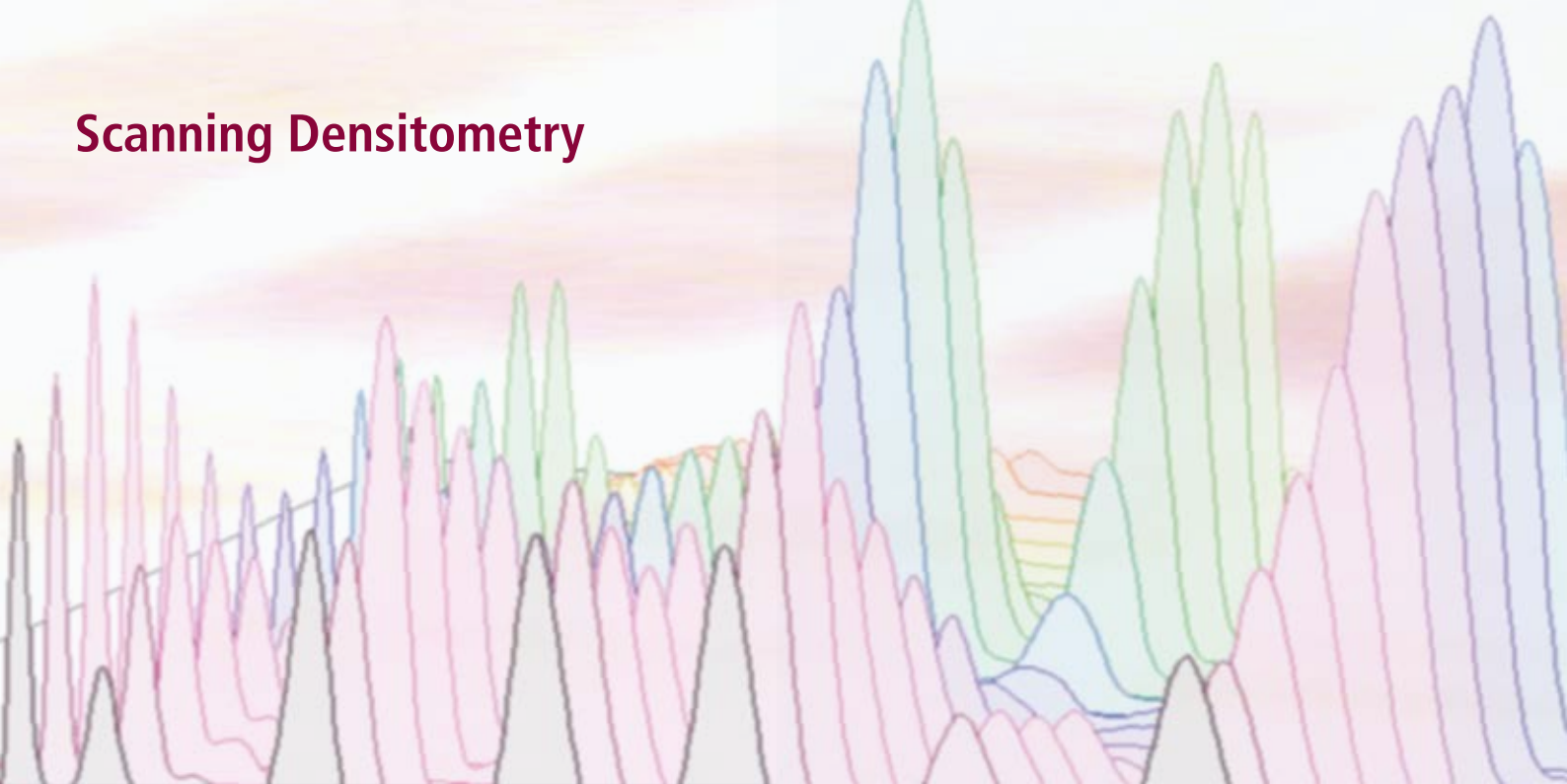
Processed wastewater containing X-ray contrast media is frequently irradiated with UV light. The HPTLC bioluminescence image shows the bioactive effect of degradation products. As can be seen, an increase of the irradiation time generates substances with a distinctively inhibitory effect on the luminescent bacteria. In a cuvette test, this inhibitory effect would have been masked by degradation products.

The example is taken from an internship report at the "Zweckverband Landeswasserversorgung" in Langenau, Germany.





# Scanning Densitometry



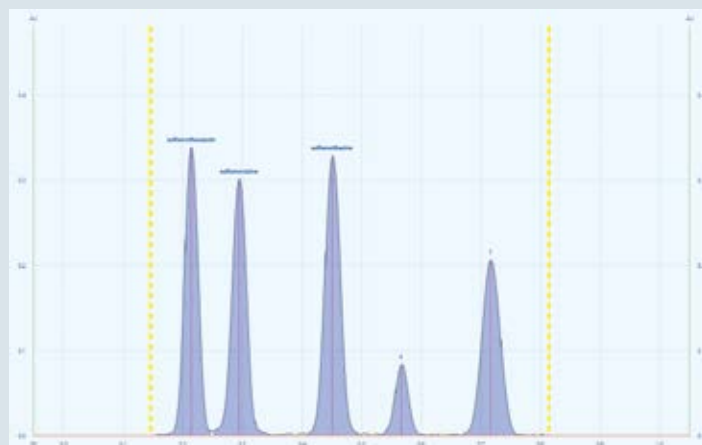
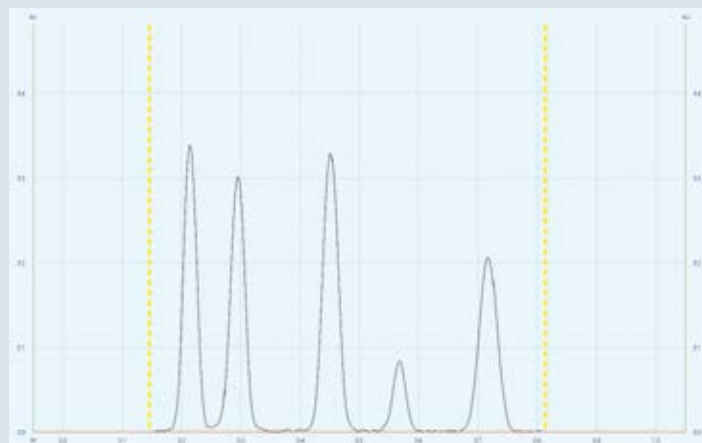
In scanning densitometry the tracks of the chromatogram are scanned with monochromatic light in the form of a slit selectable in length and width. The spectral range of the TLC Scanner 4 is 190–900 nm. Reflected light is measured either in the absorbance or in the fluorescence mode. From the acquired data quantitative results are computed with high precision and spectral selectivity.

With the TLC Scanner 4 absorption spectra can be recorded. The strengths of classical densitometry as compared with image evaluation are spectral selectivity and the higher precision of quantitative determinations.

## Recommendations

Applying samples in the form of narrow bands allows densitometric evaluation by aliquot scanning, *i.e.* scanning with a slit about  $\frac{2}{3}$  of the track width. This improves reproducibility as the center portion of the sample zone is homogeneous and positioning errors, which can occur with samples applied as spots, are avoided.

For quantitation sample zones should always be scanned with the wavelength of maximum absorbance which can be determined by spectra recording or by multi-wavelength scanning.



Automatic baseline correction and peak integration of six different sulfonamides

## CAMAG TLC Scanner 4



The TLC Scanner 4 is the most advanced workstation for densitometric evaluation of TLC/HPTLC chromatograms and other planar objects.

All functions of the TLC Scanner 4 are controlled by *visionCATS* software. Only positioning of the object to be measured is performed manually and, if desired, switching on the internal illumination to assist correct positioning. Optimal settings of the electronic amplification are automatically selected for scanning in absorbance or fluorescence mode respectively.

The 16 bit A/D converter ensures optimally adapted resolution of the measurement signal.

### Key features

- Measurement of reflected light, either in absorbance or fluorescence mode
- Object formats up to 20 × 20 cm
- Spectral range from 190 to 900 nm
- Automatic start of all lamps: deuterium, halogen-tungsten, and high pressure mercury lamp
- Data step resolution 25–200 µm
- Scanning speed 1–100 mm/s
- Spectra recording up to 100 nm/s
- Automatic selection of electronic amplification
- Rapid data transfer
- Meets all requirements to be used in a cGMP/cGLP environment
- IQ/OQ qualification and 21 CFR Part 11 ready

### Ordering information

**027.6200 CAMAG® TLC Scanner 4**, for the densitometric evaluation of TLC/HPTLC chromatograms, spectral range 190 to 900 nm, plate sizes up to 20 x 20 cm, absorbance and fluorescence mode, *visionCATS* controlled

Detailed ordering information: [www.camag.com/tlcscanner](http://www.camag.com/tlcscanner)

### Note

The TLC Scanner 4 with *visionCATS* meets all the requirements of cGMP/cGLP and can be IQ/OQ qualified. If the instrument shall be used in a 21 CFR Part 11 environment, the *visionCATS* option "21 CFR Part 11" is required.





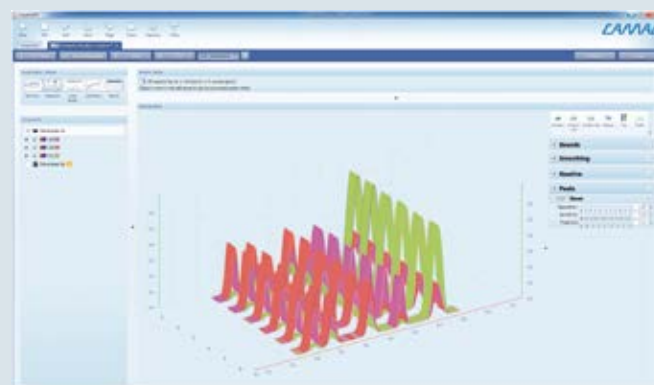
## Evaluation with visionCATS

visionCATS controls the TLC Scanner 4 and enables quantitative evaluation of the generated densitometric data. To determine the substance concentration in a sample, five different quantification functions (e.g. linear and polynomial) are available. Several scanning steps (e.g. scanning the plate after development and scanning the same plate after derivatization) and up to five different evaluations can be performed (with data obtained from single wavelength, multiple wavelengths or a combination of measurements in absorption and fluorescence detection mode).

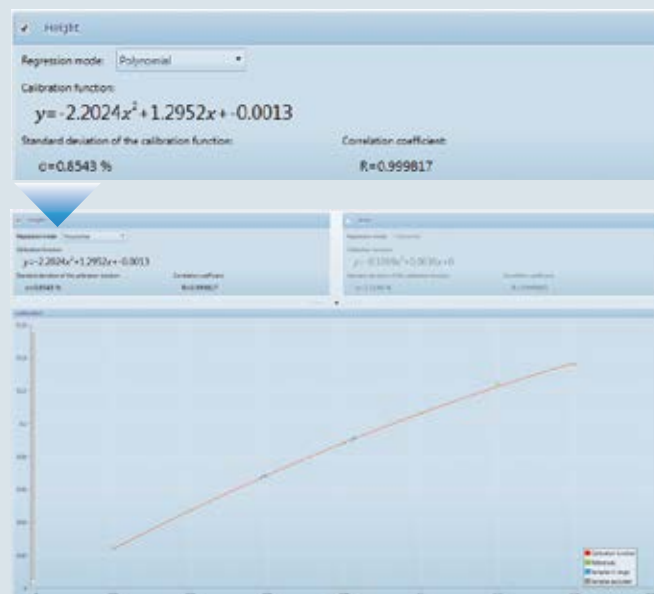
### The Scanner Ultimate Package:

- **Multi Wavelength:** this feature offers the possibility to perform a multi-wavelength scan with up to 31 selected wavelengths or a combination of measurements in absorption and fluorescence detection mode.
- **Scanner Quantification:** this feature allows to quantify each individual substance on the plate. Five different quantification functions are available for evaluation to determine the concentration of the substance in a sample. In one analysis file up to five evaluation steps can be performed in multiple plate states (e.g. plate after development and same plate after derivatization).
- **Spectrum Scanning:** this feature includes the measurement of the spectrum of each individual substance on the plate including the evaluation of the substance purity by comparison with reference standard.

Peak Assignment: the separated compounds are assigned. For quantification, data from the multi-wavelength scan at the optimum wavelength for each compound is used.



3D View and Peak Integration: densitograms are displayed in 3D, top or front view. Several peak integration and baseline correction settings can be selected.



Calibration Curve: for evaluation the best fitting calibration model is used. Quantitation can be done via peak height or area.

# Hyphenation of TLC/HPTLC with MS

## CAMAG TLC-MS Interface 2



The elution-based TLC-MS Interface 2 is a highly convenient and versatile instrument allowing for rapid and contamination-free elution of TLC/HPTLC zones with direct transfer to a mass spectrometer.

Through the pioneering concept of hyphenating High-Performance Thin-Layer Chromatography with Mass Spectrometry unequivocal substance identification is possible. The TLC-MS Interface 2 can be installed plug & play with any LC-MS system without adjustments or mass spectrometer modifications. Depending on the MS system, a substance can be identified within a minute via its mass spectrum, or for an unknown substance zone, the respective sum formula can be obtained. Furthermore, interesting zones can be eluted into vials for further investigations with, e.g., NMR, (ATR-)FTIR, ESI-MS, and MALDI-MS.

The chromatogram zones are eluted from the HPTLC plate with methanol or another suitable solvent with the flow speed appropriate for the LC-MS system. The round elution head is used for circular zones and the

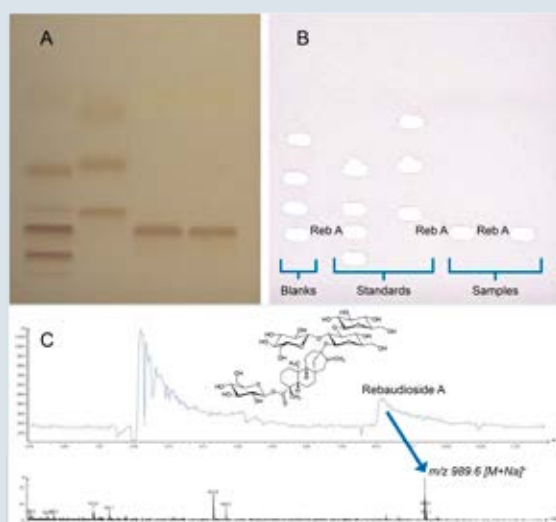
oval elution head for zones in the form of bands. After elution the eluate is either directly transferred to the mass spectrometer or collected in a sample vial for further offline analysis.

The TLC-MS Interface 2 features a modified elution head and an easily accessible, exchangeable filter, arranged in front of the valve. Cleaning is facilitated as compared to the previous version, making it highly efficient. By pushing a button, the elution path is cleaned of matrix particles with compressed air, increasing the lifetime of the filter and preventing the system from becoming blocked. These filters can be easily replaced without any modification to the elution head.

Plate positioning is significantly simplified: together with the positioning scale and the adjustable plate-stopper, the integrated crosshair laser enables accurate and reproducible positioning of the plate. Alternatively the coordinates determined by the TLC Visualizer 2 or the TLC Scanner 4 can be used to precisely position the HPTLC plate.

### Key features

- Rapid and contamination-free elution of selected zones
- Direct transfer to any mass spectrometer
- Plug & play installation
- Compatible with any LC-MS system
- Confirmation of known substances within a minute
- Highly effective backwashing function prevents the elution path from becoming blocked
- Easy handling ensures accurate and reproducible plate positioning
- Low solvent consumption



Characterization of separated compounds by mass spectrometry (Steviol glycosides in *Stevia* formulations\*)

A: Chromatogram for localizing the zones (derivatized with  $\beta$ -naphthol reagent)

B: HPTLC plate after elution of zones with the CAMAG TLC-MS Interface 2

C: HPTLC-ESI-MS spectra of Rebaudioside A,  $m/z$  989.6  $[M+Na]^+$

\*Morlock et al., *Journal of Chromatography, A*, 1350 (2014) 102–111

### Ordering information

022.8440 CAMAG® TLC-MS Interface 2, including oval elution head 4 × 2 mm

022.8441 CAMAG® TLC-MS Interface 2, including round elution head 4 mm

Detailed ordering information: [www.camag.com/tlc-ms2](http://www.camag.com/tlc-ms2)



# Software

*visionCATS*

Horizontal  
Developing Chamber

Stationary Phase

Nanomat

Developing Chamber

▲ Manual Input

Definition

Sample  
Application

Chromatogram  
Development

▼ Software-controlled

Samples

Automatic  
TLC Sampler ATS 4

Automatic  
Developing Chamber  
ADC 2

Standards

Linomat 5

AMD 2 System  
Automated Multiple  
Development

## visionCATS HPTLC Software

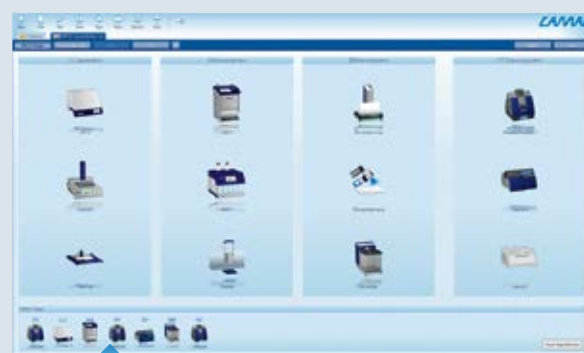
### HPTLC Analysis – made easy

*visionCATS* stands for ease of use and intuitive simplicity. The software organizes the workflow of HPTLC, controls the involved CAMAG instruments, and manages data.

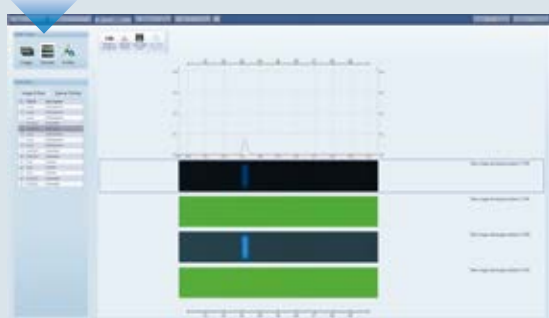
The easy to navigate user interface effectively guides the user through the chromatographic process – from definition of samples and substances to reporting of results. Simply select one of the default methods and start working: fill in the sequence table, select a mobile phase and the derivatization reagent.

If necessary modify detection parameters. Then *visionCATS* will guide you. Creating your own method is easy as well: just select the desired steps. The new sample-oriented approach allows for creating virtual plates from tracks originating from different plates, e.g. for batch-to-batch comparison or long-term stability testing.

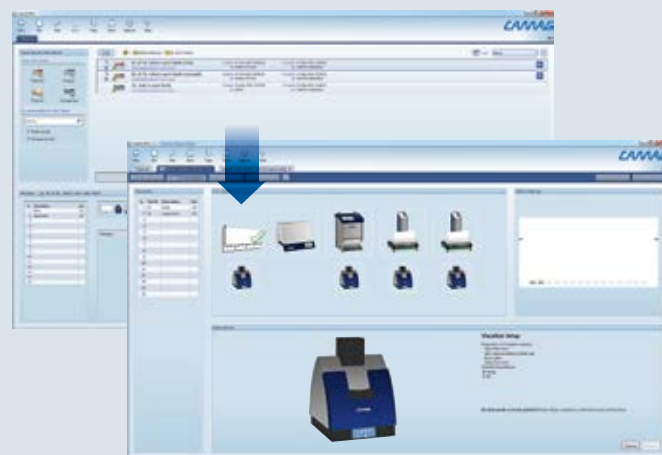
With *visionCATS* relevant samples can be located easier and faster than ever: a powerful search tool within the file explorer that includes extended preview functionalities enables the user to easily search for text and date, samples, methods, and analysis files.



HPTLC Analysis – made easy: create your own method with a few mouse clicks



Sample View: all available data related to the sample are displayed



Guided Analysis: select a method and *visionCATS* will guide you



Chromatogram  
Immersion Device

Derivatizer

Derivatization

Evaluation:  
Detection

Evaluation:  
Documentation

Report

TLC Scanner 4

TLC Visualizer 2

## Key Features

### Image Comparison Viewer

With the fully integrated Image Comparison Viewer tracks originating from the same or different plates and/or different detection modes can be compared on the same screen side-by-side.

### Image Enhancement Tools

*visionCATS* supports low-noise, high-dynamic-range imaging (HDRI) and includes a comprehensive set of Image Enhancement Tools.

### Scanning Densitometry and Spectral Evaluation

Following their chromatographic separation substances on the plate can be measured by Scanning Densitometry. Recording of UV/Vis spectra and their comparison with reference spectra allows identification.

### Quantitative Analysis

To determine the substance concentration in a sample after densitometric or image analysis, five different quantification functions (e.g. linear and polynomial regression modes) are available. Several scanning steps and up to five different evaluations can be performed in one analysis file.

### HPTLC Method Library

For seamless import of validated methods and images of standards and reference materials, *visionCATS* provides a free of charge HPTLC Method Library for licensed users.

### Regulatory Compliance

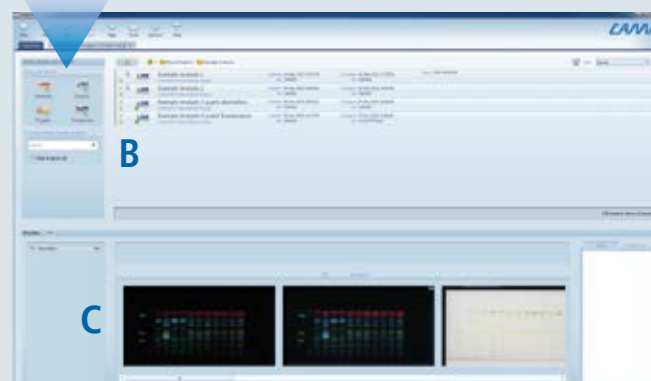
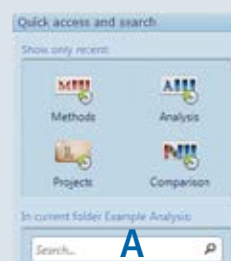
*visionCATS* supports compliance with cGMP/cGLP and 21 CFR Part 11.

### State-of-the-art Software Architecture

*visionCATS* is based on a client/server system, enabling scalability from a single workstation to a multi-user lab network.

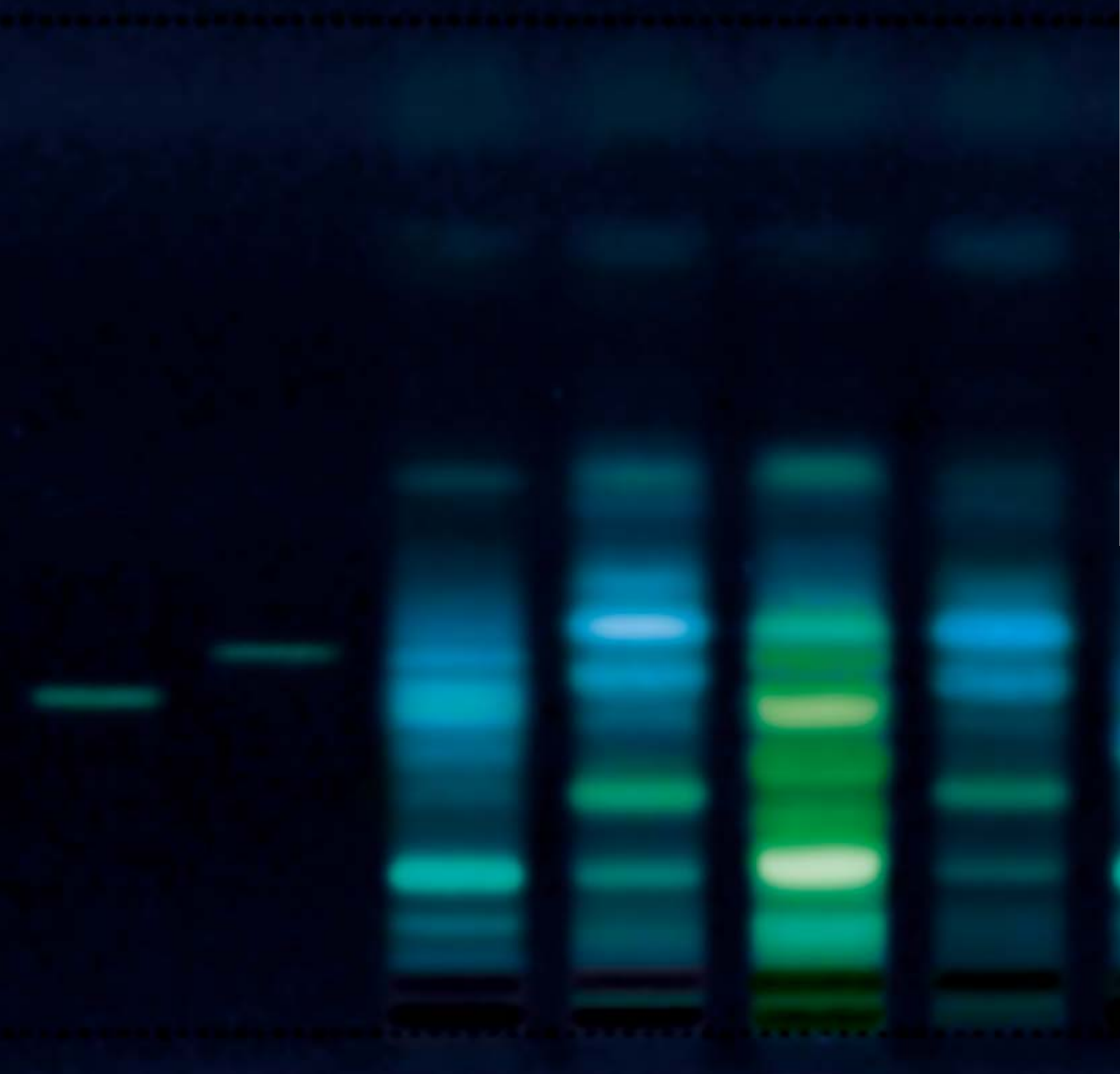


Plate Preview: provides an overview of steps and samples of an analysis or method incl. instrument parameters and thumbnails of the acquired image



File Explorer: search entry for name, ID or keyword A; file results B; preview of a selected analysis with the thumbnail of the captured images C

Ordering information: [www.camag.com/visionCATS](http://www.camag.com/visionCATS)



# Complete Systems Consumables

# HPTLC Systems

An advanced HPTLC system for more demanding tasks consists of at least one instrument for the steps sample application, chromatogram development, derivatization, and detection. The TLC Scanner 4 in conjunction with *visionCATS* software enables quantitative evaluation of the generated densitometric data.



## Ordering information

**041.1300 CAMAG® HPTLC System** for quantitative analyses including densitometric evaluation and image documentation, including *visionCATS* software for control of instruments. Suitable for laboratories with high sample throughput.



## Ordering information

**041.1200 CAMAG® HPTLC System** for quantitative analyses including densitometric evaluation and image documentation, including *visionCATS* software for control of instruments. Suitable for laboratories dealing with few samples.



## Ordering information

**041.1400 CAMAG® HPTLC System** for qualitative analyses based on image documentation and image-based evaluation, including *visionCATS* software for control of instruments. Suitable for laboratories dealing with few samples.

More preconfigured systems and detailed ordering information:  
[www.camag.com/systems](http://www.camag.com/systems)



## MERCK Precoated Layers for High-Performance Thin-Layer Chromatography (HPTLC)

| Designation                                                                                  | layer (µm) | size (cm) | quant./pkg |
|----------------------------------------------------------------------------------------------|------------|-----------|------------|
| 034.5628 HPTLC plates silica gel 60 F <sub>254</sub>                                         | 200        | 10 × 10   | 25         |
| 034.5629 HPTLC plates silica gel 60 F <sub>254</sub>                                         | 200        | 10 × 10   | 100        |
| 034.3726 HPTLC plates RP-2 F <sub>254</sub>                                                  | 200        | 10 × 10   | 25         |
| 034.3725 HPTLC plates RP-8 F <sub>254s</sub>                                                 | 200        | 10 × 10   | 25         |
| 034.3124 HPTLC plates RP-18 W F <sub>254s</sub>                                              | 200        | 10 × 10   | 25         |
| 034.3724 HPTLC plates RP-18 F <sub>254s</sub>                                                | 200        | 10 × 10   | 25         |
| 034.6464 HPTLC plates CN F <sub>254s</sub>                                                   | 200        | 10 × 10   | 25         |
| 034.2668 HPTLC plates Diol F <sub>254</sub>                                                  | 200        | 10 × 10   | 25         |
| 034.5647A HPTLC plates NH <sub>2</sub> F <sub>254s</sub>                                     | 200        | 10 × 10   | 25         |
| 034.5642 HPTLC plates silica gel 60 F <sub>254</sub>                                         | 200        | 20 × 10   | 50         |
| 034.5648 HPTLC plates silica gel 60 F <sub>254</sub> , ultra pure for pharmacopoeial methods | 200        | 20 × 10   | 50         |
| 034.1552 HPTLC plates silica gel 60 WR F <sub>254s</sub>                                     | 200        | 20 × 10   | 25         |
| 034.5548 HPTLC aluminium sheets silica gel 60 F <sub>254</sub>                               | 200        | 20 × 20   | 25         |
| 034.5445 HPTLC plates LiChrospher® Si 60 F <sub>254s</sub>                                   | 180        | 20 × 10   | 25         |
| 034.5647B HPTLC plates LiChrospher® Si 60 WR F <sub>254s</sub>                               | 100        | 20 × 10   | 25         |

## MERCK Precoated Layers for Thin-Layer Chromatography (TLC)

| Designation                                                                  | layer (µm) | size (cm) | quant./pkg |
|------------------------------------------------------------------------------|------------|-----------|------------|
| 034.5729 TLC plates silica gel 60 F <sub>254</sub>                           | 250        | 10 × 20   | 50         |
| 034.5715 TLC plates silica gel 60 F <sub>254</sub>                           | 250        | 20 × 20   | 25         |
| 034.1798 TLC plates silica gel 60 F <sub>254</sub> , with concentration zone | 250        | 20 × 20   | 25         |
| 034.5423 TLC plates RP-18 F <sub>254s</sub>                                  | 200        | 10 × 20   | 50         |
| 034.5554 TLC aluminium sheets silica gel 60 F <sub>254</sub>                 | 200        | 20 × 20   | 25         |
| 034.5559 TLC aluminium sheets RP-18 F <sub>254s</sub>                        | 200        | 20 × 20   | 20         |
| 034.5805 LuxPlate Si 60 F <sub>254</sub>                                     | 250        | 20 × 20   | 25         |

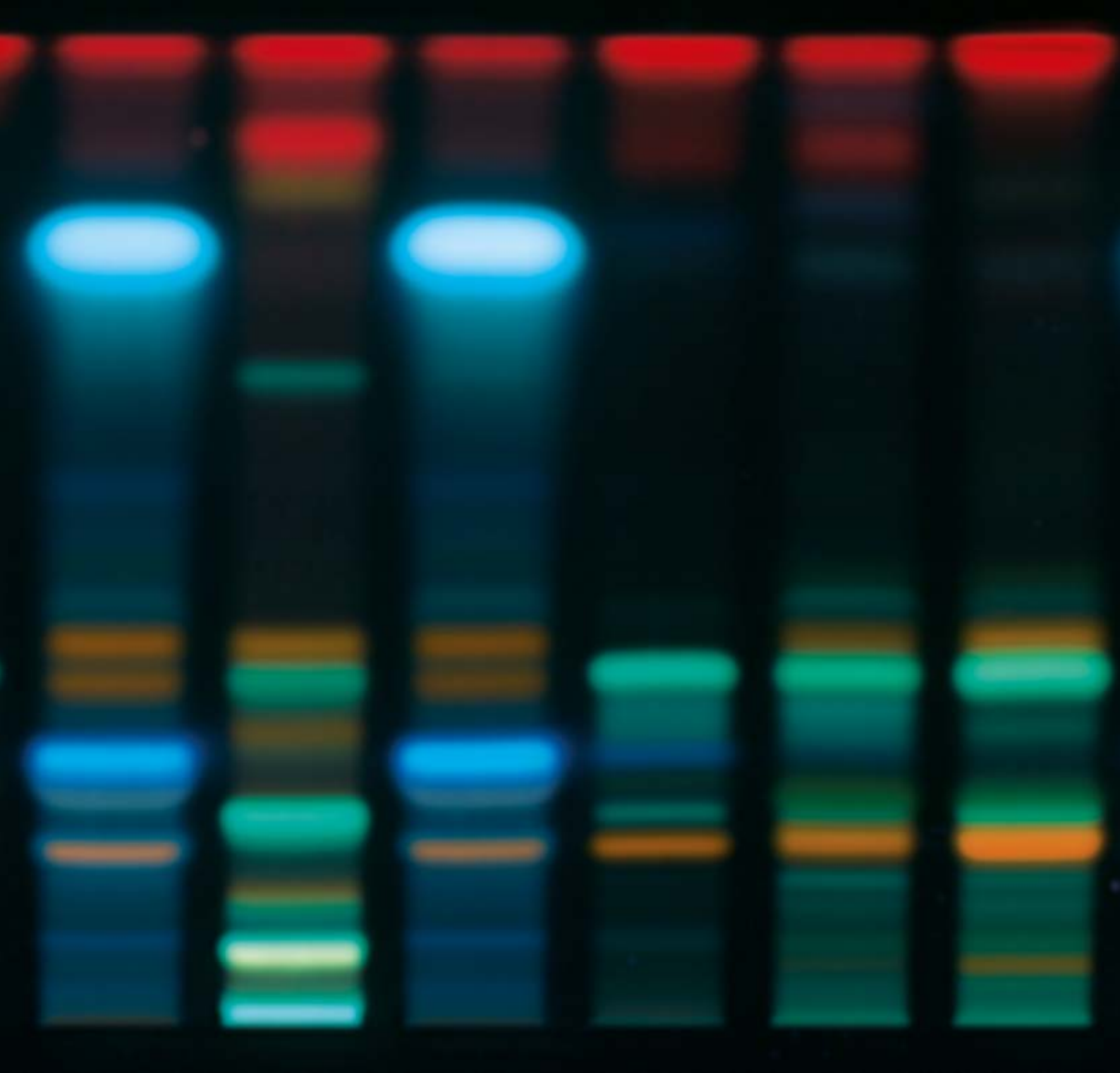
## CAMAG Test Dye Mixtures

Test dye mixtures are useful for functional checks on individual steps in the TLC/HPTLC procedure and for studying the influence of specific parameters.

### Ordering information

|          |                                                                            |
|----------|----------------------------------------------------------------------------|
| 032.8001 | Test Dye Mixture I, Toluene, 30 mL – for silica gel                        |
| 032.8002 | Test Dye Mixture II, Toluene, 30 mL – for aluminium oxide                  |
| 032.8003 | Test Dye Mixture III, Toluene, 10 mL – for HPTLC silica gel                |
| 032.8006 | Test Dye VI, powder for 30 mL – for IQ/OQ under <i>visionCATS</i> Software |
| 032.8007 | Test Dye VII (powder) – for IQ/OQ on Derivatizer                           |
| 993.0015 | Ethanol Solution standards for OQ tests, 2 vials of 10 mL with certificate |
| 993.0016 | Test Dye for AMD 2 OQ, Toluene, 10 mL                                      |





# CAMAG Services

Competent Advice  
Training Courses  
CAMAG Bibliography Service



## CAMAG HPTLC Laboratory

CAMAG HPTLC Laboratory offers analytical support with focus on HPTLC (High-Performance Thin-Layer Chromatography). The pharmaceutical, botanical and food industries are facing increasingly stringent regulation concerning product safety and quality. HPTLC is a fully reproducible, standardized, and cGMP-compliant technique. It can be the method of choice for many analytical tasks.

### 1) Feasibility studies

Following a detailed discussion of the analytical goal with the customer, the lab can evaluate whether HPTLC or TLC can offer an advantageous solution.

### 2) Consulting and training

CAMAG helps you get started! Whether you intend setting up a new lab, ensuring compliance with cGMP, or you are dealing with the authorities concerning registration, we can offer HPTLC solutions that save you time, hassle and money. Select one of our courses or let us provide customized training at your site to stay up-to-date with new developments in HPTLC technology. Let us show you how to optimally use your equipment, get reliable results, and develop and validate methods yourself.

### 3) Applied research

We offer guest residences at our laboratory for students, scholars, and researchers to engage in research projects. These are focused on, but not limited to practical aspects of HPTLC and analysis of botanicals. We publish results in journals, textbooks, through conferences and seminars as well as on our website. It is our goal to make available to the public high quality data illustrating the capabilities of HPTLC.

## Education and training

CAMAG is also your partner when it comes to education and training in the field of High-Performance Thin-Layer Chromatography. Select one of our courses to stay up to date with new developments in HPTLC methodology and technology. Let us show you how to properly use your equipment, get reliable results, and develop and validate methods yourself.

**CAMAG offers HPTLC training courses on the following topics:**

- HPTLC Today: qualitative and quantitative HPTLC; coupling techniques; gradient development; HPTLC bioautography
- HPTLC for the analysis of botanicals
- Method development and validation in HPTLC

Current training course schedule:  
[www.camag.com/courses](http://www.camag.com/courses)



## Instrument Qualification

For customers working in a cGxP regulated environment, CAMAG offers Installation Qualification (IQ) and Operation Qualification (OQ) as service.

The Installation Qualification (IQ) is performed on site and at the time of installation. It documents that all key aspects of the installation comply with the manufacturer's specifications, codes, safety and design parameters.

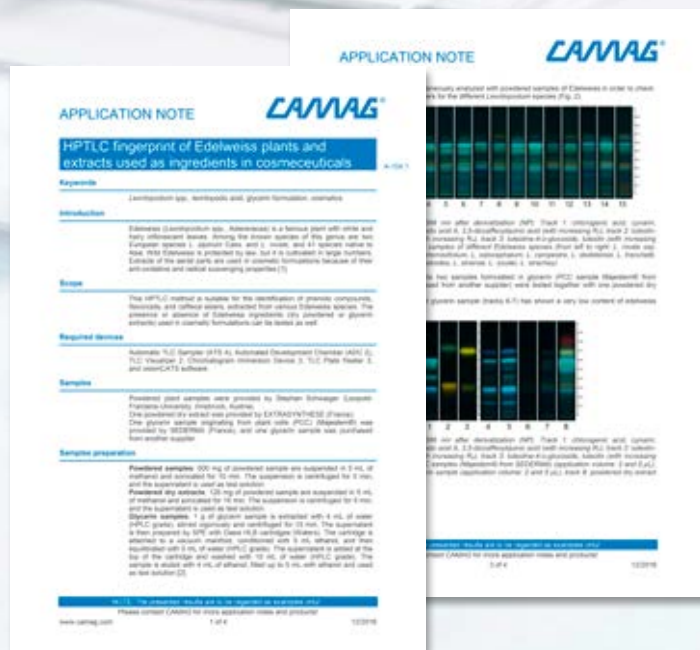
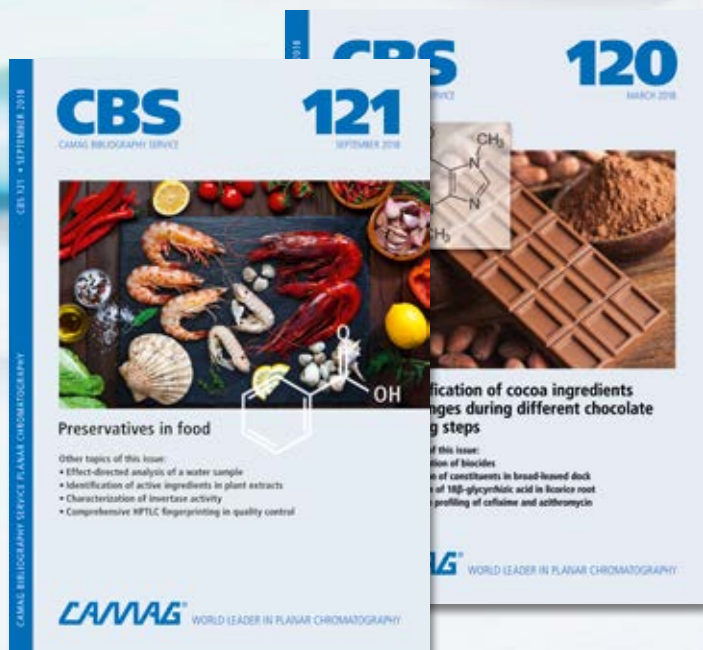
The Operation Qualification (OQ) is performed subsequent to installation and is repeated at certain intervals recommended by the manufacturer or defined by the customer. It documents that all modules of the equipment perform consistently throughout the specified operating ranges.

A Performance Qualification (PQ) is an ongoing process which documents that the instrument or system is suitable for the given task. Accordingly, only the customer himself can perform PQs, employing his substances and following his specific task descriptions and test procedures (SOPs).

**CAMAG offers qualification procedures for the following products:**

- AMD 2 System Automated Multiple Development
- Automatic Developing Chamber 2 (ADC 2)
- Automatic TLC Sampler 4 (ATS 4)
- DigiStore 2
- Derivatizer
- Linomat 5
- TLC Scanner 3
- TLC Scanner 4
- TLC Visualizer
- TLC Visualizer 2
- TLC-MS Interface
- TLC-MS Interface 2
- UV Lamp 4
- VideoScan
- *visionCATS* HPTLC Software
- winCATS – Planar Chromatography Manager





## CAMAG Bibliography Service (CBS) Planar Chromatography

CAMAG has been publishing the customer magazine CAMAG Bibliography Service CBS since 1965. The CBS is issued two times per year and focuses on recent examples of HPTLC in practice. Typical examples from research and industry, mainly written from customers, are demonstrated in each issue. Articles come from a broad variety of application fields.

## Cumulative CAMAG Bibliography Service (CCBS)

With the CCBS Online Search, you can directly search for information within the CAMAG website. The CCBS covers more than 11'000 abstracts of TLC/HPTLC publications between 1982 and today. The database covers most relevant scientific journals in the field of Planar Chromatography including also various non-English publications in German, French, Spanish, Portuguese, and Chinese. The CCBS features additional practical information for the analyst in the lab, for example details on the mobile phase or the detection. With CCBS the analyst is able to find relevant TLC/HPTLC publications which might be helpful for solving a particular analytical question.

Visit [www.camag.com/ccbs](http://www.camag.com/ccbs) and choose your preferred search option:

- Full text search
- Browse and search by CBS classification
- Alphabetical Search
- Search by CBS edition

## Application Notes

HPTLC is the method of choice for many analytical tasks – a broad range of Application Notes is available for download. Registered users can download the Application Notes for free.

CAMAG Laboratory develops and validates HPTLC methods for herbal drugs, food, cosmetics, forensics, and other application fields. By following these methods, using the recommended instruments and software, reproducible results are guaranteed. A standardized methodology according to the International Association for the Advancement of HPTLC is followed.



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# CAMAG – Global Presence



CAMAG markets its products in Switzerland directly from the headquarters, in Germany and the United States through their subsidiaries. In more than 70 other countries CAMAG is represented by selected companies.

CAMAG distributors regularly send their product specialists for education and training to our headquarters. Furthermore CAMAG organizes training courses overseas, *e.g.* in the Far East. The task of CAMAG product specialists is to advise customers in system selection and application competence and in the operation of their CAMAG systems. Service engineers of our distributors are regularly trained in Muttenz.

To our customers and distributors a comprehensive web-based information offer is available: [www.camag.com](http://www.camag.com) for product and company information, [www.camag-laboratory.com](http://www.camag-laboratory.com) for applications.

CAMAG is a flexible, customer-oriented and scientifically sound company, which in its 60 years company history has profiled as a valued partner in all areas of Planar Chromatography.

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