



Guide to visionCATS (version 3.0)





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1 General Introduction

1.1 The *visionCATS* concept: HPTLC made easy!

visionCATS is CAMAG's current HPTLC software. It stands for ease of use and intuitive simplicity. The software organises the workflow of HPTLC and controls all involved CAMAG instruments and the CAMAG[®] HPTLC PRO. The easy-to-navigate user interface guides effectively through the chromatographic process – from analysis definition to analysis reporting.

As state-of-the-art software, *visionCATS* is based on a client-server system offering enormous flexibility to the number of instruments and users that are working together, enabling access to the same data for all members of a work group. The sample-oriented approach allows for creating virtual plates with tracks originating from different plates, for example, 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 and a file explorer that includes extended preview functionalities enable highly comfortable searches for samples, methods, and analysis files.

The default settings implemented in the software were chosen according to the general chapters of the USP (chapter <203>) and the Ph. Eur. (2.8.25). This enables analysts, who work in a cGMP regulated environment, to run standardized HPTLC without the need for modifications in the software settings. In addition, *visionCATS* provides a Method Library with procedures that are in full compliance with chapter <203> (see chapter 4). All HPTLC methods of the USP Dietary Supplement Compendium (DSC 2015) are included. Other library methods of identification originate from the European Pharmacopoeia and the HPTLC Association. Methods developed by CAMAG are in the library as well.

visionCATS was developed for working in routine labs with validated methods. The primary focus is on following a standardized methodology (supported by the default settings according to the general chapters of the pharmacopoeias) to obtain reproducible and reliable analytical results. Data (digital images /scan data) should be qualified by a System Suitability Test (SST). *visionCATS* supports compliance with cGMP/GLP and 21 CFR Part 11.

1.2 Key features

• Supports qualitative and quantitative HPTLC Analysis

visionCATS organizes the workflow, controls the involved CAMAG instruments, and manages data.

Comparison Viewer

The sample-oriented approach allows for creating virtual plates from tracks originating from different plates. In the Comparison Viewer samples can be compared on the same screen side by side using selected racks of images (HPTLC fingerprints) or peak profiles of the tracks (generated from images and/or or obtained by scanning densitometry). Furthermore UV spectra of individual substances/peaks can be compared.

• Image enhancement tools

visionCATS supports low-noise, high-dynamic range imaging (HDRI) and includes a comprehensive set of Image Enhancement Tools (chapter 8).

Method Library

visionCATS provides a free of charge HPTLC Method Library for licensed users (see chapter 4)

• Flexible Reporting

visionCATS contains a fully configurable reporting system for analysis and comparison files (<u>http://hptlcmethods.cloudapp.net/300/administration/report/report.html</u>).

1.3 Benefits

- Enhanced usability
 - Focus on usability and modern appearance
 - One-click solution with semi-automatic settings
 - 3 levels for the user interface (easy, medium and expert). All levels are in reach of one mouse click.
- Guidance on workflow
 - While executing a method, the user receives information about the status of process, required actions, and the subsequent steps of the analysis.
 - The parameters for a step can still be edited/modified before the step is executed.
- State-of-the-art Architecture
 - *visionCATS* features a client/server architecture, enabling scalability from a single workstation to a multi-user lab network
 - Easily extendable
 - Easy to install (plug & play) and to service
- Compliance
 - User management (different contents/rights, passwords) for data security
 - Backup (with schedule assistant) for data safety
 - 21 CFR Part 11 (System logger, E-Signature, options related to deletion, motivated change management; for further information see online help at: <u>http://hptlcmethods.cloudapp.net/300/administration/21CFR_Part11.html</u>
 - Qualification (IQ/OQ)

 System Suitability Test (based on R_F values of marker compounds) to check and ensure that the analysis was performed appropriately. The test is passed when the detected peaks are positioned within the range established during method development.

2 Getting started

2.1 Main window

| Ξ | A | visionCATS by CAMAG® - Licensed to CAMAG with SN 100001 | - | - 8 × |
|------------|--|--|---|----------------------------|
| + | Explorer | | · · · · · · · · · · · · · · · · · · · | Instruments 7 × |
| | Quick access and search | • 🕥 -> 📫 - Demo Project - 🛅 Example Analysis | Sort: Name 🔻 🔺 | - |
| Ele | In current folder Example Analysis: | Comparison Viewer Created: 30-Jan/2017 16:47:36 Changed: 21-Jup 2019 07:05:32 by: visionCATSuser by: visionCATSuser by: visionCATSuser | • | 1 22 |
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| Tege | | Example Analysis 4 quant fluorescence Crewted: 06-Jan-2015 14:17:29 Dearget: 29-Aug-2019 13:42:19 by: CAMAG by: visionCATSuser | | 260732 |
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| Analysis | Example Analysis 4 quant fluorescence load | ded 📃 🔬 Default: 🚺 User: visio | onCATSuser Server: CM429MB-W10.camag.ch 😐 | Used memory: 329 MB |

Elements of the Main window:

- A Main Toolbar
- B Explorer and search window shows available projects / files (and their status)
- C Preview window (shows preview of a selected file)
- D Optional: Instruments window (shows all installed instruments and their connection status)
- E System Status Bar

visionCATS was developed for routine work. Users work in "project folders". In each folder, a method file is created first, which is based on a validated method documents and the standard operating procedure of the lab (*e.g.* template Ph. Eur. 2.8.25). All analyses performed with the method are typically stored in the same project folder. Comparison files generated from the analyses belong here as well.

To create a new general method template, a new method, or a comparison file an existing folder needs to be selected from the explorer or an additional one created (New folder): Select "New" / "New folder" from the main toolbar.



Then in the open folder a new method/comparison can be created (selection at the "New menu"). For creating own methods go to chapter 6.

3 Working with method templates

visionCATS supports routine work according to the general chapters on HPTLC of the USP and Ph. Eur. For this, different method templates are provided.

On CAMAG's website method templates are available for download.

Download zip-folder at: https://www.camag.com/downloads

The downloaded method template(s) can be imported into the *visionCATS* database, selecting / creating an appropriate folder, *e.g.* templates.



The templates include HPTLC standard parameters for al steps of qualitative analyses (no scanning densitometry). To create a method for an individual project based on the template, right click the template and select "copy to" or press Ctrl+Shift+C and then save the method with the name of the selected project. Next, open the new method and add information about SST, developing solvent (mobile phase) and derivatization and change any parameters that are not according to the standard template.

After saving the new method file is ready to use.

| + | Capitorer 🗠 | - Template USP 20 | | | | | | | |
|------------|----------------------------|-------------------|-----------------|-----|---|----------------|---|-------------|-----|
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4 Working with methods from the Method Library

The CAMAG Method Library is a repository of methods that can be downloaded directly a *visionCATS* installation. Each method package includes three files:

- An instrument method (A) ready to use in *visionCATS* in two versions (one for Linomat 5 and another one for the ATS 4)
- A method document (B) in a form (e.g. docx) which may serve as an SOP. This file contains a description of the System Suitability Test (SST) and acceptance criteria for passing samples
- An Image Comparison file (C) with reference images against which each analyzed sample can be compared and evaluated, based on acceptance criteria specified in the method document.



For download of methods from the library and method transfer validation see chapter 5.

5 Method transfer

5.1 Downloading a method from the Method Library

To download a method, click "Tools" on the Main Toolbar and select Method Library.

| + | Project Explorer | F9 |
|---------------------|--|-----|
| New | Instruments | F10 |
| | System Log | F11 |
| File | Edit Substances / Vials | F12 |
| Edit | 😥 Edit global lists | F8 |
| | Full System Log | |
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| Toola | F | |
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| Help | | |
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The overview page of the Method Library will open.

| Connected to CAMAG Library Tyy Import Packages (.pxf) | Plant name | | Date filter | Show | all | Advanced s | search > | | |
|--|-----------------------------|---------------------------------------|---|---------------------|------------------------------|------------|----------|----------------------------|-----------------------|
| M | Plant Other Plant part | * | From 31-Mar-2014 | O Show | only Local Library | | | | |
| | | wing new versions | To 22-May-2019 🛱 | O Show | only CAMAG Library | | | | |
| General | | | | | | | | Downloads | |
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| | عمار لمنتار لينما إيسنا ليم | 8 9 • | н | | | Page 1 | of 15 😴 | Name Alpinia katsumadai | Progress Completed |
| Latin plant name Adhatoda vasica | Plant part leaf | English drug name Malabar nut tree | Description This method identifies dried M nut tree leaf (Justicia adhatodi syn.: Adhatoda vasica Nees.) b HPTLC fingerorint and discrimi | a L. V | Date 14-Jul-2015 11:43:39 | Download | Deploy | | |
| Aesculus hippocastanum | seed | Horse Chestnut | This method identifies dried Ho Chestnut seed (Aesculus hippocastanum L) by HPTLC fingerorint and discriminates Ir | orse v1 | 05-Feb-2015 12:28:06 | 7 | 3 | | |
| Alpinia katsumadai | seed | Katsumada galangal | This method identifies dried Katsumada galangal seed (Alp katsumadai Hayata) by HPTLC fingerprint and discriminates d | v1 inia | 10-Jul-2015 09:15:35 | * | 21 | | |
| Alpinia officinarum | rhizoma | Lesser galangal | This method identifies dried Le galangal rhizome (Alpinia officinarum Hance) by HPTLC fingerorint and discriminates d | sser _V] | 10-Jul-2015 09:25:52 | 7 | 3 | | |
| Alpinia oxyphylla | fruit | Sharp-leaf galangal | This method identifies dried Sh leaf galangal fruit (Alpinia oxyp Miq.) by HPTLC fingerprint and discriminates dried Ginger rhiz | arp- v1 hylla | 10-Jul-2015 11:52:17 | ¥ | | | |
| Althaea officinalis | leaf | Marshmallow | This method identifies dried Marshmallow leaf (Althaea officinalis L.) by HPTLC fingerp and discriminates dried root of | v1 rrint | 10-Jul-2015 08:18:11 | ¥ | | | |
| Althaea officinalis | root | Marshmallow | This method identifies dried Marshmallow root (Althaea offcinalis L.) by HPTLC fingerp and discriminates dried leaves | v1 rint | 10-Jul-2015 08:25:32 | 포 | | | |
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CAMAG continually adds new methods. Please note that new methods generated with the most recent version of *visionCATS* are not compatible with previous *visionCATS* versions. To have access to all methods an update to the latest version of *visionCATS* is required.

The selected method is downloaded by clicking on the arrow button (1). The download progress is shown at (2). When download has finished the method can be imported to the database by clicking on (3).

| Import Packages (. Export license in | Fidera | Plant name Plant part | v ng new versions | Date filter From 31-Mar-2014 Count filters To 22-May-2019 Count filters | 0 | | y Local Library y CAMAG Library | dvanced se | | | | | |
|---|-------------|--------------------------|---------------------------------------|--|------------------------|--------|------------------------------------|------------|--------|---|--------------------|-------------------|---|
| General | | | | | | | | | | | Downloads | | |
| | 2 3 4 | 5 6 7 | 8 9 + | н | | | | Page 1 | of 15 | 2 | Name | 0 B/s Progress | _ |
| Latin plant name Adhatoda vasica | Pla leaf | nt part | English drug name Malabar nut tree | Discription This method identifies dried M nut tree leaf (Justicia adhatod syn: Adhatoda vasica Nees.) E HPTLC fingerint and discrim | alabar v1 i L, v | ersion | 0515 14-Jul-2015 11:43:39 | Download | Deploy | | Alpinia katsumadai | 0.0% | |
| Aesculus hippocast | anum see | d | Horse Chestnut | This method identifies dried H Chestnut seed (Aesculus hippocastanum L) by HPTLC fingerprint and discriminates in | irse y1 | | 05-Feb-2015 12:28:06 | ¥ | 31 | | | | |
| Alpinia katsumadai | see | d | Katsumada galangal | This method identifies dried Katsumada galangal seed (Alp katsumadai Hayata) by HPTLC fingerorint and discriminates d | inia ^{v1} | 1 | 10-Jul-2015 09:15:35 | ±1 | | | | | |
| Alpinia officinarum | rhiz | oma | Lesser galangal | This method identifies dried La galangal rhizome (Alpinia officinarum Hance) by HPTLC fingerorint and discriminates d | sser y1 | | 10-Jul-2015 09:25:52 | * | ₽ 3 | | | | |
| Alpinia oxyphylla | fruit | | Sharp-leaf galangal | This method identifies dried SI leaf galangal fruit (Alpinia oxyg Miq.) by HPTLC fingerprint and discriminates dried Ginger rhis | arp- v1 hylla | 1 | 10-Jul-2015 11:52:17 | 4 | | | | | |
| Althaea officinalis | leaf | | Marshmallow | This method identifies dried Marshmallow leaf (Althaea officinalis L) by HPTLC finger and discriminates dried root of | v1 rint | | 10-Jul-2015 08:18:11 | ¥ | | | | | |
| Althaea officinalis | root | | Marshmallow | This method identifies dried Marshmallow root (Althaea offcinalis L.) by HPTLC fingerp and discriminates dried leaves | v1 int | | 10-Jul-2015 08:25:32 | 4 | | | | | |
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In the next step the suitable version of the instrument method (ATS 4, Linomat 5, or both) are selected at (1), then the destination folder is selected at (2), or if a new folder is created at (3).

| Explorer | Method Library X | | | · · · · · · · · · · · · · · · · · · · |
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| General | | | Develop | K |
| Athanola vasica | 7 3 4 Plant | | Very In the package, deploy the CAMAG Method Files for the following application instruments: Very Very Very Very Very Very Very Very Very | 0 Bis Drogess staumate Completed |
| Assocutus hippoca | stanum seed | Horse Ches | Select the folder in which you want to deploy the package : | |
| Alpenia kataumad | 4 | Katsumada | Alpina officinarum | |
| Alpinia officinanz | n shizom | a Lanner gale | Blue cohosh Sowella resin | |
| Alpinia oryphylla | fut | Sharp leaf | Cannabie NP | |
| Althans officinals | - | Marstonali | When Policy Compared Folder When the destination index ready contains an item with the same name: () Key both files (the imported file will be renamed) | |
| Althana officinalis | . Post | Marshmale | O bont import | |
| | | | | |
| | | | | |
| 103 distinct method | a on CAMAG Server 107 | methods on CAMAG Server 23 de | ativit methoda diverbiaded 27 mathoda diverbiaded | |

Note: For a direct access to the Method Library an internet connection is needed. The companies' firewall needs to allow access to <u>http://hptlcmethods.cloudapp.net</u>. visionCATS communicates via Port 10501 (default). For lab PCs with no Internet access methods can be downloaded with the Standalone Downloader as PXF files and later imported using the Import Packages (.pxf) button. You can find the Standalone Downloader installer in the visionCATS server's installation folder (the default installer file is located at C:/Program Files (x86)/CAMAG/visionCATS/MethodCollectionMainInstaller.exe). When opening the Standalone Downloader for the first time, you will be prompted to import your license file. The Standalone Downloader has a user interface and functionalities similar to the Method Library tool of visionCATS, except that it downloads the files to the user-definable destination folder.

5.2 Performing a method transfer validation

Method transfer to your lab is very simple: transfer validation stipulates that the SST (System Suitability Test) must pass! After downloading and importing a method from the Method Library you can access the imported files from the Explorer window. Each method includes a method document that describes the entire procedure and the R_F values for the SST. Execute the method as described and the check the SST according to Section 9. Unless otherwise stated in the method document CAMAG recommends as general acceptance criterion that the R_F should not vary more than $\Delta 0.05$.



Check out our case study *Identification of fixed oils by HPTLC* to see how method transfer is done: <u>https://www.camag.com/article/identification-fixed-oils-hptlc</u>

6 Creating a new method

To create a new method or method template from scratch, a folder needs to be selected or an additional one created (New folder) from the main toolbar (selection at the New menu).



Then in the open folder a new method can be created (selection at the "New menu"). After entering the name (usually the same as the project folder) click "ok". A new window open, where the required steps can be selected by clicking on the instrument icons. The steps will be added to the bottom area and (later) executed in the order from left to right. Steps can be deleted if needed or rearranged in the bottom area by dragging/dropping them.



With a click on "Finish Step Definition" the settings window will open

| HPTLC-Steps | Chrometography Data | SST | æ | | Report | L |
|-----------------|---------------------|-----|---|---|--------|---|
| Sequence | HPTLC Steps | | | | | |
| fr. Description | Vol. | | | | | |
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Use the device icons to change settings like solvent type for application, SST requirements, developing solvent (mobile phase), etc. All settings that are fix for the respective method must be entered. Default setting for each step including the plate layout are in compliance with $\langle 203 \rangle / 2.8.25$.

| | Module preparation | > | | | | | | |
|----------|--|---|--------------------------------|-------|----------------------------|---|---------|----|
| | | | Pre-drying | | Drying time [min] 5 🛟 | | | |
| Les rest | Plate preparation | < | Activation | > | | | | |
| 100 | | | Pre-conditioning | > | | | | |
| | | | Solvent 1-Butanol, a | cetic | acid, water (66:17:17) 🔹 😢 | | | |
| | Development | < | Conditioning | > | | | | |
| | | | Ving | | Drying time [min] 5 🛟 | | | |
| N | otes : | | | | | | | |
| | | | | | | _ | OK Cano | el |

By clicking on "Execute Method" the analysis can be performed.

For each analysis the sequence table is opened first by clicking on it. Enter for each sample and standard (reference) a unique Vial ID, enter the application volume, select the rack position (for HPTLC PRO Module APPLICATION and ATS 4), select type (sample or reference) and tick tracks used for the SST. In the "Description" additional information on the sample/reference can be added.

| HPTLC-Steps 2 | Chromatography | 3 | Data | Sequence Table Defin | nition | | | |
|-------------------------------------|----------------|-----------|--------------|--|---------------|---------------------|--------------------|------------|
| Sequence Ir. Vial ID Description | _ | HPTLC Ste | ips | Add Add Delete overspot sequence contents | Remove Insert | Cut Copy Paste Inse | rt Left Center | |
| | | | | Tr. Vial ID | Description | | Vol. (µl) Positior | n Type SST |
| | | | | 1 | | | | |
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| | | | | Sequence table notes: | | | | OK Cance |

After clicking "OK", visionCATS will guide you through the different process steps.

6.1 Creating a qualitative method (*e.g.* identification of a herbal drug)

All process steps are selected as descripted in the method document. It is recommended to start with a Documentation (TLC Visualizer) step. This will be recognized by the software as "Clean Plate" image(s) and subtracted automatically from the image captured after development.

A typical scenario could be:

First capture an image of the empty plate, then apply the samples/references, develop the plate, capture an image of the developed plate, derivatize the plate, and capture an image of the derivatized plate.

The related HPTLC process includes those steps:



The sequence and number of process steps can vary depending on the method. Each step can be also be performed multiple times (*e.g.* an image prior to and after derivatization or with different capture settings, different ATS 4 for different dosage speeds for application of samples prepared with different solvents, two or more derivatizations).

Note: visionCATS supports different options for each process step. Samples can be applied either by HPTLC PRO Module APPLICATION, ATS 4 (Automatic TLC Sampler), by Linomat 5 (semi-automatic TLC Sampler) or manual with capillaries (Nanomat). Development can either be performed isocratic with the HPTLC PRO Module DEVELOPMENT, ADC 2 (Automatic Developing Chamber), by gradient with AMD 2 (Automated Multiple Development), or manual with a tank (Twin Trough Chamber, Flat Bottom Chamber, Horizontal Developing Chamber dimensions). Derivatization can be done by immersion (with the

Chromatogram Immersion Device), by automated spraying (with the Derivatizer) or by manual spraying, Data Acquisition can be done with the documentation system (TLC Visualizer/TLC Visualizer 2) and by scanning densitometry (TLC Scanner 3/TLC Scanner 4).

6.2 Creating a quantitative method

For quantitative methods two different options are available for Data acquisition. Either peak profiles can be generated from captured images or densitograms can be recorded with the TLC Scanner.

A typical scenario could be

First capture an image of the empty plate, then apply the samples/references, develop the plate, capture an image of the developed plate, scan the developed plate in absorption and/or fluorescence mode, derivatize the plate, and capture an image of the derivatized plate.



The sequence and number of process steps can vary depending on the method. Each step can be performed multiple times (*e.g.* a scanner step prior to and after derivatization).

7 Performing an analysis

The *visionCATS* workflow is based on instrument methods (either derived from method templates, provided by CAMAG, or created from scratch). A method is required before an analysis can be performed. Between different methods, HPTLC is a very flexible technique. For high reproducibility of analytical results flexibility must be limited within a method.

By a click on "Execute Method" a window opens and the name of the respective analysis file can be entered. By default the name is created with the method name combined with date and time.

| Ξ | | | | | | visionCATS by | / CAMAG [®] − Licensed to CA | MAG with SN 100001 | | | |
|----------|---|----------------------|----------------------|-----|---|-------------------------|---------------------------------------|--------------------|--|------------|------|
| + New | Explorer | MIII ID of Malabar n | . ATS4 [read-only] 🗙 | | | | | | | | |
| | HPTLC-Steps | Chromatograph | y Data | SST | | | | | | Report | Log |
| File | Sequence | | HPTLC Steps | | | | | | | | |
| | Tr. Description 1 Vanicine 2 Vasicinone 3 4 5 6 7 8 9 10 11 12 13 14 15 5 | Vol. 8.0 8.0 | | | | | | | | | |
| | | | Preview | | | | | | | | |
| | | | | | - | Vasicine Vasicinone | | | | Execute Me | thod |

After confirming the name by clicking on OK the Chromatography tab of the analysis file will open. In there, all settings and parameters can still be edited (not recommended). For routine work only information in the sequence table (A) must be entered after clicking on it. More information on the sequence table is available at:

http://hptlcmethods.cloudapp.net/300/method_analysis_file/chromatography/sample_sequence.html?highlight=sequence%20table

visionCATS is guiding through the analysis. In (B) plate layout parameters could still be edited (this should rather be done in the method). In (C) the entire HPTLC process is displayed. All steps can still be edited as long as they have not been executed). Completed steps are marked with a green arrow (B). In (D) a preview of the plate layout is shown. When executing a step, progression and instrument status are also displayed in this area. (E) provides instructions for the analyst, available instruments, and displays generated data during the execution of a step.



8 Data view

After all steps of an analysis have been executed, the Data View will open. The last captured image will be displayed. (A) allows to switch between three different views: Images (images of the entire plate), Tracks (track-oriented view), and Profiles (image profiles and/or densitograms). (B) displays the sequence of all captured images within this analysis. By clicking on either the entire plate is shown in the respective detection mode. (C) is the Data View Toolbar, containing general tools, R_F and track tools, and image enhancement tools. More information on the Data View Toolbar at:

http://hptlcmethods.cloudapp.net/300/method_analysis_file/dataview/data_view_toolbar.html #lbl-dataviewtoolbar

In (D) "Remarks" can be added to the analysis file (*e.g.* import an image of MS data obtained by HPTLC-MS) that will be displayed in the report and a "Report" can be generated (per default a full report of the whole analysis will be generated, including all settings, run time, results, etc.). Custom report templates can be saved and set as default. More information at:

http://hptlcmethods.cloudapp.net/300/administration/generalsettings/report.html#lbl-reportsconfig

With a click on "Log" (D) (requires option 21 CFR Part 11) the Analysis log file will be displayed.



8.1 Tools

The Toolbar in the Data view contains all tools for image editing and image data processing (general tools (A), R_F tool and select tracks for comparison (B), image enhancement tools (C), annotations (D)).



All icons are explained at:

http://hptlcmethods.cloudapp.net/300/method_analysis_file/dataview/data_view_toolbar.html #lbl-dataviewtoolbar

The most relevant icons are in section (B) and (C):

(B): " R_F Tool" displays lines at R_F 0 and R_F 1 (area of interest)



" $R_{\rm F}$ " Tip adds an $R_{\rm F}$ value to a zone of interest

| 0.9 | | 0.9 |
|-----|----------|-------|
| 0.8 | | - 0.8 |
| 0.7 | | - 0.7 |
| 0.6 | | - 0.6 |
| 0.5 | | - 0.5 |
| 0.4 | RF: 0.36 | - 0.4 |
| 0.3 | | - 0.3 |
| 0.2 | | - 0.2 |
| 0.1 | | - 0.1 |

"Sequence allows" selecting single or multiple tracks for image comparison (and together with "Adjust sequence position" to optimize the track position and width, if needed).



By a click on "Export for Comparison" a new window opens. File name, tracks, and detection modes (images and profiles) for a Comparison file can be selected. Further information is available in Section 14.

| | Sector and the sector s | |
|-----------|--|--|
| Add tract | ks and steps to a comparison Steps to export Step Developed 12 Developed 11 Developed 11 Derivatized 1 R 366 R TWhite | ● Create a new comparison ○ Open an existing comparison Enter the name of the new comparison file: I To add in the following folder: |
| | All tracks selected to export Link image and profile comparison tracks | *** Common horsetail herb ** Equisetum ** Equisetum 3 ** New Folder Current Folder OK Cancel |

By a click on "Generate Profile" image profile are generated (for each (pixel) line of the track *visionCATS* calculates the luminance from detected RGB values). Plotting the luminance as a fuction of R_F values generates peak profiles, which can be used for image-based quantitative evaluations. Data are accessible in the track and profile view, and in an evaluation tab.



(C): Image Enhancement tools



SpotAmp increases the contrast of the zones. After selecting the SpotAmp tool click on the background once or up to four times (maximum contrast 4.0). Original data are displayed after a click on "Reset changes".

White Balance allows to re-define "what is white" by clicking on the part of the captured image with a white background.

Normalize Exposure allows a normalization of the entire plate to one reference track. The default is set on track 1 and can be changed in the general settings. This feature is active for High Dynamic Range Images (HDRI) captured under UV 366 nm (sequence of images with different exposure times summarized in one). A click on "Show exposure range" displays the selected track for normalization and allows a manual change. This feature was implemented for comparing samples originating from different plates in fluorescence mode.

Clarify virtually changes the illumination setting after capturing to make weak zones better visible (for HDR images).

8.2 Export of Images

For export of images different options are available. Access the window "Export Image" by clicking on the icon at the tool bar or by right mouse-click on the captured image.

Images can be exported with or without annotations to a local disk or copied to clipboard for copy-paste to another file (*e.g.* a Word file).

| 1 HPTLC-Steps 2 Chromatography | 3 Data 4 Spectrum 5 SST 6.1 Evaluation 🖀 + | Remarks | Report | Log |
|-------------------------------------|---|--------------|--------|-----|
| Data Type Images Tracks Profiles | | <u>.</u> = 6 | | ľ |
| Overview | | | | |
| Chronological Illumination | | | | |
| Derivatized 1a (2) | Export image Please choose what you want to export in the image: | | | |
| | Export everything | | | |
| Developed 1a (3) | Export plate only | | | |
| | Export interesting area | | | |
| | Export interesting area with annotations | | | |
| | ⊙ Options | | | |
| Clean 1a (2) | Cancel | | | |
| | | | | |
| | Manufacture of the second se | | | |

9 Working with SST

The SST (System Suitability Test) is a test for assessing quality and reproducibility of the chromatographic system. CAMAG has implemented an SST according to the recommendations of the HPTLC Association. Methods from the HPTLC Association feature a set of standards with defined R_F positions. For instrument methods from the *visionCATS* Method Library the ΔR_F was set to 0.05. This is a recommendation for analyses performed on different days or in different laboratories. Other criteria can be defined.

For the SST a tick on the respective track(s) in the sequence table (Chromatography tab) is required.

| Add | Add | Remove | 4 Liii Insert | | Move up | Move down | Cut | Сору | Paste | Insert copied | ₹ L | | nter 👻 | | |
|-----|---------|--------|---------------------|-------|---------|-----------|-----|------|-------|------------------|-----|---------|----------|-----------|-----|
| Tr. | Vial ID | | Desc | ripti | on | | | | | | Vo | I. (µl) | Position | Туре | SST |
| 1 | R12345 | | Vasic | ine | | | | | | | | 8.0 | A1 | Reference | ✓ |
| 2 | R12346 | | Vasic | inor | ne | | | | | | | 8.0 | A2 | Reference | ✓ |
| 3 | S12345 | | Samp | ole u | nknov | vn | | | | | | 8.0 | A3 | Sample | |

After performing an analysis the SST can be validated in the SST tab.

| SST Table | | | | | | |
|---|---------------------|-------------------------------------|------|--------------|-------------|--------|
| Add Add M Image: Constraint of the constraint | | | | | | |
| | | | | | | |
| Substance | RF | ΔRF | Step | λ | Min. height | Status |
| Substance Vasicinone | R ⊧ 0.530 | ∆ <i>R</i> ⊧ 0.050 | Step | λ R 254 ▼ | | Status |

Example:

In the SST tab, the acceptance criteria for the SST are added (pre-set in the method template or set in the performed analysis). Then the detection mode(s) (wavelength(s)) for validating the SST is/are selected. For SST on images a profile must first be generated ("Generate Profile"). Then click on "Check" for computed evaluation (maximum of a peak within the window will be detected if AU is larger than 0.1) and indicated by a green (passed) or red (failed) arrow.

Note: It is possible to manually mark as passed an SST definition via the check box on the banner.

| d Delete Profiles Ch Substance | eck | RF | ΔRF | Step | | λ | Min. height | Status | Descript |
|-----------------------------------|---------|---------------|--------------|---------------|-------|--------------|-------------|----------|----------|
| Curcuminoid a | | 0.400 | 0.050 | | i1a ▼ | 🔲 RT White 🔻 | | ✔ Passed | |
| Curcuminoid b | | 0.280 | 0.050 | 🎒 Derivatized | i1a ▼ | RT White | 0.100 | ✓ Passed | |
| | | | | | | | • • • • | | |
| T View | | | | | | | | _ | |
| | Track 1 | l at waveleng | gth: RT Whit | e | | S | ubstances | ès 🖬 🔠 | |
| 0.0 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | - AU | | 1 ℝ | |
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| , () | | | | | | | | | |
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| E | | | | | | _ | | | |
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10 Scanning Densitometry

visionCATS controls the TLC Scanner 3 and TLC Scanner 4. Scanning densitometry generates spectrally selective quantitative responses for the individual tracks of the HPTLC plate as "Peak profiles from densitometry" (PPD). Several scanning steps (*e.g.* after development and/or after derivatization) can be programmed (single wavelength, multiple wavelengths and measurements in absorbance and/or fluorescence mode). For any detected peak on the plate, a UV-VIS spectrum can be recorded. For evaluation of the data, *visionCATS* provides five different calibration functions (*e.g.* linear and polynomial). Based on spectral data, peak purity can be determined.

The optical system



Any of the three light sources, high pressure mercury lamp, deuterium lamp, or halogentungsten lamp can be positioned in the light path by a motor drive. **(1)** (Further information can be found in the TLC Scanner Manual)

10.1 Single-wavelength scan

For a single-wavelength scan a scanner step is added to the HPTLC process in the method template.

| Explorer VIII Demo me HPTLC-Stops Chroma | | 557 🗄 | | * Report Log |
|---|--|---------------------------------|----------------------|-------------------------|
| | vication | Development | Derivatization | Con Data Acquisition |
| HPTIC PR | O Module ATTON | HTTL: PRO Module DEVELOPMENT | | |
| | | | 2 | |
| AT | 24 | ADC 2 | Derivatzation dip | Visualizer/Visualizer 2 |
| Line | 21 1415 | AMD 2 | Devivalization spray | Scannar 4 |
| Nane | a and a second s | Tranber | Demostrat | Bosnet 3 |
| HPTLC Steps | | | | |
| ä 🗓 I | i i i | li, | | Finish Step Definition |

By a click on "Finish Step Definition" you will get to the Chromatography tab. A click on the Scanner icon opens the Scanner settings.



There, "Scanner type" (Scanning mode), "Measurement mode" and "Lamps" can be chosen. Default is set to single-wavelength scan in absorbance at 254 nm. If another value is entered, *e.g.* above 400 nm, then the firmware will switch on the lamp for this range (deuterium lamp for UV range and tungsten for VIS range). If you select fluorescence mode, then you can select the wavelength for excitation (either deuterium/tungsten or selection of a spectral line of the mercury lamp). For fluorescence detections between different cut-off filters can be chosen, *e.g.* for excitation with 366 nm the filter K400 is well suited for filtering the light to be detected (only light above 400 nm can pass the filter).

| Scanner 4 Setting | js | | | | | | | 🗲 Deriva | tized 1b |
|-----------------------|--------------|---|---|-------------------|------------|--------|----------------------|-------------------|----------|
| Scanner type | Single λ | Ŧ | | | | | | | |
| Optimization for | Resolution | Ŧ | | | | | | | |
| Measurement mode | Fluorescence | Ŧ | | Filter: | | * | | | |
| Detector mode | Automatic | Ŧ | < | Quick scan start: | 4.9 🎍 | mm | Analog offset: | 10 % 🔻 | |
| | | | | Quick scan end: | 73.1 🍦 | mm | Sensitivity: | Automatic 🔻 | |
| | | | | Quick scan track: | All tracks | v | 0 adjust position Y: | 4.9 🌻 mm | |
| | | | | | | | 0 adjust track: | Track 1 🔹 🔻 | |
| Speed/resolution/slit | HPTLC * | | | Scanning speed: | 20 mm/s | | Slit: | 5 x 0.2 mm, micro | |
| | | | < | Data resolution: | 100 µm/st. | | | | |
| | Partial scan | | | Start: | 4.9 🛔 | mm | End: | 73.1 🍦 mm | |
| Wavelength selection | | | | | | | | | |
| Selection: User | | | | | Wavelength | (s) ap | | 254 nm | |
| Lamp: Mercury | | | | | | | 265 nm | 280 nm | |
| , | | | | | | | 297 nm | 302 nm | |
| | | | | | | | 313 nm | 🗸 366 nm | |
| | | | | | | | 405 nm | 436 nm | |
| | | | | | | | 546 nm | 577 nm | |
| | | | | | | | 579 nm | | |
| | | | | | | | | | |

In certain cases, changes to the default settings for scanning speed, data resolution and slit size makes sense. The wider the slit, the higher the light intensity while resolution of peaks is reduced. Especially for small peaks data resolution set to 25 μ m will lead to better results.

10.2 Multi-wavelength scan

If multiple analytes in the same analysis absorb at different wavelengths or if a subsequent measurement in fluorescence and absorbance is needed, a multi-wavelength scan can be performed.

| Scanner 4 Settings | | | | | | | 🗲 Derivat | |
|------------------------|------------------|-------|---------------------------------|------------|----|----------------------|-------------------|---------|
| Scanner 4 Setungs | | | | | | | C Derivat | IZEG ID |
| Scanner type | Multiple λ 🔹 🔻 | | | | | | | |
| Optimization for | Resolution • | | | | | | | |
| Measurement mode | Advanced 🔻 | | Filter: | | T | | | |
| Detector mode | Automatic 🔹 | < | Quick scan start: | 4.9 🕴 | mm | Analog offset: | 10 % 🔻 | |
| | | | Quick scan end: | 73.1 🍦 | mm | Sensitivity: | Automatic 🔻 | |
| | | | Quick scan track: | All tracks | v | 0 adjust position Y: | 4.9 🌻 mm | |
| | | | | | | 0 adjust track: | Track 1 🛛 🔻 | |
| Speed/resolution/slit | HPTLC . | | Scanning speed: | 20 mm/s | v | Slit: | 5 x 0.2 mm, micro | |
| | | < | Data resolution: | 100 µm/st | Ŧ | | | |
| | Partial scan | | Start: | 4.9 🌲 | mm | End: | 73.1 🍦 mm | |
| Wavelength selection | | | | | | | | |
| Wavelength(s) applied: | 205 ‡ nm Absor | bance | ▼ Lamp: Deut | erium | * | Filter: K400 💌 | | |
| Reorder 🕂 | 366 ▼ nm Fluores | cence | Lamp: Mercu | ry | Ψ. | Filter: K400 🔻 | | |
| | | | | | | | | |
|] | Deuterium | | | Tungst | en | | [| |
| | | | 500 | 600 | | 700 800 | 900 | |
| 200,0 | 300 0 | 400 | 500 | 600 | | 700 800 | 900 | |

11 Image profiles

With the Visualizer Ultimate Package Peak Profiles from images (PPI) can be obtained with a mouse-click. The software calculates for each track the resulting luminance (in the middle of the track) and plots it as a function of the R_F value.



To generate PPI, just click on the icon in the Data view toolbar.



PPI are available in the Tracks view (A), in the Profiles view (B), and in the evaluation tab ((C) for quantitative evaluation).



12 Evaluation

After all steps of an analysis have been executed, an evaluation tab can be added (click on "plus" to open an evaluation tab). Up to five different evaluations can be performed for each analysis file. *visionCATS* supports different user levels. In chapter 12.1 the "Easy mode" is described.

| | | | | | | | _ | | | | | | |
|---|--------|-----------|----------------------------|-------|----------------|-----|----------------------------------|-------------------|--------|--------------------|---------|--------|------------------|
| E |] Expl | orer | Am Ex | ample | Analysicence | * X | | | | | | | ۰ |
| 1 | н | PTLC-S | teps 2 C | hroma | tography 3 | | Data 4 Spe | ectrum 5 | SST | 6.1 Evaluation 📋 🕂 | Remarks | Report | Log |
| | | | | | | | | | | | | | |
| | Eval | uation \$ | Steps | | | | Substance table | | | | | | |
| | | _ | Substan | (e) | | | | 1 | _ | | | | |
| | | | m | 1/12 | | | Reuse substances | | - | | | | |
| | Def | nition Ir | tegration Subst. assign | Cali | vation Results | | Name | Sinensetin | | | | | Advanced options |
| | | | | | | | RF | | 0.330 | | | | |
| | | | | | | | ∆R≠ | | 0.020 | | | | |
| | Seq | uence | | | | | λ | | Ŧ | | | | |
| | Tr. | Vial ID | Description | Vol. | Туре | | Calibration type | Area | Ŧ | | | | |
| | 1 | S107 | Orthosiphon | | Sample * | | Calibration mode | Polynomial | Ŧ | | | | |
| | 2 | R351 | sinensetin sta | 1.0 | Reference * | | Range deviation | | 5.00 % | | | | |
| | 3 | S107 | Orthosiphon | 5.0 | Sample * | | | | | | | | |
| | 4 | R351 | sinensetin sta | 2.0 | Reference * | | | | | | | | |
| | 5 | S107 | Orthosiphon | 5.0 | Sample * | | | | | | | | |
| | 6 | R351 | sinensetin sta | 4.0 | Reference * | | | | | | | | |
| | 7 | R351 | Sinensetin co | 5.0 | Sample * | | | | | | | | |
| | | | sinensetin sta | 6.0 | Reference * | | | | | | | | |
| | 9 | S107 | Orthosiphon | 5.0 | Sample * | : | References Samples | | | | | | |
| | | | sinensetin sta | | Reference * | | Concentration unit type: | Mass / volume 🔻 | r | | | | |
| | | | Orthosiphon | | Sample * | | | Sinenseti | | | | | A |
| | | | sinensetin sta | | Reference * | | | | | | | | |
| | | | Orthosiphon | | Sample * | | R3518_150106 | _ M 100.000 _ Hg/ | | | | | |
| | | | sinensetin sta | | Reference * | | | | | | | | |
| | 15 | R351 | Sinensetin co | 5.0 | Sample * | | | | | | | | |
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12.1 Basics of quantitative evaluation in visionCATS

visionCATS guides you through the evaluation process:

| Evaluation Steps | |
|---|------------|
| | |
| Definition Integration Subst. Calibration assign. | on Results |

In "Definition" the substances to be analyzed and their concentration in the reference vials are defined. The quantity applied of each reference will be calculated from the application volume and the defined concentration. At (A) (red arrow) substances can be added and named. At (B) the concentration of the reference solution(s) is (/are) entered.

| Lustion Steps The importer and the steps Line importer and the steps Li | Substance table Reuse substances Name RF AR A Calibration mode Range deviation | Sinensetin 0.330 0.020 Area Polynomial 5.00% | | | | Advanced |
|--|---|--|---|--|---|----------|
| Visit Deter Bater Calibration Results Visit D Description Vol. Type Stort, Cothosphon 5.0 Sample * R351 Sinnessetin sta 1.0 Reference * Stort, Cothosphon 5.0 Sample * | Name RF ΔRF Α λ Calibration type Calibration mode | Sinensetin 0.330 0.020 v Area v Polynomial v | | | - | Advanced |
| Intern Pregration Reache exergin, Cellivation Reache Veal ID Description Vol. Type 5107. Crthosphon 5.0 Sample v 5107. Orthosphon 5.0 Sample v | Name RF ΔRF Α λ Calibration type Calibration mode | Sinensetin 0.330 0.020 v Area v Polynomial v | | | | Advanced |
| wenne Vial ID Description Vol. Type 5107. Orthosphon 5.0 Sample * R351 sinensetin sta 1.0 Reference * 507. Orthosphon 5.0 Sample * | Rr ΔRr Α λ Calibration type Calibration mode Calibration mode | Area * Polynomial * | | | | options |
| Vial ID Dasciption Vol. Type 5107 Orthosiphon 5.0 Sample * R351 sinersetin sta 1.0 Reference * 5107 Orthosiphon 5.0 Sample * | ΔRr A λ Calibration type Calibration mode | Area v Połynomial v | | | | |
| Vial ID Dasciption Vol. Type 5107 Orthosiphon 5.0 Sample * R351 sinersetin sta 1.0 Reference * 5107 Orthosiphon 5.0 Sample * | λ Calibration type Calibration mode | Area * Polynomial * | | | | |
| Vial ID Dasciption Vol. Type 5107 Orthosiphon 5.0 Sample * R351 sinersetin sta 1.0 Reference * 5107 Orthosiphon 5.0 Sample * | Calibration type Calibration mode | Area + Polynomial + | | | | |
| S107 Orthosiphon 5.0 Sample * R351 sinersetin sta 1.0 Reference * S107 Orthosiphon 5.0 Sample * | Calibration mode | Polynomial * | | | | |
| R351 sinersetin sta 1.0 Reference + S107 Orthosiphon 5.0 Sample + | | | | | | |
| S107 Orthosiphon 5.0 Sample * | Range deviation | 5.00 % | | | | |
| | | | | | | |
| R351 sinersetin sta 2.0 Reference * | | | | | | |
| | | | | | | |
| \$107 Orthosiphon 5.0 Sample * | | | | | | |
| R351 sinensetin sta 4,0 Reference * | | | | | | |
| R351 Sinensetin co 5.0 Sample * | | | • | | | |
| R351 sinersetin sta 6.0 Reference * \$107 Orthosiphon 5.0 Sample * | References Sample | | | | | |
| S107 Orthosiphon 5.0 Sample * R351 sinersetin sta 8.0 Reference * | : United the | | | | | |
| S107 Orthosiphon 5.0 Sample * | Concentration unit typ | e: Mass / volume 🔻 | | | | |
| R351 sinersetin sta 10.0 Reference * | 1 B | Sinensetin | | | | |
| S107 Orthosiphon 5.0 Sample * | : R3518_150106 | 🖬 100.000 _ng/ml 💌 | | | | |
| R351 sinersetin sta 12.0 Reference * | | | | | | |
| R351 Sinensetin co 5.0 Sample * | | | | | | |
| | | | | | | |

Use the tab "Samples" to define the sample reference amounts.

| References Samples | | | | | | | | | | | |
|--------------------|------------|------|--------------|------------------|------------|--|--|--|--|--|--|
| | Amount | Volu | ume solution | Reference amount | Related to | | | | | | |
| S10795_150106_01 | 500.000 mg | | 500.00 ml | 1.000 g | ٧ | | | | | | |
| S10795_150106_02 | 500.000 mg | | 500.00 ml | 1.000 g | | | | | | | |
| S10795_150106_03 | 500.000 mg | | 500.00 ml | 1.000 g | v | | | | | | |
| R3518_150106_02 | 500.000 mg | | 500.00 ml | 1.000 g | v | | | | | | |

Continue with the next step "Integration". The profiles (PPD or PPI) are displayed here. On the left side different detection modes can be selected.



In "Easy mode" you can directly continue with the next step "Substance Assignment" and position the substances at the proper R_F (moving each substance name to the expected R_F position).

| HPTLC-Steps 2 Chromatography 3 | Data 4 | Spectrum 5 | SST | 6.1 Evaluation | + | | | | | Remarks | Report | L |
|--|-----------------------|-----------------|-----------------|----------------|----------------|----------|-------------------|--------------|---------------------|-------------|-----------|--------|
| | | | | | | | | | | | | |
| valuation Steps | Substance Tab | e | | | | | | | | | | |
| Substance | Display all H | ide all | | | | | | | | | | |
| Vefnition Integration Subst. Calibration Results | Name | Sinensetin | | | | | | | | | | |
| assign. | RF required | 0.330 | | | | | | | | | | |
| | R# found | 0.328 | | | | | | | | | | |
| verview | ∆ <i>R</i> ≠ required | 0.020 | | | | | | | | | | |
| Developed 1b | ∆R≠ found | 0.015 | | | | | | | | | | |
| Developed 1b | λ required | | | | | | | | | | | |
| 2 366 | λ found | 366 nm | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | • | | | | | | | |
| | Substance Ass | ignment | | | | | | | | | | |
| | | All tracks at u | vavelength: 366 | | Substan | | - 1 D | | | | | |
| | | Partitions at v | avelengut. 000 | | Jubia | neo 🥪 | ≌+ | | | | | |
| | | | | | | 11 | 51 2 R 3 f | 5) 4 (R) 5 I | 151 6 R 7 15 | ର ୫.୮୧ ବ୍ୟସ | 10 R 11 S | 12 🖪 1 |
| | 0.0 RF | 0.1 0.2 | 0.3 | 0.4 | 0.5 AU | | | | | | | |
| | 1.0 | | | | | | | | | | | |
| | 1 0.9 | | | | | | | | | | | |
| | | | | | | | | | _ | | | |
| | | | | | | | | | | | | |
| | 0.7 | | | | | | | | | | | |
| | 0.6 | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | 0.5 | | | | - | | | | | | | |
| | 0.4 | | | | | | | | | _ | | |
| | | | | | Sinensetin @ 3 | 66 🔻 🗞 😰 | | | | | _ | _ |
| | 0.3 | | | | * | | | | | | | |
| | 0.2 | | | | | | | | | _ | | |
| | | | | | | | | | | | | |
| | 0.1 | | | | | | | _ | _ | | | |
| | 0.0 | | | | | | | | | | | |
| | | | | | 0.5 AU | | | | | | | |

In the next step "Calibration" the best fitting regression mode is selected (and applied for evaluation via peak height or area).



In the final step "Results" a summary of the quantified amounts/concentrations of each substance in the sample(s) is shown.

| HPTLC-Steps 2 Chromatography 3 | Data | 4 Spectru | m 5 | SST 6.1 Evaluation | 1 + | | | Remarks | Report | Log |
|--|----------------|--|--------------|----------------------|------------|------------------|---------------------|---------|--------------|-----|
| Evaluation Steps | | Results | | | | | | | | |
| Definition Interpretion Substance Interpretion Results | Engel Speed | Colleges all Reset to Colleges all Reset to Colleges all Reset to Colleges all Coll | v | | | | | Sort: | Track number | • |
| | ^ | Sinensetin @ 36 | 6 nm | (8 samples assigned) | | | | | | |
| Overview | | Sample 'R3518 | 150106_02' | 102.5 ng/ml | CV=0.30 % | (2 applications) | 102.5 µg in 1.000 g | | | |
| ✓ Toggle selection for all substances ✓ Sinensetin @ 366 nm | | ∧ Volume: 5.0 µ | | 102.5 ng/ml | CV=0.30 % | (2 replicas) | | | | |
| | | Track 7 | R¢ 0.332 | 102.3 ng/ml | 511.7 pg | | | | | |
| | | Track 15 | R# 0.330 | 102.8 ng/ml | 513.8 pg | | | | | |
| | | Sample 'S1079 | 5_150106_01' | 96.90 ng/ml | CV=0.13 % | (2 applications) | 96.90 µg in 1.000 g | | | |
| | | Volume: 5.0 µ | | 96.90 ng/ml | CV=0.13 % | (2 replicas) | | | | |
| | | Track 1 | RF 0.314 | 96.99 ng/ml | 484.9 pg | | | | | |
| | | Track 9 | R# 0.335 | 96.82 ng/ml | 484.1 pg | | | | | |
| | | Sample 'S1079 | 5_150106_02 | 70.99 ng/ml | CV=0.19 % | (2 applications) | 70.99 µg in 1.000 g | | | |
| | 1 | ∧ Volume: 5.0 μ | | 70.99 ng/ml | CV=0.19 % | (2 replicas) | | | | |
| | | Track 3 | RF 0.317 | 70.90 ng/ml | 354.5 pg | | | | | |
| | | Track 11 | R# 0.335 | 71.09 ng/ml | 355.5 pg | | | | | |
| | | Sample 'S1079 | 5_150106_03' | 61.85 ng/ml | CV=0.16 % | (2 applications) | 61.85 µg in 1.000 g | | | |
| | | ∧ Volume: 5.0 µ | | 61.85 ng/ml | CV=0.16 % | (2 replicas) | | | | |
| | | Track 5 | R€ 0.325 | 61.92 ng/ml | 309.6 pg | | | | | |
| | | Track 13 | Rr 0.333 | 61.77 ng/ml | 308.9 pg | | | | | |

12.2 Manual Mode

In the "Integration" step you can select one of three user levels. Default is the "Easy mode". On the right hand side a switch to "Manual mode" can be done. In the "Manual mode" advanced baseline and peak detection features are enabled, and the peak table is shown. Use this mode for manual peak integration.

| Explorer III Example Analysicence | | • | |
|---|---|---|---|
| 1 HPTLC-Steps 2 Chromatography 3 | Data 4 Spectrum 5 SST 61 Evaluation 🖹 + | Remarks Report Log | |
| Evaluation Depo | Peaks table The The The Sector (1995) and (1996) there is 19 metrics (1996) gifteent types in the latt grane (1996) the table(1996) | | llug AA 220m v Line v mode v View v 220m v Line v Ø Bounds |
| Overview | Integration | | Smoothing |
| University Image: Second | | to Particle P | ✔ Basatives ▲ ▲ LS Interactives ▲ ✔ Packs ■ ■ Ø Packs ■ ■ ▲ Ø Oct (/ Sazes (legacy) / Sauss) ▲ ■ ▲ Ø Saparation … … 0.10 € Breaksdig ● … … 0.10 € |

To add a peak select the "Add manually a peak" icon.



Ctrl + Mouse-scroll allows zooming-in.



12.3 Expert Mode

In the "Integration" step you can select one from three user levels. Default is the "Easy mode". If "Expert mode" is selected, all available features are accessible. Use this mode to configure advanced features such as smoothing parameters, (blank) track subtraction and dual wavelength subtraction.



Bounds

"Bounds" limit the evaluated data to those between the start and end bound (in R_F unit). "Clip outside" will hide data outside the bounds.

Smoothing

Profile raw data usually have some noise, which can adversely affect the peak detection. Smoothing can remove this noise. There are three smoothing algorithms available: Savitzky Golay (SG, which is the default), Moving average (MA), Gauss (newly implemented for
visionCATS). For each algorithm, a window or width can be adjusted to specify the degree of noise filtered.

Baseline

By default background correction is done with the slowest slope algorithm (automatic baseline detection, works on most data). "Interactive" allows manually adding baseline segments for background correction. To display the detected baseline use the button "Display Baseline".



Profile Subtraction

Dual Wavelength: select a base wavelength in the combi box. For each track PPD of the base wavelength will be subtracted from the PPD(s) of the other wavelength(s) selected.

Track: select a base track in the combi box. The PPD of this (blank) track will be subtracted from the PPD of other tracks.

Peaks

For peak detection two algorithms are available: Optional Quadratic Interpolation (OQI) and Gauss (default). For both peak detection algorithms the "Separation", "Sensitivity" and "Threshold" parameters can be adjusted.

12.4 Peak table export

In "Manual" and "Expert" modes the peak table can be exported of either a single track or of all tracks as csv files (comma separated values) for evaluation in Excel or other software.



12.5 Related substances

The feature "Related substances" allows determining the impurities within a sample (e.g. in an active pharmaceutical ingredient). For calculating the percentage of an impurity (impurities) a calibration curve of the main substance is generated. In the "Definitions" step of the "Evaluation" on the right hand side (A) "Advanced Options" are available. If "Related substances" is selected, the drop down list in (B) allows assigning the main and the related substance(s). There are three modes for evaluation: classic, by dilution (fixed volume), and by volume (fixed concentration). The classic mode (default mode) calculates the quantity of impurity/impurities based on the concentration of the main substance. In "By dilution" and "By volume" mode one track is defined as reference concentration/application. This can be defined in the "Related substances parameters". The result is then calculated in % impurity. Different calibration levels of the main substance can be generated by applying individual standards (same volume/by dilution) or a single standard (different volumes). For this feature, two example files are available for download (Example Analysis 8 related substances (volume) & related substances (dilution) in the provided Example Analysis 9 zip-folder: https://www.camag.com/downloads).

| xploter Analysilume) (r | ead-only] × | | |
|--|--|---------------------------------------|------------------------|
| HPTLC-Steps 2 Chromatography 3 | Data 4 Spectrum 5 S | T 6.1 Evaluation 📋 🐳 | Remarks Report Log |
| valuation Steps | Substance table | | |
| | Reuse substances | + | - II. |
| Definition Integration Subst. Calibration Results | Name Salicylic acid | Acetylsalicylic acid | Advanced a splice |
| assign. | Rr 0.00 | 0.000 | A |
| | ΔRr 0.010 | 0.010 | ✓ → Related substances |
| Sequence | λ | · · · · · · · · · · · · · · · · · · · | Internal standard |
| r. Vial ID Description Vol. Type | Calibration type | Height 👻 | L internal standard |
| | Calibration mode | Polynomial v | Reproducibility |
| | Range deviation | 0.00% | |
| | Related substances Related substance | Main substance V B | Limit test |
| | | | |
| R627 Acetylsalicyfic 5.0 Sample * | | | |
| | | | Υ |
| R627 Acety/salicyfic 2.0 Reference * | | | |
| R627 Acety/salicyfic 4.0 Reference * | References Samples | | |
| R627 Acetylsalicylic 6.0 Reference * 0 R627 Acetylsalicylic 8.0 Reference * | | | |
| 1 R627 Acetylsalicylic 80 Reference * | Concentration unit type: Mass / volume * | | |
| 2 | Salicylic acid | Acetylsalicylic acid | |
| 3 | : V R627-01 | ₽ 62.500 <u>µg/ml ▼</u> | |
| | | | |

Via the advanced mode (A) the "Related substances" feature can be accessed. Then in (B) "Main" and "Related" substance(s) are selected.

| | | Data 6. Spectrum | 5 SST 0.1 Evaluation 1 | | Remarks Report La |
|---------------------------------|------------------------------|---|---|---|-------------------|
| valuation Steps | | Substance table | | | |
| and the life (and | | Seuse substances | + | | · 23 |
| ladviter imparter Sales. Caller | ratur. Reads | | lic acid Acetylsalicylic acid | | Marine |
| | | Rv Aller | 0.000 0.000 0.010 0.010 | | 0 |
| iquence | | λ 230 m | | | Δ |
| Vial ID Description Vol. | - | Calibration type | Huight + | | |
| Tarto Description Free | 1794 | Calibration mode | Polynomial * | | |
| | | Range deviation | 0.00 % | | |
| | | Related substances Relate | | | |
| R627 Acetylasicolic. 5.0 | Sample + | | Related substances parameters | | |
| Net/ Activision 3.0 | sampa • | | Related substances mode: By dilution (fixed volume) * | | |
| R627 Acetylusicylic 50 | Reference + | | Allowed impurity amount: 2.00 % | - | |
| | Reference + | | Sample to use as reference concentration: R627 * | B | |
| | Reference * | References Samples | | | |
| | Reference + | Concentration unit type: Mass / | without the second s | | |
| were a semilar 20 | | | Saley | | |
| | | w R627-25 | | | |
| | | v R627-0.5 | | | |
| | | G R627-1 A | | ок | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | ₩627-1.5 ₩627-2 ₩ | ⁴¹ 2.00000 % | | |
| | | | H 2.0000 % | | |
| | | | # 2,0000 % | | |
| | | | 4 20000 % | | |
| e. | | ₩ 1627-2 | | | |
| Refere | ences Sa | | | | - |
| Refere | ences Sa | ₩ 1627-2 | # 20000% | | - |
| | _ | , MIZ72 " | С | | - |
| | _ | ₩ 1627-2 | С | | |
| | _ | mples it type: Mass / vo | C lume • | id la | |
| | _ | mples it type: Mass / vo | C lume 👻 licylic acid Acetylsalicylic ac | id | |
| Conce | entration ur | mples it type: Mass / vo | C lume ¥ lícylic acid Acetylsalicylic ac | id | |
| | _ | mples it type: Mass / vo | C lume 👻 licylic acid Acetylsalicylic ac | id a | |
| Conce | entration ur R62725 | mples it type: Mass / vo | C lume licylic acid Acetylsalicylic ac 0.25000 % | id | |
| Conce | entration ur | mples it type: Mass / vo | C lume ▼ licytic acid Acetylsalicytic ac 0.25000 % | id a | |
| Conce | R62725 R627-0.5 | mples it type: Mass / vo | C lume Iicylic acid Acetylsalicylic aci 0.25000 % 0.50000 % | id | |
| Conce | entration ur R62725 | mples it type: Mass / vo | C lume licylic acid Acetylsalicylic ac 0.25000 % | id a second | |
| Conce | R62725 R627-0.5 R627-1 | mples it type: Mass / vo 5a 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | C lume ▼ licylic acid Acctylsalicylic ac | id | |
| Conce | R62725 R627-0.5 | mples it type: Mass / vo | C lume Iicylic acid Acetylsalicylic aci 0.25000 % 0.50000 % | id 1 | |
| Conce ~ ~ | R62725 R627-0.5 R627-1 | mples it type: Mass / vo 5a 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | C lume ▼ licylic acid Acctylsalicylic ac | id | |

At (A) the "Related substances parameters" can be accessed. Default is the classic mode. For the "Related substances by dilution" one sample track is defined as reference concentration (B). In (C) the concentration of the individual standard solutions is entered.

| HPTLC-Steps 2 Chromatography 3 | Data 4 Spectrum | 5 55 | f 6.1 Evaluation 🟦 🐘 Remark | is Report | Log |
|--|------------------------------------|------------|--|-----------|----------|
| raluation Steps | | | | | |
| | Reuse substances | | 6 | | 113 |
| adultan Integration Subst. Collimation Results | Name Salicylic | acid | Acetylsalicylic acid | | Advanced |
| anajar. | RI | 0.000 | 0.000 | | |
| | ΔRr | 0.010 | 0.010 | | |
| quence | λ | | • | | |
| Vial ID Description Vol. Type | Calibration type | | Height * | | |
| | Calibration mode | | Polynomial * | | |
| | Range deviation | | 0.00 % | | |
| | Related substances Related | | | | |
| R627 Acetylsalicylic 5.0 Sample + | | | Itances parameters | | |
| Net / Activolityle 3.0 Sample * | | | bstances mode: By volume (freed concentration) * B | | |
| R627 Acetylsalicylic 2.0 Reference + | | Allowed in | purity amount: 2.00 % | | |
| R627 Acetylsalicylic 4.0 Reference + | | Reference | application Track 11, 10.0 µL applied + corresponds to 2.00 % of impurities. | | |
| R627 Acetylsalicylic 6.0 Reference + | References Samples | | | | |
| R627 Acetylsalicylic 8.0 Reference * | Concentration unit type: Mass / vo | her. | | | |
| R627 Acetylsalicylic 10.0 Reference * | • | | | | |
| | R627-01 | | | | |
| | mar el | | | | |
| | | | | | |

At (A) the "Related substances parameters" can be accessed. Default is the classic mode. For the "Related substances by volume" one sample track is defined as reference application (B).

12.6 Internal standard

The feature "Internal standard" may increase the accuracy of the analytical result. By addition of an internal standard to all samples and standards the variations in the sample preparation (different extraction yields) and errors in the measurement chain are reduced/corrected. In the "Definitions" step of the "Evaluation" on the right hand side (A) "Advanced Options" are available. If "Internal Standard" is selected, in (B) with a tick, the analyte used as internal standard is selected. The "Example Analysis 7 Internal Standard" (download zip-folder at: https://www.camag.com/downloads) provides an example.

| HPTLC-Steps 2 C | hromatography 3 | Data 4 Sj | pectrum 5 SST | 6.1 Evaluatio | on 📋 🕂 | | Rei | marks Report |
|---|---------------------|------------------------|--------------------|---------------|----------|-------------|----------|---------------------------------------|
| valuation Steps | | Substance table | | | | | | |
| | | Reuse substances | | | + | | | |
| Definition Integration Subst. | | Name | | Methandienone | | | | Advan |
| Detinition Integration Subst. assign | Calibration Results | RF | 0.000 | 0.00 | 00 | | | A A |
| | | ΔRF | 0.010 | 0.0 | | | | Related substance |
| Sequence | | λ | * | | * | | | |
| | | Calibration type | | | - - | | | Internal standard |
| r. Vial ID Description | Vol. Type | Calibration mode | | | - - | | | Reproducibility |
| 1 2 S152 0.01 mg/mL | 10.0 Reference * | Range deviation | | 5.00 | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | Internal standard | ~ | | | | | 🗆 📃 Limit test |
| s S17508 1:25 diluted | 2.0 Sample * | | M | | | | <u> </u> | |
| 4 S152 0.01 mg/mL | 1.0 Reference * | | | | | | | |
| \$152 0.04 mg/mL | 2.0 Reference * | | | | | | | * |
| s S152 0.01 mg/mL | 2.0 Reference * | | | | | | | |
| + S152 0.04 mg/mL | 2.0 Reference * | | | | | • • • • • • | | |
| 5 \$152 0.01 mg/mL | 4.0 Reference * | References Samples | | | | | | |
| + S152 0.04 mg/mL | 2.0 Reference * | | | | | | | |
| 7 \$17508 1:25 diluted | 2.0 Sample 🔻 | Concentration unit typ | | | | | | |
| 3 S152 0.01 mg/mL | 1.0 Reference * | | Methyltestosterone | Methandienone | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | v S15211-10 | / | 10.000 µg/ml | v | | | |
| 9 S152 0.01 mg/mL | 6.0 Reference * | v \$15206-250 | 40.000 µg/ml * | 1 | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | | | | | | | |
| 10 S152 0.01 mg/mL | 8.0 Reference * | | | | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | | | | | | | |
| 11 S17508 1:25 diluted | 2.0 Sample v | | | | | | | |
| 12 S152 0.01 mg/mL | 1.0 Reference * | | | | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | | | | | | | |
| 13 S152 0.01 mg/mL | 10.0 Reference * | | | | | | | |
| + \$152 0.04 mg/mL | 2.0 Reference * | | | | | | | |
| 14 \$17508 1:25 diluted | 2.0 Sample 🔻 | | | | | | | |

12.7 Reproducibility

The feature "Reproducibility" evaluates the deviation of the peaks height/area of a certain substance applied on several tracks. For each substance, the reproducibility test calculates:

- the average (height or area)
- the coefficient of variation (CV)
- the deviation of each value from the average.

| Sequence Seq | | 2 Chromatography 3 | Data 4 | pectrum 5 SST 6.1 Evaluation a + | Remarks Report Lo |
|--|-----------------|----------------------------|----------------------|---------------------------------------|----------------------|
| a w w w w w w w w w w w w w w w w w w w | valuation Steps | | Substance table | | |
| Name Condentation Name< | | Subtance | Reuse substano | n + | * B |
| No. 0.000 Apr. 0.000 | | Subst. Calibration Results | Name | | |
| Service A Control (Service) | | assign. | Re | 0.000 | |
| Val Domptoni Val Type Area 1 Grad Ania 2.5 Sample 1 Grad Ania 2.5 Sample <t< td=""><td></td><td></td><td>ΔR_F</td><td>0.010</td><td>Related substances</td></t<> | | | ΔR_F | 0.010 | Related substances |
| Vet II completes Vet II Completes Vet II Completes Vet II Completes Vet II Completes Vet III Completes Vet IIII Completes Vet IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII | quence | | λ | · · · · · · · · · · · · · · · · · · · | A |
| 10 10 <td< td=""><td>Mal II Decel</td><td>tion Mat Time</td><td>Calibration type</td><td>Area 👻</td><td>Li Libernal standard</td></td<> | Mal II Decel | tion Mat Time | Calibration type | Area 👻 | Li Libernal standard |
| 1 Orstel Ano 2.5 Sangle * Based Section 1 Orstel Ano 2.5 Sangle * Reproducibility B 1 Orstel Ano 2.5 Sangle * Reproducibility Constellation 1 Orstel Ano 2.5 Sangle * Reproducibility Constellation 1 Orstel Ano 2.5 Sangle * Reproducibility Constellation 1 Orstel Ano 2.5 Sangle * Reproducibility Reproducibility 1 Orstel Ano 2.5 Sangle * Reproducibility Reproducibility 1 Orstel Ano 2.5 Sangle * Reproducibility Reproducibility 1 Orstel Ano 2.5 Sangle * Reproducibility Constellation 1 Orstel Ano 2.5 Sangle * Reproducibility Reproducibility 1 Orstel Ano 2.5 Sangle * Constellation Reproducibility 1 Orstel Ano 2.5 Sangle * Constellation Reproducibility | | hn 2.0 Sample v * | Calibration mode | | V Reproducibility |
| 1 forst Mar 25 Bangle 2 2 forst Mar 25 Bangle 2 1 forst Mar 25 Bangle | 1 Erste Ba | | | | |
| 1 Orote Maria 2.5 Sample • 3 Orote Maria 2.5 Sample • 3 Orote Maria 2.5 Sample • 1 Orote Maria 2.5 Sample • 3 Orote Maria 2.5 Sample • 1 Orote Maria 2.5 Sample • | 1 Erste Ba | hn 2.0 Sample + | Reproducibility | B | 🗆 🔜 Limit test |
| 1 Ont Mar 23 Sangle + 1 Ont | 1 Erste Ba | hn 2.0 Sample v | | | |
| 1 Orotalian 2 3 Saryole - 1 Orotalian 2 Saryole - 2 Orotalian 2 Saryole - 1 Orotalian 2 Sary | 1 Erste Bi | hn 2.0 Sample v | | | |
| Tork Name 2 a Sample * Tork Name 2 a Sample * Tork Name 2 a Sample * Constantion 2 a Sample * Concentration with type Mass / volume * Concentration with type Mass / volume * Concentration with type Mass / volume * | 1 Erste Ba | hn 2.0 Sample v | | | |
| 1 Orto Markov 2, 2 Sample * 1 Orto Markov 2, 2 Sample * 2 Orto Markov 2, 2 Sample * 1 Orto Markov 2, 2 Sample * Concentration unit type Mark / volume * Concentration unit type Mark / volume * | 1 Erste Ba | hn 2.0 Sample v | | | |
| to the land 2 a fample v Concentration unit type Mass / volume v Concentration unit ty | 1 Erste Ba | hn 2.0 Sample v | | | |
| 1 Brst Eilwin 2.0 Sample * Consentiation unit type: Mass / volume * 1 Crist Eilwin 2.0 Sample * | 1 Erste Bi | hn 2.0 Sample v | References Samp | | |
| 1 Cirita Bahn 2.0 Sample v Duritoritan | 1 Erste Ba | hn 2.0 Sample v | Concentration unit t | a Mass / unluma - * | |
| 1 Erste sann 2,0 Sample * | | | | | |
| | | | | | |
| 1 finis fath 2.0 Sample + : ↓ 2 * 10.000 symm.** | | | ~ 2 | <u>A</u> 10.000 <u>µg/ml ▼</u> | |
| | 1 Erste Ba | hn 2.0 Sample v | | | |

At (A) the "Reproducibility" feature can be accessed. Use the check box at (B).

| Explorer X= 00_Sc4_Y | × | | | | | | | | | | | | | | | | | | | | |
|---|-------------|----------------------|----------------------|-------------|---------|--------------|------------|--------------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|---------|--------|--------|
| 1 HPTLC-Steps 2 Chromato | graphy 3 | Data 4 | Spectri | um 5 | SST | 6.1 Evaluat | ion 📋 6.2 | Evaluation 1 | 1 + | | | | | | | | | ş | Remarks | Repor | rt Lo |
| Evaluation Steps | | Results | Rep | oducibility | Α | | | | | | | | | | | | | | | | |
| Definition Integration Subst. Calibration | ion Results | Epert Real | | | | | | | | | | | | | | | | | | | |
| | | A Qualifi | cation @ | 600 nm | CV=0.5 | 57 % Average | =0.051 (pe | eak area) | | | | | | | | | | | | | |
| Overview | | | | | | 1.50 % - | | | | | | | | | | | | | | | |
| Toggle selection for all substan | | Track | Re | Peak area | | | | | | | | | | | | | | | | | |
| | .05 | S Track 1 | Rr 0.406 | 0.05147 | 0.74 % | | | | | | | | | | | | | | | | |
| Qualification @ 600 nm | | S Track 2 Track 3 | R¥ 0.406 R¥ 0.407 | 0.05160 | 0.98% | 1.00 % - | | + | | | | | | | | | | | | | |
| | | S Track 3 | Ry 0.407 Ry 0.407 | 0.05146 | 0.00% | | | | | | | | | | | | | | | | |
| | | S Track 5 | R# 0.407 | 0.05088 | -0.41 % | | - T | | + | | | | | | | | | | | | |
| | | S Track 6 | R+ 0.407 | 0.05093 | -0.33 % | 0.50 % - | | | | | | | | | + | | | | | | |
| | | S Track 7 | Rr 0.407 | 0.05098 | -0.23 % | | | | | | | | | | | | | | | | |
| | | S Track 8 | Rr 0.407 | 0.05117 | 0.15% | | | | | | | | | - | | | | | | | |
| | | S Track 9 | R# 0.407 | 0.05135 | 0.50 % | 0.00 % - | | | _ | + | _ | | | _ | _ | | - | | _ | _ | _ |
| | | S Track 10 | R≠ 0.407 | 0.05133 | 0.46 % | | | | | | | | | | | | | | | | |
| | | S Track 11 | R≠ 0.407 | 0.05108 | -0.02 % | | | | | | | _ | - + - | | | | | | | | |
| | | S Track 12 | Rr 0.408 | 0.05076 | -0.66 % | -0.50 % - | | | | | + | | | | | | | | | + | |
| | | S Track 13 | | 0.05068 | -0.82 % | -0.30 % | | | | | | | | | | | | - | | | + |
| | | S Track 14 | Rr 0.408 | 0.05087 | -0.44 % | | | | | | | | | | | | | - T- | | | |
| | | S Track 15 | Ry 0.408 | 0.05077 | -0.63 % | | | | | | | | | | | | | | - 1 | | |
| | | | | | | -1.00 % | Tr. 1 | Tr. 2 | Tr. 3 | Tr. 4 | Tr. 5 | Tr. 6 | Tr. 7 | Tr. 8 | Tr. 9 | Tr. 10 | Tr. 11 | Tr. 12 | Tr. 13 | Tr. 14 | Tr. 15 |

In the Results tab, the "Reproducibility" results are displayed in a separate tab (A).

12.8 Limit test

The feature "Limit test" determines whether the quantity or concentration of a substance in one or more samples is above or below a specified limit. The "Limit test" is a fail/pass check for each individual sample track.

| Advanced | | | | | | | | | | | |
|----------------------|---|----------|----------------------------|------------------------|--|---------------------------------|-----|--|--|--|--------------------------------|
| Adversed Adversed | | | | | | stance table | | | Steps | aluation | va |
| Advanced | | | | | † | euse substances | | | Substance | | |
| | | | | | | e Decursin | 1 | Calibration Results | markin Baber | | |
| | | | | | 0.270 | | | | assign. | | |
| Related substances | | | | | 0.010 | | | | | | |
| _ | | | | | * | | 1 | | | quence | 1q |
| Internal standard | | | | | * | aration type Height | | Vol. Type | Description | Malin | |
| Reproducibility | | | | | Ψ | aration mode Linear-1 | | 2.0 Reference * | | | |
| | | | | | | no deviation | _ | 2.0 Reference * | | | |
| 🖊 🗔 Limit test 🛛 🗛 | | | | | B / | t test | | 2.0 Sample * | Angelica giga | \$133 | |
| | | | | | | | _ | 2.0 Sample * | Angelica giga | \$133 | |
| | | | | Limit test parameters | | | | 2.0 Sample * | Angelica giga | \$133 | |
| v | | | | Limit test parameters | | | | 2.0 Sample * | Angelica giga | \$133 | |
| | | | | | | | | 2.0 Sample + | Angelica giga | \$133 | |
| | | Ŧ | ntration | Mode: Conce | | | | 2.0 Sample + | Angelica giga | \$133 | |
| | | - | 5-141111-2 | Reference vial: S1249 | | ences Samples | : 8 | 2.0 Sample + | | | |
| | | | | | dume v | entration unit tune: Mass / vol | | | | | |
| | | ove 🔿 At | Below 🔘 Abov | Limit test pass if: 🔘 | | | | | | | |
| | | - | | 0 | | | | | | | |
| | | |)% | Allowed deviation: 5.0 | | | | | | | |
| | | | | | | S12495-141111 15.00 | | | | | |
| | _ | | 5-141111-2 Below 💿 Abov | Limit test pass if: | Jume × Decursin 000 µg/ml × 000 µg/ml × | R13277-14111 20.00 | - | 2.0 Sample * 2.0 Sample * 2.0 Sample * 2.0 Sample * 2.0 Sample * | Angelica giga Angelica giga Angelica giga Angelica giga Angelica giga Angelica giga | \$133 \$133 \$133 \$133 \$133 \$133 | 8 9 10 11 12 13 |

At (A) the "Limit test" feature can be accessed. Use the check box at (B). Set the criteria at (C).

| HPTLC-Steps 2 Chromatography 3 | Data 4 | | 5 SST | 6. | 1 Evaluation 📋 | 6.2 Evalu | ation 📋 | 6.3 Ev | aluation | f 6.4 | Evaluation | 8 6 | .5 Evaluati | on 🖹 | |
|--|------------------------------|------------------|--------------|---------|----------------|--------------------------|---------|------------------|------------|-------|------------|-----|-------------|------|--|
| aluation Steps | Results | Limit test | A | | | | | | | | | | | | |
| efinition integration Subst. Calibration Results | Engent Strange | | | | | | | | | | | | | | |
| | Decursi | n @ 366 nm | Cor | centrat | ion min.=15.0 | | | | tion | | | | | | |
| verview | | | | | Limit | est passed est failed | Lir | nit forence c | concentrat | 100 | | | | | |
| Toggle selection for all substances | | | | | | | - | renembere | | 2011 | | | | | |
| Decursin @ 366 nm | Track | R. | Concentratio | Status | 16 | | | | | | | | | | |
| | S Track 3 | R# 0.241 | 18.84 µg/ml | | 14 | | | | | | | | | | |
| | S Track 4 | R# 0.241 | 25.15 µg/ml | ~ | 14 | | | | | | | | | | |
| | S Track 5 | R# 0.239 | 22.38 µg/ml | ~ | 12 | | | | | | | | | | |
| | S Track 6 | R# 0.239 | 16.05 µg/ml | ~ | | | | | | | | | | | |
| | S Track 7 | R# 0.239 | 23.57 µg/ml | ~ | 10 | | | | | | | | | | |
| | S Track 8 | R# 0.239 | 26.94 µg/ml | ~ | 7 | | | | | | | | | | |
| | : S Track 9 | R≠ 0.238 | 12.33 µg/ml | x | - 8 b | | | | | | | | | | |
| | Track 10 | R≠ 0.238 | 23.81 µg/ml | ~ | | | | | | | | | | | |
| | Track 11 | R≠ 0.236 | 23.63 µg/ml | ~ | 6 | | | | | | | | | | |
| | S Track 12 | R≠ 0.238 | 23.43 µg/ml | ~ | | | | | | | | | | | |
| | Track 13 | <i>R</i> ≠ 0.238 | 13.36 µg/ml | x | 4 | | | | | | | | | | |
| | S Track 14 | RF 0.236 | 15.44 µg/ml | ~ | 2 | | | | | | | | | | |
| | | | | | 2 | | | | | | | | | | |
| | | | | | 0 | | | | | | | | | | |

In the Results tab, the "Limit test" results are displayed in a separate tab (A).

12.9 Export results

The results table can be export as csv. file for *e.g.* further evaluation in Excel.

| xplorer Amalysicence | × | | | | | |
|--|---|--------------------------|---------------------|-----------|------------------|-------------------|
| HPTLC-Steps 2 Chromatography 3 | Data 4 Sj | ectrum 5 | SST 6.1 Evaluatio | n 🖹 🕂 | | |
| valuation Steps | Results | | | | | |
| valuation Steps | Hesults | | | | | |
| Entration Integration Subst. Calibration Results | Expect of Colleges all Asset to Original way | Export multa (CPV) | | | | |
| | Sinensetin (|) 366 nm | (8 samples assigned | 1) | | |
| Werview | Sample 'R: | 1518_150106_02' | 102.5 ng/ml | CV=0.30 % | (2 applications) | 102.5 µg in 1.000 |
| Toggle selection for all substances | Volume: | 5.0 µl | 102.5 ng/ml | CV=0.30 % | (2 replicas) | |
| 🗹 Sinensetin @ 366 nm | Track 7 | Rr 0.332 | 102.3 ng/ml | 511.7 pg | | |
| | Track 15 | R# 0.330 | 102.8 ng/ml | 513.8 pg | | |
| | Sample 'S' | 0795_150106_01' | 96.90 ng/ml | CV=0.13 % | (2 applications) | 96.90 µg in 1.000 |
| | Volume: | 5.0 µl | 96.90 ng/ml | CV=0.13 % | (2 replicas) | |
| | Track 1 | Rr 0.314 | 96.99 ng/ml | 484.9 pg | | |
| | Track 9 | Rr 0.335 | 96.82 ng/ml | 484.1 pg | | |
| | Sample 'S | 0795_150106_02 | 70.99 ng/ml | CV=0.19 % | (2 applications) | 70.99 µg in 1.000 |
| | Volume: | 5.0 µl | 70.99 ng/ml | CV=0.19 % | (2 replicas) | |
| | Track 3 | Rr 0.317 | 70.90 ng/ml | 354.5 pg | | |
| | Track 11 | Rr 0.335 | 71.09 ng/ml | 355.5 pg | | |
| | Sample 'S' | 0795_150106_03 | 61.85 ng/ml | CV=0.16 % | (2 applications) | 61.85 µg in 1.000 |
| | Volume: | 5.0 µl | 61.85 ng/ml | CV=0.16 % | (2 replices) | |
| | Track 5 | R# 0.325 | 61.92 ng/ml | 309.6 pg | | |
| | | | 61.77 ng/ml | 308.9 pg | | |

12.10 Export graphs

All graphs (calibration curves, spectra, limit test results, reproducibility results) can be exported by a right mouse click. The graphs can be exported with different resolution and saved to a destination folder or to clipboard.



Example of an exported calibration curve (white background for all exported graphs)

13 Spectrum Scan

After single- or multi-wavelength scan spectra of selected peaks can be recorded. For this, another scanner step is added.



In the Scanner settings the range can be selected, e.g. from 190 to 400 nm.

| Scanner 4 Settings | | | | | | | 🗲 Derivatized 1 |
|---|---|------------------|-----------------|-------------|----------------|------------|-----------------|
| Scanner type Optimization for Measurement mode Detector mode | Spectrum Resolution Absorbance Automatic | ▼ ▼ ▼ ▼ | | | | | |
| Speed/slit | HPTLC - | | Spectrum speed: | 20 nm/s 🔹 | | Slit: 5 x | 0.2 mm, micro 🔹 |
| Spectrum parameters | | < | | | | | |
| Reference spectrum: | per plate | * | < | X: | 10.0 mm | Y: 10.0 mm | |
| | | Purity | | Distance to | o peak center: | 0.5 mm | |
| Lamp: Deuterium & Tur | n▼ Start λ: | 190 ‡ End | A: 400 🗘 | | | | |
| 2 | | | | | | | |
| Deuterium | | | Tungste | en . | | | |
| | \sim | | | | | | |
| 290 300 90 | 400,00 | 500 | 600 | 700 | 800 | 900 | |

There are two options available: the classical spectrum scan of selected peaks or spectrum scan for purity testing. Purity testing is selected by a tick.

| Spectrum parameters | | | | | | | | | | |
|---------------------|-----------|--------|---|------|------|---------|---------|----|------|----|
| Reference spectrum: | per plate | v | , | | X: | 10.0 | mm | Y: | 10.0 | mm |
| | - I | Purity | ` | Dist | ance | to peal | center: | | 0.5 | mm |

In this case for each peak a spectrum will be recorded at 3 positions comparing beginning, middle, and end of a peak (peak pure or co-elution of a compound).

By clicking on "continue" in the "Chromatography" tab (to start the scanner step for spectrum scan) the "Spectrum" tab (A) is opended. *visionCATS* will guide you through all required steps (B). First the substance(s) and peaks are selected for spectrum scan (C).

| HPTLC-Steps 2 Chromatograph | hy 3 Data 4 Spectrum 6 SST + | Remarks Report I |
|-----------------------------|--|------------------|
| etrum Steps | Spectrum Definition Table Spectrum Table Spectr | |
| Developed 1b | | |
| 254 | | |
| 🍘 Developed 1a 🌗 | | |
| | Substance Positioning | |
| | All tracks at wavelength: 254 Substances | |
| | | 7 1 |

Then spectra will be recorded (Execution) and the data window opens (Spectrum data, (A)). At the rights side (B) the results (correlation) for either overlay of spectra obtained at the same R_F on different tracks or overlay within one zone (start – middle – end) for purity testing are shown. Maxima can be displayed as well.



14 Working with Comparison files

Comparison files allow comparing sample tracks and/or spectra of substance peaks from the same and/or from different plates. This section uses the data of the "Comparison Viewer" demo file (download zip-folder at: <u>https://www.camag.com/downloads</u>) as an example.

14.1 Image Comparison

For Image Comparison select the tracks of interest from your analysis file (A), (B), (C). The tracks can be exported to an existing or a new Comparison file. By a direct click on (C) all tracks are selected for Comparison.



Clicking on (C) opens a new window, asking for selecting the detection modes and steps for Comparison.



In (A) the steps of the captured images (e.g. before and after derivatization) and the detection modes (UV 254 nm, UV 366 nm, white light) can be selected. In (B) the tracks can be selected.

Then the selected tracks will be exported to an already open, existing Comparison file or the software will ask for the name of a new Comparison file. New tracks are added to the end (right side) of existing Comparison files. If several detection modes have been selected for "Export to Comparison" then (A) allows to switch between them.



Switch within detection modes at (A)

14.2 Profile Comparison

For Profile Comparison tracks of interest are selected from the analysis file in the Data View tab (either Image tab or Profile tab). The tracks can be exported to an existing or a new Comparison file.



By clicking on "Export to Comparison" a new window opens (see next image) asking for selecting the detection modes (A) and steps (B) for Comparison. In (C) the tracks can be selected.



The selected tracks for profile comparison will be exported to the open existing Comparison file or the software will ask for the name of the new Comparison file. New tracks are added to existing Comparison files at the end. There are different display modes: "Overlay" and "Stack" view.



Overlay view; Reference tracks can be moved up at (A) for Comparison.

| Explorer III Test Steroides29_02 | X ne Comparison Viewer* X | • |
|--|---|--|
| Image Profile | Spectrum | Remarks Report Log |
| View Tracks View Wavelengths | Overlay Stack 03 02 03 04 03 03 02 03 | Tools |
| Overview Comparison tracks Comparison tracks Compare Hele at C | 4.00 ···· | S15211 Adebandenon 1.15 m. |
| On Test Steroides_20161129_02 | Samples | References |
| Wavelength/illumination selection | | 1 Statut Samples Sampl |
| Count • Developed 1a 254 nm 5 | | 2 5 1000 Texamo (4 any 10 ar) 515001 between (4 any 10 ar) Chlorodelhydomethyles. 2 10101 a 10101 a Chaves (4 any 10 ar) 10100 Chlorodelhydomethyles. 2 10101 a Chaves (4 any 10 ar) 3 1 |
| | 6.482 5.680 6.462 | 3 817370 Clostebol 1.15 mg/10_ |
| | 8.00 | 4 S15206 Methylhestosteron 1.3 |
| | | |
| | | + 100% · |

Stack view

14.3 Spectrum Comparison

To compare spectra obtained from different substance zones click on "Export to Comparison" (A) at the "Spectrum data" in the "Spectrum" tab of your analysis file.



A new window will open asking for selecting the substances (recorded spectra of substance zones) for Comparison.



15 Other features

15.1 Quick scan / Partial scan

visionCATS allows a manual control of the TLC Scanner 3 and 4. Prior to the scanning densitometric measurement of all sample tracks, a quick scan is performed to adjust the photomultiplier (adjust offset of the detector). In certain cases, the sample matrix (especially in fluorescence detection mode) can have a larger signal response then the target. To focus on the target zone, the quick scan can be performed at a defined area of interest (on a single track, with a start and end position within the entire R_F range) (A). Furthermore, a partial scan can be performed for scanning densitometric measurement of all sample tracks with defined start and end position within the entire R_F range (B). This will lead to larger scaling of small peaks, needed for trace analysis.



15.2 Dual Wavelength scan and (blank) track subtraction

Background signals from matrices, solvent, etc. can cause problems during quantitative evaluation. In the "Integration" tab of the evaluation within the "Export mode" a "Profile subtraction" is available. Either a blank track (pure solvent applied) can be subtracted or in the case of a multi-wavelength scan two scanned wavelengths can be subtracted (Dual wavelength).



15.3 AMD 2 settings

Chromatographic separation of complex samples is a challenging task for every laboratory, particularly when the components span a wide polarity range. The AMD (Automated Multiple Development) offers a convenient and most efficient solution. It employs stepwise elution over increasing solvent migration distances with a gradient that can be designed according to the requirements of the sample.

The principle

- Multiple development over increasing solvent migration distances
- Each successive run uses a solvent of lower elution strength than the previous
- Between runs the layer is dried under vacuum

The result

- Extremely narrow bands due to gradient elution with simultaneous focusing effect
- Enhanced separation capacity with base line separation of up to 40 components over a separation distance of 80 mm
- Highest resolution that can be attained with a planar chromatography system

To perform a polarity gradient, an AMD step is added to the method template (HPTLC steps).



Then the parameters are entered in the "Chromatography" tab after selecting the instrument icon by a mouse click. In (A) the number of bottles is selected and the solvent are defined. In (B) the gradient is entered. In (C) a graph of the gradient can be seen.



16 Reports

Beginning with *visionCATS* version 2.4, custom report templates can be generated in addition to the provided templates. To change the content and design (global css) basic knowledge on html programming is required. Further information can be found in the *visionCATS* online help at: <u>http://hptlcmethods.cloudapp.net/300/administration/report/styles.html</u>

17 Export of Data

For *visionCATS* the option "Export of Data" can be purchased. This option allows exporting the raw data (all data points obtained by scanning densitometry or from captured images). There are two different ways for "Export of Data" with this option: raw data without filtering (smoothing algorithms) and baseline correction, and export of filtered and baseline corrected data. The data are exported as csv. files allowing an evaluation in Excel or MathLab, etc.

Table 1 Export of Data

| Data export | Exportable data and their format | Scanning mode |
|---------------|---------------------------------------|----------------------------------|
| Scanner | | |
| Raw data (no | AU values of all tracks at SWL or | SWL: all tracks at X nm |
| filter, no | MWL (later for spectra too) from 3D | MWL: all tracks at X1, X2, X2 |
| baseline) * | view profiles | X _n nm |
| Filtered and | AU values of all tracks at SWL or | SWL: all tracks at X nm |
| baseline | MWL (later for spectra too) from 3D | MWL: all tracks at X1, X2, X2 |
| corrected * | view profiles | X _n nm |
| *Metadata | Track position, wavelength(s), | |
| | Position of blank measurement, Vial | |
| | ID & sample name (if peaks are | |
| | assigned), peak detection algorithms | |
| | (just the name of the used algorithm) | |
| Format of | and parameters .csv | |
| exported data | .csv | |
| Data export | Exportable data and their format | Detection mode |
| Visualizer | Exportable data and then format | Detection mode |
| Raw data (no | AU values of all tracks at WRT, 366 | WRT, 366 nm, 254 nm (all tracks) |
| filter, no | nm, 254 nm from 3D view profiles | (|
| baseline) * | | |
| Filtered and | AU values of all tracks at WRT, 366 | WRT, 366 nm, 254 nm (all tracks) |
| baseline | nm, 254 nm from 3D view profiles | |
| corrected * | | |
| *Metadata | Track position, | |
| | Vial ID & sample name (if peaks are | |
| | assigned), images tools (clarify, | |
| | exposure normalization, Spot Amp, | |
| | etc), plate layout, track sequencer, | |
| | peak detection algorithms (just the | |
| | name of the used algorithm) and | |
| | parameters, plate layout, track | |
| | sequencer | |
| Format of | .csv | |
| exported data | | |



Example of a graph re-drawn in Excel

To get unmodified raw data, the csv. file is exported from the "Data" tab (Profiles).



To get filtered/baseline corrected data (use of peak detection algorithms, smoothing algorithms, etc.), the csv. file is exported from the "Evaluation" tab (Integration).



18 21 CFR Part 11

For *visionCATS* the option "21 CFR Part 11" can be purchased. This option includes: System logger, E-Signature, options related to deletion, motivated change management. For further information, see online help at:

http://hptlcmethods.cloudapp.net/300/administration/21CFR_Part11.html

19 Example Files

Several Example Analysis files can be downloaded at: <u>https://www.camag.com/downloads</u>

After importing the downloaded files to your own *visionCATS* database, making a copy is recommended. Imported files are in "read only" mode. No changes can be saved. Copied files can be used for training purposes.

20 Online help

The current version of the *visionCATS* online help can be accessed at: <u>http://hptlcmethods.cloudapp.net/300/index.html</u>

21 Case studies with application tutorial videos

21.1 HPTLC-Fingerprint of *Ginkgo biloba* flavonoids (qualitative example) <u>https://www.camag.com/article/hptlc-fingerprint-ginkgo-biloba-flavonoids</u>

21.2 Identification of fixed oils by HPTLC (qualitative example, including method transfer validation)

https://www.camag.com/article/identification-fixed-oils-hptlc

21.3 In-process control during chemical synthesis (qualitative example, including HPTLC-MS)

https://www.camag.com/article/process-control-during-chemical-synthesis-ergolinepsychedelics-hptlc

21.4 Quantitative determination of steviol glycosides (quantitative example)

https://www.camag.com/article/quantitative-determination-steviol-glycosides