



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

Explorer		visionCATS by CAM.	AG [®] − Licensed to CAMAG with SN 10000			- 5
Quick access and search	• • •	• 🛅 Demo Project • 🛅 Example Analysis			Sort: Name	
In current folder Example Analysis:	NII	Comparison Viewer Home/Demo Project/Example Analysis/	Created: 30-Jan-2017 16:47:36 by: visionCATSuser	Changed: 21-Aug-2019 07:05:32 by: visionCATSuser		
Search P	× AIII	Example Analysis 1 Home/Dem Priset/Example Analysis/	Created: 16-Dec-2013 15:17:47 by: CAMAG	Changed: 08-Jan-2018 08:27:25 by: visionCATSuser	Notes: Plate HX004754	TLC
<u>۳</u>	AIII	Example Analysis 2 Nome/Demo Project/Example Analysis/	Created: 04-Apr-2012 18:35:21 by: CAMAG	Changed: 20-Nov-2015 11:30:35 by: Melanie Broszat		
	· · · · · · · · · · · ·	Example Analysis 3 quant absorption	Created: 06-Jan-2015 10:51:11 by: CAMAG	Changed: 06-Feb-2015 12:29:48 by: Melanie Broszat		TLC
	AIII	Example Analysis 4 quant fluorescence Home/Perro Project/Example Analysis/	Created: 05-Jan-2015 14:17:29 by: CAMAG	Changed: 29-Aug-2019 13:42:19 by: visionCATSuser		
Advanced search	All All	Example Analysis 5 MWL sulfonamides Home/Demo Project/Example Analysis/	Created: 07-Jan-2015 11:33:51 by: CAMAG	Changed: 12-Jul-2019 14:34:44 by: visionCATSuser		
	AIII	Example Analysis 6 spectrum scan Home/Demo Project/Example Analysis/	Created: 14-Sep-2015 09:23:00 by: Eliezer Ceniviva	Changed: 28-Feb-2018 13:10:19 by: visionCATSuser		ALC: TLC
	×≞ _A⊞	Example Analysis 8 related substances (volume) Home/Demo Project/Example Analysis/	Created: 01-Feb-2017 08:39:04 by: Eliezer Ceniviva	Changed: 07-Feb-2017 08:28:24 by: visionCATSuser		
	[™] → [™]	Example Analysis 9 related substances (dilution) Home/Demo Project/Example Analysis/	Created: 01-Sep-2016 13:26:30 by: Eliezer Ceniviva	Changed: 20-Oct-2016 11:44:52 by: Ellezer Ceniviva		
	AIII	Example Analysis SWL MWL Spectrum Sulfonamides	Created: 29-Sep-2015 08:06:21 by: Melanie Broszat	Changed: 29-Sep-2015 08:12:13 by: Melanie Broszat		•
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Tr. Vial ID Description Vol. 1 \$10795		ŶÊØŴ C			Notes E-S	ignature
2 R3518_1 sinensetin standard 1.0 3 \$10795 Orthosiphon \$.0	Images Sca	ns Spectra SST Evaluation				
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6 R3518_1 sinensetin standard 4.0 7 R3518_1 Sinensetin control 5.0		ter .	Zhelkein	len.		
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Elements of the Main window:

A Main Toolbar

B Explorer and search window shows available projects / files (and their status)

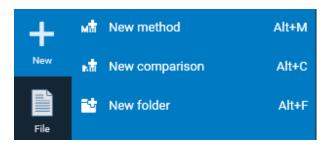
C Preview window (shows preview of a selected file)

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.

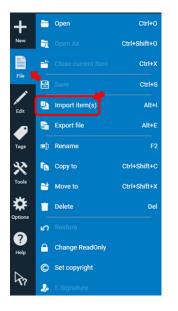


Then in the open folder a new method/comparison can be created (selection at the "New menu"). For creating own methods go to chapter 6.

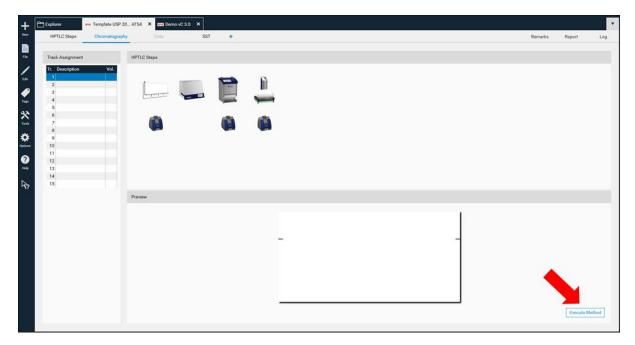
3 Working with method templates

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. Go to <u>https://www.camag.com/downloads</u> and download the ZIP-folder "Method Templates".

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



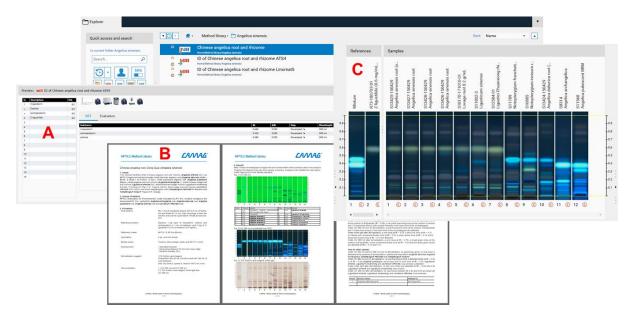
The templates include HPTLC standard parameters for all steps of qualitative analyses (no scanning densitometry). To create a method for an individual project based on the template, right click the template and select "copy to" or press Ctrl+Shift+C and then save the method with the name of the selected 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.



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.

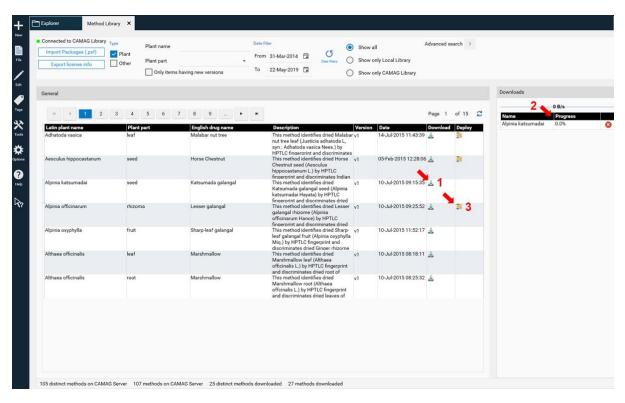
+	Project Explorer	F9
New	 Instruments 	F10
	System Log	F11
File	Edit Substances / Vials	F12
Edit	😥 Edit global lists	F8
	i) Full System Log	
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Tools	▶	
Options		
?		
Help		
A ?		

The overview page of the Method Library will open.

	Plant name Plant] Other Plant part	* wing new versions	Date filter From 31-Mar-2014 Cour filters To 22-May-2019	O si	how all how only Local Library how only CAMAG Library	Advanced s	search >			
General								Downloads		
H 4 1 2	3 4 5 6 7	8 9 •	н			Page 1	of 15 😴	Name	0 B/s Progress	
Latin plant name Adhatoda vasica	Plant part leaf	English drug name Malabar nut tree	Description This method identifies dried Mi nut tree leaf (Justicia adhatoda syn.: Adhatoda vasica Nees.) b HPTLC fingerprint and discrimi	labar v1 L, /	raion Date 14-Jul-2015 11:43:39	Download	Deploy	Alpinia katsumadai	Completed	
Aesculus hippocastanum	seed	Horse Chestnut	This method identifies dried Ho Chestnut seed (Aesculus hippocastanum L) by HPTLC fingerorint and discriminates In	rse v1	05-Feb-2015 12:28:06	¥	3			
Alpinia katsumadai	seed	Katsumada galangal	This method identifies dried Katsumada galangal seed (Alp katsumadai Hayata) by HPTLC fingerorint and discriminates di	v1 nia	10-Jul-2015 09:15:35	*	31			
Alpinia officinarum	rhizoma	Lesser galangal	This method identifies dried Le galangal rhizome (Alpinia officinarum Hance) by HPTLC fingerorint and discriminates di	sser v1	10-Jul-2015 09:25:52	¥	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 rhizi	arp- v1 hylla	10-Jul-2015 11:52:17	Ŧ				
Althaea officinalis	leaf	Marshmallow	This method identifies dried Marshmallow leaf (Althaea officinalis L.) by HPTLC fingerp and discriminates dried root of	٧1	10-Jul-2015 08:18:11	¥				
Althaea officinalis	root	Marshmallow	This method identifies dried Marshmallow root (Althaea offcinalis L.) by HPTLC fingerpr and discriminates dried leaves		10-Jul-2015 08:25:32	*				

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).



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 Metho	d Library X					•
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e e <u>e</u> <u>e</u> <u>e</u> <u>Esting plant danam</u> Adhabada seaara Algonia katsumada Algonia officinarum Algonia officinarum Algonia officinarum Algonia officinarum	3 4 5 6 Plant part had seed seed seed future fruit had	7 8 8 Popular Coglina de Malabar en Katsureadu Lasseer gala Sharp-kat r Marshynali		numents: ad Ceptor D D D D D D D D D D D D D	O Biv <u>Hone</u> <u>Program</u> Ajoria kataurudu Completed	
100 dataset methods on CA	MAG Server 107 methods on CA	MAG Server 27 Bastroo		ancel		

Note: For a direct access to the Method Library an internet connection is needed. The companies' firewall needs to allow access to http://hptlcmethods.cloudapp.net. 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

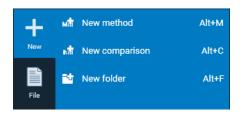
Method transfer to your lab is very simple: transfer validation stipulates that the SST (System Suitability Test) must pass! After downloading and importing a method from the Method Library, you can access the imported files from the Explorer window. Each method includes a method document that describes 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 $\Delta 0.05$.

Explorer Method Libra	×			
Quick access and search	🔹 🕑 \varkappa 📫 🔸 Method library 🖢 New Folder		Sort: Name 💌	
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Search P	ID of Katsumada galangal seed Linomat5	Created: 09-Sep-2011 14:06:41 Changed: 29-Aug-2019 15:04:55 by: admin by: visionCATSuser		w
۵۰ 💄 🕤	Katsumada galangal seed	Created: 15-Jun-2015 16:52-04 Changed: 29-Aug-2019 15:05:01 by: Anita Anital by: visionCATSuser		
	1			
Advanced search				

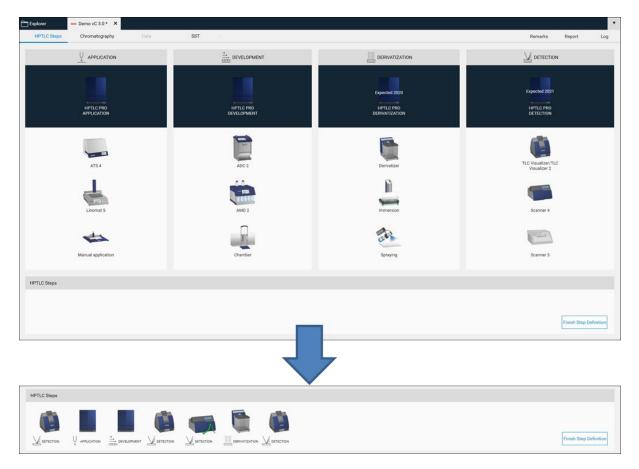
Check out our case study *Identification of fixed oils by HPTLC* to see how method transfer is done: <u>https://www.camag.com/article/identification-fixed-oils-hptlc</u>

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

Explorer	Mem Demo vC 3.0 *	×								٠
HPTLC Steps	Chromatograph	y Data	SST	+			Rei	marks	Report	Log
Track Assignmen	ıt	HPTLC Steps								
Tr. Description 2 3 3 4 5 6 7 8 9 10 11 12 13 14	Val		<mark>.</mark>							
15		Preview								
					-	-				
									Execute Me	thod

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 <203> / 2.8.25.

HPTLC Steps	HPTLC PRO DEVELOPM	1ENT s	ttings		← Develope	d1 🔿
	Plate preparation	<	Pre-drying Drying time Activation > Pre-conditioning >	ne [min] 1 🛟		1
-	Development	<	Solvent 1-Butanol, acetic acid, water (66:17:17 Conditioning Drying Drying time			
	Notes :				ОКС	ancel

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

For each analysis, the Track Assignment table is opened first by clicking 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.

Explorer Au Demo vC 3.0_20	0203_01 × MIII Demo vC 3.0	x
1 HPTLC Steps 2 Chromatograph	hy 3 Data 4	Track Assignment
Track Assignment Tr. Vial ID Description Vol.	HPTLC Steps	Ad Add Deise Renove Inset V Move op Move dowr V Cot Copy Parks Legend V Center V
		Tr. Vial ID Description Vol. (µ) Position Type SST 1
	Instructions	Track Assignment notes: OK Cancel

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 with different 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.

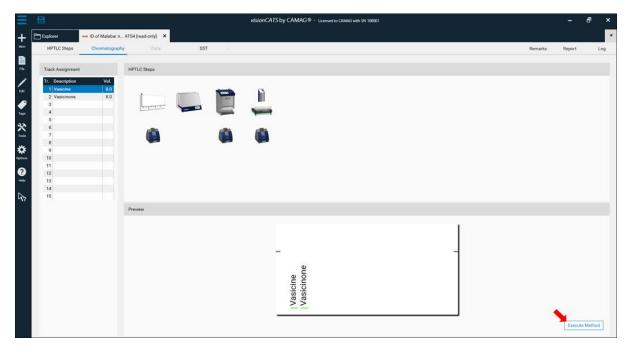


The sequence and number of process steps can vary depending on the method. Each step can be performed multiple times (*e.g.* a scanner step prior to and after derivatization).

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 clicking "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.



After confirming the name by clicking "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 Track Assignment table (A) must be entered after clicking it. More information on the Track Assignment table 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.

Explorer Am ID of Malabar n 1 HPTLC Steps 2 Chromatograp	L95246 X IIID of Malabar n ATS4 (read-only) X yy 3 Data 4 Spectrum 5 SST +	₹ Remarks Report Log
Track Assignment	HPTLC Steps	Plate Progress
Tc. Val. ID Description Vol. 1 R123 Vasicinee 8.0 2 R123 Vasicinee 8.0 3 S123 Sample unkn 8.0 4 A A A 5 A A B 7 B B B 9 10 11 12 13 13 14 15		D
		•
	Instructions	
	E	TLC Visualizer/TLC Visualizer 2 Setup Preparation of TLC Visualizer 7 Includes: Open front door Set L-stop according to plate size Inser tplate from first Close front door Selected diminantions: RT White R 254
		No instruments currently available! Please check connections, instrument power and functions.

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 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#lbldataviewtoolbar

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-reports-config

By clicking "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:

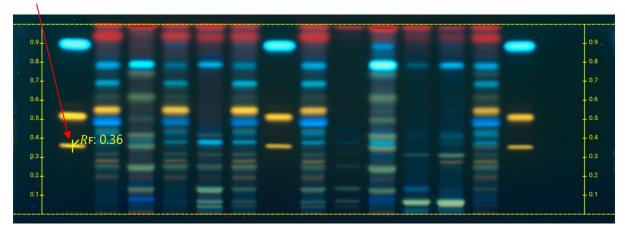
http://hptlcmethods.cloudapp.net/300/method_analysis_file/dataview/data_view_toolbar.html#lbldataviewtoolbar

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

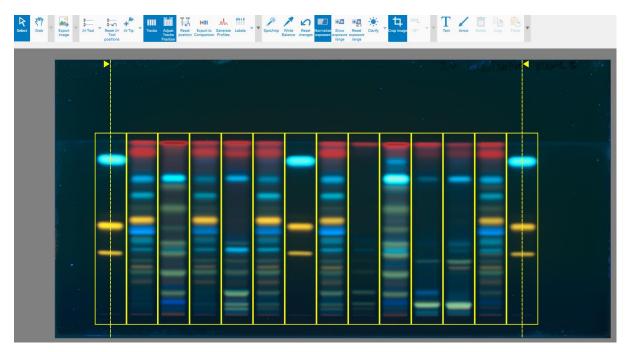
(B): " R_F Tool" displays lines at R_F 0 and R_F 1 (area of interest)



"RF" Tip adds an RF value to a zone of interest



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



By clicking "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.

Add tracks and	steps to a comparison	and a second
•	Steps to export Step Images Profiles Developed 1 Illuminations Wavelengths	Create a new comparison Open an existing comparison Enter the name of the new comparison file: I To add in the following folder:
	Developed 1b 350 nm Illuminations Wavelengths Wavelengths X 366 RT White	# > Demo Project > New Demo Files *** Bupleurum 2 *** Bupleurum comparison file
	All tracks selected to export Link Image and profile comparison tracks	← Common horsetail herb Æquisetum Æquisetum Æquisetum 3

By clicking "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.

	xplorer	AIII	Equisetum 3 🗙												
1	HPTLC-Steps	2	Chromatography	3	3	Da	ata	4	Spect	rum	5	SST	6.1 Eva	aluation 🗎	+
D	ata Type				b	*	Q	*	mm R _F			[=]]] (⊲/⊵	▲ 23 _↓	
	uuu				View		Zoom	Line	Unit	Collapse	Export	Export to Normal Comparison		Reset normalize range	
		Tracks	Profiles		А	AU.									

(C): Image Enhancement tools



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

White Balance allows to re-define "what is white" by clicking 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). By clicking "Show exposure range" the selected track for normalization and allows

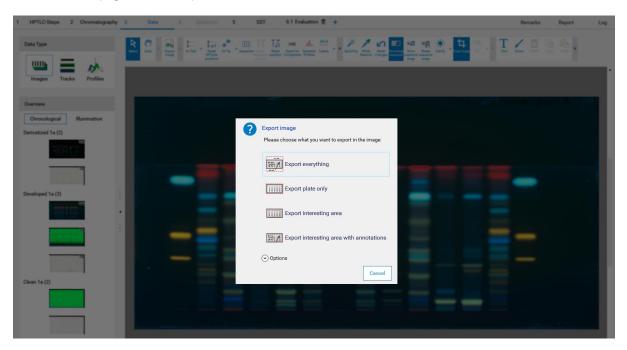
a manual change are displayed. This feature was implemented for comparing samples originating from different plates in fluorescence mode.

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 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).



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 Track Assignment table (Chromatography tab) is required.

Add	Add Delete	Remove	insert	▼ Move up	Move down	Cut	Сору	Paste	Insert copied		nter 🔻		
Tr.	Vial ID		Descri	iption						Vol. (µl)	Position	Туре	SST
1	R12345		Vasici	ne						8.0	A1	Reference	<
2	R12346		Vasici	none						8.0	A2	Reference	
3	S12345		Samp	le unknow	/n					8.0	A3	Sample	

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

SS	ST Tak	ble												
-		L G	enerate Profiles	Check	₹									
	Subs	stance				RF		Δ <i>R</i> f	Step		λ		Min. height	Status
	Vasio	cine					0.370	0.050	🐞 Developed 1a	•	R 254	•	0.100	🏠 Not computed

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 "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.

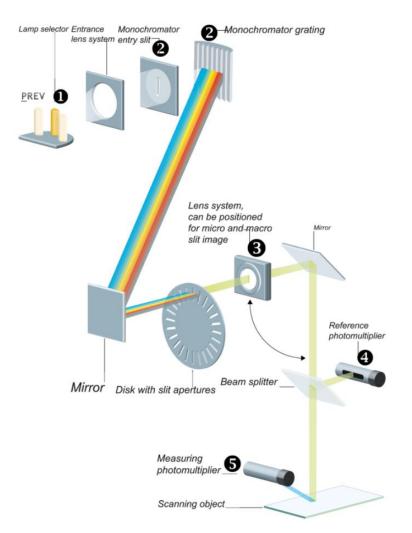
Note: It is possible to mark an SST definition as passed manually via the check box on the banner.

Substanc	e	eck	RF	ΔRF	Step		λ		Min. height	Status	Descriptio
Curcumin			0.400	0.050		atized 1a		RT White 🔻	0.100	✓ Passed	Descriptio
Curcumin	oid b		0.280	0.050	🍓 Deriv	atized 1a	•	RT White 🔻	0.100	✔ Passed	
									• • • •		
ST View											
		Track	1 at waveleng	gth: RT Whit	е			Sub	stances	i 🖓 🚺 🗰	4
10	0.1	0.2	0.3	0.4	0.5	0.6	5			1 ℝ	
0.0		012	0.5		015			AU.			
, }											
3											
/											
								_			
			>					Curcumin	oid a		
	>							Curcumin	oid b		
2											
I											

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.

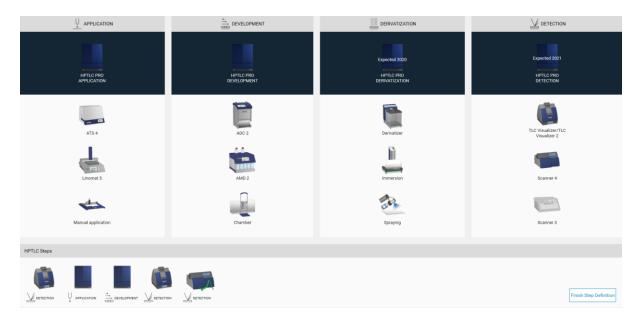
The optical system



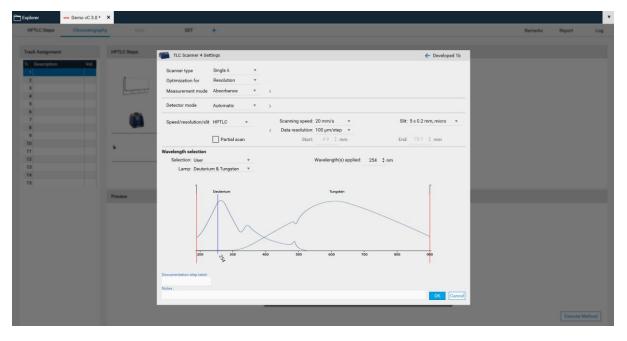
Any of the three light sources, high-pressure mercury lamp, deuterium lamp, or halogen-tungsten lamp can be positioned in the light path by a motor drive. **(1)** (Further information can be found in the TLC Scanner Manual)

10.1 Single-wavelength scan

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



By clicking "Finish Step Definition", you will get to the Chromatography tab. Clicking 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	gs							🗲 Derivatize	d 1b
Scanner type	Single λ	*							
Optimization for	Resolution								
Measurement mode	Fluorescence	*		Filter:		*			
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	Ψ.	0 adjust position Y:	4.9 🍦 mm	
							0 adjust track:	Track 1 🔹	
Speed/resolution/slit	HPTLC	Ŧ		Scanning speed:	20 mm/s	*	Slit:	5 x 0.2 mm, micro	Ŧ
			<	Data resolution:	100 µm/st	•			
	Partial scan	n		Start:	4.9 🌲	mm	End:	73.1 🌲 mm	
Wavelength selection									
Selection: User		Ŧ			Wavelength	n(s) app		254 nm	
Lamp: Mercury		w.					265 nm	280 nm	
							297 nm	302 nm	
							313 nm 405 nm	✓ 366 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.

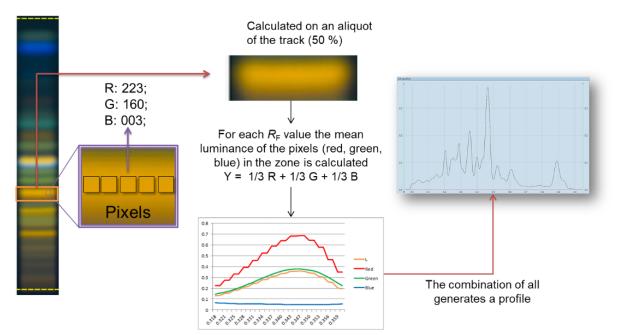
10.2 Multi-wavelength scan

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					🗲 Derivatized 1b
Scanner type Optimization for	Multiple λ ▼ Resolution ▼				
Measurement mode	Advanced 🔻		K400 🔻		
Detector mode	Automatic 🔻	 Quick scan start: Quick scan end: 		Analog offset: Sensitivity:	
		Quick scan track	All tracks 🔻	0 adjust position Y:	
0		Seepping aroud	20 mm/a =	0 adjust track:	
Speed/resolution/slit	HPTLC V	Scanning speed: < Data resolution:	20 mm/s ▼ 100 µm/st ▼	Sit	5 x 0.2 mm, micro 🔹
Wavelength selection	Partial scan	Start:	4.9 🍦 mm	End:	73.1 🌻 mm
Wavelength(s) applied:	205 ‡nm Absorba			▼ Filter: K400 ▼	
Reorder 🕂	366 ▼ nm Fluorescer	nce 🔻 Lamp: Mercu	ıry	▼ Filter: K400 ▼	
	Deuterium		Tungsten	700 800	
200,200	300 300	00 500	600	700 800	900

11 Image profiles

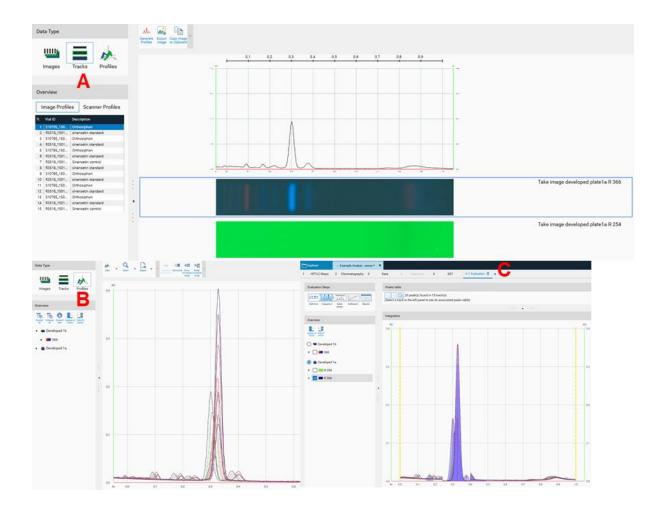
With the Visualizer Ultimate Package Peak Profiles from images (PPI) can be obtained with a mouseclick. The software calculates for each track the resulting luminance (in the middle of the track) and plots it as a function of the R_F value.



To generate PPI, just click 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 "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.

efinition Integration Subst. Ca	albration Results	Reuse substances	+		
efinition Integration Subet. Ca	albration Results				î 🖽
assign.		Name	Sinensetin		Advanced
		RF	0.330		
		ΔRF	0.020		
ack assignment		λ	· ·		
Malup Description 161	2	Calibration type	Area 👻		
	I. Type .0 Sample +	Calibration mode	Linear-2 *		
R3518 sinensetin stan 1.		Range deviation	5.00 %		
	.0 Sample *				
R3518 sinensetin stan 2					
	.0 Sample +				Ψ
R3518 sinensetin stan 4					
R3518 Sinensetin cont 5	.0 Sample +	References Samples			
R3518 sinensetin stan 6	.0 Reference *	References Samples			
S1079 Orthosiphon 5	.0 Sample +	Concentration unit type	:: Mass / volume 🔻		
R3518 sinensetin stan 8	1.0 Reference *		Sinensetin		
S1079 Orthosiphon 5	.0 Sample +	B3518 150106	a 100.000 _ng/ml ▼		
R3518 sinensetin stan 10					
	.0 Sample +				
R3518 sinensetin stan 12					
R3518 Sinensetin cont 5	.0 Sample +				

12.1 Basics of quantitative evaluation in *visionCATS*

visionCATS guides you through the evaluation process:

Evaluatio	n Steps			
			\ <u></u>	
Definition	Integration	Subst. assign.	Calibration	Results

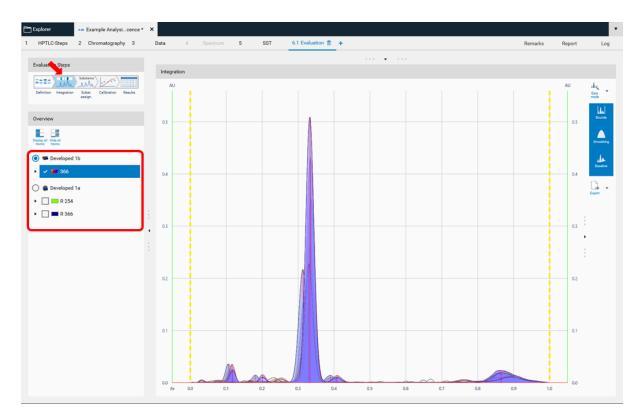
In "Definition" the substances to be analyzed and their concentration in the reference vials are defined. The quantity applied of each reference will be calculated from the application volume and the defined concentration. At (A) (red arrow) substances can be added and named. At (B) the concentration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

aluation Steps		Substance table				
and a company of the particular of the particula		oubstance table				
Substance		Reuse substances	+			î 🖽
efinition Integration Subst. (Calibration Results	Name	Sinensetin			Advanced
assign.		RF	0.330			
			0.020			
ack assignment		x A	*			
Vial ID Description W	ol. Type	Calibration type	Area 🔻			
	5.0 Sample *	Calibration mode	Linear-2 *			
R3518 sinensetin stan		Range deviation	5.00 %			
	5.0 Sample +					
	2.0 Reference *					
S1079 Orthosiphon	5.0 Sample +					Ŧ
R3518 sinensetin stan	4.0 Reference *			•••• •••		
R3518 Sinensetin cont	5.0 Sample +	References Samples				
R3518 sinensetin stan	6.0 Reference *					
	5.0 Sample *	Concentration unit type:	Mass / volume 🔻			
	8.0 Reference *	В	Sinensetin			
	5.0 Sample + :	 R3518_150106 	€ 100.000 ng/ml ▼			
R3518 sinensetin stan 1						
	5.0 Sample +					
R3518 sinensetin stan 1						
R3518 Sinensetin cont	5.0 Sample v					

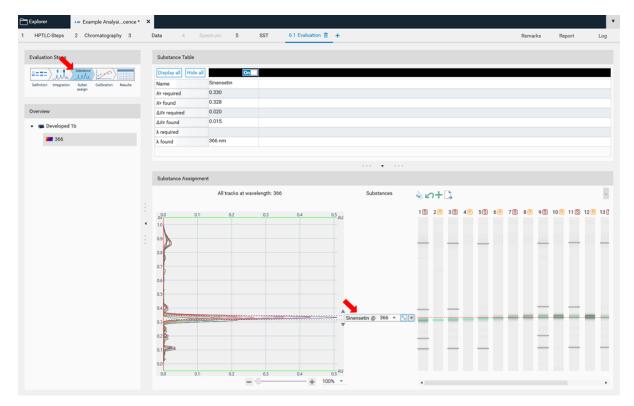
Use the tab "Samples" to define the sample reference amounts.

References Samples	_						
	Amount		Volume solution		Reference an	nount	Related to
S10795_150106_01	500.000 mg	٧	500.00	ml	1.000	g `	
S10795_150106_02	500.000 mg	v	500.00	ml	1.000	g ,	
S10795_150106_03	500.000 mg	٧	500.00	ml	1.000	g .	
R3518_150106_02	500.000 mg	v	500.00	ml	1.000	g ,	

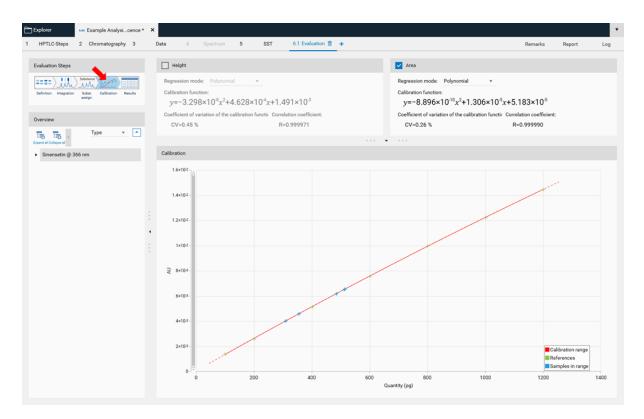
Continue with the next step "Integration". The profiles (PPD or PPI) are displayed here. On the left side, different detection modes can be selected.



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).



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

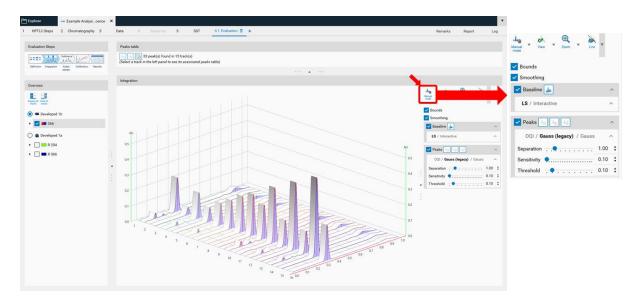


In the final step "Results", a summary of the quantified amounts/concentrations of each substance in the sample(s) is shown.

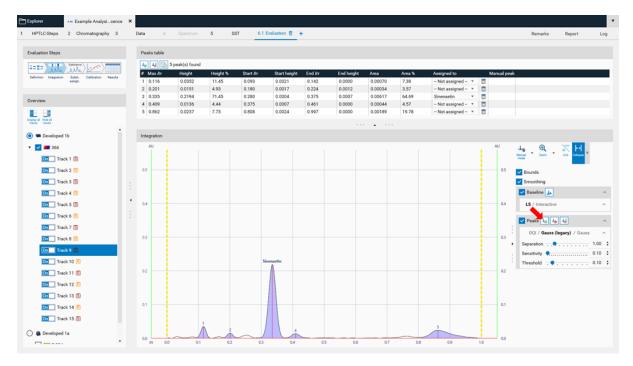
Explorer Amalysicenc	e* ×									
HPTLC-Steps 2 Chromatography 3	D	ata 4 Spe	setrum 5	SST 6.1 Evaluatio	• 📋 +			Remarks	Report	L
Evaluation Steps		Results								
Definition Integration Substance		Export al Collegee all Reset to default view	Export (CSV)					Sort:	Track number	v
		 Sinensetin @ 	366 nm	(8 samples assigned	i)					
Overview		 Sample 'R35 	518_150106_02'	102.5 ng/ml	CV=0.30 %	(2 applications)	102.5 µg in 1.000 g			
Toggle selection for all substances		∧ Volume: 5	5.0 µl	102.5 ng/ml	CV=0.30 %	(2 replicas)				
 Sinensetin @ 366 nm 		Track 7	RF 0.332	102.3 ng/ml	511.7 pg					
		Track 15	R# 0.330	102.8 ng/ml	513.8 pg					
		 Sample 'S10 	0795_150106_01'	96.90 ng/ml	CV=0.13 %	(2 applications)	96.90 µg in 1.000 g			
		∧ Volume: 8	5.0 µl	96.90 ng/ml	CV=0.13 %	(2 replicas)				
		Track 1	RF 0.314	96.99 ng/ml	484.9 pg					
		Track 9	Rr 0.335	96.82 ng/ml	484.1 pg					
	:	 Sample 'S10 	0795_150106_02	70.99 ng/ml	CV=0.19 %	(2 applications)	70.99 µg in 1.000 g			
	`	∧ Volume: 8	5.0 µl	70.99 ng/ml	CV=0.19 %	(2 replicas)				
	1	Track 3	RF 0.317	70.90 ng/ml	354.5 pg					
		Track 11	RF 0.335	71.09 ng/ml	355.5 pg					
		 Sample 'S10 	0795_150106_03'	61.85 ng/ml	CV=0.16 %	(2 applications)	61.85 µg in 1.000 g			
		∧ Volume: 5	5.0 µl	61.85 ng/ml	CV=0.16 %	(2 replicas)				
		Track 5	R# 0.325	61.92 ng/ml	309.6 pg					
		Track 13	RF 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.



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

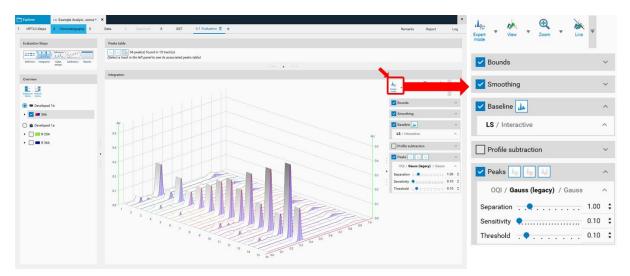


Ctrl + Mouse-scroll allows zooming-in.

IPTLC-Steps 2 Chromatography 3	Data 4	Spectrum	5 S	ST 6.1	Evaluation 📋	+						Remarks	Report	L
aluation Steps	Peaks table													
	443	6 peak(s) found												
	# Max Rr	Height	Height %	Start RF	Start height	End Rr	End height	Area	Area %	Assigned to	Manual p	2eak		
finition Integration Subat. Calibration Results assign.	1 0.116	0.0352	11.13	0.093	0.0021	0.142	0.0000	0.00070	7.18	- Not assigned - *	1			
	2 0.201	0.0151	4.79	0.180	0.0017	0.224	0.0012	0.00034	3.47	- Not assigned - *	İ			
erview	3 0.253	0.0089	2.82	0.226	0.0013	0.278	0.0004	0.00028	2.84	- Not assigned - *				
	4 0.335	0.2194	69.43	0.280	0.0004	0.375	0.0007	0.00617	62.85		T			
8 8	5 0.409	0.0136	4.32	0.375	0.0007	0.461	0.0000	0.00044	4.44	- Not assigned - *				
ny ali Hide ali ka tracka	6 0.862	0.0237	7.51	0.808	0.0024	0.997	0.0000	0.00189	19.22	- Not assigned - *				
Developed 1b														
2 566	Integration													
On Track 1 (S)												Hanual Zoom -	ж н.	
On Track 2 R												Manual Zoom mode	Unit Collapse	
On Track 3 (S)												Bounds		
	1.1											Smoothing		
On Track 4 R														
On Track 5 (S)	· _											🗹 Baseline 🔔		
												LS / Interactive		
On Track 6 R					2									
0n Track 7 S								3				. 🔽 Peaks 🛵 🎝		
On Track 8 R	/													
Track 8 🔣												OQI / Gauss	legacy) / Gauss	1
On Track 9 S				_								Separation		1.0
On Track 10 🔞				~			_					Sensitivity 🔍		0.1
On Track 11 🖏					0.2					0.3		Threshold , •		0,1
On Track 12 関														
On Track 13 🖏										and the second sec		3		
On Track 14 🔞														
On Track 14 R														

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.

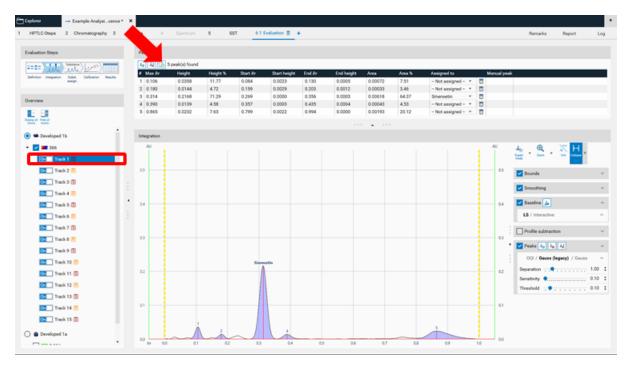
Track: select a base track in the combi box. The PPD of this (blank) track will be subtracted from the PPD of other tracks.

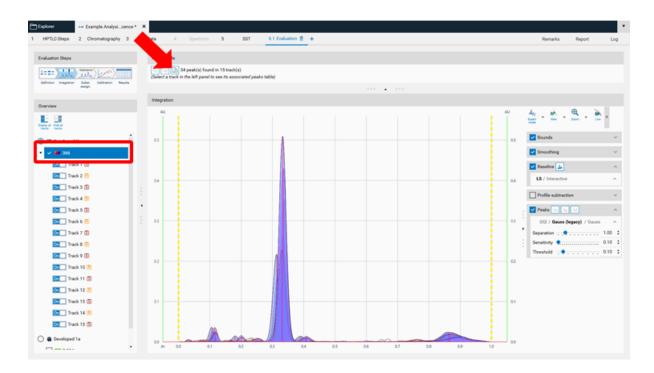
Peaks

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 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) & Example Analysis 9 related substances (dilution) in the provided zip-folder: https://www.camag.com/downloads).

HPTLC	Steps 2 Chr	omatography 3	Data 4 S;	ectrum 5 SS1	6.1 Evaluation	+	Remarks Report Log
Evaluation	Steps		Substance table				
	Mu Substance		Reuse substances			+	^ III
Definition		Calibration Results	Name	Salicylic acid	Acetylsalicylic acid		Advanced epticies
	assign.		RF	0.000	0.000		
			ΔRF	0.010	0.010		V 🗔 Related substances
Track assi	gnment		λ	v	Ŧ		
ViaLID	Description	Vol. Type	Calibration type		Height v		Internal standard
. viai itz	Description	voi. Type	Calibration mode		Polynomial v		Reproducibility
			Range deviation		0.00%		
			Related substances	Related substance v	Main substance 🔹	IB	Limit test
						-	
R627	Acetylsalicylic a	5.0 Sample *					Y
						•••• • •••	
R627	Acetylsalicylic a	2.0 Reference *	: References Samples				
R627	Acetylsalicylic a	4.0 Reference *					
		6.0 Reference *	Concentration unit type	e: Mass / volume 🔻			
		8.0 Reference *		Salicylic acid	Acetylsalicylic acid		
	Acetylsalicylic a	10.0 Reference *	R627-01	<u>a</u>	@ 62.500 µg/ml ▼		
2							
3							
4							

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

Explorer Am Example Analysition) [read-	only] ×						
1 HPTLC Steps 2 Chromatography 3	Data 4 Spec	ctrum 5 SST 6.1 Eve	luation 1		Remarks	Report	Log
Evaluation Steps	Substance table						
	Reuse substances		+		_	1	
Definition Integration Subst. Calibration Results	Name	Salicylic acid Acetylsalicylic a					franced plane
	Rs ARs	0.000	0.000				ter.
Track assignment	λ	230 nm * 230 nm	*				Δ
Tr. Vial ID Description Vol. Type	Calibration type	Height	*			Ľ	
	Calibration mode Range deviation	Polynomial	*				
2	Related substances	Related substances parameters					
4			By dilution (fixed volume) *				
5 R627 Acetylsalicylic ac 5.0 Sample +		Allowed impurity amount: Sample to use as reference					
6 7 R627 Acetylsalicylic ac 5.0 Reference +	References Samples	Sample to use as relevent.	e concentration. No27	B			
8 R627 Acetylsalicylic ac 5.0 Reference +	_			В			
9 R627-1 Acetylsalicylic ac 5.0 Reference * 10 R627 Acetylsalicylic ac 5.0 Reference *	Concentration unit type:						
11 R627-2 Acetylsalicylic ac. 5.0 Reference *	v R627-25						_
12	 R627-0.5 						
13	 R627-1 			ок			
15	 R627-1.5 	H 1 50000 1					
	v R627-2	E 2.00000 1					
References Sa	mples	~					
References 3a	mpies	С					
							٣
Concentration un	nit type: Mass /	volume 🔻					
		Salicylic acid	Acetylsalicylic acid				
✓ R62725	6	6	0.25000 %				
~ R627-0.5	6	6	0.50000 %				
~ R627-1	<u>.</u>	<u>.</u>	1.00000 %				
✓ R627-1.5	<u></u>	<u>_</u>	1.50000 %				
✓ R627-2		<u>.</u>	2.00000 %				
V 1027 2			2.00000 /0				

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.

1 1	Explorer Am Example Analysilume) [re	rad-only] ×									٠
Image: Service in the service in th	1 HPTLC Steps 2 Chromatography 3	Data 4 Spr	amus 5 SST	6.1 Evaluation				Remarks	Report	Lo	9
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Max DOG DOG Track assignment Note Dorogram May A - Calibration type Hogle Calibration roode Polynomial Readed substances Related substances Matrix Do Beference + Staff Able Related substances Matrix Do Beference + Staff Able Related substances Matrix Do Beference + Staff Able Related substances Matrix Able Related Su				alteylic acid						Advenued .	
Adv 0.010 0.010 A * <											
Vote Overage/or Vote Test Calibration type Hog/k Calibration roads 2 Acceptual cycle S. 00 Sample Related substances Related substances <td< td=""><td></td><td>Δ.Rv</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>		Δ.Rv									
Note: Calibration mode Paymental 0	Track assignment	λ								A	
Caldwardsmore mode Pulyacessi * Caldwardsmore mode Pulyacessi	D. Market Market and Torres	Calibration type	Height							C. A	
Related substances Related s	a. You to testingtion was type	Calibration mode	Polyno	nial +							
6 7 Ref2-: Actifytaligicka: 20 Reference * 8 R62-: Actifytaligicka: 40 Reference * 10 R62-: Actifytaligicka: 40 Reference * 13 R62-: Actifytaligicka: 40 Reference * 14 R62-: Actifytaligicka: 40 Reference * 14 R62-: Actifytaligicka: 40 Reference * 14 R62-: Actifytaligicka: 40 Reference * 15 R62-: Actifytaligicka: 40 Reference * 16 R62-: Actifytaligicka: 40 Reference * 17 R62-: Actifytaligicka: 40 Reference * 18 R62-: Actifytaligicka: 40 Reference * 19 R627-01 10 R627-01 10 R627-01 10 R627-01	2 3 4 5 RAZZ Activitaticals as Samula +		Related substanc		xed concentration) *	в					
8 R827- Activitativities 4.0 Reference * Services Ser	6		Reference applica	tion Track 11, 10.0 µL	applied * corresponds	to 2.00% of impuritie	15.				
10 R027- Actriptuiligite a. 60 Reference * 11 R027- Actriptuiligite a. 100 Reference * 12 P627-01 13 Note: P627-01		References Samples									
11 R627- Antifuligific a. 130 Reference * : - R627-01 13 14 CK	9 R627 Acetylsalicylic a 6.0 Reference +	Concentration unit type:									
12 962701 13 0K											2
		₩ R627-01									
	14						OK.				

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.

Explorer All Exp	xamples Analysndard [r	read-only] ×				
1 HPTLC Steps 2 C	Chromatography 3	Data 4 Spe	ctrum 5 SST	6.1 Evaluation	- +	Remarks Report Log
Evaluation Steps		Substance table Reuse substances			-	·
Definition Integration Subst		Name	Methyltestosterone	Methandienone		Advanced
assign	n.	RF	0.000	0.000		A options
		ΔRF	0.010	0.010		Related substances
Track assignment		λ	*	Ψ		<u> </u>
		Calibration type		Height v		 Internal standard
Tr. Vial ID Description	Vol. Type	Calibration mode		Linear-2 v		Reproducibility
2 S1521 0.01 mg/mL	10.0 Reference *	Range deviation		5.00 %		
+ S1520 0.04 mg/mL	2.0 Reference *	Internal standard				🗆 📰 Limit test
3 S17508 1:25 diluted	2.0 Sample v					
4 S1521 0.01 mg/mL	1.0 Reference *					Ψ
+ \$1520 0.04 mg/mL	2.0 Reference *				····	
5 S1521 0.01 mg/mL	2.0 Reference *	References Samples				
+ \$1520 0.04 mg/mL	2.0 Reference *					
6 \$1521 0.01 mg/mL	4.0 Reference *	Concentration unit type:	Mass / volume 🔻			
+ S1520 0.04 mg/mL	2.0 Reference *		Methyltestosterone	Methandienone		A
7 S17508 1:25 diluted	2.0 Sample *	× \$15211-10	/	10.000 µg/ml v		
8 S1521 0.01 mg/mL	1.0 Reference *		40.000 µg/ml ▼			
+ S1520 0.04 mg/mL	2.0 Reference *	S15206-250	40.000 pg/m	/		
9 S1521 0.01 mg/mL	6.0 Reference *					
+ \$1520 0.04 mg/mL	2.0 Reference *					
10 S1521 0.01 mg/mL	8.0 Reference *					
+ S1520 0.04 mg/mL	2.0 Reference *					
11 S17508 1:25 diluted 12 S1521 0.01 mg/mL	2.0 Sample * 1.0 Reference *					
+ S1520 0.04 mg/mL	2.0 Reference *					
13 \$1521 0.01 mg/mL	2.0 Reference + 10.0 Reference +					
+ S1520 0.04 mg/mL	2.0 Reference *					
14 S17508 1:25 diluted	2.0 Sample *					
15	2.0 Gample V					

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.

	Steps			Substance table								
	integration Sub	t. Calibration Res	lite lite	Reuse substances	Qualification	+						Advanced A
	assi	n.		RF		0.000						
				ΔR_F		0.010					🍃 Related substar	nces
ack assig	gnment			λ		Ŧ						
ViaLID	Description	Vol. Type		Calibration type	Area	*				U 🗄	📙 Internal standar	
	Erste Bahn			Calibration mode						~ [Reproducibility	A
	Erste Bahn	2.0 Sample		Range deviation								
	Erste Bahn	2.0 Sample		Reproducibility		B					🜏 Limit test	
	Erste Bahn	2.0 Sample	-									
1 8	Erste Bahn	2.0 Sample									Ψ	
1 1	Erste Bahn	2.0 Sample						· ···				
1 8	Erste Bahn	2.0 Sample	· .	References Sample								
1 1	Erste Bahn	2.0 Sample	•	References Sample	•							
1 8	Erste Bahn	2.0 Sample	•	Concentration unit typ	e: Mass / volume	Ŧ						
1 1	Erste Bahn	2.0 Sample			Qualifica	ation						
1 1	Erste Bahn	2.0 Sample	• 1	~ 2	a 10.000 ⊨	µg/ml 🔻						
	Erste Bahn	2.0 Sample	•									
1 8	Erste Bahn	2.0 Sample	*									
1 1	Erste Bahn	2.0 Sample	v.									

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

HPTLC-Steps 2 Chromatography 3	Data	4		m 5	SST	6.1 Eva	luation 📋	6.2 Ev	aluation 🏦	+									1	Remarks	Repor	rt L
		-			_					- 1												
Evaluation Steps		Results	Peer	oducibility																		
L'andation oteps		Nesuns	Nepr	Jobolionity	A																	
Definition Integration Subst. Calibration Results																						
	~	Qualific	ation @ (i00 nm	CV=0.5	57 % Aver	age=0.051	l (pea	k area)													
Overview						1.50 %		. (p. c. c														
	Tra	ak.	Re	Deak area	Deviation	1.50 %	ê															
 Toggle selection for all substances 			R≠ 0.406	0.05147	0.74%	-																
Qualification @ 600 nm		Track 2	Ry 0.406	0.05160	0.98 %	1.00 %																
		Track 3	R# 0.407	0.05146	0.71 %	1.00 %			+													
		Track 4	R≠ 0.407	0.05109	0.00 %			1		1												
	(5)	Track 5	R≠ 0.407	0.05088	-0.41 %					- T												
	(5)	Track 6	R≠ 0.407	0.05093	-0.33 %	0.50 %	-									+	+					
	(5)	Track 7	R≠ 0.407	0.05098	-0.23 %																	
		Track 8	R≠ 0.407	0.05117	0.15%										+							
	S	Track 9	R# 0.407	0.05135	0.50 %	0.00 %			_	_	+							+				
	5	Track 10	R# 0.407	0.05133	0.46 %																	
	S	Track 11	R≠ 0.407	0.05108	-0.02 %								+	- T -								
	S	Track 12	R≠ 0.408	0.05076	-0.66 %	-0.50 %						+									+	
	• 5	Track 13	R⊧ 0.408	0.05068	-0.82 %	2.50 %													-			+
	(7)	Track 14	Rr 0.408	0.05087	-0.44 %																	
	0																					

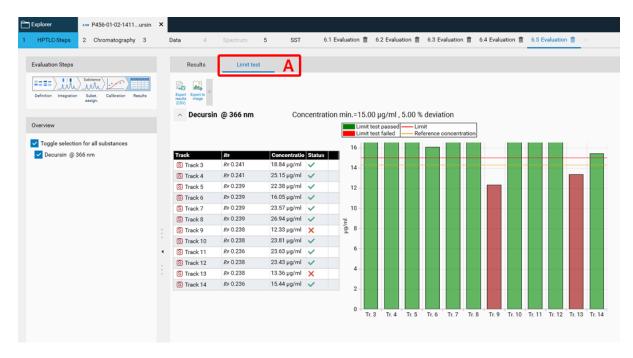
In the Results tab, the "Reproducibility" results are displayed in a separate tab (A).

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.

HPTLC Steps 2 Chromatography 3	Data 4 Sj	bectrum 5 SST	6.1 Evaluation 📋 6	2 Evaluation 📋 6.3 Ev	aluation 📋 6.4 Eva	aluation 🏦	6.5 Evaluation	1 🗄 👘 🛨		Remarks Rep	ort
aluation Steps	Substance table										
	Reuse substances	Ť -	-								^ III
efinition Integration Subst. Calibration Results	Name	Decursin									Advances
assign.	RF	0.270									
	ΔRF	0.010								🗆 🗔 Related	d substances
ack assignment	λ	Ψ								🗆 🧮 Interna	I at a set of a set
Vial ID Description Vol. Type	Calibration type	Height *									i stanoaru
R1327 Decursin 0.02 m 2.0 Reference *	Calibration mode	Linear-1 🔻								Reprod	lucibility
S1249 Angelica gigas B 2.0 Reference *	Range deviation		1						1	_	
S1331 Angelica gigas (2.0 Sample 🔻	Limit test	🖂 🖬 🖌								🗸 🗔 Limit te	est A
S1332 Angelica gigas (2.0 Sample 🔻											
S1332 Angelica gigas (2.0 Sample 🔻			Limit	test parameters	С	1					v
S1332 Angelica gigas (2.0 Sample 🔻					<u> </u>						
S1332 Angelica gigas (2.0 Sample v	References Samples	3		Mode	Concentration			*			
S1332 Angelica gigas (2.0 Sample v											
S1333 Angelica gigas (2.0 Sample v S1332 Angelica gigas (2.0 Sample v	Concentration unit typ			Reference vial:	S12495-14111	1-2		Ψ.	_		
S1332 Angelica gigas (2.0 Sample *		Decursin		imit toot poop if:	O 8-1		h	\sim	_		
S1332 Angelica gigas (2.0 Sample v	R13277-14111	20.000 µg/ml ¥	L	imit test pass if:	 Below 	A	bove () At			
S1332 Angelica gigas (2.0 Sample v	✓ \$12495-141111	I 15.000 μg/ml τ		lowed deviation:	5.00 %						
S1333 Angelica gigas (2.0 Sample 🔻			A	lowed deviation:	5.00 %						
								_			

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



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

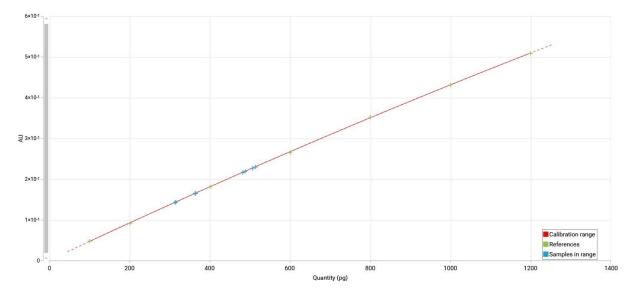
12.9 Export results

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

	×						
HPTLC-Steps 2 Chromatography 3	Dat	a 4 Spe	ectrum 5	SST 6.1 Evaluation	n 📋 🕂		
Evaluation Steps		Results					
Definition Integration Subst. Calibratio Results		and all Collapse all Reset to default vie	Export results (CSV)				
		 Sinensetin @ 	366 nm	(8 samples assigned)		
Overview		 Sample 'R3 	518_150106_02'	102.5 ng/ml	CV=0.30 %	(2 applications)	102.5 µg in 1.000 g
Toggle selection for all substances		∧ Volume:	5.0 µl	102.5 ng/ml	CV=0.30 %	(2 replicas)	
✓ Sinensetin @ 366 nm		Track 7	<i>R</i> = 0.332	102.3 ng/ml	511.7 pg		
		Track 15	<i>R</i> = 0.330	102.8 ng/ml	513.8 pg		
		 Sample 'S10 	0795_150106_01'	96.90 ng/ml	CV=0.13 %	(2 applications)	96.90 µg in 1.000 g
		Volume: 3	5.0 µl	96.90 ng/ml	CV=0.13 %	(2 replicas)	
		Track 1	RF 0.314	96.99 ng/ml	484.9 pg		
		Track 9	RF 0.335	96.82 ng/ml	484.1 pg		
	:	 Sample 'S10 	0795_150106_02'	70.99 ng/ml	CV=0.19 %	(2 applications)	70.99 µg in 1.000 g
	`	Volume:	5.0 µl	70.99 ng/ml	CV=0.19 %	(2 replicas)	
	-	Track 3	<i>R</i> € 0.317	70.90 ng/ml	354.5 pg		
		Track 11	<i>R</i> F 0.335	71.09 ng/ml	355.5 pg		
		 Sample 'S10 	0795_150106_03'	61.85 ng/ml	CV=0.16 %	(2 applications)	61.85 µg in 1.000 g
		^ Volume: 3	5.0 µl	61.85 ng/ml	CV=0.16 %	(2 replicas)	
		Track 5	<i>R</i> ⊧ 0.325	61.92 ng/ml	309.6 pg		
		Track 13	RF 0.333	61.77 ng/ml	308.9 pg		

12.10 Export graphs

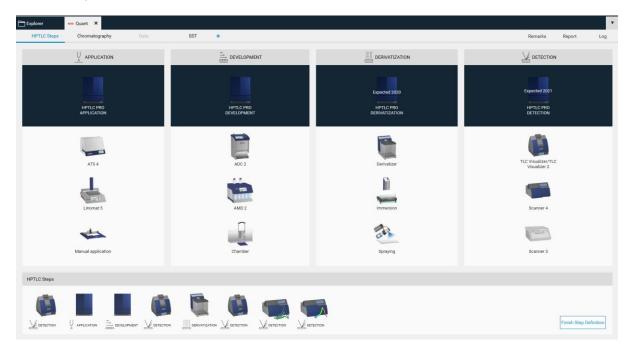
All graphs (calibration curves, spectra, limit test results, reproducibility results) can be exported by a right mouse click. The graphs can be exported with different resolution and saved to a destination folder or to clipboard.



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.

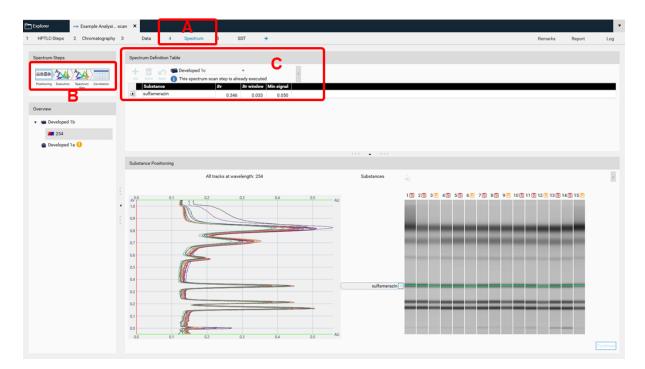
Scanner 4 Settings				🗲 Derivatized 1c
Scanner type Optimization for Measurement mode Detector mode Speed/slit	Spectrum Resolution Absorbance Automatic HPTLC •	Spectrum speed: 20 nm,	/s •	Slit: 5 x 0.2 mm, micro 🔹 🔻
Spectrum parameters Reference spectrum:	per plate	× (X: 10.0 mm Y:	10.0 mm
Lamp: Deuterium & Tur	n ▼ Start λ: 1	Purity D	istance to peak center:	0.5 mm
Deuterium		Tungsten		
	\sim			
200 300	40000	500 600	700 800	000

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.

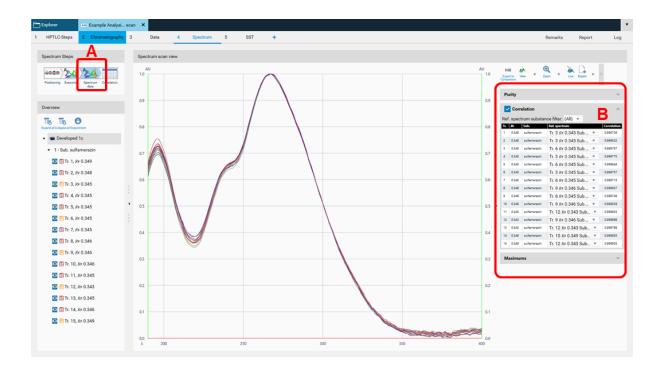
Spectrum parameters										
Reference spectrum:	per plate	Ŧ	,		X:	10.0	mm	Y:	10.0	mm
	~	Purity	`	[Distance	to peal	center:		0.5	mm

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 "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).



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.

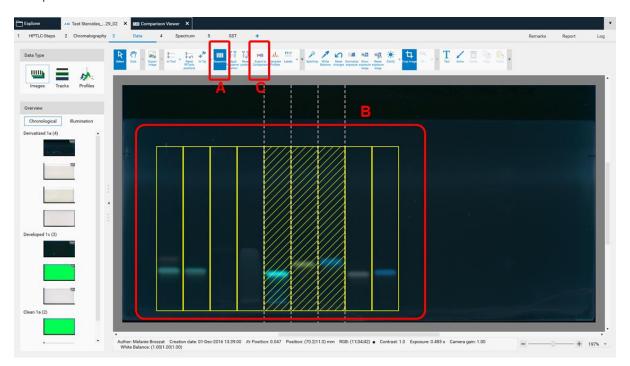


14 Working with Comparison files

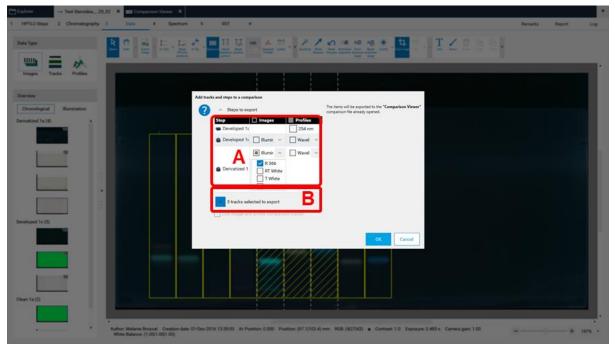
Comparison files allow comparing sample tracks and/or spectra of substance peaks from the same and/or from different plates. This section uses the data of the "Comparison Viewer" demo file (download zip-folder at: <u>https://www.camag.com/downloads</u>) as an example.

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 directly clicking (C) all tracks are selected for Comparison.

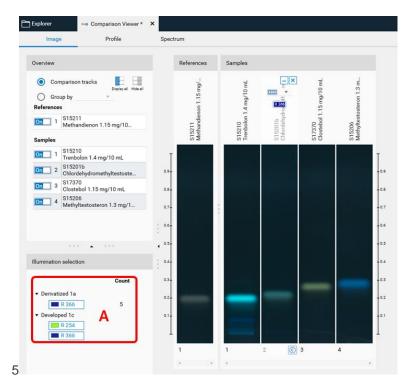


Clicking (C) opens a new window, asking for selecting the detection modes and steps for Comparison.



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.



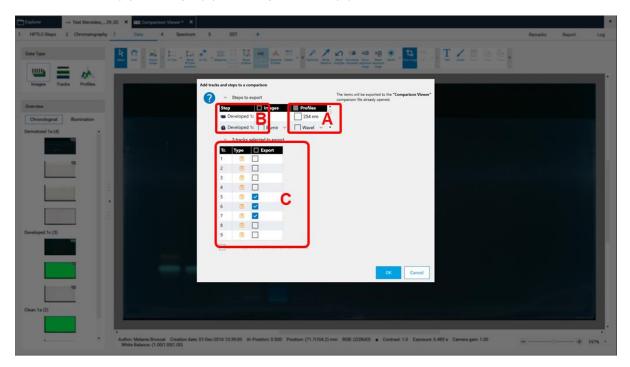
Switch within detection modes at (A)

14.2 Profile Comparison

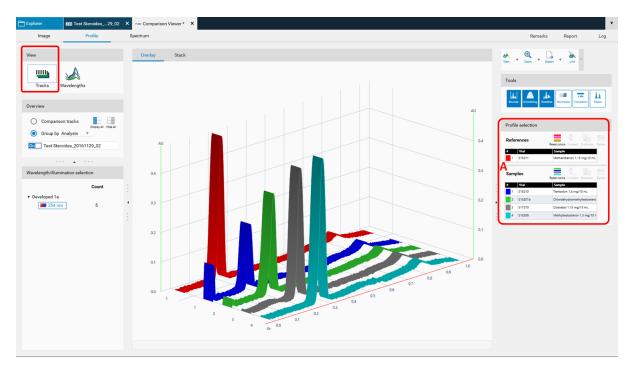
For Profile Comparison tracks of interest are selected from the analysis file in the Data View tab (either Image tab or Profile tab). The tracks can be exported to an existing or a new Comparison file.

mi <mark>102 hard Thermont, 21,07, 31 and Comparison Sovier™ X</mark> LC-Deps 2 Okonstopre/ry 3 Dura 4 Spectrum 5 557 ⊕	• Renaña Report Log
	<u>2</u> ★ <mark>9</mark> 2 · T ∠ 2 2 2 ·
ee motogoal Burnaution	
le le	ophere in Stat Sension, 20,00 X BD Compariso View* X
	HPTLCOxpe 2 Chromotography 3 Club 4 Spectrum 5 557 + Remarks Report
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	Brendered for
	Boulpells (0)
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	Indi 4 10
	The K 6
	1 had 7 = 61

By clicking "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.



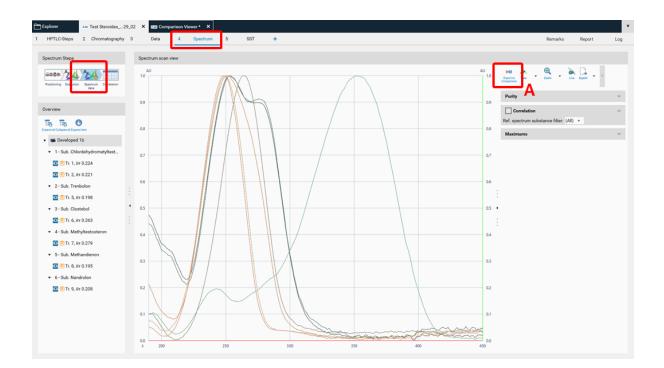
Overlay view; Reference tracks can be moved up at (A) for Comparison.

Explorer III Test Steroides29_02	NIII Comparison Viewer* X	ĺ	•
Image Profile	Spectrum		Remarks Report Log
View	Overlay Stack		Eport Prot
Tracks Wavelengths	References 0.092	1 ^	Tools
Overview		S15211 Methandienon 1.15 m	Bounds Smoothing Baseline Normalize Correction Pasks
Comparison tracks		*	Profile selection
On Test Steroides_20161129_02	Samples	1 ^	References Reset colors Unsate: Dustante Dustante Visit Visit Strapile 1 515211 Methandienon 1.15 mg/10 mL
Wavelength/illumination selection	\vee	\$15210 Trenbolon 1.4 mg/10	Samples
Count • Developed 1a 254 nm 5	9.42 9.42 9.42	2 S15201b Chlordehydromethylte	Vial Sample 1 515210 Treholon 1.4 mg/10 mL 2 515210 Cholodehydionerbythastostero 3 517270 Clostebol 1.5 mg/10 mL 4 515205 Methytestosteron 1.3 mg/10 r
	2,42	⁻ 3 S17370 Clostebol 1.15 mg/10	
	2,42	4 S15206 Methyltestosteron 1.3_	
	0.1 02 03 04 03 06 07 08 09		
	× 0	+ 100% -	

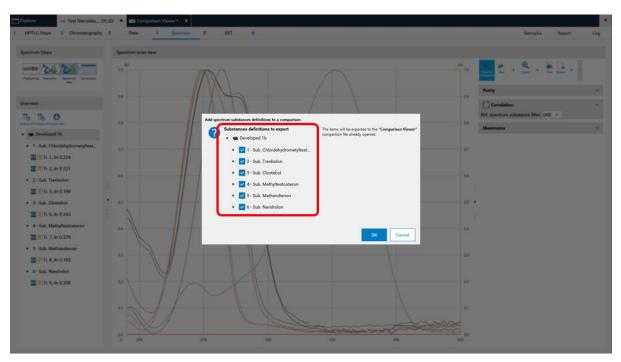
Stack view

14.3 Spectrum Comparison

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



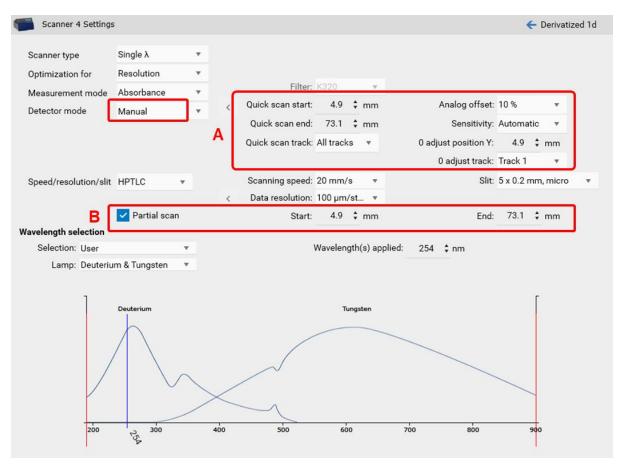
A new window will open asking for selecting the substances (recorded spectra of substance zones) for Comparison.



15 Other features

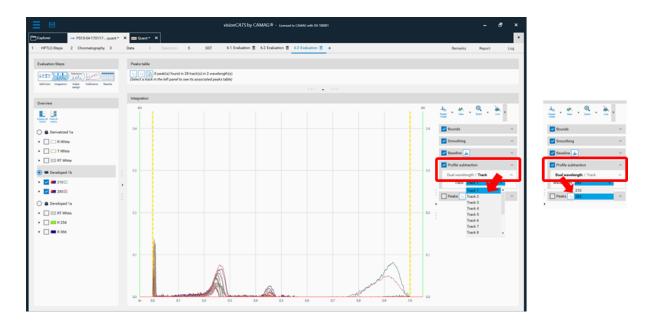
15.1 Quick scan / Partial scan

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.

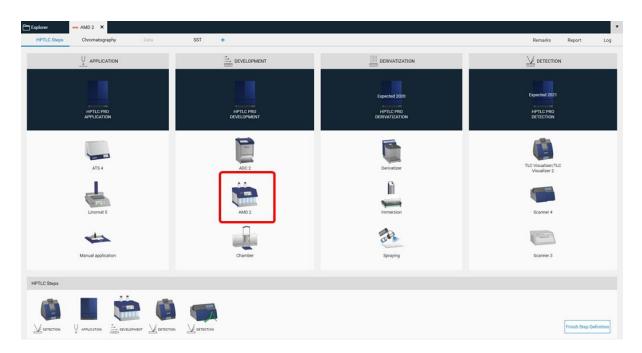
The principle

- 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

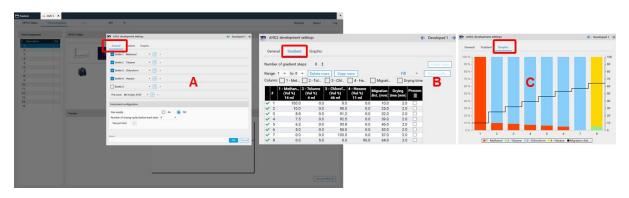
The result

- 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.



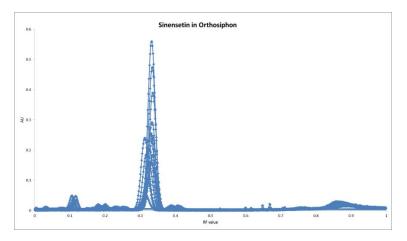
16 Reports

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 http://hptlcmethods.cloudapp.net/300/administration/report/styles.html

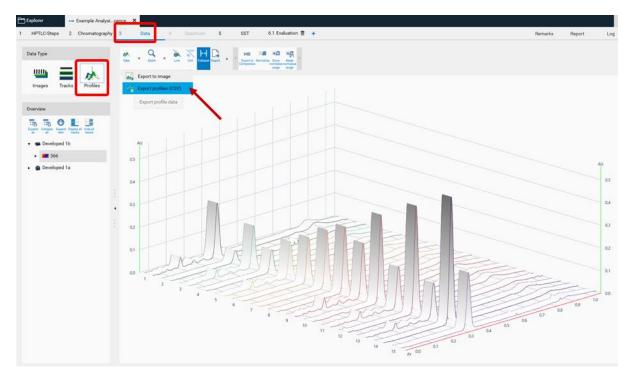
17 Export of Data

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.

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 X ₁ , X ₂ , X ₂ ,
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) and parameters	
Format of	.CSV	
exported data Data export	Exportable data and their format	Detection mode
Visualizer	Exportable data and their format	Detection mode
Raw data (no filter, no baseline) *	AU values of all tracks at WRT, 366 nm, 254 nm from 3D view profiles	WRT, 366 nm, 254 nm (all tracks)
Filtered and baseline corrected *	AU values of all tracks at WRT, 366 nm, 254 nm from 3D view profiles	WRT, 366 nm, 254 nm (all tracks)
*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		

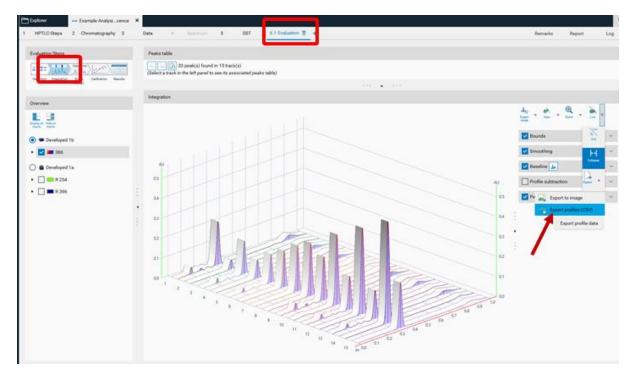


Example of a graph re-drawn in Excel



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 https://www.camag.com/downloads

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 http://hptlcmethods.cloudapp.net/300/index.html

21 Case studies with application tutorial videos

21.1 HPTLC-Fingerprint of *Ginkgo biloba* flavonoids (qualitative example)

https://www.camag.com/article/hptlc-fingerprint-ginkgo-biloba-flavonoids

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-ergoline-psychedelics-hptlc

21.4 Quantitative determination of steviol glycosides (quantitative example)

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