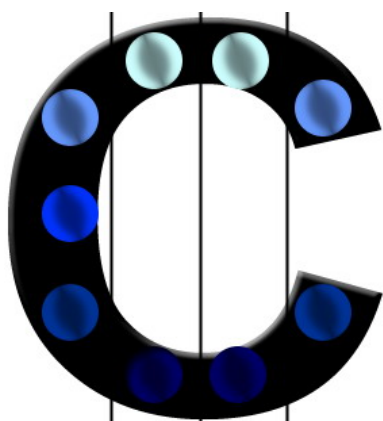


Christhin

Quantitative Analysis of Thin Layer Chromatography
Version 1.00 for Christhin 0.1.36
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Chromatography
Riser Thin

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This is the first edition of the Christhin documentation, and is consistent with version 0.1.36 of Christhin.

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*Dediqué largos años a aprender el
orden y la configuración de las manchas*

El aleph (La escritura de dios)
Jorge Luis Borges

Preface

Christhin (Chromatography Riser Thin) is software developed for the quantitative analysis of data obtained from thin-layer chromatographic techniques (TLC). Once installed on your computer, the program is very easy to use, and provides data quickly and accurately.

This manual describes the program, and reading should be enough to use it properly.

Specifically, the manual contains the following chapters:

Chapter 1 presents an approach to the technique of thin layer chromatography (TLC), explaining what is, their purposes, advantages, and the materials and methods used in classical chromatography, as well as how to analyze quantitatively the results.

Chapter 2 refers to the use of the software, describing in simple steps the methodology to follow for proper operation of it, and through which will yield the desired results.

Chapter 3 specifies the way in which Christhin software and its various components perform the tasks necessary to accomplish its purpose, describing them in great detail.

Chapter 4 contains details of Octave, explaining what is, the way in which it is running and operating systems within which it can work properly. This software is essential to the functioning of Christhin.

If user's intent is to use the software without knowing in detail its operation, the *quick guide* will be very useful, and also presents descriptive images easy tracking. You may have some questions that have been asked (and answered) a few times before in the *FAQ* document.

The software can be downloaded from its website, and is distributed under the terms of the GNU General Public License. Before any installation problems or questions regarding the operation, feel free to contact for help. Also we will find very useful tips to add, remove or edit contents of this manual, as well as software.

If you find Christhin useful let us know, we are always interested in knowing which way the software is used.

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Chapter 1

A brief introduction about Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a chromatographic method on the plane. It employs a flat, thin layer, at the same time the support, and covers a surface (glass, plastic). Mobile phase or eluent moves through the support or stationary phase by capillary action, allowing components of a mixture be separated by a difference in polarity.

Is a fast, good resolution technique and with greater sensitivity than paper chromatography (allows detection of smaller amounts of analyte). This technique is widely used in industrial laboratories, and as a result of the many different application areas, it is estimated that as many TLC analysis are performed as HPLC analysis.

The advantages of following this procedure are speed and low cost of thin-layer experimental trials. The TLC is a useful tool for quality control and purity of products.

With respect to the final result of applying the TLC technique, comparing the relative intensities of the different spots presents, belonging each to a different compound, a quantitative estimate of the composition of the sample can be made. [4]

1.1 Materials and methods

Characteristics thin layer separations are performed on glass or plastic plates which are covered with a thin, adherent layer of finely divided particles; this layer forms the stationary phase. [4, 5]

1.2 Plate Preparation

Is generally used as stationary phase powdered silica gel, which is mixed with water in suitable proportions in order to prepare a homogeneous paste.

It is distributed evenly over a glass plate and subjected to a drying oven for the time necessary, to remove humidity. The square glass plates to be used have a size of between 16 to 20 *cm.* sideways and seek a suitable stationary layer thickness, from 0.25 to 0.75 *mm.* approximately. [1]

1.3 Preparation of the tray

The tray must have appropriate measures in order to introduce inside it the plate where the run will be done; it is placed also inside it the solvent mixture serving as mobile phase. [3]

As to the solvents used, alternatives may be varied, but generally are mixtures of hexane, di-ethyl-ether and acetic acid.

1.4 Development

The sample application is the most critical aspect of thin-layer chromatography, especially when dealing with quantitative measures. In general, the sample should be diluted to achieve a good advance and must be applied at 1 or 2 *cm.* from the lower end of the plate, thus avoiding to take direct contact with the solvent. For efficient separation, the patch must have a minimum diameter (0.5 *mm* or less), so that the application is conducted through a capillary, or a specific chromatographic syringe.

During the test, the sample is transported by the mobile phase through the stationary phase by diffusion. For the assay, is marked with a pencil a line of 1 or 2 *cm.* from one end, and there is deposited the drop of the sample. Once the solvent to evaporate from the sample, we proceed to placing the plate in a closed vessel saturated with solvent vapors, which makes the assay (tray).

The end where the drop was deposited is introduced into the solvent avoiding direct contact; the mobile phase ascends the plate by capillary effect exerted between the fine particles of silica gel. When scrolling, the solvent passes through the point of application of sample, dissolves and drags it through plate, distributing the sample among the mobile phase that is displaced and stationary phase according to the degree of polarity of the components. When the solvent reaches two-thirds of the length of the plate, this is removed from the tray and dried.

1.5 Location of compounds of interest

To locate the components on the board is sprayed over it a solution of iodine as it reacts with organic compounds to give dark products, or simply the board is placed in a tray similar to that containing the mixture of solvents, but containing instead solid iodine.

1.6 Quantification

For quantitative analysis of TLC chromatography, plate must be digitally processed by a digital-optical media, such as a scanner, and then process the information with an appropriate software. The software **Christin** allows processing of images obtained by TLC, collecting information on the color intensity of the image, and relating this to the concentration of the compounds present in the sample analyzed.

Chapter 2

How to use Christhin

To use the software Christhin, we recommend following the steps listed below:

1. Install the program. This can be done in two different ways:
 - After installing the software Octave, from the main window of it run the command *pkg install program name*, where instead of program name places the file name containing the Christhin's software, which is by default (the file downloaded from internet) *christhin-0.1.36.tar.gz*.
 - After installing the software Octave, run the *setup.exe* file that contains the material downloaded from internet, then follow the steps in the installation wizard, taking into account the detail of first specify the folder where you want to install the software, and then specify the folder where the software Octave is installed. Thus, the execution of the program allows immediate use of the software.
2. It is essential for the proper functioning of software, that the plate where chromatography was performed (once already revealed), is scanned and taken to image format type. This must be done prior to program execution.
3. Run the software, which immediately asks you to select the image to be processed. After selecting the image, click *Ok* to continue.
4. If the scan of the plate was defective, the program allows rotating the image in order to make corrections; instead, not being necessary this correction is placed a rotation angle of zero degrees. The rotation must always be done by ensuring that the seed points of the sample remaining in the bottom of the image, position essential for the proper functioning of the software in the following steps.

5. Select the rectangular area where the desired run is placed. This is done through a single click in the top left, and another on the opposite side, being formed an *imaginary* rectangle. Remember to include in it both the solvent front as the point of sowing.
6. After selecting the appropriate area, you press *enter* to confirm. On screen will then appear a new image containing only the selected runing inside the *imaginary* rectangle. On this image, select the seed point and the solvent front with a single click in each case. This step is intended to collect information to calculate the value *Rf* (Ratio of Front). After selecting both two points (which can be selected interchangeably), press *enter* to confirm.
7. Screen now shows a chromatogram which contains the information of the run, being the peak area proportional to the intensity of the spots on the run, and therefore proportional to the amount of each component in the sample chromatographed. In it, select by clicking the start of each *peak* and the end with another click, depending on how many of them possessing the image being analyzed. Make the selection without considering the height of the respective click (not give importance to the selected value on the y-axis, but the set value on the x-axis), as the program locates the click automatically over the curve.
8. After selecting all the peaks, press *enter*, and this completes the analysis of the run; the program lets you enter comments if necessary (if not carrying comments, press *Ok* only).
9. The program offers the possibility to analyze another run, because in general this kind of chromatography are performed several runs simultaneously on the same plate. If it is necessary to consider another run, select *Yes*, and repeat steps 3 to 6 inclusive; not being necessary to consider another run, select *No*, which ends the program execution.
10. As a result, in the same location of the processed image, the program creates a folder with the same name of the image, in which is saved the image in grayscale, the chromatogram which allowed the analysis, and a text file containing the comments entered, the percentage of area that represents each peak selected and the *Rf* value for each compound. Each peak in the chromatogram has an assigned number, which corresponds to the number that contains the percentage of area in the text file.

Chapter 3

Operation of the Software Christhin

The software operation is carried out by a basic function, called **christhin** in collaboration with two other functions that have specific tasks, called **imgchop** and **pickdef**.

3.1 Christhin

This function is the main structure of the software, and the same is executed immediately upon being opened the program. The software asks the user to select the image to be processed, an image that contains the interest chromatography. The same is converted of a scale RGB, the color scale, to a grayscale image, thus facilitating further processing. Is allowed rotation of the image if needed, to ensure that seed points remaining always at the bottom of the image. The grayscale image is stored within a folder created by the program, with the same name that has the image analyzed.

Then the function **christhin** uses the function **imgchop** in order to obtain the area of interest where the run is going to be analyzed (see Section 3.3). Once generated the area of analysis, we proceed to select the seed point and the solvent front point, in a separate image created by the program for this purpose. In addition, the function generates based on the selected area the corresponding chromatogram. This is done taking into account the color intensity of spots in the image, and therefore uses the grayscale image.

Thus, the function **christhin** uses the function **pickdef** to calculate the percentage of each compound present in the sample, as well as their corresponding *R_f* value (see Section 3.2).

Finally, through a number of commands, the program gives the images a specific format, color, font size and scale. Also save these images along with a text file in the folder mentioned above. The text file contains the percentage

of each peak selected, and the *Rf* value presented by each compound in the sample.

3.2 **Imgchop**

When used, the function **imgchop** allows the selection of the rectangular area in which is located the run that is going to be analyzed. The selection is done by clicking the top left and the other in the bottom right, forming an *imaginary* rectangle, containing the running of interest, besides the seed point and solvent front. If the selection fails, the program issues an error message and asks again the selection. Thus, collects information about the location of the *imaginary* rectangle where there is the run to be analyzed.

3.3 **Pickdef**

When used, the function sets first the output data, such as color and font size in graphics, as well as the nomenclature of each axis in the graphics created and their respective scales.

Then, selects the beginning and end of each peak in the chromatogram, previously generated within function **christhin**. The selection is made with a single click at the beginning and one at the end, and regardless of the y-axis value of the selection, but the x-axis value, because the program automatically places the selection on the curve of the chromatogram. If the selection of the peaks is wrong, sometimes the program issues an error message, requesting the peaks to be selected again.

After selecting the peaks, the function calculates the area under the curve of each peak (using numerical integration known as the **trapezoid method**).

We also calculates the value of *Rf* of each compound in the sample, making use of the values obtained when the user is prompted to mark the point of seeding and the front of solvent in the function **christhin**. (Remember that the value of *Rf* is the ratio between the distance traveled by the compound of interest and the distance traveled by the solvent front, giving an idea of the degree of retention of the compound, directly related to its polarity).

Finally, it calculates the percentage of each compound in the analyzed sample, taking into account the percentage of area corresponding to each compound relative to the sum of all areas resulting from the selected peaks.

Thus, the function **pickdef** allows the calculation of the percentage of each compound in the sample, as well as the corresponding *Rf* value.

Chapter 4

About Octave

Text extracted from GNU Octave documentation. [2]

Octave is a high-level interactive language, primarily intended for numerical computations that is mostly compatible with Matlab¹. Octave can do arithmetic for real, complex or integer-valued scalars and matrices, solve sets of nonlinear algebraic equations, integrate functions over finite and infinite intervals, and integrate systems of ordinary differential and differential-algebraic equations.

Octave uses the GNU readline library to handle reading and editing input. By default, the line editing commands are similar to the cursor movement commands used by GNU Emacs, and a vi-style line editing interface is also available. At the end of each session, the command history is saved, so that commands entered during previous sessions are not lost. The Octave distribution includes a 650+ page Texinfo manual. Access to the complete text of the manual is available via the `doc` command at the Octave prompt.

4.1 Running Octave

On most systems, Octave is started with the shell command `octave`. Octave displays an initial message and then a prompt indicating it is ready to accept input. You can begin typing Octave commands immediately afterward.

If you get into trouble, you can usually interrupt Octave by typing Control-C (written C-c for short). C-c gets its name from the fact that you type it by holding down CTRL and then pressing c. Doing this will normally return you to Octave's prompt. To exit Octave, type `quit`, or `exit` at the Octave prompt.

On systems that support job control, you can suspend Octave by sending it a SIGTSTP signal, usually by typing C-z.

¹Matlab is a registered trademark of The MathWorks, Inc.

4.2 The platforms Octave

Octave runs on various Unices—at least Linux and Solaris, Mac OS X, Windows and anything you can compile it on. Binary distributions exist at least for Debian, Suse, Fedora and RedHat Linuxes (Intel and AMD CPUs, at least), for Mac OS X and Windows' 98, 2000, XP, Vista, and 7.

Two and three dimensional plotting is fully supported using gnuplot and an experimental OpenGL backend.

The underlying numerical solvers are currently standard Fortran ones like LAPACK, LINPACK, ODEPACK, the BLAS, etc., packaged in a library of C++ classes. If possible, the Fortran subroutines are compiled with the system's Fortran compiler, and called directly from the C++ functions. If that's not possible, you can still compile Octave if you have the free Fortran to C translator `f2c`.

Octave is also free software; you can redistribute it and/or modify it under the terms of the GNU General Public License, version 3, as published by the Free Software Foundation, or at your option any later version.

Appendix A

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