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quanTLC, an online open-source solution for videodensitometric quantification

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ABSTRACT

The image is the key feature of planar chromatography. Videodensitometry by digital image conversion is the fastest way of its evaluation. Instead of scanning single sample tracks one after the other, only few clicks are needed to convert all tracks at one go. A minimalistic software was newly developed, termed quanTLC, that allowed the quantitative evaluation of samples in few minutes. quanTLC includes important assets such as open-source, online, free of charge, intuitive to use and tailored to planar chromatography, as none of the nine existent software for image evaluation covered these aspects altogether. quanTLC supports common image file formats for chromatogram upload. All necessary steps were included, *i.e.*, videodensitogram extraction, preprocessing, automatic peak integration, calibration, statistical data analysis, reporting and data export. The default options for each step are suitable for most analyses while still being tunable, if needed. A one-minute video was recorded to serve as user manual. The software capabilities are shown on the example of a lipophilic dye mixture separation. The quantitative results were verified by comparison with those obtained by commercial videodensitometry software and opto-mechanical slit-scanning densitometry. The data can be exported at each step to be processed in further software, if required. The code was released open-source to be exploited even further. The software itself is online useable without installation and directly accessible at http://shinyapps.ernaehrung.uni-giessen.de/quanTLC.

1. Introduction

Quantitative evaluation is an important part of the chromatographic analysis. In planar chromatography, this evaluation is performed using data obtained by either opto-mechanical slit-scanning densitometry of sample tracks or videodensitometry of digitally converted chromatographic images. The latter were recorded by a flat-bed scanner [1–4], camera [5] or light emission diodes [6]. The performance of such approaches has recently been compared [6,7], showing that videodensitometry is a viable option for quantification. Instead of scanning single sample tracks one after the other, as known in conventional densitometry, only few clicks are needed to convert all tracks at one go in videodensitometry, leading to performance data comparable to slit-scanning densitometry [8].

Per se, the videodensitometric analysis is fast and comparatively low-cost. It is the method of choice for laboratories limited in the budget, due to the ubiquitous access to a smart phone, digital camera or flat-bed scanner for image recording and documentation. So far, nine different software have been reported for videodensitometric analyses, *i.e.*, ImageJ [7], VideoScan [8], Sorbfil TLC Videodensitometer [9], Macherey Nagel TLC scanner [10], JustTLC [11], TLC Analyzer [12], qtlc [13], TLSee [14] and Matlab's Imaging Processing Toolbox [15]. However, advantageous assets such as open-source, online, free of charge, intuitive to use and tailored to planar chromatography were only partially covered by some of the existent software for image evaluation (Table 1). For example, TLC Analyzer was written in Matlab and is available free of charge, but a quantification of the data was not integrated in the software. Or the open-source R package qtlc is very rudimental in its functionality and does not have a graphical user interface, which means that it is difficult to operate for a non-programmer. Or the software is not free of charge (VideoScan, Sorbfil TLC Videodensitometer, Macherey Nagel TLC scanner, JustTLC, TLSee and Matlab's Imaging Processing Toolbox). Or other software is not easy to operate (ImageJ, qtlc, and Matlab's Imaging Processing Toolbox) or less tailored to the chromatographic evaluation (ImageJ and Matlab's Imaging Processing Toolbox). So far, only two software (ImageJ

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Table 1
Comparison of nine existent software for image evaluation with the newly developed quanTLC software regarding important software assets for the HPTLC user.

Software name	Manufacturer or Developer	Important software asset for the HPTLC user				
		Open-source	Online	Free of charge	Tailored	Quantitative
ImageJ	U.S. National Institute of Health, Bethesda, MD, USA	x		x		x
VideoScan	CAMAG, Muttenz, Switzerland				x	x
Sorbfil TLC Video- densitometer	Jsc Sorbpolymer, Krasnodar, Russia				x	x
Macherey Nagel TLC scanner	Macherey Nagel, Düren, Germany				x	peak integration only
JustTLC	Sweday, Sodra Sandby, Sweden				x	peak integration only
TLC Analyzer	A. Victoria, I. Hess [12]			x	x	
qtlc	I. D. Pavicevic [13]	x		x	x	via R
TLSee	AlfaTech, Genova, Italy				x	peak integration only
Matlab's Imaging Processing Toolbox	MathWorks, Natick, MA, USA					via Matlab
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and qtlc) have been written as open-source software with an open-source code.

This clearly underlines that sophisticated software comprising important assets (open-source, free of charge, online, *i.e.* easily accessible, intuitive, minimalistic, yet tailored to quantitative image evaluation) would be beneficial in the field of videodensitometry. Recently, rTLC [16] was introduced as online open-source software for multivariate data analysis in planar chromatography, based on the R programming language [17]. This software is tailored and focused on the chemometric evaluation of planar chromatograms. Other open-source software explored the potential and possibilities of unsupervised artificial neural network for denoising of chromatograms and feature extraction [18]. Exploring further the new options that offer open-source technologies, newly developed software, termed quanTLC, is demonstrated in this study. It is a minimalistic, yet sophisticated solution for videodensitogram evaluation and its capabilities are discussed and shown on the example of a lipophilic dye mixture separation.

2. Material and methods

2.1. Chemicals and materials

Dye mixture III composed of 6 lipophilic dyes, i.e., Oracet Violet 2R, Solvent Blue 22, Sudan Red G, Solvent Blue 35, Oracet Red G, and Dimethyl Yellow (in ascending hR_F order) [19], was purchased from CAMAG, Muttenz, Switzerland. HPTLC plates silica gel 60 F_{254} (10 cm × 10 cm format, 0.2 mm layer thickness) and toluene (gradient grade) were obtained from Merck, Darmstadt, Germany.

2.2. Planar chromatography

The lipophilic dye mixture III was diluted 1:10 in toluene and applied on an HPTLC plate silica gel 60 F_{254} as 8-mm bands using the Automated TLC Sampler 4 (CAMAG). The applied sample volume range was 0.5–2.5 μL/band. The track distance was 10 mm. The distance from the lower edge was 7 mm and from the side edge 25 mm. Development was performed with 4 mL of toluene up to a migration distance of 60 mm in the Twin-Trough Chamber, 10 cm × 10 cm (CAMAG). The chromatogram was documented under white light illumination using the DigiStore 2 Documentation System (CAMAG).

2.3. Newly developed quanTLC software

Videodensitometric quantification was performed with the newly developed quanTLC software. It was written in the R programming language [17]. The code was released open-source to be exploited even further. The shiny package [20] was used to create a user interface in HTML, directly accessible from an internet browser. Thus, the software itself is online useable without installation and directly accessible at http://shinyapps.ernaehrung.uni-giessen.de/quanTLC. In the form of an R package, instructions for installation and operation are available on GitHub at http://github.com/dimitrif/quanTLC

2.4. Verification of results by software comparison

Densitometry was performed via absorbance measurement with the multi-wavelength scan at 450, 500, 530, and 620 nm of the TLC Scanner 4 and winCATS software, version 1.4.6 (CAMAG) [19]. The scanning speed was 20 mm/s with a measuring slit dimension of 3.0 mm × 0.3 mm. Videodensitometry was performed via the VideoScan software (CAMAG). Parameters used for integration in each of the software are summarized (Table 2). Polynomial regression was selected in all software and applied to all dyes.

3. Results and discussion

3.1. Description of the workflow in quanTLC

In the newly developed software quanTLC, the videodensitometric analysis was structured in five steps, *i.e.* data input, preprocessing, integration, statistical data analysis and data export. A one-minute video serves as illustration (Video S1). As data input, the user had to upload a chromatogram as file. JPEG, PNG, BMP and TIFF are accepted as graphic file formats. As further data input, the plate dimension as well as distances of sample application and chromatographic development were required to extract the individual videodensitograms automatically. The pixel values across a line were averaged to achieve a mean pixel value for each hR_F value. The software is also able to deal with an application from both sides, used for example in high-throughput horizontal development.

Preprocessing improved the compatibility of the videodensitograms for quantitative analysis [21,22]. Several preprocessing options were integrated in quanTLC to be selectable, *i.e.*, negative peak inversion, smoothing with the Savitzky-Golay algorithm [23], baseline correction via the R package baseline [24] and warping for peak alignment with the parametric time warping (ptw) and dynamic time warping algorithms (dtw) via the R packages ptw and dtw [25–27]. During selection of the preprocessing, visual inspection of the resulting videodensitograms in real time helped to choose the best options.

Automatic peak integration was necessary to increase reproducibility and minimize user action. Peak integration was done via the R package pracma [28]. Peak starts and peak ends were detected based on the succession of increasing and decreasing steps before a peak. A minimum peak height was set to remove all peaks of a smaller intensity.

For calibration, quantification and statistical data analysis, the user had to select the standard tracks and input their values (amount per band or target concentration). By clicking on a peak

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