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RESEARCH ARTICLE

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Open-source add-on kit for automation of zone elution in planar chromatography

Tim T. Häbe | **Gertrud E. Morlock**

Chair of Food Science, Institute of Nutritional Science, and Interdisciplinary Research Center (iFZ), Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany

Correspondence
G. E. Morlock, Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany.
Email: gertrud.morlock@uni-giessen.de

Dedicated to Dr. Heinrich Luftmann

Rationale: High-throughput capacity, maximal efficiency and automation are missing key features for thin-layer chromatography/high-performance thin-layer chromatography-mass spectrometry (TLC/HPTLC-MS) hyphenations. So far, commercial interfaces have been operated without an automated positioning system. Hence, a 3D-printed, fully automated, open-source add-on user interface was built for elution-head-based TLC/HPTLC-MS.

Methods: Precise plate movement, batch-wise zone elution, and cleaning processes were automated and synchronized with MS data acquisition. The elution head cleaning was made adjustable in intensity and interval, and remaining elution solvent or particles were thoroughly blown out of the MS transfer line. An adjustable short gas beam across the elution zone was integrated to avoid contamination or leakage by released layer particles, or zone distortion by residual eluent flow when lifting the elution head.

Results: By clicking on zones on the chromatogram, these were selected for consecutive automated positioning below the elution head, sealing and elution. Mean spatial deviations of the positioning on 294 target zones were determined to be 160 μm for track-wise and 190 μm for randomized zone positioning order. Reproducibility of the elution of butyl paraben zones (5.4%, 10 ng/band, *n* = 70) and its quantitative performance (R^2 = 0.992–0.999, 5–50 ng/band) were proven.

Conclusions: The stand-alone electronic system design and the compact footprint were achieved by a minimalistic positioning system and internally installed valves. The open-source OC_manager software opened new perspectives in terms of combining MS data evaluation with previous HPTLC results. The successfully demonstrated TLC/HPTLC-MS automation presents a highly efficient, user-independent hyphenation for a broad range of application fields.

1 | INTRODUCTION

The coupling of mass spectrometry (MS) with planar chromatography (preferably high-performance thin-layer chromatography, HPTLC) was largely developed during the past 15 years. This paved the way for its usage in a broad range of quite different fields. Many different approaches for desorption or elution, ionization and transfer of substance zones into the mass spectrometer had been reported for planar chromatography.[1] However, in 2004, an elution head-based interface was described by Luftmann that seemed practical in its operation.[2] By pressing the aluminum foil against a fixed elution head, a substance zone was isolated within a round cutting edge and subsequently eluted. This elution-head-based interface was minimalistic in every respect and thrilling for analysts seeking for more information on a zone. It was suited for any liquid ionization technique. In the same year of 2004, another elution head device was reported by Prosek et al[3] which was more complex. TLC plate positioning was performed manually and a special cutting device had to be used to prepare the elution zone similarly to the Eluchrom.[3,4] Computer control was used for two syringe pumps and three injection valves only to perform a complex elution process and sampling loop filling before transfer to the source of the mass spectrometer.[3]

In 2006, the Luftmann interface, termed ChromeXtractor, was successfully modified. Its functioning on glass plates was an important step forward, as its operation was not limited to aluminum foils anymore.[5] In the following years we studied the Luftmann interface under the following aspects: functionality on extra thin[6] as well as preparative layers,[7] influence of different cutting edges,[8] quantitative performance[9] capability of detection down to the 20 pg/zone,[10] and its employment in a wide range of applications.

In 2007, a fully automated Luftmann prototype utilized a plate-positioning system, elution head activation by a stepper motor and an electronic six-port valve.[11] An image-based target zone selection was used to perform the batch-wise workflow of multiple, precise zone elutions. Caffeine standard amounts of 50–500 ng/band were measured with a determination coefficient R^2 of 0.9973 by a triple quadrupole mass spectrometer. The repeated analyses of pharmaceuticals and food products were <5.6%. Unfortunately, the large size and the high material and manufacturing costs of this device (ca. 24 kEuro) has been a hindrance with regard to a broader usage.[11]

Though the way was paved by manual and even automated prototypes (showing the proof of principle, application and validation), by fundamental knowledge and promising data in more than 20 research papers on the elution-head-based interface, it has been very difficult to get the potential of the HPTLC/MS technique recognized by manufacturers. First in 2009, a commercial interface was launched on the market (TLC-MS Interface, CAMAG).[12] Support by application notes/videos was given first in 2015, when an application tutorial video was published (www.youtube.com/watch?v=gTN6fcTrHGM), followed by the application note A-100.1 in 2016, showing the confirmation of a zone assignment by HPTLC/MS.

This pressed-on-layer elution head principle was not intellectually protected in the USA and was thus taken over by another manufacturer in 2015 (Plate Express, Advion). In the same year, the CAMAG system was to a certain extent upgraded to the TLC-MS Interface 2. After difficult years of non-recognition and rejection, the idea to bring forward the benefit through zone characterization by HPTLC-MS[13] was finally translated. The intention to support analysts worldwide with a tool that can provide deeper information about TLC/HPTLC zones has become a reality. Two commercially available elution-head-based TLC-MS interfaces are now well accepted in the market. The elution head even became the core part of the dried blood spot sampling automation platform DBS-MS 500 from CAMAG.

Both commercial interfaces are operated by manual plate positioning and manual activation of each single zone elution process. The base plate of the upgraded TLC-MS Interface 2 was enlarged and equipped with a ruler for eased manual plate positioning. The filter frit of the elution head was removed and replaced by a replaceable filter fitting directly on the still manually operated six-port valve. The elution head cleaning process was improved. Instead of blowing a gas against the cutting edge of the elution head while simultaneously moving the drawer underneath it (to capture dried particles within the cutting edge that felt down), a parallel gas flow was installed, directed through the six-port valve with connected lineout capillary of the elution head. Unchanged from the previous interface is the pneumatic elution head movement and its activation by a toggle switch as well as the activation of the pneumatically driven elution head cleaning at the push of a button.

The Advion interface exploits the same elution head principle, but two-parted to replace the elution head independently from the capillaries towards the automated six-port valve. The elution head movement and contact pressure are controlled by a stepper motor (as used in Luftmann et al.[11]) with distance control of a compression spring. After manual zone positioning, the interface is activated by contact closure, leading to zone sealing, elution and the subsequent cleaning process. Elution period and contact pressure are controlled by software. This interface represents the current state of automation.

A SciFinder search found around 100 research studies discussing the use of the two brands of elution head-based interfaces (keywords surveyed 17.10.2018: TLC-MS: 248; HPTLC-MS: 68; TLC-ESI-MS: 20; HPTLC-ESI-MS: 45; TLC-MS Interface: 66). As sales figures of both companies were not available, the number of devices in research and industry was estimated to be more than 10 times higher. Among the different techniques of HPTLC-MS, the elution head-based concept can be seen as the technique with the highest detectability. Its drawback is the manual operation for each zone elution, if compared to scanning techniques like MALDI-TOFMS,[14] DESI-MS,[15] and DART-MS.[16]

Automation and standardization was recognized as a crucial asset for existing interfaces. A commercial solution seemed not to be available for target zone selection by click-on-the-image and automated self-positioning, as well as advanced automation of the elution and cleaning processes. An open-source solution was strived for and investigated as an option for the TLC/MS Interface 2. It was aimed to exploit cost-effective electronics, open-source and rapid do-it-yourself prototyping techniques. A first working prototype (Figure S-1, supporting information) was presented in July 2017 at the International Symposium for HPTLC in Berlin. A minimalistic and compact final design with additional hardware and software features has since been developed for a streamlined target zone elution with online transfer into the MS system (Figure S-2, supporting information).

2 | EXPERIMENTAL

2.1 | Hardware construction

The TLC/MS Interface 2 (CAMAG) was digitized (Figure 1A) as a 3D model with OpenSCAD software (http://openscad.org). All functional and housing parts were 3D printed with polylactic acid (PLA; ColorFabb, Belfeld, The Netherlands) on a Prusa i3 MK2 3D printer (Prusa Research, Praha, Czech Republic). For control of basic and advanced pneumatic functions (Figure 2A), four SY3000 series magnetic valves (SMC, Egelsbach, Germany) were installed. For solvent control, the 2/6-port valve TitanHP MHP9900 with driver board 7770016 (IDEX, Lake Forest, Illinois, US) was integrated (Figure 2B). A NEMA14 stepper motor and mechanical endstop sensor (both eMotion-Tech, Toulouse, France) were mounted partially below the interface base for the belt-driven *x*-axis movement. Two similar units in parallel mode at both rear sides of the interface base were used to move both *y*-carriages guiding the *x*-axis with attached plate carrier for 20 × 10 cm plates. This plate carrier was optionally equipped with an adapter for any other plate size, typically for 10 × 10 cm plates (Figure S-3, supporting information). Attached to the rear cover, receptacles of each automation unit with connection to a communication port were installed.

2.2 | Firmware and electronic boards

The interface was controlled by an Arduino Mega 2560 (https://arduino.cc) and a Ramps 1.4 shield (http://reprap.org/wiki/RAMPS_1.4) via the Marlin firmware (http://marlinfw.org). To provide sufficient processing power and an integrated system, a small size next unit of computing (NUC) PC system was extended by a 3D printed housing to install the modified Arduino (Figure S-4, supporting information) and Ramps boards together with the NUC_6CAYH board in a just 11 × 11 × 11 cm^3 unit (Figure S-5, supporting information) with internal power and USB connections. All board modifications, e.g., removing triacs, resistors and other connectors to enable the integrated USB and power connection as well as the installation of the communication ports, can be performed with medium soldering experience.

2.3 | Software for interface control

To create G-code files and send them to the Arduino, the dedicated open-source software OC_manager (Figure 3, https://github.com/OfficeChromatography/OC_manager) was expanded by the TLC/MS user interface. The software was written in R (R Core Team) and used the R package Shiny and the package Reticulate to call the Python software Printrun (http://www.pronterface.com) for communication with the Arduino. The software was hosted on the NUC system and user control was available via an in-plane switching (IPS) display on the autoTLC-MS Interface.

2.4 | Bill of materials and assembly

All necessary parts and their assembly are summarized in a list of printjobs (Figure S-6, supporting information), a bill of materials (Figure S-7, supporting information), a list for pin assignments (Figure S-8, supporting information) and the assembly instruction (Instruction S-1, supporting information). Along with all SCAD files, firmware and OC_manager software, this has been made available as an open-source package for reproduction and self-mounting (https://github.com/OfficeChromatography/autoTLCMS).

2.5 | Chemicals

Bidistilled water was obtained from a Destamat Bi 18E system (Heraeus, Hanau, Germany). Ethanol and methanol (MS grade) were obtained from Th. Geyer (Renningen, Germany) and azophloxine (hist.) and butyl paraben (BE, ≥99.0%) from Sigma Aldrich (Schnelldorf,

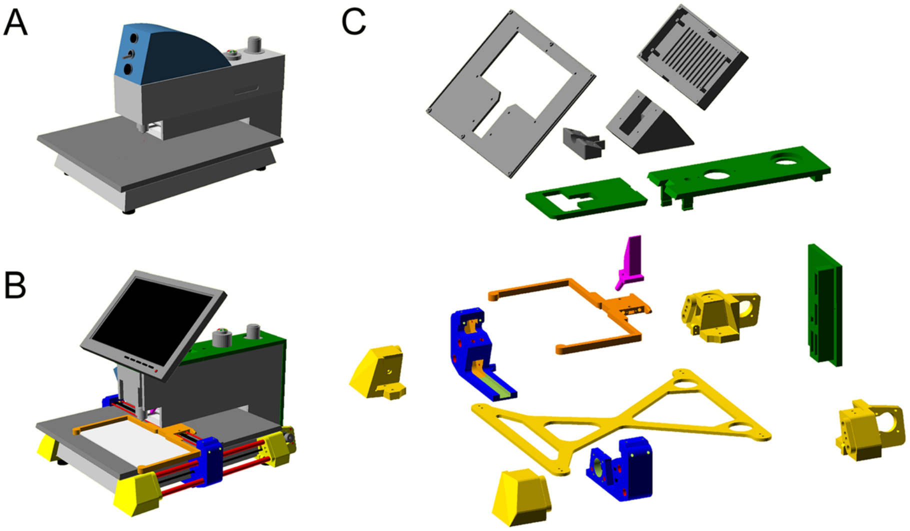


FIGURE 1 First version of the 3D-printed parts for the hardware modifications of the TLC-MS Interface 2: A, Original interface for manual plate positioning and manual elution/cleaning procedure; B, modified autoTLC-MS Interface; and C, its 3D-printed parts. Original and display parts (grey), *y*-movement components (yellow), *xy*-carrier (blue), *x*-carrier with plate holder (orange), nozzle for elution zone cleaning (magenta) and valves/electronic parts (green). [Color figure can be viewed at wileyonlinelibrary.com]

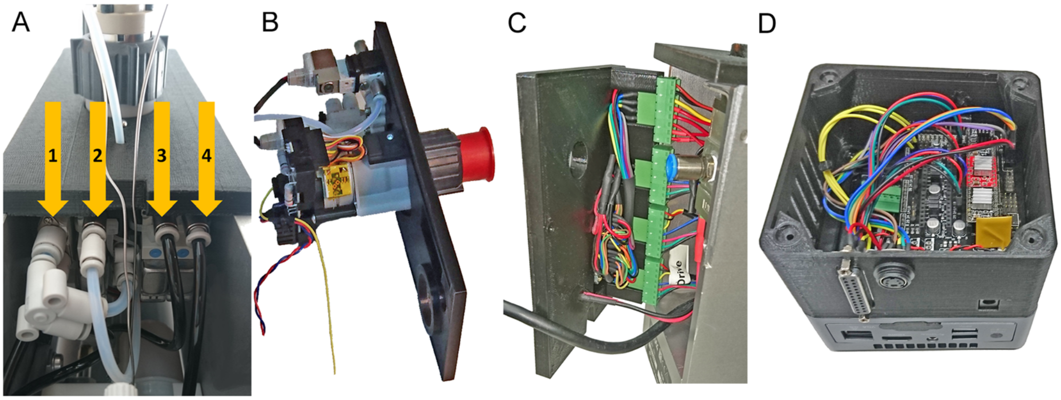


FIGURE 2 Internal features for automation: Pneumatic valves A, for control of the elution head (1), head cleaning (2), cleaning drawer (3) and elution zone cleaning (4); automatic 2/6-port valve and B, its driver board. C, Receptacles of each automation unit and connection towards the NUC housing. D, The Arduino mega 2560 with ramps 1.4 mounted inside the expanded NUC housing [Color figure can be viewed at wileyonlinelibrary.com]

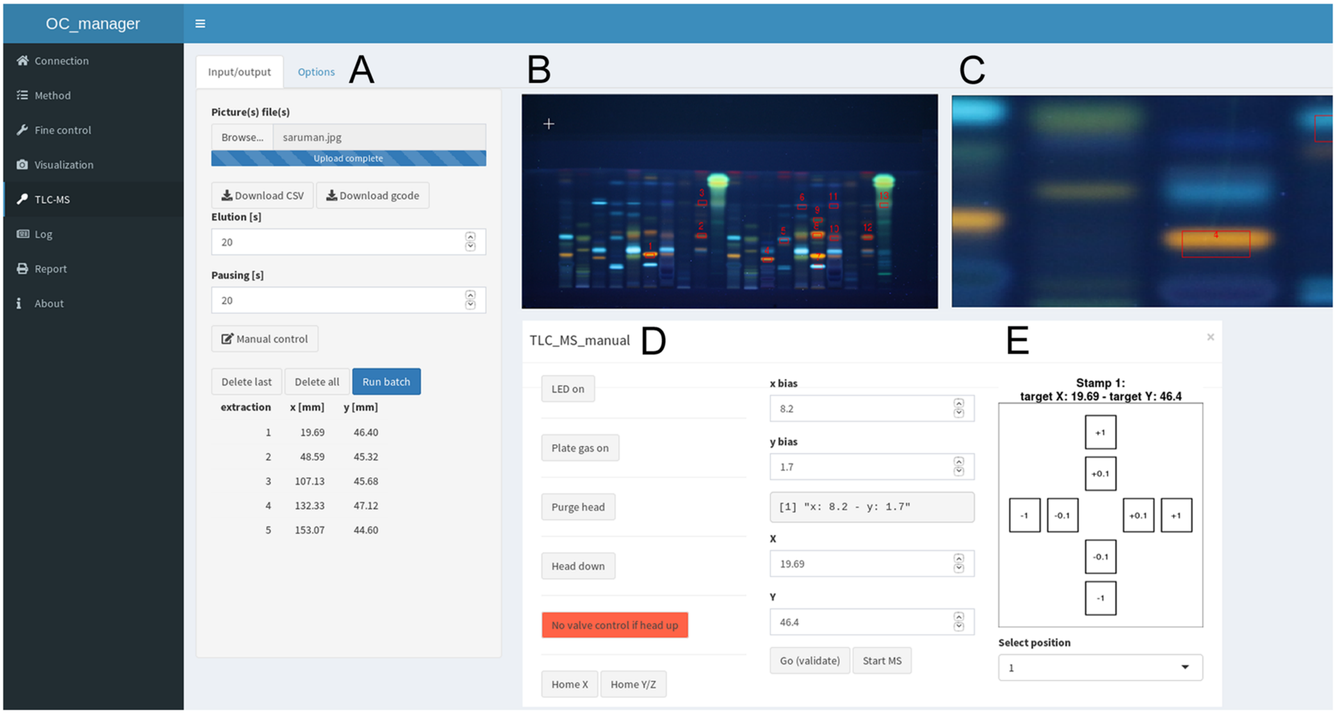
Germany). HPTLC plates (silica gel 60 F_{254}; prewashed with methanol/water 7:3, v/v and dried at 120 °C for 15 min)[17] as well as MS-grade plates with reduced layer thickness (only heated at 120 °C for 15 min) were obtained from Merck (Darmstadt, Germany).

2.6 | Standard solutions

An azofloxine solution was prepared by dissolving 5 mg in 100 mL H_2O (50 ng/μL) and a stock solution of BE by dissolving 10 mg in 10 mL ethanol and subsequent 1:100 dilution in ethanol (10 ng/μL).

2.7 | Spray-on application

All application patterns were applied with the FreeMode option of the Automatic TLC Sampler 4 (ATS 4, CAMAG). The azofloxine pattern with 294 bands was applied as 6-mm bands (each 1 μL) spaced 8 mm in the x-direction (2 mm distance between bands) and 6 mm in



Picture(s) file(s): saruman.jpg

Elution [s]: 20

Pausing [s]: 20

extraction	x [mm]	y [mm]
1	19.69	46.40
2	48.59	45.32
3	107.13	45.68
4	132.33	47.12
5	153.07	44.60

TLC_MS_manual

x bias: 8.2

y bias: 1.7

```
[1] "x: 8.2 - y: 1.7"
```

X: 19.69

Y: 46.4

Stamp 1:
target X: 19.69 - target Y: 46.4

Select position: 1

FIGURE 3 Two screenshots combined in this illustration to display the OC_manager software input/output functionalities for A–C, automated operation versus D, E optional input for manual control via software buttons. Upload of chromatogram or up-/download of coordinates, setting of elution and pausing period and generated batch list of assigned target positions (A), chromatogram with assigned target positions (B), zoom view for precise assignment (C), manual control of all functions (D), and correction of the positioning (E) [Color figure can be viewed at wileyonlinelibrary.com] the *y*-direction. For precise assignment of the middle of the stamp position after stamping, an 80 mm long band (10 μL) was applied vertically over each column of bands. All plates were documented before and after autoTLC-MS elution at UV 254 nm and white light illumination using the TLC Visualizer and winCATS software (version 1.4.7, CAMAG). For plate documentation and preparation of images for zone selection any calibrated digital camera can be used. If necessary, the image size and resolution can be adjusted in the OC_manager source code.

2.8 | Evaluation of positioning

The mean deviation (target shift) of the spray-on application and the subsequent elution by the autoTLC-MS Interface were calculated via the absolute target coordinates and the coordinates obtained by digital image evaluation. CorelDraw X5 (Corel Corporation, Ottawa, Canada) was used to mark the application or elution areas and to export these coordinates.

2.9 | Mass spectrometry

For high-resolution mass spectrometry (HRMS), a Q Exactive Plus system with enhanced resolution mode and heated electrospray ionization (HESI) source was used; solvent delivery (methanol at 0.2 mL/min) was provided by a Dionex UltiMate 3000 LPG-3400XRS pump and data evaluation was performed with Thermo Xcalibur 3.0.63 (all Thermo Fisher Scientific, Dreieich, Germany). MS-source settings were: spray voltage −3.5 kV, sheath gas 20 AU; aux gas 10 AU at 270 °C; probe heater 200 °C; S-lens RF level 50 AU. The start signal for data acquisition was provided by a 5-V relay, directly controlled by the Ramps 1.4. Data acquisition was performed in single ion monitoring (SIM) mode at *m/z* 192.5–193.5 with FT resolution 70,000. Quantitative data for BE was obtained via the extracted ion chromatogram (EIC) out of the SIM experiments at automatic gain control (AGC) target 5×10^5 and max. injection time 10 ms (10 ng/band) and AGC target 1×10^6 and max. injection time 50 ms (5–50 ng/band).

3 | RESULTS AND DISCUSSION

3.1 | Internal features including valves

The digitalization of the TLC/MS Interface 2 (Figure 1A) enabled us to select suitable sites as base or bottom support for the additional mechanic parts of the automation unit. This was crucial in order to provide the best fitting and the possibility to simulate all instrument movements. The add-on parts for automation were designed based on the basic parts of the original interface (Figure 1B). All functional and housing parts (Figure 1C) were 3D printed with polylactic acid on a Prusa i3 MK2 3D printer. The original blue and silver top covers (Figure 1A) as well as the complete mechanic user interface were removed. This included the pneumatic valve with toggle switch to activate the head movement, a button to switch on the positioning LED as well as a push button to pneumatically activate the gas flow through the elution head outlet and to move the cleaning drawer. Instead, all pneumatic functions were then controlled by four magnetic valves (Figure 2A). The newly automated control of the elution head operated analogously to the previous mechanic valve. The previous simultaneous activation of the elution head cleaning and the cleaning drawer by a simple T-piece was now controlled by two individual magnetic valves. This enabled a 0.5-s delayed activation of the cleaning drawer to avoid spitting of residual solvent from the elution head cutting edge onto the HPTLC plate or in the front of the cleaning drawer. Additionally, the elution head cleaning was now regulated in terms of duration and pulsation of the gas beam through the outlet capillary. Thus, this optimized cleaning procedure further minimized the risk of cross-contamination and zone distortion, especially necessary for HPTLC-HRMS.

These functionalities were expanded by a new elution zone cleaning using a gas flow controlled by a fourth magnetic valve. The gas flow was directed by a nozzle on the rear side of the elution head (Figure 1C, magenta) directly onto the HPTLC plate. Layer particles or dust settled during plate movement were removed from the target zone when auto-positioned beneath the elution head. After elution, microliter volumes of residual eluent migrated radially from the imprint area. Depending on the solvents used, the too slow evaporation caused distortion of nearby zones. This was avoided by a gas flow directed on the target zone in the moment the elution head moved upwards. This gas flow interval was selected as short as possible to avoid evaporation of substances with a high volatility, and was provided with an adjustable timing via the magnetic valve. It was also necessary for a precise time-out in relation to the cleaning drawer movement to ensure that no layer material was blown out of the drawer, as observed for the simultaneous activation (status quo).

The mechanic 2/6-port valve was replaced by the electric 2/6-port valve TitanHP MHP9900 with integrated actuator (Figure 2B). This enabled a precise and defined timing for the automated elution and subsequent cleaning process. Thus, the elution period is selectable and highly reproducible. In combination with the automated positioning and batch-wise elution of zones, this accuracy was important for the quantitative evaluation and assignment of the resulting MS data.

On the rear side of the instrument, the battery pack for the LED cross (manually pushed on for indicating the imprint position) was removed, as the 5-V LED is now powered and controlled by the integrated system. This free space next to the pressure regulator of the pneumatic system was now available to mount an electronic port assembly to connect all individual automation units (Figure 2C). On this connector, the link to the advanced NUC system is generated (Figure 2D and Figure S-5, supporting information). This enabled an easy replacement or repair of individual units and a more modular compilation of the electronic system. All these modifications and units mounted inside the 3D-printed top and rear covers (Figure 1C, green) represented the internal features of the device (Figure 2).

3.2 | External features including positioning system

The external features comprised the 3D-printed housing and mounting post for the optional IPS display (Figure 1C, grey) and all parts of the plate-positioning system. The original base of the TLC-MS Interface 2 had only few options for a solid mounting of an automated positioning system. The space between the upper and lower parts of the device measured just 8 mm. Thus, an individual system had to be designed as common industrial positioning systems did not fit or would have excessively raised the footprint and costs of the device.

The stainless-steel rods, stepper motors and endstop sensors for the *y*-axis were mounted by four 3D-printed parts (Figure 1C, yellow) fixed on each corner of the interface base (Figure 1B, yellow). Utilizing the threaded holes of the four pedestals underneath the instrument and a mounting frame (Figure 1C, yellow) for the upper fixation, a solid and compact design was realized with discrete automation units. The original pedestals were mounted beneath these corner parts for a good leveling of the device.

To realize an *x*-axis freely moveable beneath and along the upper part of the device, two challenges had to be solved. First, the spring for retracting the cleaning drawer, which was positioned partially below the drawer, had to be remounted at an elevated position. Nevertheless, the 10-mm maximum space between the lower and upper parts of the device was still limited by the steel parts of the instrument body. Thus, at a given safety distance of 2 mm, a 3D-printed *x*-carrier with attached plate holder (Figure 1C, orange) was limited to 8 mm height including the bearings and timing belt fixation for the *x*-axis, meaning that the *x*-axis rods had to be 3 mm with 5 mm collar bushings mounted in the *x*-carrier for lowest dimensions and costs. The plate holder on the *x*-carrier was designed as a flexible 8 mm high clamp. The plate was slid below the holder and the flanks kept the plate tight into position when pushed over plate edges. This was the simplest one-piece solution strong enough for reproducible positioning and user-friendly handling.

For mounting the *x*-axis rods and for guiding the tightened timing belt, two *xy*-carriers were designed to move along the *y*-axis on both sides of the device (Figure 1C, blue) connected by a flat traverse (Figure 1C, yellow) below the device to avoid increased friction in the bearings due to distortion caused by the tightened timing belt. The *x*-axis stepper motor was mounted on the right carrier below the hollowed interface base. Thus, the smallest footprint was achieved and all timing belts were guided inside the 3D-printed parts or between the axis rods to comply with safety issues.

3.3 | Electronics and firmware

The Arduino Mega 2560 and the Ramps 1.4 shield were used, as they are common open-source tools to control 3D printer setups similar to the autoTLC-MS Interface. All stepper motors and endstops were connected in the regular manner for 3D printers, for endstops and all other 5-V units, the ground potential was merged inside the electronic port. The LED cross (for check of the positioning) was directly powered from the Ramps when activated. The same was applied for the pneumatic valves. As the 2/6-port valve needed a 24 V supply and a voltage drop as signal, a 5-V relay was added and controlled by the Ramps. Except the high definition multimedia interface (HDMI) and power supply connection, all electronic wiring was merged in a standard 25-pin Sub-D connection. All functions were then controllable by simple G-code commands.

Using the advanced NUC system in the stand-alone mode (Figure 2D), both boards were modified before integration in the 3D-printed housing. To reduce the board height and to enable an internal 12-V supply and internal USB connection, the 11-A fuse, all triacs and their resistors/LEDs for unneeded heating options were removed. Herewith all screw connections of the board were isolated and were then used for a user-friendly and detachable internal USB connection from the NUC board towards the Arduino. All three boards were internally connected in parallel, and thus, only one 12-V power supply attached to the Arduino was needed to run the whole integrated system. These modifications resulted in a compact 11 × 11 × 11 cm^3 system (Figure S-5, supporting information) that delivered enough processing power to control the whole device.

The Marlin firmware (http://marlinfw.org) of the Arduino was modified for the mechanic dimensions used, especially for the parallel movement of the two *y*-axis motors (assigned as *y*- and *z*-motor in the firmware) with two independent endstops per side. This allowed the alignment after missing motor steps or any other minor deviations after disabling the motors to ensure a parallel operation of both *y*-carriages.

3.4 | Target zone selection by OC_manager

The OC_manager software was expanded by the TLC/MS functionalities to control the automated positioning and zone elution process. There are two input options to generate a batch list for zone elutions (Figure 3A). The chromatogram captured at white or UV light, also after derivatization or bioassay application, can be uploaded. The software rasters the image and a full view frame is given (Figure 3B) as well as a zoomable frame for precise target zone selection, and, when selected, a batch list with their coordinates (Figure 3C). If coordinates are already known or a certain zone pattern had to be stamped, e.g. for validation, the coordinates can be uploaded via comma-separated values (CSV) file (Figure 3A). In both cases, the coordinates are implemented into the G-code file. The elution period and interval (for rinsing the capillary towards the MS system) are set. Options for positioning, inter-elution cleaning and pre-/post-batch cleaning can be defined. This tailored G-code file is then transferred via Printrun to the Arduino and executed by the autoTLC Interface.

3.5 | Open-source autoTLC Interface

All SCAD files, firmware, assembly instruction, bill of material, self-mounting of the autoTLC Interface (Figure 4) and the OC_manager software (Figure 3) are available as open-source packages (https://github.com/OfficeChromatography/autoTLCMS and https://github.com/OfficeChromatography/OC_manager). The total hardware costs are 17 kEuro: material for open-source automation unit (2 kEuro) and the TLC-MS Interface 2 (15 kEuro, CAMAG). A tutorial video showing its operation is available at https://www.youtube.com/watch?v=JEh-BBGdvYI&feature=youtu.be.

3.6 | Verification of the automated positioning

The OC_manager software is equipped with several software functions to control individual actuators, valves and other features by mouse clicks. The LED cross activation and all valves are controllable via software buttons. After homing both axes, the plate holder can be moved to any position. *x*/*y*-Bias values can be set to adjust the dimension of the plate holder to that of the previously used image capture device. As sub-millimeter precision was required and the sizes of HPTLC plates slightly differed, a fine tuning for each plate had to be performed (Figure 3E). Therefore, the plate was marked at the top left position. This mark was selected by click-on-the-image and the plate holder was automatically driven to the marked coordinates, so that the mark was positioned beneath the elution head. This automated positioning (coordinates) was verified by activation of the positioning LED cross. In case it was necessary, a manual correction via the software buttons was performed, moving the mark exactly under the positioning LED cross. By confirming the overlaid positions of image and actual plate, the bias for positioning was automatically fixed. After verification of the positioning system, the autoTLC Interface was ready for the validation study.

3.7 | Validation of the autoTLC-MS Interface

The red azo dye azophloxine was selected and its solution was multifold sprayed on the plate as homogenous band patterns including cross-over lines via the FreeMode option of the ATS4. This plate was used for validation of the positioning of the autoTLC-MS Interface.

The mean deviation of the application of 294 bands (Figure 5A) was determined to be 250 μm (*n* = 294) via digital image evaluation (Figure 5B). This deviation of spray-on application was used as reference value. Taking the generated imprint after stamping, the two positioning modes of the autoTLC-MS Interface were evaluated. The mean deviation for track-wise positioning on 294 azophloxine zones was determined to be 160 μm (Figure 5C) and for a randomized positioning order to be 190 μm (Figure 5D). By this, the high-throughput positioning was successfully validated via the elution head imprint position.

Next, 70 butyl paraben application zones of 10 ng/band each were eluted online into the HRMS system and recorded in the SIM mode and evaluated by the EIC. The repeatability was determined to be 5.4% (*n* = 70, Figures 6A and 6B), which proved the reliable high-throughput capability of the autoTLC-MS Interface. The calibration performance was evaluated using butyl paraben ranged 5–50 ng/band (Figures 6C and 6D). Determination coefficients R^2 of six 10-point calibrations were between 0.9924 and 0.9986 (*n* = 6) and the

FIGURE 4 The autoTLC-MS Interface, a 3D-printed add-on package for automated plate positioning (mounted on the base part of the TLC-MS Interface 2) with integrated automatic 2/6-port valve and four magnetic valves for pneumatic functions. The IPS display and the communication port were mounted on the top and on the rear side of the interface and can be connected to the advanced NUC system (not displayed) [Color figure can be viewed at wileyonlinelibrary.com]

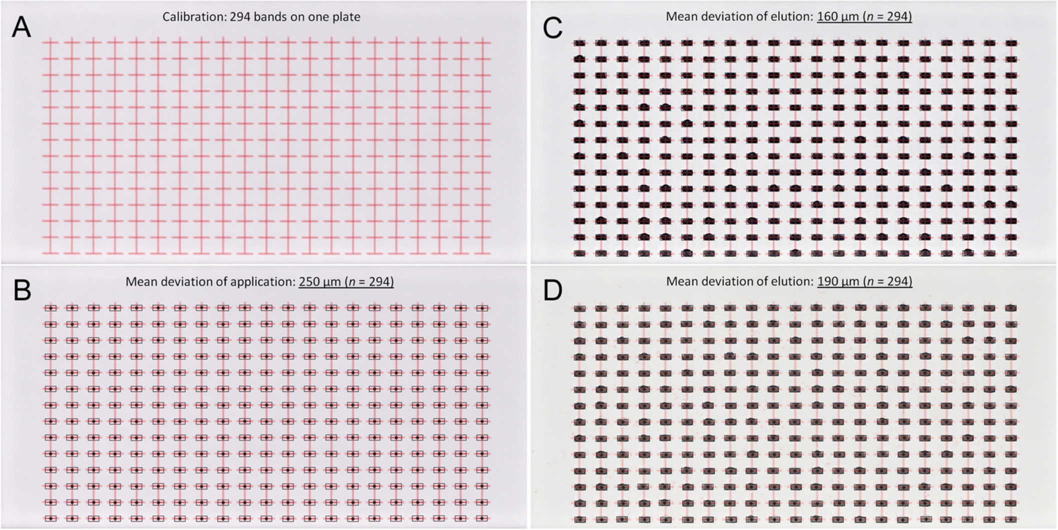


FIGURE 5 A, Calibration pattern to determine the mean deviation (target shift) of the positioning system. For 294 target zones on one plate, the mean deviation of spray-on application (ATS4) was determined to be 250 μm (*n* = 294, B), for the track-wise positioning on all zones to be 160 μm (*n* = 294, C) and for the randomized positioning order to be 190 μm (*n* = 294, D) [Color figure can be viewed at wileyonlinelibrary.com]

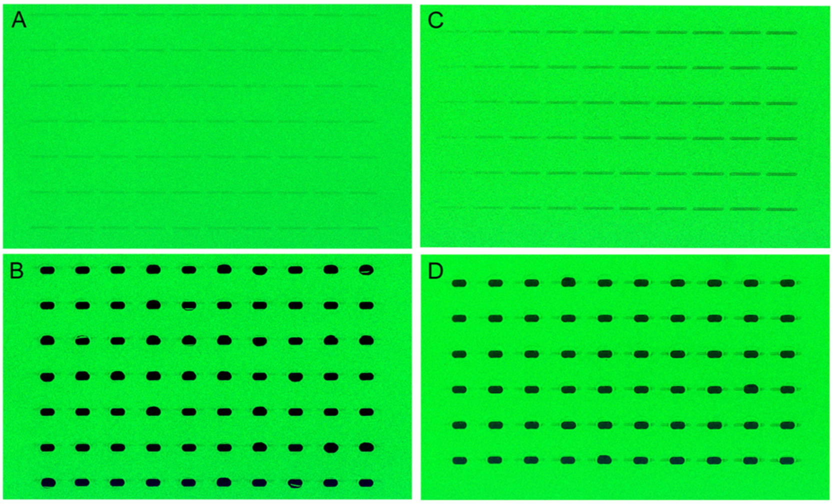


FIGURE 6 Validation of the autoTLC-MS Interface determined via butyl paraben zones applied on an HPTLC layer, eluted and recorded by HRMS in the SIM mode: Repeatability of 70 1-min elutions (A and B, 10 ng/band each, 30 s elution, 20 s post-run and 10 s cleaning/positioning) to be 5.4% as well as 10-point calibration (C and D, *n* = 6, 5–50 ng/band) with R^2 = 0.9924–0.9986 and RSD = 1.0%–5.2% (B/D: imprint after elution) [Color figure can be viewed at wileyonlinelibrary.com]

relative standard deviations were between 1.0% and 5.2% for the ten different calibration levels.

4 | CONCLUSIONS

Based on cost-effective electronics and rapid prototyping techniques, an open-source add-on kit was developed for the TLC-MS Interface 2. Using the toolbox of 3D printing and RepRap[18] environment, a very compact plate-positioning system was combined with an image-based software solution. This provided the platform to control and synchronize all steps from positioning, elution, advanced cleaning to MS data acquisition. The good repeatability of the positioning was proven as well as the good calibration performance, when automated positioning and elution was used for HRMS recordings. This validation data also proved that cross-contamination was kept at a minimum, if present at all.

The open-source automation of the elution head-based interface and its user-friendly operation will boost the application of this technique in research and routine analysis. Furthermore, this part of the OC_manager platform can be used to implement various further functionalities. It may provide the platform for easy mass spectra readout and processing in a facilitated workflow to automatically define target zones and to combine all possible datasets from HPTLC plates for an intelligent evaluation. For example, quanTLC[19] for peak detection and videodensitometric quantification or superior image evaluation by artificial neural networks[20] or powerful cluster analysis of the recorded HPTLC-MS spectra[21] were recently developed modern tools that are helpful for analysts in the field of TLC/HPTLC.

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ORCID

Tim T. Häbe https://orcid.org/0000-0001-8329-4857

Gertrud E. Morlock https://orcid.org/0000-0001-9406-0351

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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