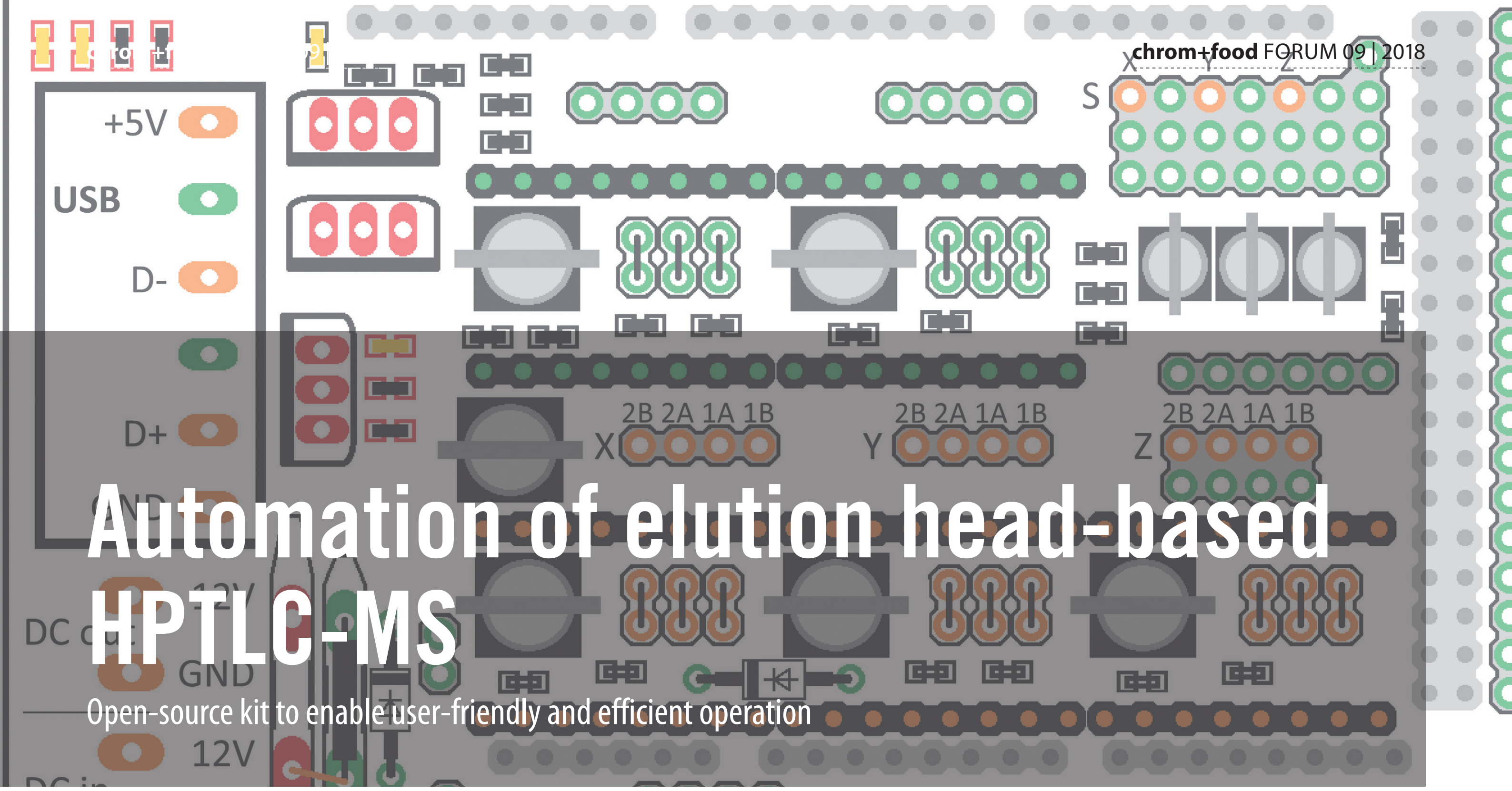

Automation of elution head-based HPTLC-MS

Open-source kit to enable user-friendly and efficient operation

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The TLC-MS Interface 2 was automatized by an open-source 3D printed add-on hardware controlled by open-source software (OC_manager) to provide an image-based user interface. High reproducibly, user-friendly high-throughput handling and reduced cross contamination was achieved.

The forerunner model of the current commercially available elution head-based interface in high-performance thin-layer chromatography mass spectrometry (HPTLC-MS) was described by Luftmann 14 years ago [1]. In the same year of 2004, another approach was reported with a semi-quantitative setup by Prosek *et al.* [2]. The latter used a special preparation of the elution zone, and two syringe pumps and three injection valves were computer controlled. This resulted in a complicated elution process and filling of a sampling loop before MS injection. For both initial approaches, the HPTLC plate positioning was manually performed. The limitation of the Luftmann approach to aluminum foils [1] was solved in 2006, when reporting the successful elution from glass plates by a modification of the elution head [3]. In 2007, a fully automated model was demonstrated with a plate positioning system, a stepper motor-driven elution head and an electronic 6-port valve [4]. Target zones were selected in the chromatogram (plate image) by clicking on these, and their batch-wise elution was precisely performed. Caffeine standards of 50–500 ng/band were measured by a triple quadrupole mass spectrometer with excellent quantitative performance. [4] Though this successful proof-of-principle of a fully automated user-friendly HPTLC-MS system was given in 2007, this promising automatization has not been commercialized due to the high overall costs and the large instrument footprint. Instead, a manually-operated TLC-MS interface was launched by Camag in 2009 (upgraded in 2015), and later imitated by Advion in 2015. The pneumatic cylinder of the Camag elution head and its pneumatic cleaning were manually activated. The Advion interface used almost the same elution head principle, *i. e.* the contact pressure of the elution head is controlled by a stepper motor and the distance by a compression spring. For both interfaces, marking of zones (if UV-light needed), plate positioning and activation of each elution needed user intervention, and thus, it still was a time-consuming process. Detection limits reached by elution head-based HPTLC-MS were lower if compared to other HPTLC-MS techniques (*e. g.,* MALDI-TOFMS [5], DESI-MS [6] and DART-MS [7]), as the whole compound zone can be transferred to MS. However, the manual operations required for each zone elution were inconvenient. As many analytical scientists demand for a user-friendly and efficiently automatized hyphenation, an open-source kit was developed for automation of the Camag TLC-MS Interface 2.

Rapid-prototyping techniques cost-effective electronics and open-source software were employed for streamlined target zone selection, positioning and advanced automation of elution and cleaning functions. After digitalization of the CAMAG TLC-MS Interface (Fig. 1a), all parts for the autoTLC-MS Interface were designed and simulated prior to 3D printing of most parts for plate positioning and housing with mount for the LCD display (Fig. 1b). The height of the plate positioning was limited to just 8 mm between the upper and lower part of the device. Thus, a tailor-made positioning system was designed to avoid excessive costs or an enlarged footprint. An 8-mm high plate holder with x-carrier was designed for a user-friendly plate handling and stabile, reproducible positioning. The plate was slid below the holder and its flanks were pushed over the plate edges for a tight fitting. An x-motor/belt-driven system moved the x-carrier between two y-carriers, also belt-driven by two motors at the rear corner posts. The whole system was easily attached by the corner posts screwed at the originally threaded

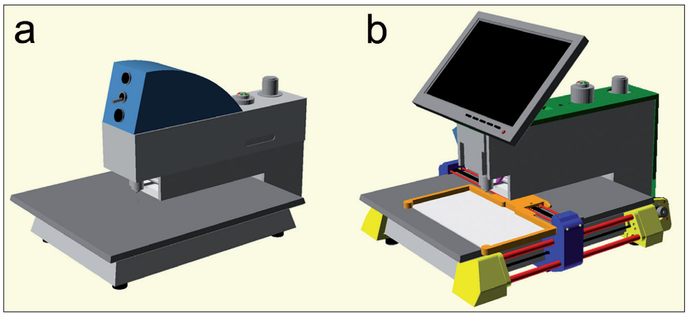

Fig. 1: Digitized original TLC-MS Interface 2 (Camag) with manual user interface (a) as basis to design and simulate all customized add-on parts and functionalities of the autoTLC-MS Interface with digital user interface (b).

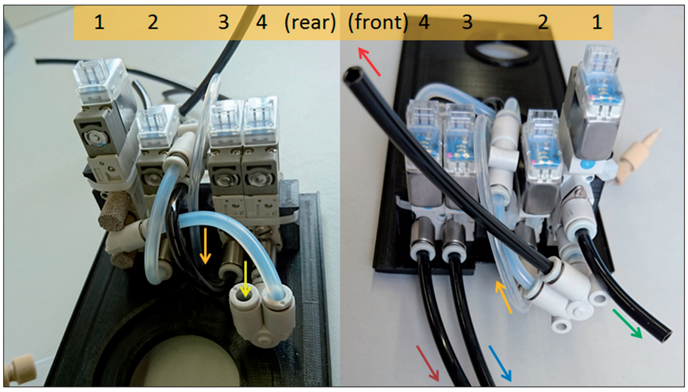

Fig. 2 Timing of elution and cleaning were synchronized and precisely adjusted by four pneumatic valves that control the gas flow (yellow) to the elution head (1, up: red, down: green), head cleaning (2, orange), cleaning drawer (3, blue) and gas flow for elution zone cleaning (4, brown).

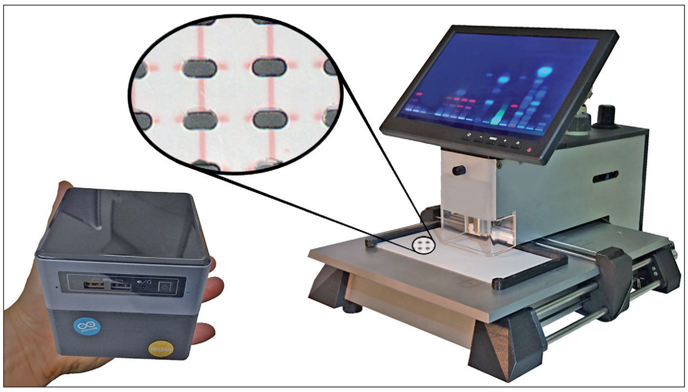

Fig. 3 High processing power for image-based targeting and control via LCD-display for stand-alone automation at highest precision: 3D printed extension of the PC system for the open-source electronics enabled an embedded system with small footprint (11^3 cm^3).

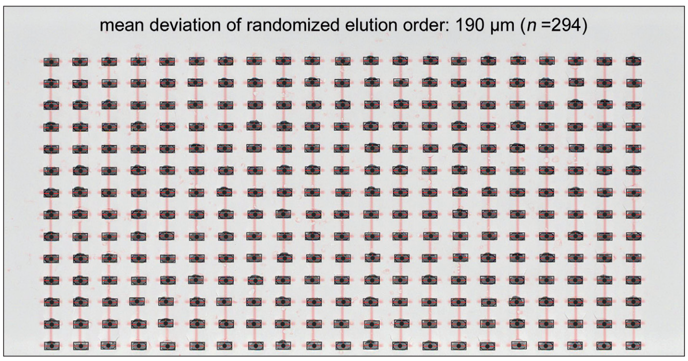

Fig. 4 Zone pattern (294 target zones on one plate) to determine the mean deviation of the positioning (target shift) to be 190 µm (n = 294) for a randomized elution order.

holes of the pedestals below the device. On the upper side, a custom-fit mounting frame was placed below the base plate of the interface to lock the corner posts. This design preserved the small footprint of the device and ensured that all timing belts were guided inside the 3-D printed parts or between the axis rods to increase safety and user-friendly access for maintenance.

Inside the interface, four magnetic valves for the pneumatic system (Fig. 2) and an automatic 6-port valve were mounted for the elution process, which was synchronized with the pneumatic functions and crucial for precise elution times, assignment of resulting MS data and high-throughput processing of the target zones. In contrast to the original manual pneumatic valve, the elution head was now electronically controllable. Likewise, head cleaning and cleaning drawer activation were now automated to remove solvents and residual layer material inside the cutting edge of the elution head. Instead of the their previous simultaneous activation (resulting in spitting of residual solvent from the elution head onto the HPTLC plate or in front of the cleaning drawer), the electronic activation was now independent and a simple 500 ms delay between both provided an optimal cleaning procedure. The duration and pulsation of the cleaning gas through the outlet capillary was now adjustable to optimize the cleaning procedure. Especially for HPTLC-high-resolution (HR)MS, cross-contamination was a major issue, observed for zone elutions with the previous interface. The cleaning functions were expanded by a fourth magnetic valve to provide a gas flow for elution zone cleaning. A nozzle, fixed on the rear side of the elution head, directed this gas flow directly onto the HPTLC plate. Thus, layer particles and dust were removed around the target zone before elution. After elution, residual solvent in the elution zone was quickly removed. As nearby zones were often distorted by residual solvent spreading into the layer material, this improvement was very important for elution of nearby zones.

The complete mechanic user-interface was removed and replaced by an LCD display, a wireless mouse/keyboard and an integrated system for image-based target zone selection and control of the interface (Fig. 3). The open-source boards Arduino Mega 2560 and the Ramps 1.4 shield were modified and mounted in a 3D printed extension of a NUC (Intel) PC (Fig. 3). All motors and valves were then controlled by these boards via GCODE commands. This only 11^3-cm^3 system enabled the stand-alone control of the whole device with enough processing power for image-based target zone selection and batchwise automation of the whole elution and cleaning process, as well as the synchronization with MS data acquisition.

These functions were operated by the extended open-source OC_manager software. Input data were chromatograms under white light or UV light illumination, also after micro-chemical derivatization or effect-directed detection. The import of target zone positions was also possible via CSV file obtained by other detection modes like densitometry. In all input modes, the coordinates of the batch are combined with defined elution times and pausing times between each elution to exclude cross-contaminations. Additionally, settings for the cleaning processes were appended and the complete GCODE transferred to the Arduino board to execute all steps.

The positioning and elution was evaluated for different elution modes. As endurance test, 294 bands of azophloxine were applied via ATS4 with Freemode option (CAMAG) on one HPTLC plate and eluted in a randomized order (Fig. 4). Digital image evaluation was used to determine the mean positioning deviation to be 190 µm (n = 294). This way, the suitability of the autoTLC-MS Interface for reliable high-throughput applications was proven. The HRMS (Q Exactive Plus, Thermo Fisher Scientific) quantification of butyl paraben (EIC data) in a working range of 40–180 pg/band resulted in a determination coefficient R^2 of 0.995 and an LOQ of 40 pg/band. A good quantification performance in the low picogram-range was possible for this automated hyphenation by well-adjusted scan parameters.

Summary

A cost-effective open-source add-on kit was presented for the TLC-MS Interface 2. Exploiting techniques of 3D printing and the RepRap environment [8], this compact design was combined with a digital image-based user interface. The synchronized and standardized workflow with advanced cleaning options provided a high-throughput performance and an enhanced quantitative performance in the picogram-range depending on the MS system. The operation was performed by the open-source OC_manager software. Datasets from different HPTLC detection techniques were processed in an easy operation. Further functionalities can still be implemented like quanTLC for videodensitometric peak detection and quantification [9] as well as image evaluation by artificial neural networks [10] for a non-supervised assignment of zones to be eluted. A download link for all open-source software and hardware as well as a link to a video about the autoTLC-MS Interface is available on our website www.uni-giessen.de/food.

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