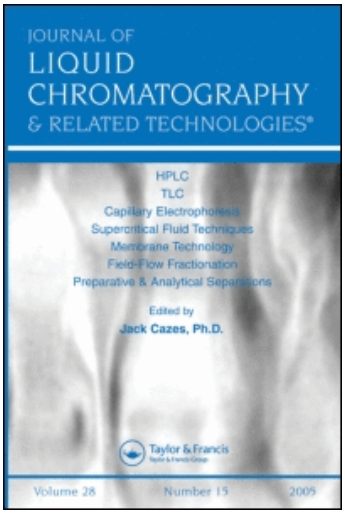

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Efficacy of a Modified Printer for Application of Reagents in Planar Chromatography

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Abstract: A commercially available printer was modified for application of reagents in planar chromatography. Optimal settings of the printer driver regarding utmost reagent transfer were investigated. The spatial resolution of the printer was ascertained to be 0.5 mm by visual inspection and 1.5 mm by scanning. On the example of post-chromatographic derivatization of taurine, the efficacy of printing was evaluated. Therefore, the ninhydrin reagent was adjusted regarding surface tension and viscosity. Repeatability ($n = 5$) showed RSDs of better than $\pm 1.1\%$ and polynomial calibrations correlation coefficients of better than 0.9996. The results obtained for analysis of the energy drink sample 'Red bull' were as good as such obtained by dipping. However, several advantageous were involved by printing, such as very low reagent consumption by a cheap, versatile and clean workstation.

Keywords: Printing of reagents, HPTLC, Planar chromatography, Printer, Method comparison, Taurine

INTRODUCTION

The immense possibilities of post- or pre-chromatographic derivatization are a great advantage of high-performance thin-layer chromatography (HPTLC) over other chromatographic methods. For pre-chromatographic derivatization the starting zones are oversprayed by the reagent and the plate is usually

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heated then. This is a much more efficient way, since it is automated, in contrast to pre-chromatographic derivatization in the vessel one by one.[1] Post-chromatographic derivatization can be performed in three different modes, i.e., the reagent can be applied onto the plate by exposure to vapor, by immersion (dipping), or by spraying.[2] Thereby, the most homogeneous application quality is obtained by evaporation of vapors, like iodine, bromine, or hydrochloric acid, onto the plate or, secondly, by automated dipping. Spraying is mostly performed manually by various spraying devices, either electro-pneumatic or simple glass sprayers with a rubber bulb pump. This way, the reagent application can never be done uniformly and, thus, satisfy the specification for quantitative HPTLC regarding precision. However, a user-friendly fully-automated spraying device, called ChromaJet DS 20 (Desaga, Nümbrecht, Germany), was introduced around 2000 which overcame this drawback and showed a better homogeneity of the reagent application and, thus, an improved precision. This was demonstrated for the analysis of primula saponins.[3] The precision of about 4% obtained by manual spraying was reduced to ca. 2% by automated spraying. The device is able to apply even reagents from different reservoirs to predefined areas (e.g., lanes). It uses a pressurized 'spraying gun' which produces aerosols that are removed by ventilation. Hence, all these approaches are based on application of the reagent solutions as an aerosol with reagent particles in the range of 0.3–10 μm. However, a general disadvantage of spraying an aerosol is the contamination of the whole interior part of the device, despite the grade of automation. Moreover, an increased exposure to reagents and the health concerns implicated affect adversely the reputation of the spraying technique.

On this background, a new technique for spraying was considered which is unharmful, homogenous, sensitive, selective, user-friendly, fast, and cost-effective. Drop on demand printers are commercially available, are cheap, and are commonly used in daily life. Instead of printing on papers, printing on adsorbents would be an option due to the planar nature of the stationary phase. Indeed, the idea of printing solutions onto a plate is not new. The patent of Nyiredy[4] describes a kind of device for fully automatic TLC that covers even more than only the printing step on the layer. However, the experimental proof was missing so far. Only the paper of Nilsson et al. described a kind of modified ink-jet printer for application of monoclonal antibodies onto nylon membranes.[5] Thereby, the ink-jet reservoir was replaced by a syringe. Thus, the usage of printer systems for application of reagents in planar chromatography has not been demonstrated up to now. In this study,[6,7] the employment of commercially available printers for printing on HPTLC plates and foils was shown for the first time and the advantages and limitations of this new technique were investigated. As an example for the post-chromatographic derivatization, taurine (2-aminoethanesulfonic acid) was detected with the ninhydrin reagent whereby printing was compared to dipping.

EXPERIMENTAL

Chemicals

Ink cartridges BCI 3eBk (black, 28 mL) were obtained from Canon (Krefeld, Germany) and empty cartridges from Kopyform (Beindersheim, Germany). Taurine (>99%) and ethanol (HPLC quality) was purchased from Merck (Darmstadt, Germany). Polypropylene glycol (≥99.5%) was obtained from Acros Organics (Geel, Belgium). Ultrapure water (18 MΩ/cm^2) was generated from a Synergy System (Millipore, Schwalbach, Germany). Chromatography was performed on silica gel 60 F_{254} HPTLC glass-backed plates from Merck, 10 cm × 10 cm, 200 μm in thickness. Alternatively, HPTLC aluminum foils (Merck) and polyester foils (Machery & Nagel, Düren, Germany), both silica gel 60 F_{254} of 250 μm thickness, were used. The energy drink was acquired from the local market.

Standard and Derivatization Solutions

50 mg taurine was weighed into a 10 mL volumetric flask and filled up to 10 mL with ultra-pure water (5 μg/μL). For determination 1) of the repeatability this solution was diluted 1:6.25 with water (800 ng/μL) and 1:125 (40 ng/μL), 2) of the functional correlation 1:125 (40 ng/μL), 3) of the limit of detection (LOD) 1:250 (20 ng/μL), and 4) of samples 1:37.5 (133 ng/μL). The solutions stored refrigerated and protected from light were at least stable for 10 days. For derivatization 0.8 g ninhydrin was dissolved in 50 mL ethanol and water 7:3 (v/v). This ninhydrin solution (1.6%) was modified for printing by addition of 4 mL polypropylene glycol (8%).

Sample Preparation

The energy drink sample was degassed for 20 min in an ultrasonic bath. For quantification of taurine, 2 mL of the sample were diluted to 25 mL with water. For determination of the recovery rate (calculated via standard addition), the samples were spiked with 0.4% taurine at the beginning.

Chromatography

The HPTLC plates were washed (pre-chromatography) with methanol and dried for 15 min at 120°C if quantification was intended. Sample and standard solutions were applied with the Automatic TLC Sampler 4 (ATS 4, CAMAG Muttenz, Switzerland) using the following settings: band length

6 mm, track distance 4 mm, distance from left plate edge 10 mm and from the lower plate edge 8 mm, resulting in 8 tracks per plate, dosage velocity 120 nL/s, as application volumes 10 μL of the energy drink sample and different volumes of the taurine standard solutions were applied, i.e., 1 μL (800 ng/band) and 6 μL (240 ng/band) for the repeatability test, and 1 to 8 μL (133–1064 ng/band) for quantification of samples, 1 to 3 μL of the standard solution for functional correlation (40–120 ng/band) and 1–5 μL (20–100 ng/band) for LOD.

Chromatography was performed in a 10 × 10 cm twin trough chamber (CAMAG) up to a migration distance of 80 mm (from the lower plate edge) using a mixture of ethanol and water 3:2 (v/v) as mobile phase. Then, the plate was dried for 3 min in a stream of warm air.

For post-chromatographic derivatization of taurine, the Canon printer cartridge was filled with ca. 28 mL of the modified ninhydrin solution. This solution was printed by the Canon Pixma iP 3000x printer onto the layer, according to the R_F-selective pattern, as a 9 cm × 15 mm area at the position of 35–50 mm from the bottom edge of the plate with a side distance of 5 mm each. Subsequently the HPTLC plate was heated at 100°C for 2 min and the pink-brownish coloring of taurine (MD 42 mm, hR_F 47) was visible.

Detection was performed by TLC Scanner 3 (CAMAG) with a slit dimension of 4 × 0.45 mm and a scanning speed of 100 mm s^{-1}. Absorbance of taurine was measured at 525 nm. For digital documentation, the DigiStore 2 Documentation System (CAMAG) consisting of illuminator Reprostar 3 with digital camera Baumer optronic DXA252 was applied in the visual range in the reflectance mode.

The printing patterns were preferably generated by CorelDRAW software (Corel, Unterschleißheim, Germany) or optionally by Microsoft Word 2003 (Microsoft Deutschland, Unterschleißheim, Germany) WIN PLT 7.1 (HG-Software, Kaltenkirchen, Germany) and Adobe PhotoShop (Adobe Systems, München, Germany). All other instruments were controlled via the software platform winCats 1.4.1 Planar Chromatography Manager (CAMAG).

RESULTS AND DISCUSSION

Modifications of the printer

In initial experiments, the general utility of printers was investigated for planar chromatographic plates/foils as a carrier instead of paper. The standard paper tray was used for aluminum or polyester foils, whereas the CD tray of the printer was employed for glass plates with a limited format of up to 10 × 10 cm. To allow printing on plates, the CD plug-in position had to be removed in the middle part of the CD tray. Two types of printer were investigated, i.e., a piezoelectric-driven (Epson Stylus D88) and a bubble jet printer

(Canon Pixma iP 3000x). However, the Epson Stylus D88 uses chip-controlled cartridges and almost refused printing of colorless liquids, even after resetting of the chip. Additionally, re-placing of the cartridges was tedious because it was just allowed as automated procedure which insisted on an intermediate rinsing step. Therefore, further focus was laid on the more flexible Canon printer. For a better filling and exchange of the Canon Pixma iP 3000x cartridges, the printer was completely uncovered (Fig. 1a). The cartridges (Fig. 1b) were filled with inks or reagents with an in-house built filling station (Fig. 1c) which worked under slight vacuum (membrane vacuum pump).

As usually for manual sprayers or the ChromaJet DS 20, the tubes had to be rinsed with a rinsing solvent after usage to avoid clogging, contamination, or cross-over. In the case of the printer, the printing head was purged by a rinsing cartridge filled with a suitable solvent, preferably methnol.

Optimization of the Printer Driver Settings

The standard loading of paper with ink was around 1 mg/cm^2 which were, indeed, not sufficient for application of reagents onto HPTLC foils and plates. Arising questions regarding the printer driver, such as addressing the right cartridge(s) and achieving maximum reagent output/transfer with which software had to be clarified first. The paper feed, paper type, color balance, intensity, brightness, and print type selected had an influence on the reagent loading onto the plate. Therefore, the loading at different settings with different software was investigated gravimetrically by printing a 64 cm^2 black square on a paper and measuring the consumption of the ink in the cartridge. The best settings for the Canon printer are summarized in Table 1. The best software for constructing the printing pattern was CorelDraw because better than 95% of the loading was obtained by a single cartridge (not mixed from several cartridges).

The different color inks of the two printers investigated showed a surface tension between 30 and 32 mN/m, a viscosity ranging from 2.2 to 3.8 mm^2/s, and a density of 1.04 to 1.08 kg/L. Thus, the use of reagent solutions instead of inks required an adjustment of the viscosity and surface tension. This was necessary to prevent either uncontrolled dropping of the reagent or clogging of the print head. For this purpose, polypropylene glycol was used. The addition investigated ranged from 5% to 20%, whereby the best adjustment was obtained by addition of 8% polypropylene glycol. Additionally, the reagent transfer rate was improved to up to 30%, which was advantageous because the reagent loading of the plate influenced the capability of detection.

Further on, detectability was dependent on the reagent concentration. The highest printer-suited concentration of ninhydrin was established to be 1.6% (Figure 2a). Too high concentrations could cause crystallization of the

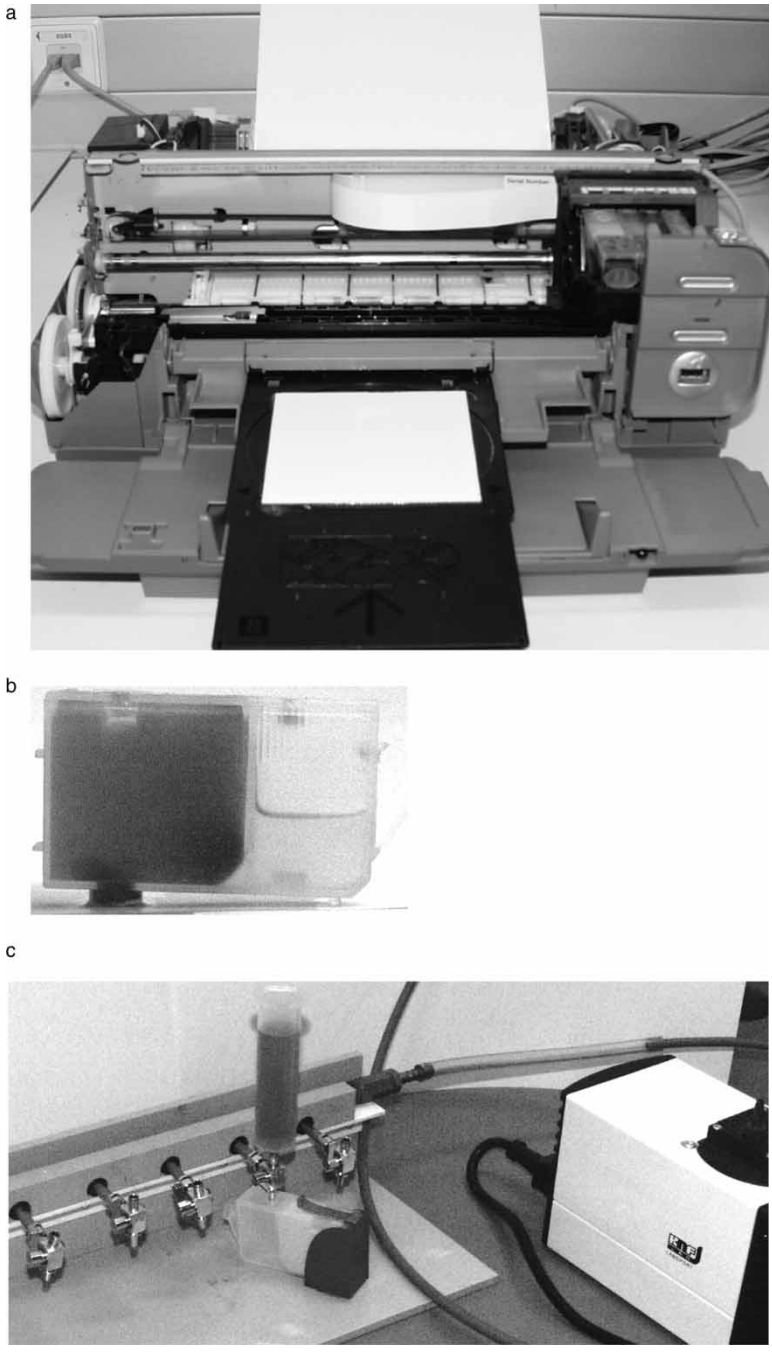

Figure 1. Work station for printing: (a) Modified reagent printer Canon Pixma iP 3000x, (b) cartridge, and (c) cartridge filling station working under slight vacuum.

Table 1. Best settings for the Canon printer regarding utmost plate loading; determined gravimetrically

Canon Pixma iP 3000x	
Paper selection	Normal paper
Print quality	High
Image type	None
Change of color quality	Printing color +50, other cartridges −50
Intensity	+50
Paper feed	Upper feed

substance in and clogging of the print head. As the reagent output was limited by the printer driver, multiple print repetitions were performed to check the impact on detectability (Figure 2b). The signal intensities of taurine (peak height) were best if a 4-fold printing on the same area was performed (Figure 3). Further repetitions had no significant influence on the signal intensity. This clearly showed the limitation of the plate loading by the given printer driver.

All printer settings and parameters investigated were summarized in a mind map (Figure 4), whereby the best options regarding plate loading are marked bold. Moreover, items demonstrating the efficacy of printing are included in the same figure.

Efficacy of Printing

Derivatization patterns can be assigned track-selective, substance(hR_F)-selective, or starting zone-selective, which is required for pre-chromatographic derivatization (Figure 5). By printing, the utmost flexibility was

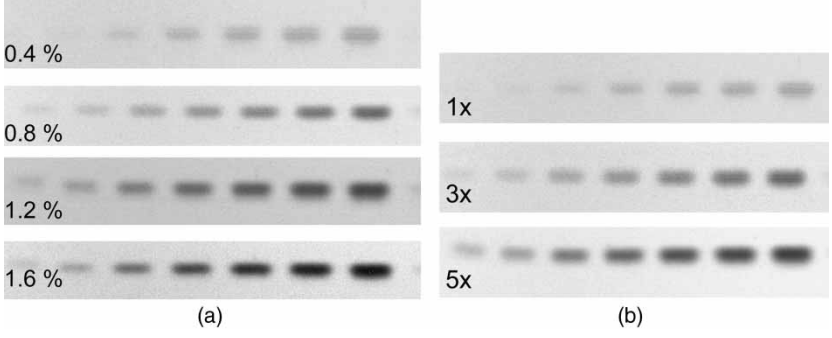

Figure 2. Influence of the plate loading on detectability (a) by increasing reagent concentrations from 0.4%, 0.8%, 1.2% to 1.6% ninhydrin, and (b) by multiple print repetitions of 1, 3, and 5 times.

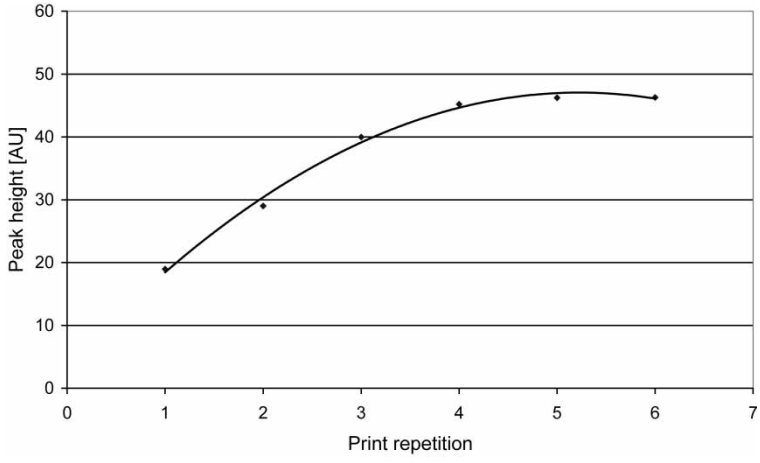

Figure 3. Peak height intensities depending on increased plate loading achieved by print repetitions up to 4 × further repetitions do not significantly improve the signal intensity.

obtained for comparison of different tracks. The individual tracks of a sample, which was applied multiply on the plate, could be developed under identical conditions, however, derivatized by different reagents side by side. The different detections related to the same sample could be matched at a glance.

The spatial resolution of the Canon printer was highly satisfying for the application of the different patterns. Even very narrow substance-selective windows could be assigned because the spatial resolution of the printer was ascertained to be 0.5 mm by visual inspection. By using the TLC Scanner 3, a baseline separation was obtained for two lines printed at a 1.5 mm-distance (Figure 6). Of course, such narrow substance windows would imply an automated developing chamber with an integrated humidity control of the plate, which guarantees good repeatability. Using such climate-controlled chambers, it was shown on the example of heterocyclic aromatic amines that the RSD ($n = 6$ plates) of the migration distance was better than $\pm 1.3\%$ ($\leq \pm 0.5$ mm) if the mean value of 14 tracks per plate was considered or better than $\pm 2.7\%$ ($\leq \pm 1.1$ mm) if just a single track on the plate was compared over 6 plates.[8]

The consumption of reagents based on gravimetric measurements was calculated to be 10 $\mu L/cm^2$. This very low consumption is especially important in the case of expensive reagents. Additionally, the work place was clean over the whole period. This was considered as an important improvement compared to conventional spraying, either automated or manual.

On the example of taurine, post-chromatographic derivatization with ninhydrin was performed by printing. After heating of the plate, the

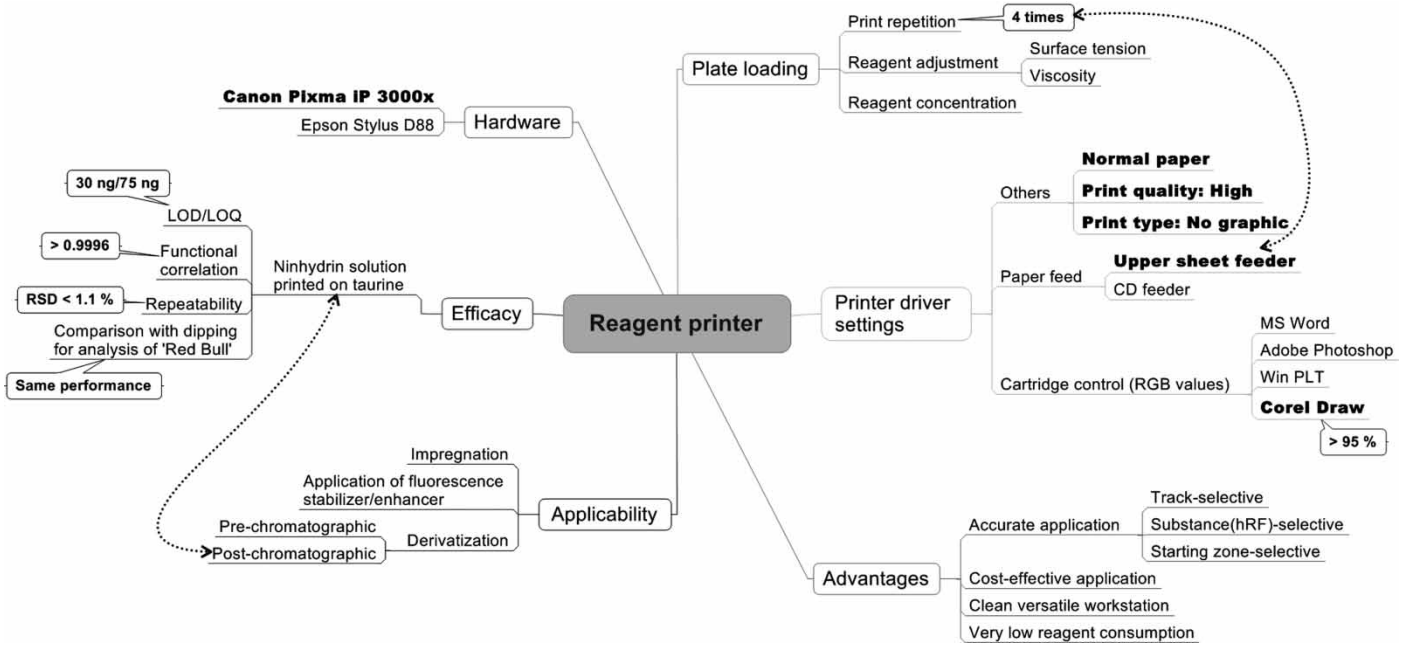

Figure 4. Mind map of all printer parameters investigated; the best settings and choices are marked bold.

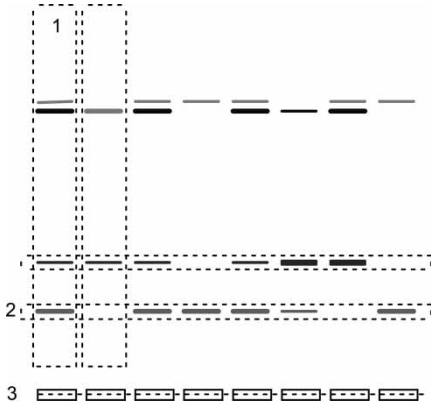

Figure 5. Increased possibilities for application by printing a **1** track-selective, **2** substance(hR_F)-selective or **3** starting zone-selective pattern (for pre-chromatographic derivatization).

pink-brownish color was quantified by absorbance measurement at 525 nm. The repeatability ($n = 5$, Figure 7a) of the post-chromatographic derivatization of taurine (800 ng/band) with ninhydrin printed on the plate showed an RSD of $\pm 1.0\%$ (peak height) and $\pm 1.1\%$ (peak area). At a

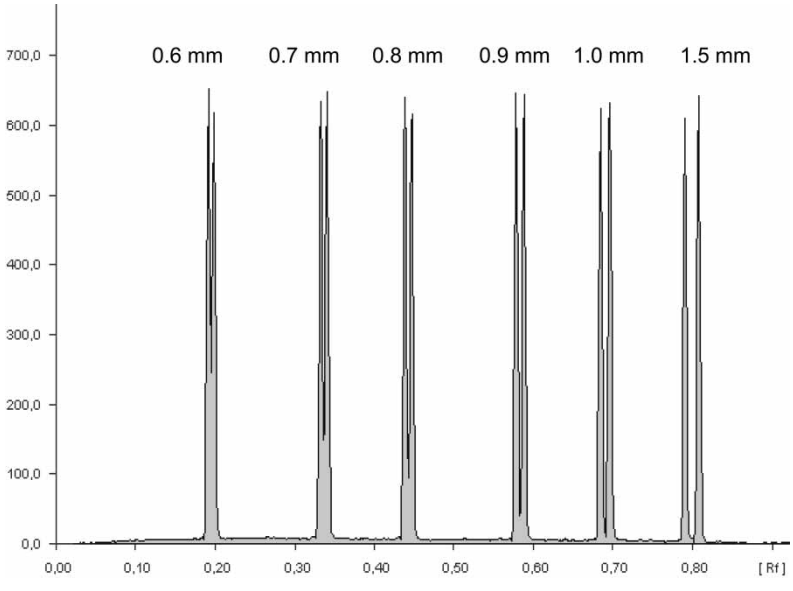

Figure 6. Spatial resolution of the Canon Pixma iP 3000x printer: two lines were printed with black ink in different distances of 0.5 to 1.5 mm and scanned by TLC Scanner 3.

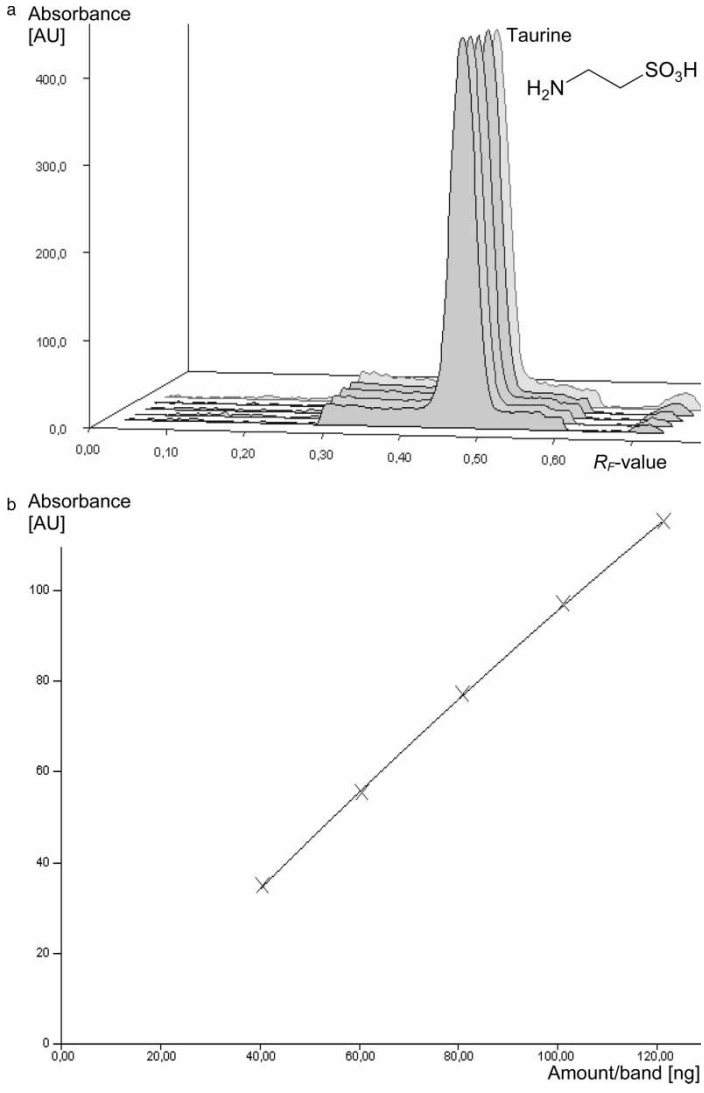

Figure 7. Validation of the post-chromatographic derivatization of taurine with ninhydrin by printing: (a) Repeatability (RSD, $n = 5$) of the taurine standard solution was $\pm 1\%$ (800 ng/zone each, absorbance measurement at 525 nm), (b) polynomial calibration $y = -0.001x^2 + 1.182x - 11.419$ with a correlation coefficient of 0.9999 and a RSD of $\pm 0.87\%$ (peak height, 40–120 ng/band).

lower amount of taurine (240 ng/band), repeatability was similar and showed an RSD of $\pm 1.1\%$ ($n = 5$). The polynomial calibration ($y = -0.001x^2 + 1.182x - 11.419$) had a correlation coefficient of 0.9999 and an RSD of $\pm 0.87\%$ (peak height, 40–120 ng/band), even when the

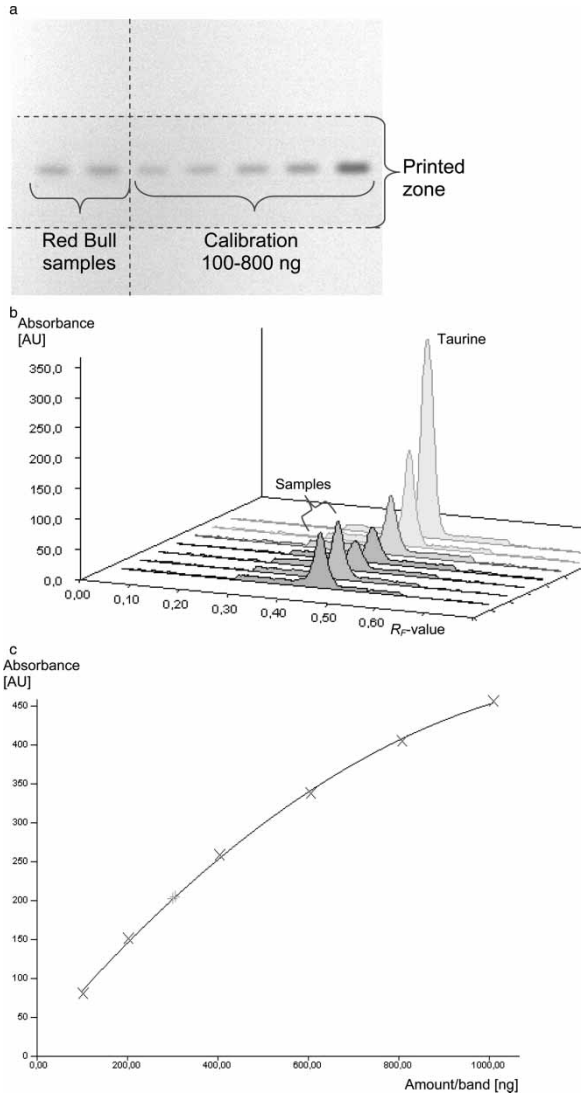

Figure 8. Post-chromatographic derivatization of taurine in the energy drink 'Red Bull' with ninhydrin solution (1.6%) printed 4 times as area: (a) HPTLC plate image (section), (b) absorbance scan at 525 nm and (c) polynomial calibration of taurine with a correlation coefficient of 0.9996 (peak height, 0.1–1 μg/band).

working range was started near the LOD (Figure 7 b). The validation data showed that printing offered a homogeneous reagent transfer and reliable data. Encouraged by this results, the new derivatization method was compared to dipping.

Table 2. Comparison of the results for taurine determination in the energy drink 'Red Bull' (content label 0.4%) obtained by dipping in and printing of the ninhydrin solution

	Dipping[9]	Printing
Functional correlation		
RSD	±0.9%	±1.0%
Correlation coefficient r	0.9998	0.9996
LOD	41 ng	30 ng
LOQ	82 ng	75 ng
Repeatability (RSD, $n = 5$)	±0.9%	±1.0%
Taurine found	0.37% ($n = 4$)	0.35% ($n = 2$)
Recovery rate ($n = 3$)	103% ± 3.0%	98% ± 2.8%

Comparison of Printing with Dipping Regarding 'Red Bull' Samples

For post-chromatographic derivatization of taurine in the energy drink sample 'Red Bull', the ninhydrin solution (1.6%) was printed 4 times as area onto the aluminum foil. The printing area can clearly be seen (Figure 8a and b).

Obtained by printing, the LOD of taurine was established to be 30 ng/zone (S/N 3) and the LOQ 75 ng/zone (S/N 10). The calibration in the working range of 0.1 to 1 μg/band was polynomial and showed a correlation coefficient of 0.9996 (Figure 8c). The recovery rate ($n = 3$) was established by standard addition to be 98% ± 2.8% at 0.4 g/100 mL. A spiking level of 0.4% taurine was chosen, which is equivalent to the label claim. The values obtained were compared with a given method based on dipping[1] (Table 2).

CONCLUSION

The selective derivatization of distinct areas on a plate, the immense spatial resolution (1.5 mm by scanning), and the very low consumption of reagents (~10 μL/cm^2) are major advantages of this new application technique. Compared to manual and automated spraying devices, the printer avoids the formation of aerosols and keeps the working place clean. As no ventilation impacts the reagent application, it offers a homogeneous reagent transfer all over the plate and, hence, a good precision (RSD of better than ±1.1%). Commercially available printers are rather cheap, lightweight, and versatile devices that could be used in a lab on-site, not only for printing reagents. The comparison of printing with dipping on the example of the detection of taurine with ninhydrin solution showed comparable results and proofed the general functioning of printing.

In further investigations, focus will be laid on the modification of the printer driver to obtain an improved reagent output, besides the development of print-suited reagent formulations and the circumvention of chip-controlled ink cartridges. The selective and defined application of fluorescence stabilizer/enhancer solutions or plate impregnation solutions could be further fields of application.

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