Esmaiili and Shetab-Boushehri *Journal of Analytical Science and Technology* (2017) 8:22
DOI 10.1186/s40543-017-0131-z

Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

Comparative verification study of silica gel-coated TLC and HPTLC plates' performances in separation of opium alkaloids on the basis of their physicochemical properties

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Abstract

Background: Thin layer chromatography (TLC) has been a conventional analytical method which is currently used for separation and detection of drugs in biological and non-biological samples. High-performance thin layer chromatography (HPTLC) has been claimed to have several advantages over TLC due to its smaller adsorbent particle size. Opium is the dried latex obtained from the opium poppy containing several alkaloids. Its precise and accurate detection in biological and non-biological samples is a matter of great importance in forensic and clinical toxicology.

Methods: In the present work, quality of commercial silica gel-coated TLC and HPTLC plates from the two best-known international manufacturers (Merck and Macherey Nagel) in separation of opium alkaloids were investigated and compared in the fluorescence quenching mode to propose the best plate for separation and identification of opium alkaloids. Particle size, zeta potential, specific surface, density, and scanning electron photomicrographs of silica gels, development time for 10 cm migration distance, number of theoretical plates (N), and resolution (R) were compared between all plates.

Results: Merck TLC plate showed the best separation of opium alkaloids. Although spherical-particle silica-coated Merck HPTLC plate showed narrower particle size distribution, it did not show better separation than Merck TLC plates.

Conclusions: On the basis of performance and price of TLC and HPTLC plates and great importance of precise and accurate detection of opium in forensic and clinical toxicology, the present work proposes conventional verified analytical TLC plates (for example Merck-TLC plate) for detection of opium alkaloids in biological and non-biological samples.

Keywords: Opium alkaloids, TLC, HPTLC, Physicochemical properties

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Background

Opium is an air-dried latex obtained by incision from the unripe seed pods/capsules of *Papaver somniferum* (Papaveraceae). It varies in color from yellow to dark brown and has a characteristic odor and a bitter taste. It is a non-homogeneous, sticky, tar-like and dark brown material when fresh, and fragile and hard when aged. It contains about 40 alkaloids, which constitute about 25% of the opium, up to 25% water, meconic acid, some lactic and sulfuric acids, albumin, mucilage, sugar, and some resinous and waxy-like substances. The principal active alkaloids of opium are morphine (4 to 21%), codeine (0.7 to 3%), thebaine (0.2 to 1%), papaverine (0.5 to 1.3%), and noscapine (2 to 8%) (Moffat et al. 2011).

Addiction to opium has been a worldwide problem for a long time (Mehendale et al. 2013; Kalantari Meibodi et al. 2015; Ebrahimi et al. 2011). Poisoning with opium, its alkaloids, and opioids are very common in some countries (Westermeyer et al. 1991). Thus, their precise and accurate detection in biological and non-biological samples is a matter of great importance in forensic and clinical toxicology (Ahadi et al. 2011).

Thin layer chromatography (TLC) is one of the most favorite and conventional methods for screening and detection of drug abuse (Shetab Boushehri et al. 2009). Simultaneous development of drugs and poisons on one TLC plate has made it a rapid method (Ahadi et al. 2011; Shetab Boushehri et al. 2009). Modern computer-controlled scanning instruments and automated sample application and development instruments permit accuracy and precision in quantification that are, in many cases, comparable with those obtained with high-performance liquid chromatography (HPLC) and gas chromatography (GC). There is a wide range of layers and developing solvents (acidic, basic, completely aqueous, aqueous-organic). Solvents that interfere with HPLC UV detection can be used in TLC because the mobile phase can be removed from the plate before detection. Every sample is separated on a fresh layer, so that problems result from retaining and cross-contamination of samples and sorbent regeneration procedures are avoided. Low mobile-phase consumption results in reduced costs of solvent purchase and disposal. Because layers are normally disposable, sample preparation methods are less required, and complex, impure samples can be applied to the layer without concern for the interfering peaks and retained compounds that shorten the life of HPLC columns. Simultaneous sample cleanup and separation of target compounds are often achieved with TLC (Sherma 2003).

In the present work, quality of commercial silica gel--cotaed TLC and HPTLC (high-performance TLC) plates from two best-known international manufacturers (Merck and Macherey Nagel (MN)) in separation of opium alkaloids were investigated and compared in the fluorescence quenching mode to propose the best plate for separation and identification of opium alkaloids. Our objective was not to detect opium alkaloids by TLC and HPLC; rather, we investigated whether TLC plates or HPTLC plates are better in separation of opium alkaloids on the basis of physicochemical properties of their silica gel coatings.

Methods

Materials

Merck TLC plates (Art. No.: 1.05735.0001, silica gel 60 UV_{254} pre-coated, 20 × 20 cm, 200 μm layer thickness, plastic-backed, mean particle size: 10–12 μm, particle size distribution: 5–20 μm), Merck HPTLC plates (Art. No.: 1.05548.0001, silica gel 60 UV_{254} pre-coated, 20 × 20 cm, 200 μm layer thickness, aluminum-backed, mean particle size: 5–6 μm, particle size distribution: 4–8 μm), Merck Lichrospher® HPTLC plates (Art. No.: 1.05586.0001, silica gel 60 UV_{254} pre-coated, 20 × 20 cm, 100 μm layer thickness, aluminum-backed, mean particle size: 3–5 μm, particle size distribution: 6–8 μm), MN TLC plates (Art. No.:805,023, silica gel 60 UV_{254} pre-coated, 20 × 20 cm, 200 μm layer thickness, polyester sheet, particle size distribution: 5–17 μm), MN HPTLC plates (Art. No.:818,143, silica gel 60 UV_{254} pre-coated, 20 × 20 cm, 200 μm layer thickness, aluminum sheet, particle size distribution: 2–10 μm) were gifts from the respective companies. Crude opium powder, morphine hydrochloride, codeine phosphate hydrate, papaverine hydrochloride, noscapine hydrochloride hydrate were gifts from Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran. Acetone, toluene, ethanol, and concentrated ammonia solution (32%) were from Merck, Germany.

Solutions

Opium and opium alkaloids reference standards were prepared as previously described (The Japanese pharmacopoeia 2006). Briefly, 0.1 g of powdered opium was added to 5 ml of diluted ethanol (70% *v*/v) and dissolve by treating with ultrasonication for 10 min. Sufficient diluted ethanol (70% v/v) was added to make 10 ml final volume. The solution was centrifuged (5000 rpm, 5 min) and the supernatant was used for spotting. 25 mg of morphine hydrochloride hydrate, 12 mg of codeine phosphate hydrate, 2 mg of papaverine hydrochloride, and 12 mg of noscapine hydrochloride hydrate were separately dissolve in 25 ml of diluted ethanol (70% v/v), and the resulting solutions were used as the standard solutions 1, 2, 3, and 4 respectively.

Instruments

Flat bottom TLC chamber for development of 20 × 20 cm TLC plates (CAMAG, Switzerland), a dual-wavelength (254/366 nm) UV cabinet (CAMAG, Switzerland), Linomat 5 (CAMAG, Switzerland), a TLC Scanning Densitometer (Shimadzu CS-9000, Japan) and 4 decimal place analytical balance (Sartorius, Entris124-1S, Germany) were used in this study.

Measurements

2 × 2 cm piece of each of 5 plates (Merck TLC, HPTLC, Lichrospher® HPTLC, and MN TLC and HPTLC plates) in quintuplicate plate samples were cut and their silica coating were scraped. The scraped silica samples were used for determination of density, particle size, zeta potential, specific surface, and particle shape.

Mass of each scraped silica sample was measured by weighting with the 4 decimal place analytical balance and the respective density was calculated by division of mass by the volume of silica coating (2 cm × 2 cm × layer thickness in cm). The density for each plate silica sample was repeated in quintuplicate plates and individual measurements were averaged.

Particle size and zeta potential of scraped silica in distilled water were determined by a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK) and a Zetasizer (Nano ZS, Malvern Instruments Ltd., Malvern, UK), respectively. The particle size and zeta potential for each plate silica sample were repeated in quintuplicate plates and individual measurements were averaged.

Specific surfaces of scraped silica by BET method were determined by Nano SORD (Toseye Hesgarsazan Asia Co., Iran). The specific surface for each plate silica sample was repeated in quintuplicate plates and individual measurements were averaged.

Scraped silica samples were photographed by a field-emission scanning electron microscope (FE-SEM) (Hitachi, Model S-4160, Japan) to study the morphology of silica particles.

Initial zones were applied as bands by spraying of 10 μl of standards with a Camag Linomat 5 fitted with a 100-μL syringe and operated with the settings: band length 6 mm, rate of application 4 μl s^{-1}, table speed 10 mm s^{-1}, distance between bands 10 mm, distance from the plate edge 10 mm, distance from the bottom of the plate 1.5 cm.

Plates were developed with a mixture of acetone, toluene, ethanol (99.5% *v*/v), and ammonia water (28% *w*/w) (20:20:3:1) as mobile phase to a distance of 10 cm and then air-dried in a flat bottom Camag TLC chamber containing a common filter paper saturation pad in the back wall. The tank was left to equilibrate for 20 min before insertion of the spotted plate with the layer support facing the saturation pad (The Japanese pharmacopeia 2006). The development distance was 10 cm for all TLC and HPTLC plates. Chromatography for each plate was repeated on quintuplicate plates and individual measurements were averaged.

Development time for each plate was measured for quintuplicate plates and individual measurements were averaged.

After development, plates were dried in a stream of cold air for 10 min. Chromatograms were viewed under 254-nm UV light in the Camag UV-viewing cabinet. Chromatograms of the samples were scanned at 254 nm in the single-wavelength, single-beam mode with the TLC Scanning Densitometer with a slit setting of 7 mm length and 1 mm height.

Data for calculation of number of theoretical plates (*N*; efficiency) and resolution (*R*) values of seven adjacent largest peaks including migration distance from the origin to the mobile phase front (*l*), migration distance from the origin to the center of each solute zone (*z*), and chromatographic zone width in the direction of mobile phase migration (*w*) were obtained by ruler of PhotoShop CS6 software on each densitogram. *N* for each spot and *R* for two adjacent largest peaks were measured in quintuplicate plates and individual measurements were averaged. The equations below were used to calculate *N* and *R*, respectively, as previously described (Halkina and Sherma 2006):

$$N = \frac{16 \times l \times z}{w^2}$$

$$R = \frac{z_1 - z_2}{0.5(w_1 + w_2)}$$

Subscripts 1 and 2 indicate two adjacent largest peaks.

Statistical analysis

Densities, particle sizes, zeta potentials, specific surfaces, development times, *N*, and *R* values of seven adjacent largest peaks of plates were compared by one-way analysis of variance (ANOVA) followed by Scheffe Post-Hoc on SPSS statistical package. *P* values less than 0.05 were statistically considered significant.

Results and discussion

Densities of scraped silica gel samples from all 5 plates were shown in Table 1. Density of Merck-TLC plate silica (0.331 ± 0.061 g cm^{-3}) was significantly ($p < 0.05$) greater than that of Merck-Lichrospher plate (0.137 ± 0.046 g cm^{-3}) but lower than those of MN-TLC (0.518 ± 0.036 g cm^{-3}; $p < 0.05$) and MN-HPTLC (0.498 ± 0.004 g cm^{-3}; $p < 0.05$) plates silica. Density of Merck-HPTLC plate silica (0.319 ± 0.008 g cm^{-3}) was significantly ($p < 0.05$) greater than that of Merck-Lichrospher

Table 1 Physicochemical properties of silica gel-coated Merck-TLC, Merck-HPTLC, Merck-Lichrospher, MN-TLC, and MN-HPTLC plates

	Plate				
Property	Merck-TLC	Merck-HPTLC	Merck-Lichrospher	MN-TLC	MN-HPTLC
Density ($g.cm^3$)	0.331 ± 0.061	0.319 ± 0.008	0.137 ± 0.046	0.518 ± 0.036	0.498 ± 0.004
Specific surface ($m^2.g^{-1}$)	236.18 ± 6.35	239.60 ± 25.01	425.58 ± 123.87	366.45 ± 3.22	372.87 ± 26.06
Mean particle size (μm)	12.0 ± 0.7	6.7 ± 0.1	6.8 ± 0.3	20.1 ± 0.7	15.2 ± 0.1
Zeta potential (mV)	−31.6 ± 6.0	−35.9 ± 8.1	−40.4 ± 5.1	−12.6 ± 4.7	−19.5 ± 4.0
Migration distance (cm)	10	10	10	10	10
Development time (min)	19.33 ± 0.45	27.62 ± 0.39	19.83 ± 0.42	24.06 ± 0.67	17.92 ± 0.30
Plate number (spot 1)	85.7 ± 7.2	204.4± 115.9	172.3 ± 7.7	162.6 ± 53.6	146.0 ± 60.7
Plate number (spot 2)	625.8 ± 302.2	656.4± 158.8	396.6 ± 122.8	538.9 ± 188.2	567.3 ± 58.0
Plate number (spot 3)	1384.1 ± 662.6	999.2± 243.4	766.9 ± 322.0	505.7 ± 153.0	390.0 ± 30.8
Plate number (spot 4)	1180.8 ± 107.2	576.4± 484.4	958.7 ± 239.2	91.5 ± 8.7	750.5 ± 333.8
Plate number (spot 5)	812.9 ± 126.5	2725.3± 196.7	1366.7 ± 197.0	2893.2 ± 2097.5	1837.3 ± 369.0
Plate number (spot 6)	567.0 ± 68.6	851.2± 257.4	645.7 ± 116.9	1168.2 ± 1133.0	589.9 ± 185.5
Plate number (spot 7)	865.7 ± 546.3	1273.6± 516.2	1241.4 ± 63.6	897.1 ± 221.4	809.7 ± 76.5
Resolution (spot 1–spot 2)	0.90 ± 0.13	1.11± 0.13	0.75 ± 0.16	0.63 ± 0.52	0.98 ± 0.08
Resolution (spot 2–spot 3)	1.95 ± 0.11	1.83± 0.31	1.70 ± 0.04	1.09 ± 0.16	1.26 ± 0.27
Resolution (spot 3–spot 4)	0.88 ± 0.12	0.65± 0.33	0.75 ± 0.07	0.46 ± 0.06	0.61 ± 0.09
Resolution (spot 4–spot 5)	0.85 ± 0.11	0.71± 0.46	0.99 ± 0.02	0.53 ± 0.05	0.89 ± 0.16
Resolution (spot 5–spot 6)	1.30 ± 0.42	1.85± 0.41	1.26 ± 0.13	1.17 ± 0.24	1.54 ± 0.33
Resolution (spot 6–spot 7)	1.02 ± 0.03	1.133± 0.25	1.53 ± 0.00	1.54 ± 0.80	0.98 ± 0.07

For explanation about significant differences of each parameter between plates, please see the Results and discussion section

plate but lower than those of MN-TLC ($p < 0.05$) and MN-HPTLC ($p < 0.05$) plates silica. Density of Merck-Lichrospher plate silica was significantly ($p < 0.05$) lower than those of MN-TLC ($p < 0.05$) and MN-HPTLC ($p < 0.05$) plates silica. No significant differences between silica densities of other plates were detected.

Specific surfaces of scraped silica gel samples from all five plates were shown in Table 1. Specific surface of Merck-Lichrospher silica gel ($425.58 \pm 123.87\ m^2\,g^{-1}$) was significantly greater than those of Merck-TLC ($236.18 \pm 6.35\ m^2\,g^{-1}$; $p < 0.05$) and Merck-HPTLC ($239.60 \pm 25.01\ m^2\,g^{-1}$; $p < 0.05$) plates. No significant differences between specific surfaces of silica gel samples of other plates were detected.

FE-SEM microphotographs of scraped silica gel samples from all five plates are shown in Fig. 1. As it is seen, except Merck-Lichrospher silica which had a spherical shape, plates had irregular shape.

Particle size distribution curves and mean particle size of scraped silica gel samples from all five plates are shown in Fig. 2 and Table 1 respectively. Mean particle size of Merck-TLC plate (12.0 ± 0.7 μm, compare with respective manufacturer's claim of 10–12 μm) was significantly ($p < 0.05$) greater than that of Merck-HPTLC plate (6.7 ± 0.1 μm, compare with respective manufacturer's claim of 5–6 μm). It was significantly ($p < 0.05$) greater than that of Merck-Lichrospher plate (6.8 ± 0.3 μm, compare with respective manufacturer's claim of 3–5 μm) but significantly ($p < 0.05$) smaller than those of MN-TLC (20.1 ± 0.7 μm, compare with respective manufacturer's claim of 5–17 μm) and MN-HPTLC (15.2 ± 0.1 μm, compare with respective manufacturer's claim of 2–10 μm) plates. Mean particle size of Merck-HPTLC plate was significantly smaller than those of MN-TLC ($p < 0.05$) and MN-HPTLC ($p < 0.05$) plates. Mean particle size of Merck-Lichrospher plate was significantly smaller than those of MN-TLC ($p < 0.05$) and MN-HPTLC ($p < 0.05$) plates. Mean particle size of MN-HPTLC plate was significantly ($p < 0.05$) smaller than that of MN-TLC plate. No significant differences between mean particle sizes of other plates were detected.

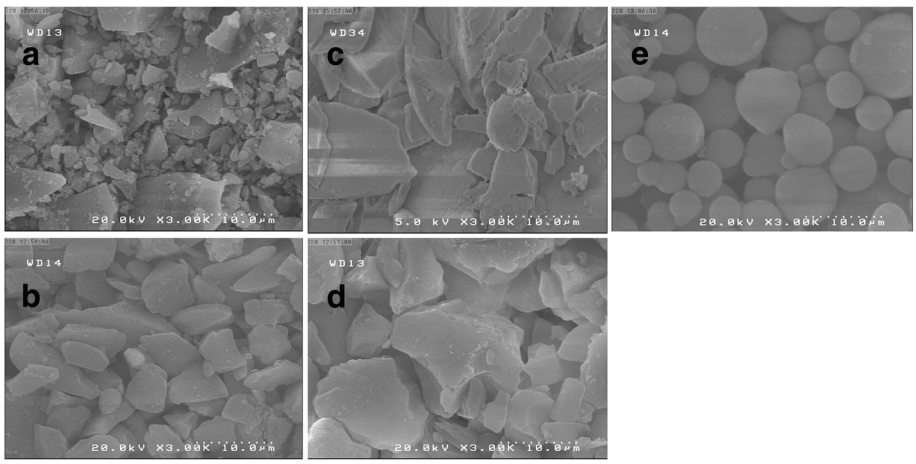

Fig. 1 Field-emission scanning electron photomicrographs of scraped silica. Magnification: 3000×. **a** Merck TLC plate. **b** Merck HPTLC plate. **c** MN TLC plate. **d** MN HPTLC plate. **e** Merck Lichrospher plate

Mean zeta potential of scraped silica gel particle samples from all five plates are shown in Table 1. Mean zeta potential of Merck-TLC plate particles (−31.6 ± 6.0 mV) was significantly ($p < 0.05$) lower than that of MN-TLC plate (−12.6 ± 4.7 mV). Mean zeta potential of Merck-HPTLC plate particles (−35.9 ± 8.1 mV) was significantly ($p < 0.05$) lower than that of MN-TLC plate. Mean zeta potential of Merck-Lichrospher plate particles (−40.4 ± 5.1 mV) was significantly lower than those of MN-TLC ($p < 0.05$) and MN-HPTLC (−19.5 ± 4.0 mV; $p < 0.05$) plates. No significant differences between mean zeta potentials of other plates' particles were detected.

Development times of all five plates are shown in Table 1. Development time of Merck-TLC plate (19.33 ± 0.45 min) was significantly shorter than those of Merck-HPTLC (27.62 ± 0.39 min; $p < 0.05$) and MN-TLC (24.06 ± 0.67 min; $p < 0.05$) plates. Development time of Merck-HPTLC plate was significantly longer than those of Merck-Lichrospher (19.83 ± 0.42 min; $p < 0.05$), MN-TLC ($p < 0.05$), and MN-HPTLC (17.92 ± 0.30 min; $p < 0.05$) plates. Development time of MN-TLC plate was significantly ($p < 0.05$) longer than that MN-HPTLC plate. No significant differences between development times of other plates were detected.

Densitograms of opium alkaloids separation by silica gel-coated Merck-TLC, Merck-HPTLC, Merck-Lichrospher, MN-TLC, and MN-HPTLC plates were concurrently shown in Fig. 3. According to retention factors (R_fs) of standard solutions 1, 2, 3, and 4, peaks 1, 4, 6, and 7 were detected as morphine, codeine, papaverine, and noscapine, respectively. *N* and *R* values of seven adjacent largest peaks of all five plates were shown in Table 1. *N* for zone four of Merck-TLC plate (1180.8 ± 107.2) was significantly ($p < 0.05$) greater than that of MN-TLC plate (91.5 ± 8.7). No significant differences between *N* of other zones of other plates were detected.

R between zones 2 and 3 (R_{2-3}) in Merck-TLC plate (1.95 ± 0.11) was significantly ($p < 0.05$) greater than that of MN-TLC plate (1.09 ± 0.16; $p < 0.05$) and MN-HPTLC plate (1.26 ± 0.27; $p < 0.05$). R_{2-3} in Merck-HPTLC plate (1.83 ± 0.31) was significantly ($p < 0.05$) greater than that of MN-TLC plate. No significant

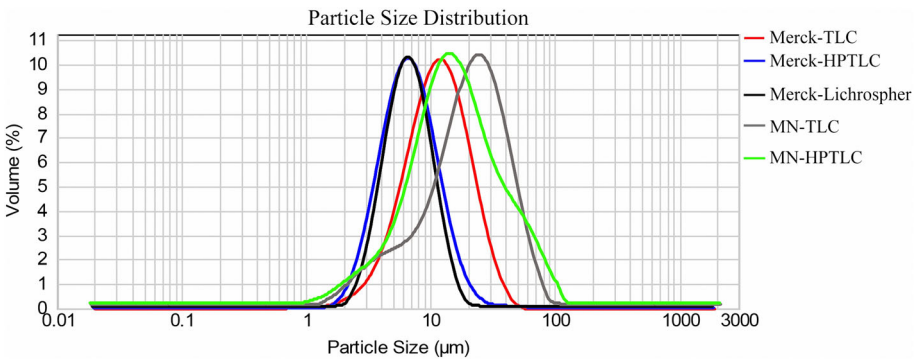

Fig. 2 Concurrent representation of particle size distributions of scraped silica

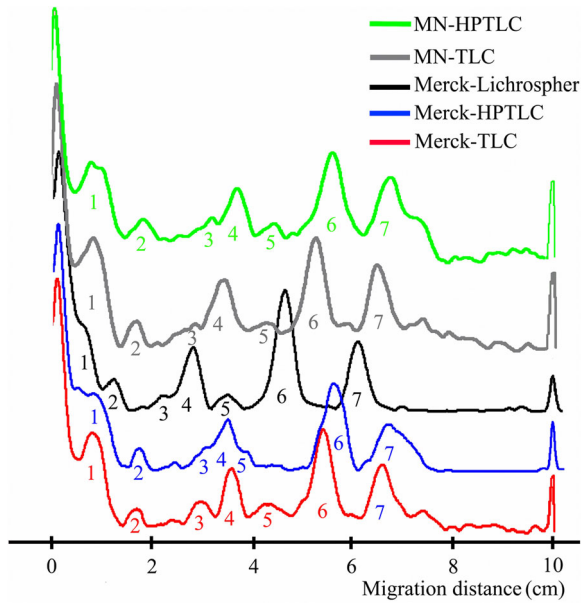

Fig. 3 Concurrent representation of densitograms obtained from separation of opium alkaloids on plates

differences between R of other two adjacent zones in other plates were detected.

TLC and HPTLC have found many applications in chemistry, toxicology, and pharmaceutical sciences (Ahadi et al. 2011; Shetab Boushehri et al. 2009). Silica gel has been one of the most widely used adsorbent in TLC so far (Ahadi et al. 2011). TLC silica gel is a porous inorganic material, which is characterized by particle size, pore size, pore volume, specific gravity, specific surface, and pH stability (Ahadi et al. 2011). Standard silica gel-coated TLC uses silica particles with particle sizes between 5 and 17 μm and the layer thickness of 0.25 mm for analytical plates. HPTLC uses adsorbent layers of 0.2 mm thickness and silica particles with a mesh size of 2–10 μm (Ahadi et al. 2011; Halkina and Sherma 2006; Campbell and Sherma 2003).

Several manufacturers as well as some literature have claimed that HPTLC is superior to classical TLC (Halkina and Sherma 2006; Camag 2017; Macherey Nagel 2017; Merck 2017). HPTLC uses adsorbents with smaller particle sizes and has been claimed to have increased sensitivity and decreased detection limit over that of TLC. HPTLC has been claimed to have some advantages over TLC including smaller spot diameter before and after development, shorter migration distances and time, and decreased R_f values (Ahadi et al. 2011; Halkina and Sherma 2006; Campbell and Sherma 2003).

In the present work, quality of five commercial silica gel-coated TLC and HPTLC plates with spherical or irregular particles in separation of opium alkaloids have been investigated and compared on the basis of their physicochemical properties. Effects of silica gel density, particle shape, size, zeta potential, and development time on N and R of seven adjacent largest peaks in the fluorescence quenching mode were studied to propose the best plate for separation and identification of opium alkaloids. The present work also aimed to study whether TLC or HPTLC is better in separation of opium alkaloids.

All scraped silica gel samples had a negative zeta potential which means that all silica gel samples had a negative surface charge. Sphericity, small particle size, and narrow particle size distribution of Merck-Lichrospher silica resulted in lower density silica gel than those for other TLC and HPTLC plates. Merck-TLC plate had a better separation of opium alkaloids than its counterpart, MN-TLC plate. It may be due to its smaller silica gel particle size and narrower particle distribution than those of MN-TLC.

Although it has been previously shown that spherical silica gel-coated preparative TLC plate had the best performance in separation of components of a test dye mixture on the basis of N and R data (Campbell and Sherma 2003), present work showed that its counterpart, analytical Merck-Lichrospher plate failed to produce a better separation of opium alkaloids than conventional Merck-TLC plate. Although we used longer development distances (10 cm) for HPTLC plates than previously applied, one similar research have showed that even in shorter conventional development distances (6 cm), Merck-Lichrospher plate failed to produce better separation than Merck-TLC plate produced in 12 cm development distance (Halkina and Sherma 2006).

Specific surface of scraped silica gel sample from small particle size Merck-Lichrospher plate was greater than that of Merck-TLC plate. This may result in higher surface energy and stronger adsorptive properties of the former than that of the latter (Ahadi et al. 2011). Lower performance of spherical silica gel-coated Merck-Lichrospher plate than irregular silica gel-coated Merck-TLC plate in long (10 cm) as well as short (6 cm) development distances may be due to this property. Smaller particle size of scraped silica gel sample from Merck-HPTLC plate may also be the reason for its lower performance than that of Merck-TLC plate.

Due to large particle sizes and very wide particle size distributions, MN-TLC and -HPTLC plates failed to produce better performance in separation of opium alkaloids than Merck-TLC plate did. In spite of MN claims about the particle sizes of its TLC and HPTLC silica gel coatings, the results of our measured mean particle size of MN TLC and HPTLC silica gel coatings varied considerably from those were claimed by MN. The results of our study about mean particle sizes of Merck TLC and HPTLC silica gel coatings were very close to those claimed by Merck itself.

The results of the present work as well as the results of previous similar research (Halkina and Sherma 2006) indicate that analytical Merck-Lichrospher plate show lower performance than conventional Merck-TLC plate in separation of opium alkaloids (our research) and dye mixture (previous research). It was found that TLC often gives the best N and R values.

Conclusions

On the basis of performance and price of TLC and HPTLC plates and great importance of precise and accurate detection of opium in forensic and clinical toxicology, the present work proposes conventional *verified* analytical TLC plates (for example Merck-TLC plate) for detection of opium alkaloids in biological and non-biological samples. More experiments are needed to generalize the results of the comparison of TLC and spherical silica gel-HPTLC plates to other drugs and pharmaceuticals.

Abbreviations

HPTLC: High-performance thin-layer chromatography; MN: Macherey Nagel; N: Number of theoretical plates; R: Resolution; SEM: Scanning electron microscopy; TLC: Thin-layer chromatography

Acknowledgements

The authors gratefully thank Merck & Co., and Macherey Nagel Incs. and their respective Iranian distributors, Merik Kimia Novin Co. and Tehran Nojhen Tajhiz Co. for supplying TLC and HPTLC plates used in the present work.

Funding

This work has not been financially supported.

Authors' contributions

This work was designed by SVSB. The experimental work was performed by KE, and analysis of the results were done by SVSB. This manuscript was written by SVSB. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Received: 28 August 2017 Accepted: 7 November 2017
Published online: 15 November 2017

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