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Development of quantitative HPTLC-densitometry methods for analyzing aminophylline, bisoprolol fumarate, griseofulvin, hydrochlorothiazide, pyrimethamine, bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, and oxybutynin Cl, following a model process developed earlier for transfer of TLC screening methods

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ABSTRACT

Transfer of thin layer chromatography (TLC) methods for semiquantitative detection of counterfeit and substandard pharmaceutical products published in the Global Pharma Health Fund (GPHF) Minilab and U.S. Food and Drug Administration (FDA) Compendium of Unofficial Methods for Screening of Pharmaceuticals to high performance TLC (HPTLC)-densitometry quantitative methods using a model process has been reported earlier in a series of papers. In this paper, HPTLC-densitometry methods developed and validated using the model process are described for analyzing the products containing aminophylline, bisoprolol fumarate, griseofulvin, hydrochlorothiazide, and pyrimethamine for which screening methods have been published in the Minilab Manual. HPTLC-densitometry methods have also been described for analyzing the products containing bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, and oxybutynin Cl, for which screening methods are not included in the Minilab Manual or FDA Compendium. These new methods use only relatively inexpensive and nontoxic solvents for preparation of sample and standard solutions and mobile phases; Merck KGaA Premium Purity HPTLC silica gel 60 F_{254} plates; semiautomated standard and sample solution application with a CAMAG Linomat 4; mobile phase development in a CAMAG twin trough chamber; automated densitometry using a CAMAG Scanner 3 for detection, assessment of peak purity and identity, quantitative assay; and validation by standard addition. Qualitative TLC screening methods based on the quantitative HPTLC-densitometry methods for the drug products not covered in the Minilab Manual or FDA Compendium were subsequently developed as supplements to the FDA Compendium and posted online with open access.

KEYWORDS: aminophylline, bisoprolol fumarate, griseofulvin, hydrochlorothiazide, pyrimethamine, bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, oxybutynin Cl, thin layer chromatography, densitometry, drug analysis.

INTRODUCTION

A model process described previously [1-3] was devised for the transfer of visual, qualitative TLC drug product screening methods from the Global Pharma Health Fund (GPHF) Minilab Manual [4]

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and U.S. Food and Drug Administration (FDA) Compendium of Unofficial Methods for Screening of Pharmaceuticals by TLC [5] to quantitative HPTLC methods suitable for support of regulatory compliance actions. This process has also been followed to develop and validate HPTLC-densitometry methods for drug products not included in these sources, after which screening methods were developed for these products as supplements to the FDA Compendium and published online with open access [5]. A recent review paper listed all of the drug products for which HPTLC-densitometry and TLC screening methods have been published using this process [6]. HPTLC-densitometry methods developed and validated for a combination pharmaceutical product containing dolutegravir, lamivudine, and tenofovir disproxil fumarate have been reported [7] since the review article was published.

This paper describes the transfer of Minilab TLC screening methods to HPTLC-densitometry methods for products of the following drugs: aminophylline (bronchodilator, CAS No. 317-34-0), bisoprolol fumarate (beta-blocker, CAS No. 105878-43-1), griseofulvin (antifungal, CAS No. 126-07-8), hydrochlorothiazide (diuretic, CAS No. 58-93-5), and pyrimethamine (antimalarial, CAS No. 58-14-0). In addition, TLC-densitometry methods are described for bupropion HCl (antidepressant, CAS No. 31677-93-7), carbamazepine (anticonvulsant, CAS No, 298-46-4), clomiphene citrate (ovulatory stimulant, CAS No. 50-41-9), mirabegron (bladder relaxant, CAS No. 223673-61-8), and oxybutynin Cl (bladder relaxant, CAS No. 1508-65-2). Supplemental FDA Compendium screening methods for these drug products, not already in the Minilab Manual or FDA Compendium, were developed and published online with open access [5].

The model process includes standard and sample solution preparation, establishment of linear and polynomial regression calibration curves by spotting 70-130% of the product's label value, assay in comparison to the label value of three individual tablets by spotting triplicate samples of each, peak purity and identity tests, and validation of the method using standard addition with triplicate analysis of 50, 100, and 150% spike levels. Only relatively inexpensive and low toxicity reagents specified for use in the Minilab TLC screening methods, including acetone, concentrated ammonium hydroxide, n-butanol, ethanol, ethyl acetate, glacial acetic acid, toluene, and methanol can be employed in the model process for transferring and developing new methods.

MATERIALS AND METHODS

Standard and sample solution preparation

Standard and sample solutions were quantitatively prepared for each drug product using the procedures described by Zeng and Sherma [8] (Table 1).

HPTLC

Detailed procedures are described by Zeng and Sherma [8] wherein they used 100% standard and sample solutions, Premium Purity silica gel 60 F_{254} plates (20 × 10 cm; Merck KGaA, Darmstadt, Germany; Catalog No. 1.05648.0001), a CAMAG (Wilmington, NC, USA) Linomat 4 bandwise applicator, twin trough chamber, and Scanner 3 controlled by winCATS software. These procedures were applied to create linear and second order calibration curves covering 70-130% of the label value of the active pharmaceutical ingredient, to assay three samples of each product in triplicate, to test the peak identity and peak purity, and to validate the new method at 50, 100, and 150% spiking levels by standard addition. Mobile phases and the respective R_f values are listed in Table 2.

All drugs were detected and scanned by inherent fluorescence quenching of the phosphor in the F plates under 254 nm ultraviolet (UV) light, or at 254 nm or 366 nm after heating the developed plate on a CAMAG plate heater to induce fluorescence quenching or fluorescence, respectively (thermochemical activation [9]).

RESULTS

Assay results for the pharmaceutical products are shown in Table 3, all of which were between 85% and 115% of the label value as required by the model process for a product of good quality. Calibration curve r-values for assays and validations were greater than 0.99; in validation (Table 4), all standard addition recoveries were between 95% and 105%; peak purity and identity r-values were at least 0.99; and all relative standard deviation (RSD) values were below 3%

Table 1. Preparation of 100% standard and 100% sample solutions.

Pharmaceutical product	100% standard solution	100% sample solution
Aminophylline (100 mg; Aurochem Laboratories (I) Pvt. Ltd., No. 333, Gundecha Ind. Complex, Akurli Road, Kandivali East, Mumbai, India)	1.67 µg/10.0 µL: dissolve 167 mg standard (Sigma-Aldrich, St. Louis, MO, USA, No. A1755-25G) in 100 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol.	1.67 µg/10.0 µL[a]: dissolve a tablet in 100 mL of methanol, then dilute 1.00 mL with 5.00 mL of methanol.
Bisoprolol Fumarate (5 mg; Unichem Laboratories Ltd., Unichem Bhavan, Prabhat Estate, Jogeshwari (West), Mumbai, India)	10.0 µg/10.0 µL: dissolve 10.0 mg standard (Sigma-Aldrich, No. 1075757-200MG) in 10.0 mL of methanol.	10.0 µg/10.0 µL: dissolve two tablets in 10.0 mL of methanol.
Griseofulvin (500 mg; Dales Pharmaceuticals Ltd., Snaygill Industrial Estate, Keighley Road, Skipton, United Kingdom)	1.25 µg/10.0 µL: dissolve 12.5 mg standard (Sigma-Aldrich, No. PHR1730-1G) in 100 mL of acetone.	1.25 µg/10.0 µL: dissolve a tablet in 100 mL of acetone, dilute 1.00 mL with 24.0 mL of acetone, and then dilute 5.00 mL with 3.00 mL of acetone.
Hydrochlorothiazide (12.5 mg + Nebivolol HCl 5 mg; Intas Pharmaceuticals Ltd., Selaqui, India)	2.50 µg/10.0 µL: dissolve 25.0 mg of standard (Sigma-Aldrich, No. H4759-5G) in 100 mL of methanol.	2.50 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol.
Pyrimethamine (25 mg + Sulfamethoxypyrazine 500 mg; Elys Chemical Industries Ltd., Road B Enterprise Rd, Nairobi, Kenya)	1.25 µg/10.0 µL: dissolve 25.0 mg standard (Sigma-Aldrich, No. 46706-250MG) in 50.0 mL of methanol, then dilute 1.00 mL with 3.00 mL of methanol.	1.25 µg/10.0 µL: dissolve a tablet in 100 mL of methanol, then dilute 1.00 mL with 1.00 mL of methanol.
Bupropion HCl (150 mg; Express Scripts, 2040 Route 130 North, Burlington, NJ, USA)	0.600 µg/10.0 µL: dissolve 60.0 mg standard (Sigma-Aldrich, No. PHR1730-1G) in 100 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol.	0.600 µg/10.0 µL: dissolve a tablet in 100 mL of methanol, then dilute 1.00 mL with 24.0 mL of methanol.
Carbamazepine (100 mg; Shanghai Fudan Fuhua Pharmaceutical Co., Ltd., 446 Zhaojiabang Rd, Shanghai, China)	2.00 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. PHR1067-1G) in 100 mL of methanol, then dilute 2.00 mL with 8.00 mL of methanol.	2.00 µg/10.0 µL: dissolve a tablet in 100 mL of methanol, then dilute 2.00 mL with 8.00 mL of methanol.
Clomiphene Citrate (50 mg; Pacific Pharmaceuticals Ltd., Mount Wellington, Auckland, New Zealand)	1.00 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. C6272-1G) in 100 mg of methanol, then dilute 1.00 mL with 9.00 mL of methanol.	1.00 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol.
Mirabegron (50 mg; Astellas Pharma Inc., Nihonbashi-Honcho, Chuo-Ku, Tokyo, Japan)	1.00 µg/10.0 µL: dissolve 100 mg standard (Toronto Research Chemical Inc., Toronto, Canada, No. M364900-1G) in 100 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol.	1.00 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol.

Table 1 continued..

Pharmaceutical product	100% standard solution	100% sample solution
Oxybutynin Cl (15 mg; Walgreens Pharmacy, 2979 Linden St., Bethlehem, PA, USA)	10.0 µg/10.0 µL: dissolve 150 mg standard (Sigma-Aldrich, No. O2881-1G) in 50.0 mL of methanol, then dilute 1.00 mL with 2.00 mL of methanol.	10.0 µg/10.0 µL: dissolve a tablet in 15.0 mL of methanol.
Oxybutynin Cl (10 mg; Kremers Urban Pharmaceuticals Inc., 902 Carnegie Center, Princeton, NJ, USA)	10.0 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. O2881-500MG) in 100 mL of methanol.	10.0 µg/10.0 µL: dissolve a tablet in 10.0 mL of methanol.

[a]Concentrations indicated for 100% sample solutions are theoretical concentrations.

Table 2. Mobile phases used for the development of plates for analysis of pharmaceutical products containing aminophylline, bisoprolol fumarate, griseofulvin, pyrimethamine, bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, and oxybutynin Cl.

Pharmaceutical product	Mobile phase[a]	R_f
Aminophylline	Ethyl acetate-toluene-methanol (30:10:6)	0.37
Bisoprolol fumarate	Ethyl acetate-methanol-ammonia (24:5:2)	0.65
Griseofulvin	Ethyl acetate-methanol (18:4)	0.56
Hydrochlorothiazide	Ethyl acetate-methanol-acetic acid (17:2:1)	0.67
Pyrimethamine	Ethyl acetate-methanol-acetone-ammonia (24:12:4:1)	0.56
Bupropion HCl	Ethyl acetate-methanol-ammonia (24:3:1)	0.78
Carbamazepine	Ethyl acetate-methanol (30:20)	0.65
Clomiphene citrate	Methanol-ethyl acetate-glacial acetic acid (14:5:1)	0.36
Mirabegron	Toluene-ethyl acetate-methanol-ammonia (7.5:21:7.5:3)	0.50
Oxybutynin Cl (15 mg)	Toluene-methanol-acetone (24:3:3)	0.40
Oxybutynin Cl (10 mg)	Toluene-ethyl acetate-glacial acetic acid (15:9:18)	0.40

[a]All solutions are shown in volume proportions.

as required by the model process. The preferred mode of regression for each pharmaceutical product was chosen during method development based on the best results obtained in terms of higher r-value for the calibration curve, assay and validation recoveries closer to 100%, and lower RSDs.

DISCUSSION

When silica gel Minilab or Compendium TLC methods are transferred to HPTLC-densitometry methods according to the previously published model process, the same solvent for sample and standard solution preparation, applied weights from 100% sample and sample solutions (in 10.0 µL for the densitometry methods instead of 2.00 µL or 3.00 µL as in the Minilab or FDA Compendium methods, respectively), mobile phases, and detection methods are tested first and then modified as necessary. In the case of the pharmaceutical products studied in this paper for which no Minilab Manual or FDA Compendium methods are available, previously published papers

describing solvents for standard and sample solutions, mobile phases, calibration curve weight ranges, and detection methods for silica gel TLC analyses of the respective drugs were consulted to assist in the development of the HPTLC-densitometry methods.

Table 3. Assay results for pharmaceutical products containing aminophylline, bisoprolol fumarate, griseofulvin, pyrimethamine, bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, and oxybutynin Cl.

Pharmaceutical product	Regression mode	Tablet 1		Tablet 2		Tablet 3	
		Assay (%)	RSD (%)	Assay (%)	RSD (%)	Assay (%)	RSD (%)
Aminophylline	Polynomial	100	0.449	102	0.392	104	0.887
Bisoprolol fumarate	Linear	110	2.42	108	0.405	102	2.39
Griseofulvin	Linear	109	0.225	108	1.36	108	1.00
Hydrochlorothiazide	Polynomial	94.5	0.963	86.1	2.54	85.1	2.02
Pyrimethamine	Polynomial	103	0.322	102	1.35	106	0.986
Bupropion HCl	Linear	103	2.69	107	2.30	103	2.93
Carbamazepine	Linear	105	0.822	107	1.10	103	0.961
Clomiphene citrate	Polynomial	110	1.67	111	0.520	109	1.59
Mirabegron	Polynomial	98.5	1.27	100	0.482	106	1.61
Oxybutynin Cl (15 mg)	Polynomial	98.1	1.49	107	1.60	94.6	1.47
Oxybutynin Cl (10 mg)	Polynomial	108	0.185	111	2.48	110	0.793

Table 4. Validation results for pharmaceutical products containing aminophylline, bisoprolol fumarate, griseofulvin, pyrimethamine, bupropion HCl, carbamazepine, clomiphene citrate, mirabegron, and oxybutynin Cl.

Pharmaceutical product	Regression mode	50% spike		100% spike		150% spike	
		Rec.[a] (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Aminophylline	Polynomial	101	1.02	101	0.799	97.7	2.17
Bisoprolol fumarate	Linear	101	2.73	95.4	1.93	93.3	3.53
Griseofulvin	Linear	102	0.451	98.9	0.797	99.9	0.542
Hydrochlorothiazide	Polynomial	102	1.91	96.7	0.775	101	1.72
Pyrimethamine	Polynomial	98.8	0.131	106	0.694	103	2.47
Bupropion HCl	Linear	97.0	2.10	103	1.95	105	2.03
Carbamazepine	Linear	102	0.568	103	2.13	102	2.52
Clomiphene citrate	Polynomial	105	1.08	102	0.609	101	1.06
Mirabegron	Polynomial	97.9	3.16	100	0.759	100	0.339
Oxybutynin Cl (15 mg)	Polynomial	100	1.77	97.8	0.984	99.9	0.734
Oxybutynin Cl (10 mg)	Polynomial	97.3	0.520	104	2.74	105	1.51

[a] Rec.= Recovery.

A TLC screening method for aminophylline is in the Minilab Manual (Volume II, Method 6.2, pp. 36-39). There is no published HPTLC-densitometry method for aminophylline. In the Minilab Manual, the 100% sample and standard solutions were prepared in water with a concentration of 5.00 µg/2.00 µL. For the HPTLC-densitometry method, the 100% sample and standard solutions were prepared in methanol with a concentration of 1.67 µg/10.0 µL. Methanol solutions could be applied to the plate more quickly with the Linomat than water solutions. Also, the Minilab mobile phase, acetone-toluene (14:7), gave diffuse bands that were unscannable. After extensive testing, ethyl acetate-toluene-methanol (30:10:6) was chosen; this combination of solvents provided excellent HPTLC-densitometry results as mobile phases for products containing cefpodoxime proxetil and efavirenz in proportions of 16:20:4 and 4:28:8, respectively, in previous research [10].

A TLC screening method for bisoprolol fumarate is in the Minilab Manual (Volume II, Supplement 2015, Method 6.91, pp. 8-11). The Minilab mobile phase, methanol-concentrated ammonium hydroxide (20:0.1), was replaced by ethyl acetate-methanol-concentrated ammonium hydroxide (24:5:2), which was modified from a published mobile phase with the same components in different proportions [11] to obtain better scans for quantification. The concentration of the 100% standard and sample solutions is 2.50 µg/2.00 µL in the Minilab method, but that was raised to 10.0 µg/10.0 µL so that the drug could be detected under 254 nm UV light by heating the plate at 180 °C for 25 min without ninhydrin staining. Figure 1 shows a densitogram of bisoprolol fumarate at 254 nm after heating; no peak appeared upon scanning before heating. It has been reported that a number of other drugs that are detected by the use of a staining reagent or iodine vapors in Minilab screening methods because they are invisible under 254 nm UV light can be detected more easily by thermochemical activation of fluorescence quenching by heating the silica gel plate after mobile phase development. These drugs were listed in the review article by Sherma and Rabel [6].

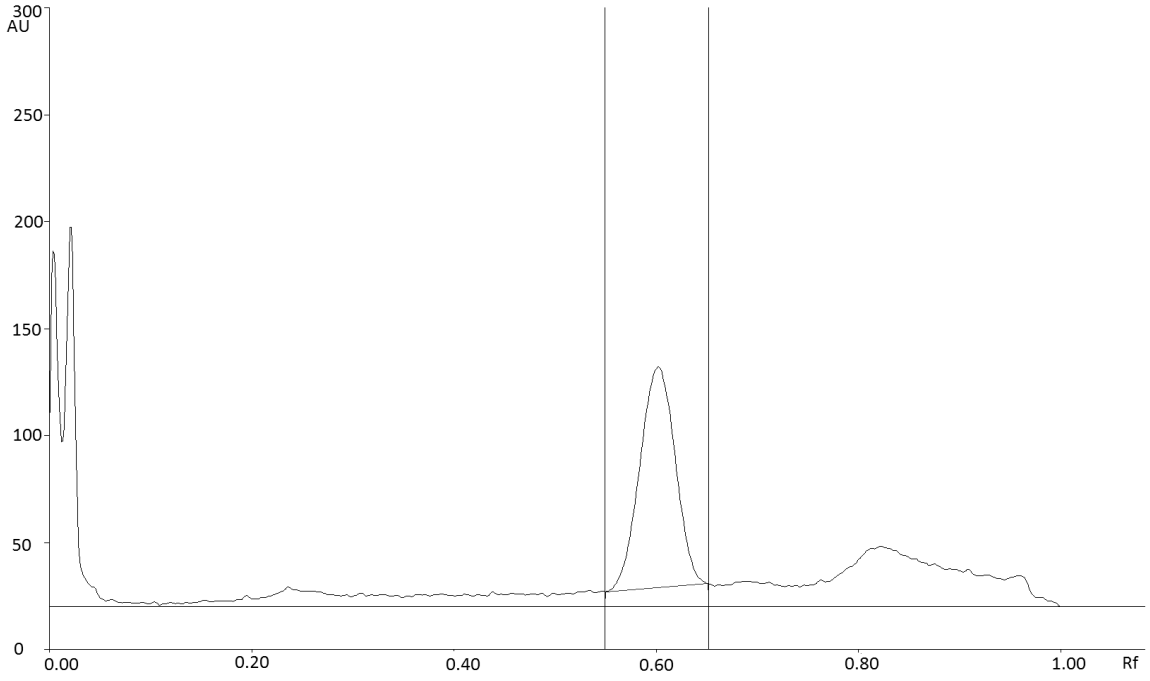

Figure 1. Densitogram of 10.0 µL of bisoprolol fumarate 100% standard solution (10.0 µg) after heating the plate and scanning at 254 nm (R_f = 0.65).

The griseofulvin Minilab method (Volume II, Method 6.18, pp. 100-103) was transferred to HPTLC-densitometry with the only change being in the concentration of 100% standard and sample solutions to 1.25 µg/10.0 µL for HPTLC-densitometry from 2.50 µg/2.00 µL for the Minilab method. The hydrochlorothiazide Minilab method (Volume II, Supplement 2015, Method 6.84, pp. 20-23) was also transferred with only a change in the concentration of the 100% standard and sample solutions to 2.50 µg/10.0 µL for HPTLC-densitometry from 4.00 µg/2.00 µL for the Minilab method.

The pyrimethamine Minilab method (Volume II, Supplement 2012, Method 6.61, pp. 16-19) was transferred directly to produce the new HPTLC-densitometry method. The analyzed product was a combination of 25 mg of pyrimethamine with 500 mg of sulfamethoxypyrazine. The two drugs were well separated after development of the plate with respective R_f values of 0.56 and 0.37. A simultaneous method for analyzing pyrimethamine and sulfadoxine in a combination product was described in an earlier paper [12].

By following the model transfer process, HPTLC-densitometry methods were developed and validated for the remaining drug products. TLC screening methods for these drugs are not published in Minilab Manual or FDA Compendium.

The optimum mobile phase for HPTLC-densitometric assay of bupropion HCl was ethyl-acetate-methanol-concentrated ammonium hydroxide (24:3:1), which was also used earlier by O'Sullivan and Sherma for assay of acetaminophen tablets [1]. The 100% standard and sample solutions were made at a concentration of 0.600 µg/10.0 µL.

The mobile phase for carbamazepine was ethyl acetate-methanol (30:20), which was used in a published paper for assays of oxacarbazepine in bulk and pharmaceutical formulation [13]. The best 100% standard and sample solution concentration was 2.00 µg/10.0 µL.

The mobile phase for the clomiphene citrate method was methanol-ethyl acetate-glacial acetic acid (14:5:1) [14]. The 100% standard and sample solutions were made at a concentration of 1.00 µg/10.0 µL.

For the mirabegron method, the best 100% standard and sample solution concentration was 1.00 µg/10.0 µL with the mobile phase toluene-ethyl acetate-methanol-concentrated ammonium hydroxide (7.5:21:7.5:3). This mobile phase was used by Zeng and Sherma for the determination of levocetirizine 2HCl reported in an earlier paper [8].

HPTLC-densitometry methods were developed and validated for 10 mg and 15 mg oxybutynin Cl tablets. For both methods a concentration of 10.0 µg/10.0 µL for 100% standard and sample solutions was chosen, and detection was possible by heating the plate [9] to induce fluorescence quenching at 254 nm (scanner deuterium lamp) and fluorescence emission at 366 nm excitation (high pressure mercury lamp). For 10 mg oxybutynin Cl tablets the mobile phase used was toluene-ethyl acetate-glacial acetic acid (15:9:18), modified from the mobile phase toluene-ethyl acetate-methanol (20:8:16) used earlier for assay of aldendazole [15]. Fluorescence was scanned with 366 nm excitation after heating at 160 °C for 30 min. For 15 mg oxybutynin Cl tablets, the method comprised the mobile phase toluene-methanol-acetone (24:3:3) [16] and scanning at 254 nm. The only HPTLC-densitometry procedure published in the literature for quantitative determination of oxybutynin Cl in pharmaceutical dosage forms employed scanning at 220 nm [17]; the two new methods described in this paper illustrate that heating of the plate causes a reaction on the silica gel surface producing one or more chromophores in the compound structure, thereby increasing the wavelengths and amounts of UV absorption.

Following the development and validation of the new HPTLC-densitometry methods for drug products not included in the Minilab Manual or FDA Compendium, qualitative TLC screening methods adequate for use in the field were developed as supplements to the FDA Compendium and posted online with open access [5]. Attempts were made to use direct transfer in terms of solvents used in sample and standard solution preparation, weights spotted on the plate (in 3.00 µL instead of 10.0 µL), mobile phases, and methods of detection, but some conditions of the HPTLC-densitometry methods had to be adjusted to improve the screening methods in terms of visual differences

between 85, 100, and 115% weights of the drug product, relative R_f values of co-formulants, if present, and spot shapes.

CONCLUSION

HPTLC-densitometry methods using a previously published model transfer process are presented for assay of five drug products for which TLC screening methods are contained in the Minilab Manual and six products that are not included. The new methods should be fully validated for parameters such as accuracy, precision, specificity, linearity, range, and robustness according to International Conference on Harmonization (ICH) guidelines [18] or by interlaboratory studies [19] if required by their future applications. Qualitative TLC screening methods were developed and published for the five products not currently included in the Minilab Manual or FDA Compendium.

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CONFLICT OF INTEREST STATEMENT

The authors of this article declare that there are no conflicts of interest.

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