Trends in Chromatography
Vol. 12, 2018

Original Article

Development of quantitative HPTLC-densitometry methods for the analysis of levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydrate, cyclobenzaprine HCl, and sulfamethoxazole + trimethoprim 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 qualitative detection of 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 levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydrate, and cyclobenzaprine HCl for which qualitative methods have not appeared in the Minilab manual or FDA Compendium, and for sulfamethoxazole + trimethoprim for which a method is published in the Minilab manual. These new methods comprise the following aspects of the model process: use of only relatively inexpensive and nontoxic solvents for sample and standard solution and mobile phase preparation, Merck KGaA Premium Purity HPTLC silica gel 60 F_{254} plates, semiautomated standard and sample solution application with a CAMAG Linomat 4, 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 these pharmaceutical products were subsequently developed as supplements to the FDA Compendium and posted online with open access.

KEYWORDS: levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydrate, cyclobenzaprine HCl, sulfamethoxazole + trimethoprim, thin layer chromatography, densitometry, drug analysis, TLC.

INTRODUCTION

The model process described previously [1-3] was devised for the transfer of visual, qualitative TLC drug screening methods from the Global Pharma Health Fund (GPHF) Minilab manual [4] 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 drugs not included in these sources, after which screening methods were developed and published online with open access as supplements to the FDA Compendium [5]. Earlier papers described HPTLC-densitometry methods developed and validated according to the model process for pharmaceutical products containing

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acetylsalicylic acid, acetaminophen, ibuprofen, and chlorpheniramine maleate [1]; mebendazole, diphenhydramine HCl, amodiaquine + artesunate, and amitriptyline HCl [2]; amodiaqine and diazepam [3]; lumefantrine + artemether [6]; albendazole, amodiaquine + artesunate, amoxicillin, and aciclovir [7]; pyrazinamide + ethambutol + isoniazid + rifampicin [8]; quinine sulfate, mefloquine, and dihydroartemisinin + piperaquine phosphate [9]; azithromycin, imipramine HCl, and sulfadoxine + pyrimethamine [10]; clarithromycin, azithromycin, and modiaquine + artesunate [11]; naproxen sodium, loperamide HCl, and loratidine [12]; cefixime, cefuroxime axetil, cephalexin H_2O, ciprofloxacin HCl, levofloxacin, and metronidazole [13]; metformin HCl, potassium clavulanate, caffeine, fluoxetine HCl, and gabapentin [14]; atenolol, chloramphenicol, furosemide, glibenclamide, penicillin V potassium, and praziquantel [15]; desloratidine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine [16]; and amiodarone HCl, carvediol, doxylamine succinate, magnesium salicylate, metoprolol succinate, nebivolol HCl, and salicylamide [17]. This paper details the development of HPTLC-densitometry methods for analyzing the following additional pharmaceutical products for which no Minilab or FDA Compendium methods have been published: levocetirizine 2HCl (antihistamine, CAS No. 130018-87-0), sertraline HCl (selective serotonin reuptake inhibitor, CAS No. 79559-97-0), atorvastatin calcium trihydrate (statin, CAS No. 344423-98-9), and cyclobenzaprine HCl (muscle relaxant, CAS No. 6202-23-9). In addition, a quantitative method is described for analyzing sulfamethoxazole (antibiotics, CAS No. 723-46-6) + trimethoprim (antibiotics, CAS No. 738-70-5), for which a simultaneous method is included in the Minilab manual (Volume II, Method 6.39, pp. 184-187). Supplemental FDA Compendium screening methods were developed and published online with open access [5] for these drug products not already in the Minilab manual or FDA Compendium, plus an optimized method for trimethoprim because the simultaneous Minilab manual method is optimized for sulfamethoxazole but not for trimethoprim.

The model process includes standard and sample preparation, establishment of linear and polynomial regression calibration curves by spotting 70-130% of the product's label value, assay in comparison to label value of three individual tablets or capsules by spotting triplicate sample 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 nontoxic reagents, including acetone, concentrated ammonium hydroxide, ethanol, ethyl acetate, glacial acetic acid, hydrochloric acid, methanol, sulfuric acid, and toluene, were used in the development of these new methods.

MATERIALS AND METHODS

Standard and sample preparation

Standard and sample solution preparation followed the guidelines described previously [1-3] unless otherwise noted. Standards, tablets ground by mortar and pestle, and capsule contents were dissolved in their respective solvents with 10 min of magnetic stirring followed by 10 min of sonication. Sample stock solutions, before further dilution or application onto the plates, were syringe filtered to remove undissolved excipients. Volumetric flasks, measuring pipets, and volumetric pipets of appropriate volume designation were used for stock solution preparation and dilution to the working solutions if necessary. Solutions were refrigerated in parafilm-sealed glass vials. Table 1 describes the sources of the sample products and procedures for 100% standard and sample solution preparation for each drug product.

HPTLC

Premium Purity silica gel 60 F_{254} plates (20 × 10 cm; Merck KGaA, Darmstadt, Germany; Catalog No. 1.05648.0001) were used without prewashing. Calibration curves were created by spotting 7.00, 9.00, 11.0, and 13.0 µL of the 100% sample solution, representing 70-130% of the label value of the active pharmaceutical ingredient. Assays were carried out by applying 10.0 µL of each sample solution in triplicate. A CAMAG (Wilmington, NC, USA) Linomat 4 was used for semi-automated bandwise standard and sample solution zone application. An application rate of 4 s/µL was used for all solutions. The band length was 6 mm, table speed 10 mm/s, distance between bands 4 mm, distance from the left edge of the plate 17 mm, and distance

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

Pharmaceutical product	100% standard solution	100% sample solution
Levocetirizine 2HCl (5 mg; Chongqing Huapont Pharmacy Co., Ltd., 69 Xingguang Ave., Chongqing, China)	5.00 µg/10.0 µL: dissolve 25.0 mg standard (Sigma-Aldrich, St. Louis, MO, USA, No. L7795-50MG) in 50.0 mL of methanol.	5.00 µg/10.0 µL[a]: dissolve a tablet in 10.0 mL of methanol.
Sertraline HCl (50 mg; CVS, generic, 2651 Easton Ave., Bethlehem, PA, USA)	10.0 µg/10.0 µL: dissolve 100 mg standard (European Pharmacopoeia, F-67081 Strasbourg, France, Code Y0000828) in 100 mL of methanol.	10.0 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol.
Atorvastatin calcium trihydrate (10 mg; Zhejiang Neo-dongguang Pharmaceutical Industry Co., Ltd., 183 Zhongxin Ave., Taizhou, Zhejiang, China)	1.25 µg/10.0 µL: dissolve 50.0 mg standard (European Pharmacopoeia, Code Y0001327) in 100 mL of methanol, then dilute 1.00 mL with 3.00 mL of methanol.	1.20 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol, then dilute 3.00 mL with 2.00 mL of methanol.
Cyclobenzaprine HCl (5 mg; CVS, generic)	0.500 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. C4542-1G) in 100 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol, and then dilute 1.00 mL with 1.00 mL of methanol.	0.500 µg/10.0 µL: dissolve a tablet in 100 mL of methanol.
Sulfamethoxazole + trimethoprim (400 mg + 80 mg; Kunming Pharmaceutical Co., 166 Keyi Rd., Kunming, Yunnan, China)	0.800 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. PHR1126-1G) in 100 mL of methanol, then dilute 2.00 mL with 23.0 mL of methanol. 0.160 µg/10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. PHR1056-1G) in 100 mL of methanol, then dilute 1.00 mL with 24.0 mL of methanol, and then dilute 2.00 mL with 3.00 mL of methanol.	0.800 µg/10.0 µL + 0.160 µg/10.0 µL: dissolve a tablet in 100 mL of methanol, then dilute 1.00 mL with 49.0 mL of methanol.

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

from the bottom of the plate 1 cm. Mobile phases used to develop plates in a CAMAG twin trough chamber and respective R_f values are listed in Table 2. Automated HPTLC-densitometry was performed using a CAMAG Scanner 3 controlled by winCATS software, with 4.00 × 0.45 mm Micro slit dimensions and a 20 mm/s scan rate. All drugs for which the methods are detailed in this paper quenched fluorescence of the phosphor in the silica gel, and were, therefore, scanned under 254 nm UV light. The winCATS software created two calibration curves using linear and second order polynomial regressions for each sample by determining the relationship between the scan areas and applied weights of standards. Sample weights were interpolated from calibration curves based on the bracketed scan areas of samples. Spectral comparison was used to test peak purity and identity. Validation of the developed methods was performed using standard addition by spiking at 50, 100, and 150% levels as described by Popovic and Sherma [3].

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. 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% as required by the model process. 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.

Table 2. Mobile phases used for the development of plates for the analysis of pharmaceutical products containing levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydtate, cyclobenzaprine HCl, and sulfamethoxazole + trimethoprim.

Pharmaceutical product	Mobile phase[a]	R_f
Levocetirizine 2HCl	Toluene-ethyl acetate-methanol-ammonium hydroxide (7.5:21:7.5:3)	0.18
Sertraline HCl	Toluene-ethyl acetate-ethanol-ammonium hydroxide (32:8:3.6:0.4)	0.34
Atorvastatin calcium trihydrate	Ethyl acetate-methanol-ammonium hydroxide (30:8:2)	0.17
Cyclobenzaprine HCl	Toluene-ethyl acetate-methanol-glacial acetic acid (16:8:14:2)	0.21
Sulfamethoxazole + trimethoprim	Ethyl acetate-methanol (30:10)	0.66 + 0.17

[a]: All solutions are shown in volume proportions.

Table 3. Assay results for pharmaceutical products containing levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydtate, cyclobenzaprine HCl, and sulfamethoxazole + trimethoprim.

Pharmaceutical product	Regression mode	Tablet 1		Tablet 2		Tablet 3	
		Assay (%)	RSD (%)	Assay (%)	RSD (%)	Assay (%)	RSD (%)
Levocetirizine 2HCl	Polynomial	98.2	0.516	101	1.59	100	2.35
Sertraline HCl	Linear	110	0.551	111	1.98	106	1.89
Atorvastatin calcium trihydrate	Linear	106	2.34	113	2.17	110	3.20
Cyclobenzaprine HCl	Polynomial	96.9	1.08	98.1	1.63	92.1	3.20
Sulfamethoxazole	Linear	104	1.31	99.9	0.478	107	1.55
Trimethoprim	Linear	112	2.95	109	1.39	110	2.65

Table 4. Validation results for pharmaceutical products containing levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydtate, cyclobenzaprine HCl, and sulfamethoxazole + trimethoprim.

Pharmaceutical product	Regression mode	50% spike		100% spike		150% spike	
		Rec.[a] (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Levocetirizine 2HCl	Polynomial	102	3.04	103	3.05	105	1.97
Sertraline HCl	Linear	100	1.40	99.4	1.86	98.5	3.27
Atorvastatin calcium trihydrate	Linear	102	1.25	105	1.10	105	0.934
Cyclobenzaprine HCl	Polynomial	103	2.80	97.0	2.55	97.0	3.42
Sulfamethoxazole	Linear	99.0	3.00	99.6	3.40	97.0	1.32
Trimethoprim	Linear	103	3.14	104	1.05	101	2.38

[a]: Rec.= Recovery

DISCUSSION

When Minilab or Compendium TLC methods are transferred to HPTLC-densitometry methods according to the previously published model process, the same solvents for sample and standard solution preparation, applied weights of sample and standard (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 described in this paper for which no Minilab manual or FDA Compendium methods are available, previously published papers describing solvents for standard and sample solutions, layers, mobile phases, calibration curve weight ranges, and detection methods for TLC analyses of the respective drugs, found by exhaustive literature searches through ISI Web of Science, Chemical Abstracts (Scifinder), and Google Scholar, were consulted to assist in the development of our methods.

For the levocetirizine 2HCl method, the sample and standard solution solvent and the mobile phase were taken from the publication by Rathore *et al*. [18]. Weights of 1.00 µg and 2.00 µg were tested first according to the linearity range in that paper [18], and the applied weight was then changed to 5.00 µg to obtain sufficiently dark zones for accurate scanning. The relatively small amount of drug contained in each tablet (5 mg) and the inability to dissolve a tablet in less than 10.0 mL of solvent limited the amount that could be applied.

For the development of the sertraline HCl method, methanol was used as the sample and standard solution solvent according to the publication by Rao *et al*. [19]. An applied weight of 5.00 µg was tested first to match the calibration curve in that paper, but it was changed to 10.0 µg to achieve better zone scans. Also, it was found that when the mobile phase was prepared exactly as described in that paper [19], ammonium hydroxide did not fully dissolve; therefore, the amount of ethanol was increased until a clear mobile phase could be produced.

The atorvastatin calcium trihydrate method uses the applied weight and the solvent suggested by Dhaneshwar *et al*. [20] and the mobile phase suggested by Rao and Sankar [21]. Four other mobile phases from the literature were also tested, namely, toluene-methanol (8:2) [20], diethyl ether-ethyl acetate (7:3) [22], toluene-methanol-glacial acetic acid (6.5:3.5:0.1, formic acid was changed to acetic acid) [23], and toluene-methanol (7:3) [24]. However, none of these could be used because after development with each, the plate showed two solvent fronts and/or bands that were too diffuse to be scanned accurately.

For the cyclobenzaprine HCl method, the standard and sample solution solvent and the mobile phase were used according to Harde *et al*. [25]. Applied weights of 2.00, 1.00, and 0.667 µg were tested according to the linearity range of that paper [25], but the optimum applied weight was found to be 0.500 µg.

For the simultaneous method of sulfamethoxazole + trimethoprim, the standard and sample solution solvent and mobile phase were selected from the sulfamethoxazole screening protocol in the Minilab manual. The mobile phase gave excellent separation of the two drug products as shown in the densitogram (Figure 1). Weights spotted in the Minilab method are 10.0 µg for sulfamethoxazole and 2.00 µg for trimethoprim, which were found to be too great to produce usable densitometry calibration curves. Optimization studies for a simultaneous method led to applied weights of 0.800 and 0.160 µg, respectively. If individual assay methods were to be developed, the best concentration of sulfamethoxazole would be lower to give less dark zones, and the best concentration of trimethoprim would be higher to give darker zones.

Following the development and validation of the new HPTLC-densitometry methods, 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.

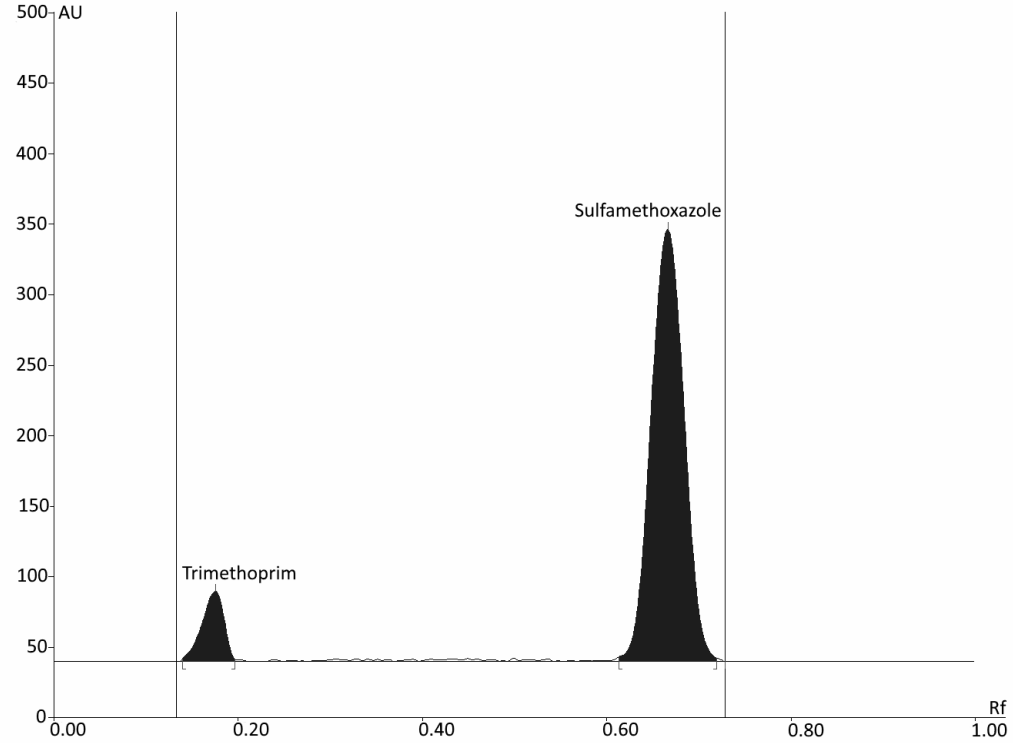

Figure 1. Densitogram of 10.0 µL of sulfamethoxazole + trimethoprim 100% sample solution, showing peaks for sulfamethoxazole (R_f: 0.66) and trimethoprim (R_f: 0.17).

CONCLUSION

HPTLC-densitometry methods for assay of levocetirizine 2HCl, sertraline HCl, atorvastatin calcium trihydrate, cyclobenzaprine HCl, and sulfamethoxazole + trimethoprim in pharmaceutical preparations were developed and validated using the model process. The methods should be fully validated for parameters such as accuracy, precision, specificity, linearity, range, and robustness according to International Conference on Harmonization (ICH) guidelines [26] or by interlaboratory studies [27] if required by their future applications. Qualitative TLC screening methods that could be used as the basis for transfer to HPTLC-densitometry did not exist in the Minilab manual or FDA Compendium for four of the drugs, and hence initial experimental parameters that comply with our model process were determined by literature searches. The supplemental FDA Compendium TLC screening methods can be converted to Minilab methods with the only changes being application of the same weights of samples and standards in 2.00 µL instead of 3.00 µL, and use of authentic drug products available to GPHF as standards rather than our commercial standards. As evidence of this suggestion, a Minilab manual TLC screening method for naproxen sodium tablets was published in Volume II, Supplement 2018, Method 6.101 that specified the same sample and standard solvent, layer, and mobile phase as supplemental Compendium methods for 220 and 500 mg tablets of this drug posted online [5] by Zhang and Sherma in 2015.

ACKNOWLEDGEMENTS

The authors thank Dr. Gerd Battermann of Merck KGaA for providing the Premium Purity glass HPTLC plates used in our experiments. Bingsong Zeng was supported by the Lafayette College EXCEL Scholar Program.

CONFLICT OF INTEREST STATEMENT

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

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