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Development of quantitative HPTLC-densitometry methods for analyzing desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl, using a model process developed earlier for the transfer of TLC screening methods

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ABSTRACT

Development of a model process for the transfer of thin layer chromatography (TLC) methods for qualitative screening of fake or counterfeit drugs, 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 by TLC, to quantitative high-performance TLC (HPTLC)-densitometry methods was described in a series of papers. In this paper, HPTLC-densitometry methods developed and validated using this model process are reported for desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl, for which qualitative screening methods have not appeared in the Minilab manual or FDA Compendium. These methods only use relatively inexpensive and nontoxic "green solvents" for sample and standard solution and mobile phase preparation, Merck Premium Purity silica gel 60 F_{254} plates, automated standard and sample solution application by a CAMAG Linomat 4, and automated densitometry by a CAMAG Scanner 3 for the assessment of peak purity and identity and quantification. Qualitative TLC screening methods based on the quantitative HPTLC-densitometry methods for these drugs were subsequently developed and posted with open access, as supplements to the FDA Compendium.

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KEYWORDS: desloratadine, etodolac, famotidine, omeprazole, oxaprozin, phenazopyridine HCl, thin layer chromatography, densitometry, drug analysis, TLC.

INTRODUCTION

The model process previously described in [1-3] was devised for the transfer of visual, qualitative TLC drug screening methods, which are published in the Global Pharma Health Fund (GPHF) Minilab [4] manual 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. Use of this model process has been reported for the development and validation of HPTLC-densitometry methods for analyzing pharmaceutical products of acetylsalicylic acid, acetaminophen, ibuprofen, and chlorpheniramine maleate [1]; mebendazole, diphenhydramine HCl, amodiaquine + artesunate, and amitriptyline HCl [2]; amodiaquine 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 amodiaquine + artesunate [11]; naproxen sodium, loperamide HCl, and loratidine [12]; cefixime, cefuroxime axetil, cephalexin hydrate, ciprofloxacin

HCl, levofloxacin, and metronidazole [13]; and metformin HCl, potassium clavulanate, caffeine, fluoxetine HCl, and gabapentin [14]. All of these drug analysis methods were transferred from a previously published GPHF Minilab or FDA Compendium method, except those for caffeine, fluoxetine HCl, amitriptyline, acyclovir, naproxen sodium, loperamide HCl, loratidine, and gabapentin, for which no methods have been published in these sources. This paper details the development of HPTLC-densitometry methods for the following additional pharmaceutical products for which no Minilab or FDA Compendium methods have been published: the antihistamine desloratadine (CAS No. 100643-71-8), the non-steroidal anti-inflammatory drug etodolac (CAS No. 41340-25-4), the H_2-antagonist famotidine (CAS No. 76824-35-6), the anti-microbial proton-pump inhibitor omeprazole (CAS No. 73590-58-6), the non-steroidal anti-inflammatory drug oxaprozin (CAS No. 21256-18-8), and the urinary tract analgesic phenazopyridine HCl (CAS No. 136-40-3). Supplemental FDA Compendium screening methods were also developed and published online with open access, for these six drug products.

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, an assay that compares the label value of three individual tablets or capsules 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 the "green" solvents and reagents acetone, concentrated ammonium hydroxide, ethanol, ethyl acetate, glacial acetic acid, hydrochloric acid, methanol, sulfuric acid, and toluene were considered for use in the development of these 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, by 10 min of magnetic stirring followed by 10 min of sonication. Sample solutions, before further dilution or application onto the plates, were syringe-filtered to remove excipients. Volumetric flasks, measuring pipets, and volumetric pipets of appropriate volume designation were used for stock solution preparation and dilution to obtain working solutions if necessary. Sample solutions were refrigerated in Parafilm-sealed glass vials. Solutions of phenazopyridine HCl retained orange, red, and brown colors after their clarification by filtration. Table 1 describes the source of the sample products as well as the methods employed to prepare the 100% standard and sample solutions 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 generated by spotting 7.00, 9.00, 11.0, and 13.0 µL of the 100% sample solution, which represents 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, zone application. An application rate of 4 s/µL was used for all solutions, except those containing omeprazole, as a rate of 15 s/µL is needed due to the use of water in solution preparation. 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 from the bottom of the plate, 1 cm. The mobile phases and their respective R_f (retardation factor: distance traveled by the zone, divided by distance traveled by the mobile phase) 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 analytical methods are detailed in this paper quenched the fluorescence of the phosphor in the layer, and were, therefore, scanned with 254 nm UV radiation. Phenazopyridine HCl was viewed on the plate in daylight as red-brown bands, but it also quenched fluorescence and was quantified by scanning at 254 nm. The winCATS software generated two calibration curves (linear and second-order polynomial regressions) for each sample, by determining the relationship between the scan

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

Pharmaceutical product	100% standard solution	100% sample solution[b]
Desloratadine (2.5 mg[a]; Merck Sharp & Dohme Corp., 1 Merck Dr, Whitehouse Station, NJ, USA)	1.00 µg/10.0 µL: dissolve 10.0 mg standard (USP, Rockville, MD, USA, Catalog No. 1173042) in 100 mL of methanol.	1.00 µg/10.0 µL: dissolve a tablet in 25.0 mL of methanol.
Etodolac (400 mg; Taro Pharmaceutical Industries Ltd., 3 Skyline Dr, Ste 120, Hawthorne, NY, USA)	0.800 µg/10.0 µL: dissolve 80.0 mg standard (USP, No. 1268706) in 100 mL of methanol, then dilute 1.00 mL with 9.00 mL of methanol, for a total volume of 10.0 mL.	0.800 µg/10.0 µL: dissolve a tablet in 50.0 mL of methanol, then dilute 1.00 mL with 99.0 mL of methanol, for a total volume of 100 mL.
Famotidine (10 mg; CVS Health, Woonsocket, RI, USA)	0.800 µg/10.0 µL: dissolve 20.0 mg standard (Sigma-Aldrich, St. Louis, MO, USA, No. PHR1055) in 50.0 mL of methanol-glacial acetic acid (9:1), then dilute 2.00 mL with 8.00 mL, for a total volume of 10.0 mL.	0.800 µg/10.0 µL: dissolve a tablet in 25.0 mL of methanol-glacial acetic acid (9:1), then dilute 2.00 mL with 8.00 mL, for a total volume of 10.0 mL.
Omeprazole (40 mg; Sandoz Pharmaceuticals, Suurstoffi 14, 6343 Rotkreuz ZG, Switzerland)	1.02 µg/10.0 µL: dissolve 25.6 mg standard (Sigma-Aldrich, No. PHR1059) in 25.0 mL of methanol, then dilute 1.00 mL with 9.00 mL of deionized water, for a total volume of 10.0 mL.	1.00 µg/10.0 µL: dissolve a capsule in 50.0 mL of methanol, then dilute 1.00 mL with 7.00 mL of deionized water, for a total volume of 8.00 mL.
Oxaprozin (600 mg; SEARLE, G.D. Searle and Co. for Searle Daypro, Inc., USA)	0.120 µg/10.0 µL: dissolve 2.50 mg of standard (Sigma-Aldrich, No. O9637) in 25.0 mL of ethanol, then dilute 1.20 mL with 8.80 mL of ethanol, for a total volume of 10.0 mL.	0.120 µg/10.0 µL: dissolve a tablet in 100 mL of ethanol, then dilute 1.00 mL with 49.0 mL of ethanol, for a total volume of 50.0 mL, then dilute 1.00 mL of this solution further with 9.00 mL of ethanol, for a total of 10.0 mL.
Phenazopyridine HCl (200 mg; Amneal Pharmaceuticals, Paterson, NJ, USA)	0.500 µg/10.0 µL: dissolve 10.0 mg of standard (Sigma-Aldrich, No. 34076) in 200 mL of methanol.	0.500 µg/10.0 µL: dissolve a tablet in 100 mL of methanol, then dilute 2.50 mL with 97.5 mL of methanol.

[a]Desloratadine (2.5 mg) co-formulated with pseudoephedrine (120 mg).
[b]Concentrations indicated for all 100% sample solutions are theoretical concentrations.

areas and the weights of standards applied. 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 with 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, with all values between 85-115% of the label value, as required by the model process. Calibration curve r-values for the assays and the validation process were over 0.99, all standard addition recoveries in the validation results (table 4) were within ± 5.00%, peak purity and identity r-values were at least 0.99, and all relative standard deviation (RSD) values were below 3.00%, also, as required by the model process. Choice between the use of linear and polynomial regression was made during method development, based on which mode gave better r-values for the calibration curve, assay and standard addition recovery values closer to 100%, and lower RSD values for the triplicate analyses.

DISCUSSION

When transferring Minilab or Compendium TLC methods to HPTLC-densitometry, according to the earlier published model process, the same

Table 2. Mobile phases used for the development of the plates for the analysis of pharmaceutical products containing desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl.

Pharmaceutical product	Mobile phase[a]	R_f
Desloratadine	Methanol-concentrated ammonium hydroxide (10:0.3)	0.25
Etodolac	Toluene-ethyl acetate-methanol (5:4:1)	0.35
Famotidine	Ethyl acetate-methanol-toluene-concentrated ammonium hydroxide (10:25:20:2)	0.40
Omeprazole	Toluene-acetone-methanol-concentrated ammonium hydroxide (7:1.5:1:0.1)	0.32
Oxaprozin	Ethyl acetate-glacial acetic acid (95:5)	0.35
Phenazopyridine HCl	Ethyl acetate-acetone-glacial acetic acid (18:4:0.1)	0.42

[a]All solutions are shown in volume proportions.

Table 3. Assay results for pharmaceutical products containing desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl.

Pharmaceutical product	Regression mode	Tablet 1		Tablet 2		Tablet 3	
		Assay (%)	RSD (%)	Assay (%)	RSD (%)	Assay (%)	RSD (%)
Desloratadine	Linear	100	0.790	101	0.431	96.5	0.403
Etodolac	Linear	107	0.385	108	1.60	105	0.193
Famotidine	Polynomial	106	2.65	107	1.49	104	1.20
Omeprazole	Polynomial	96.5	0.310	98.0	1.12	103	0.644
Oxaprozin	Polynomial	93.0	1.99	101	0.0658	94.7	2.55
Phenazopyridine HCl	Linear	107	2.20	111	1.11	108	1.06

Table 4. Validation results for pharmaceutical products containing desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl.

Pharmaceutical Product	Regression Mode	50% spike		100% spike		150% spike	
		Rec.[a] (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Desloratadine	Linear	100	2.19	102	2.05	102	1.09
Etodolac	Linear	104	0.810	104	0.291	104	0.359
Famotidine	Polynomial	103	0.342	104	0.807	104	0.873
Omeprazole	Polynomial	104	2.24	105	1.97	102	0.160
Oxaprozin	Polynomial	100	1.43	104	0.960	103	2.39
Phenazopyridine HCl	Linear	104	0.730	105	0.404	103	0.743

[a]Rec. = Recovery.

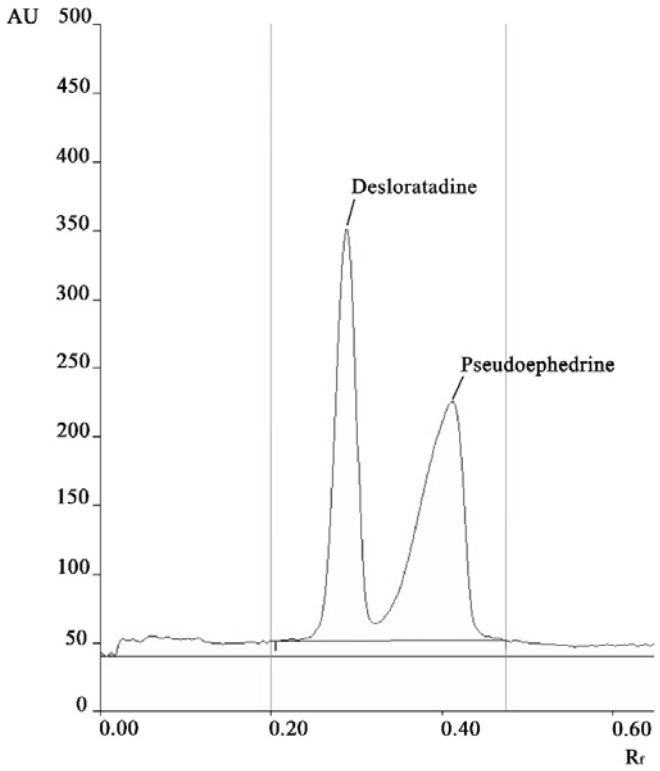

Figure 1. Densitogram of the 100% sample solution of 10.0 µL of desloratadine, showing peaks for desloratadine (R_f: 0.25) and its co-formulant, pseudoephedrine (R_f: 0.41).

solvents for sample and standard solution preparation, weight of sample and standard applied (in 10.0 µL for the densitometry methods, instead of 2.00 µL or 3.00 µL as in the Minilab or Compendium, respectively), mobile phase, and detection method are used. In the case of the pharmaceutical products described in this paper, for which no Minilab or Compendium methods exist, previously published papers describing solvents, layers, mobile phases, calibration curves, and detection methods for the TLC analysis of the respective drugs, found by exhaustive literature-searches through SciFinder® (Chemical Abstracts), ISI Web of Science, and Google Scholar, were used to assist in our method development research.

In the development of the method for analyzing desloratadine, the use of methanol as the solvent, methanol-concentrated ammonium hydroxide (10:0.3) as the mobile phase and a concentration of 1.00 µg/ 10.0 µL for 100% sample and standard-solution preparation (based on the range reported for the calibration curve) was based directly on Youssef *et al*. [15]. Desloratadine was co-formulated with pseudoephedrine in the product analyzed, but we could not obtain a commercial standard that allowed for the development of a simultaneous HPTLC-densitometry method for analyzing both drugs. Pseudoephedrine is commonly abused, and, therefore, a license, which we do not possess, is required to obtain the standard. The mobile phase used provided adequate resolution of the two drugs to allow for the analysis of desloratadine (Figure 1).

For the etodolac quantification method, the use of methanol as the solvent and toluene-ethyl acetate-methanol (5.0:4.0:1.0) as the mobile phase was based directly on Chaube *et al*. [16]. The mobile phase toluene-ethyl acetate-ethanol (6:1.5:2.5) used by Patel *et al*. [17] was also tested, but this gave lower r-values for the linear and polynomial calibration curves. The Patel *et al*. method [17] suggested the use of 100% sample and standard solutions with a concentration of 0.700 µg/10.0 µL, but the 0.800 µg/ 10.0 µL concentration we chose was more convenient to prepare from the tablet product and gave good results.

Based on Campbell and Sherma [18], the famotidine quantification method used methanol-glacial acetic acid (9:1) as the solvent, ethyl acetate-methanol-toluene-concentrated ammonium hydroxide (40:25:20:2.0) as the mobile phase, and 0.800 µg/ 10.0 µL concentrations of the 100% sample and standard solutions.

For the development of the omeprazole quantification method, the use of methanol as the solvent, toluene-acetone-methanol-concentrated ammonium hydroxide (7:1.5:1:0.1) as the mobile phase, and a standard and sample-solution concentration of 1.00 µg/10.0 µL was based on the work of Nanaware *et al.* [19]. All papers found in the literature associated with the TLC determination of omeprazole [19-21] designated densitometric scanning of zones around 300 nm, but in accordance with our model process, scans were successfully performed with 254 nm UV light.

Based on Greshock and Sherma [22], the oxaprozin quantification method employs ethanol as the solvent, ethyl acetate-glacial acetic acid (95.0:5.00) as the mobile phase, and 100% sample and standard solutions of concentration 0.120 µg/10.0 µL.

No previously published method for the densitometric determination of phenazopyridine HCl was found in our literature search. After testing the many drug analysis mobile phases we had used previously, ethyl acetate-acetone-glacial acetic acid (18:4:0.1) [7] was found to be optimal. The standard and sample solution concentrations (0.500 µg/10.0 µL) and resulting weights applied were chosen after testing multiple dilutions of stock solutions.

Following the validation of the quantitative HPTLC-densitometry methods, qualitative TLC methods were developed. Solvents used in sample and standard solution preparation, weights of analytes spotted on the plate (in 3.00 µL for the qualitative method, instead of the 10.0 µL used in the quantitative method), mobile phases, and methods of detection were transferred from the HPTLC-densitometry methods. If necessary, parameters of the quantitative methods (mobile phase or weights spotted) were adjusted to improve visual differences between 85, 100, and 115% of the drug product, relative R_f of co-formulants, if present, and spot shape.

CONCLUSION

HPTLC-densitometry methods for the analyses of desloratadine, etodolac, famotidine, omeprazole, oxaprozin, and phenazopyridine HCl in pharmaceutical preparations were developed and validated using our model procedure. The methods should be fully validated according to the International Conference on Harmonization (ICH) guidelines [23] or by interlaboratory studies [24], 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 for these drugs in the Minilab manual or FDA Compendium; so literature searches were relied upon for suggestions of experimental parameters that could be adapted for use within our model process. TLC screening methods adequate for use in the field were subsequently developed corresponding to the HPTLC-densitometry methods and posted online with open access, as supplements to the FDA Compendium on Dr. Tom Layloff's website [25]. These can be converted to Minilab methods if desired by the GPHF, with the only changes being the application of the same weights of samples and standards in 2.00 µL instead of 3.00 µL, and the use of authentic drug products available to them as standards, rather than the commercial standards we purchased from Sigma-Aldrich.

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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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