Dedicated to Professor Costel Sârbu on the Occasion of His 65th Anniversary

DEVELOPMENT OF QUANTITATIVE HPTLC-DENSITOMETRY METHODS FOLLOWING A MODEL APPROACH FOR TRANSFER OF TLC SCREENING METHODS FOR PHARMACEUTICAL PRODUCTS OF METFORMIN HCL, POTASSIUM CLAVULANATE, CAFFEINE, FLUOXETINE HCL, AND GABAPENTIN

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ABSTRACT. Transfer of thin-layer chromatography Global Pharma Health Fund Minilab kit protocols for detecting counterfeit drugs in pharmaceutical products in the field to quantitative high-performance TLC (HPTLC)-densitometry methods was carried out for potassium clavulanate and metformin HCl using a model process published earlier. HPTLC-densitometry methods were also developed following the model process for caffeine, fluoxetine HCl, and gabapentin, for which methods are not included in the Minilab manual. The model process involves use of EMD Millipore Premium Purity silica gel 60 F_{254} plates, automated sample and standard solution application with a CAMAG Linomat 4, and automated densitometry with a CAMAG Scanner 3 for determination of peak purity and identification and for quantification. Detection methods for counterfeit samples of the three drugs not covered in the Minilab manual were also developed and posted online with open access as supplemental methods for the Compendium of Unofficial Methods for Rapid Screening of Pharmaceuticals by Thin Layer Chromatography.

Keywords: thin layer chromatography, drug analysis, metformin HCl, potassium clavulanate, gabapentin, fluoxetine HCl, caffeine

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INTRODUCTION

A model process was previously described [1-3] for transfer of qualitative/semiquantitative thin-layer chromatography (TLC) screening methods for pharmaceutical products with quality defects contained in the Global Pharma Health Fund E.V. (GPHF) Minilab manual [4] or U.S. Food and Drug Administration Compendium of Unofficial Methods for Rapid Screening of Pharmaceuticals by Thin Layer Chromatography [5] to quantitative high-performance TLC (HPTLC)-densitometry methods. The model process was applied earlier to formulations containing acetylsalicylic acid, acetaminophen, ibuprofen, and chlorpheniramine maleate [1]; mebendazole, diphenhydramine HCl, amodiaquine, and artesunate [2]; amodiaqine and diazepam [3]; lumefantrine + artemether [6]; albendazole, amodiaquine + artesunate [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]; and cefixime, cefuroxime axetil, cephalexin hydrate, ciprofloxacin HCl, levofloxacin, and metronidazole [12].

The model process comprises sample and standard preparation, establishment of a linear or polynomial calibration curve covering 70-130% of the label value, assay of three samples of the pharmaceutical product relative to the label value each in triplicate, evaluation of the accuracy of the method using standard addition analysis at 50, 100, and 150% fortification levels each in triplicate, and peak purity and peak identity tests; only certain relatively nontoxic solvents can be used for standard and sample solution and mobile phase preparation. In this article, we report the use of the model process to transfer TLC Minilab methods to HPTLC-densitometry for pharmaceutical products containing the diabetes medication metformin HCl (CAS No. 1115-70-4) and antibiotic potassium clavulanate (CAS No. 61177-45-5), as well as to develop HPTLC-densitometry methods for the products containing the stimulant caffeine (CAS No. 58-08-2), the nerve pain and anticonvulsant medication gabapentin (CAS No. 60142-96-3), and the antidepressant fluoxetine HCl (CAS No. 56296-78-7) for which there are no Minilab or Compendium methods published.

RESULTS

Results of the method development for the five pharmaceutical products are displayed in Table 1 for the assay of the three tablets and in Table 2 for the validation analyses. The optimal regression mode for assays and validation of each was chosen based on the best calibration curve r-values,

assay values closer to the label value, accuracy of the standard addition validation, and lower relative standard deviations (RSDs) for the replicated analyses. Calibration curve r-values in our assay and validation experiments were at least 0.99; all validation analysis recoveries at 50, 100, and 150% spike levels were within +/- 5%; and peak purity and identity r-values were 0.99 consistent with the model process requirements. All assays were within 85-115% specification limits of the label value as specified by the U.S. Pharmacopeia (USP) for individual dosage form analysis except for the one high assay result of the potassium clavulanate tablets. RSDs for triplicate assays and validation analyses were within the required 3% except for the 100% spike level for gabapentin.

Table 1. Assay results for pharmaceutical products containing caffeine, fluoxetine HCl, gabapentin, metformin HCl, potassium clavulanate respectively

Pharmaceutical product	Regression mode	Tablet 1		Tablet 2		Tablet 3	
		Assay (%)	RSD (%)	Assay (%)	RSD (%)	Assay (%)	RSD (%)
Caffeine	Polynomial	106	0.502	113	1.39	106	0.181
Fluoxetine HCl	Linear	101	1.93	93.2	0.832	97.0	1.13
Gabapentin	Polynomial	89.8	1.98	93.5	0.733	95.5	2.20
Metformin HCl	Linear	100	0.484	106	1.40	105	0.547
Potassium clavulanate	Linear	101	1.74	117	0.914	114	0.751

Table 2. Validation results for pharmaceutical products containing caffeine, fluoxetine HCl, gabapentin, metformin HCl, potassium clavulanate respectively

Pharmaceutical product	50% spike		100% spike		150% spike	
	Rec.[a] (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Caffeine	104	1.13	102	2.33	104	2.28
Fluoxetine HCl	100	0.700	101	0.758	96.6	2.76
Gabapentin	104	1.86	103	7.42	101	1.68
Metformin HCl	105	1.88	105	0.0777	105	0.452
Potassium clavulanate	103	1.81	100	0.183	97.5	2.90

[a]Rec.=Recovery

DISCUSSION

A direct transfer of Minilab TLC methods to HPTLC-densitometry according to the earlier published process involves use of the same solvents in preparing the sample and standard solutions, application of the same weight of sample and standard in 10.00 μL as in 2.00 μL, and use of the same mobile phase and detection method.

The metformin HCl Minilab method for a 250 mg tablet (Volume II, Supplement 2014, Method 6.78, pp. 24-27) could not be transferred directly. The Minilab method involves preparation of stock standard and sample solutions in water followed by dilution with methanol to prepare the 100% standard and sample solutions. The drug was found to precipitate out upon dilution with methanol; therefore dilution was made with water instead. The Minilab mobile phase, methanol-water-glacial acetic acid (15:5:1) did not give tight bands nor symmetrical scan peaks, so the mobile phase methanol-water-1% (w/v) ammonium chloride reported in the literature [13] was adopted. After testing many mobile phases, none without ammonium chloride as a component gave good results, so it was decided that use of this non-hazardous salt was acceptable. When applying sample and standard weights specified in the Minilab unusually high scan areas (>10,000) were obtained, but calibration, assay, and validation results were within the model process requirements.

The potassium clavulanate-amoxicillin coformulations Minilab method for a product containing 62.5 mg of clavulanic acid (Volume II, Supplement 2013, Method 6.69, pp.20-23) was directly transferred for our product with a label value of 57 mg of clavulanic acid, equivalent to 67.9 mg of potassium clavulanate except for the detection method. Bands were detected by heating the plate after development at 160°C for 5 min to produce fluorescence quenching bands visible under 254 nm ultraviolet (UV) light rather than using iodine vapor. This reagentless thermochemical activation method involving simple heating of silica gel layers was first reported by our laboratory and has been applied to a variety of drugs and dietary supplements [11]. Assay recovery for one of the three tablets was slightly above the model procedure upper limit of 115%, but the standard addition validation (Table 2) and peak identity and peak purity results meeting requirements indicated the assay was reliable. It was necessary to carefully adjust the integration limits when scanning bands of potassium clavulanate at 254 nm so that streaked amoxicillin bands did not interfere (Figure 1). Potassium clavulanate was also visible as fluorescent bands after heating, but interference of the amoxicillin bands when they were scanned at 366 nm was greater than at 254 nm. The reason for the two high assay results, one greater than the 115% model process limit, is not known, but the good validation results indicate that the assays are accurate and that

the product, which was obtained without a prescription from a shop in China, contains tablets with variable active ingredient amounts. A simultaneous method for assay of the coformulation could not be developed after unsuccessfully testing many different solvents to extract both potassium clavulanate amoxicillin completely from the sample and standard and mobile phases to separate the two compounds without streaked amoxicillin bands. In addition, after weeks of considerable research we have been unable to successfully use any published HPTLC-densitometry method for determination of amoxicillin, or the similar drug ampicillin, in any pharmaceutical product alone or in a coformulation, or to develop and validate a new method.
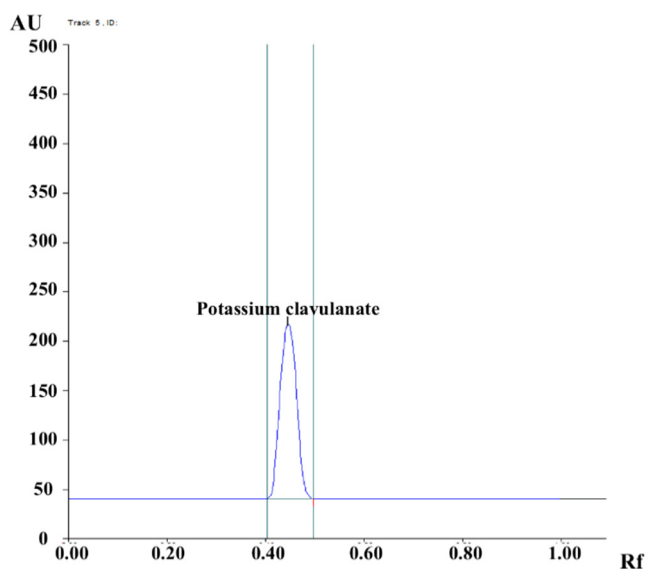

Figure 1. Densitogram of 10.0 uL of potassium clavulanate 100% sample solution, representing 3.31 ug of potassium clavulanate when interpolated from the calibration curve based on its area.

The model transfer process has also been used earlier as the basis of development of HPTLC-densitometry methods for drug products covered neither in the Minilab manual nor the Compendium, e.g., naproxen sodium, loperamide HCl, and loratidine [14]. In this paper, methods were similarly developed for caffeine, fluoxetine HCl, and gabapentin. Based on the development of these methods, corresponding TLC screening methods were devised, tested, and published in an open access online supplement to the FDA Compendium [15], from which they could be easily transferred to Minilab

TLC screening methods, if desired, by taking into account the 2.00 µL rather than 3.00 µL spotting volumes and the usual use of an authentic drug product rather than a commercial standard to prepare the standard solutions for Minilab methods.

The caffeine method for a 200 mg tablet was a direct adaptation of a previously published method [16] that used methanol solvent for standard and sample solution preparation, a similar calibration curve weight range, silica gel HPTLC plates, ethyl acetate-methanol (85:15) mobile phase, and fluorescence quenching detection. The fluoxetine HCl method for a 20 mg capsule was directly adapted from a published method for alprazolam and fluoxetine HCl in a tablet formulation [17] with use of methanol solvent for standard and sample solution preparation, acetone-toluene-ammonium hydroxide (6.0:3.5:0.5) mobile phase, and fluorescence quenching detection, but the calibration curve was prepared with weight range about four times greater in order to achieve successful band detection and scanning.

The gabapentin method for an 800 mg tablet was directly transferred from a published method for 200 mg capsules [18] in terms of the use of methanol for standard and sample solution preparation, the same weight range for the calibration curve, and the use of ninhydrin spray reagent for band detection. However, the mobile phase *n*-butanol-water acetic acid (3:3:2) [18] was modified by replacing *n*-butanol with ethanol, which are in the same selectivity group and have a similar solvent strength in Snyder's liquid chromatography solvent classification list [19], because *n*-butanol is not one of the allowed solvents for Minilab or Compendium methods. Unlike potassium clavulanate, heating the plate after development did not produce fluorescence quenching zones to eliminate for the need of a detection reagent, the use of which usually leads to poorer accuracy and precision data in method development.

Depending on the applications of the methods described in this paper, they should be fully validated for parameters such as accuracy, precision (repeatability and intermediate precision), specificity, linearity, range and robustness under relevant guidelines such as those described by the International Conference on Harmonization [20] or subjected to an interlaboratory study [21] to prove that they are suitable for their intended purpose by users.

CONCLUSIONS

HPTLC-densitometry methods were developed and validated for assay of pharmaceutical formulations of two drugs by transfer of TLC screening methods contained in the Minilab manual, and for formulations of three drugs

not included in the Minilab manual or FDA Compendium. Supplemental Compendium methods that can be easily converted to Minilab methods if desired were devised for these latter drugs and posted on an open access internet site.

EXPERIMENTAL

Standard and sample solution preparation

General preparation procedures were carried out as described earlier [1-3] unless otherwise specified. All standards and ground (by mortar and pestle) tablets or capsule contents were dissolved with the aid of 10 min each of magnetic stirring and sonication before syringe filtration to remove undissolved excipients prior to further dilution or direct application. Dilutions were made using appropriate volumetric flasks and transfer and measuring pipets. Solutions were stored in sealed vials wrapped in parafilm in a refrigerator. A description of standards and samples and their sources as well as detailed procedures for stock and 100% working standard and sample solution preparation are shown in Table 3.

Table 3. Preparation of 100% standard and 100% sample solutions

Pharmaceutical product	100% standard solution	100% sample solution
Caffeine (200 mg; CVS Pharmacy, Inc., USA)	0.400 µg/ 10.0 µL: dissolve 40.0 mg standard (Sigma-Aldrich, St. Louis, MO, Catalog No. C0750) in 100 mL methanol, then dilute 1.00 mL with 9.00 mL methanol	0.400 µg/ 10.0 µL[a]: dissolve a tablet in 100 mL methanol, then dilute 1.00 mL with 49.0 mL methanol
Fluoxetine HCl (20 mg; Aurobindo Pharma, USA Inc.)	4.01 µg/ 10.0 µL: dissolve 80.1 mg standard (Sigma-Aldrich, No. PHR1394) in 200 mL methanol	4.00 µg/ 10.0 µL: dissolve a capsule in 50.0 mL methanol
Gabapentin (800 mg; Glenmark Pharmaceuticals Inc., USA)	1.60 µg/ 10.0 µL: dissolve 100 mg standard (Sigma-Aldrich, No. PHR1049) in 100 mL methanol, then dissolve 16.0 mL in 84.0 mL methanol	1.60 µg/ 10.0 µL: dissolve a tablet in 100 mL methanol, then dissolve 1.00 mL with 49.0 mL methanol

Pharmaceutical product	100% standard solution	100% sample solution
Metformin HCl (250 mg; Shanghai Xinyi Tianping Pharmaceutical Co., Ltd, Shanghai, China)	8.00 µg/ 10.0 µL: dissolve 40.0 mg standard (Sigma-Aldrich, No. PHR1084) in 50.0 mL deionized water	8.00 µg/ 10.0 µL: dissolve a tablet with 25.0 mL deionized water, then dilute 2.00 mL with 23.0 mL deionized water
Potassium clavulanate (67.9 mg[b]; Guangzhou Baiyunshuan Pharmaceutical Holdings Co., Ltd, China)	2.72 µg/ 10.0 µL: dissolve 34.0 mg standard (Sigma-Aldrich, No. 33454) in 25.0 mL deionized water, then dilute 1.00 mL with 4.00 mL deionized water	2.72 µg/ 10.0 µL: dissolve a tablet in 50.0 mL deionized water, then dilute 1.00 mL with 4.00 mL deionized water

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

[b] The potassium clavulanate sample was a tablet of 57 mg clavulanic acid (or 67.9 mg potassium clavulanate, when adjusted for molecular weight factor) with 400 mg amoxicillin as the other active ingredient.

HPTLC

Detailed HPTLC-densitometry methods and instruments were described earlier [1-3, 6-12]. Silica gel 60 F_{254} Premium Purity HPTLC glass plates (20 x10 cm; EMD Millipore Corp., Billerica, MA, a division of Merck KGaA, Darmstadt, Germany; Part No. 1.05648.0001) were used as received. Application of 7.00, 9.00, 11.0, and 13.0 µL aliquots of the 100% standard solution of each drug [representing 70-130% of the active pharmaceutical ingredient (API) content based on label value] and triplicate 10.0 µL aliquots of 100% sample solution were applied using a CAMAG (Wilmington, NC, USA) Linomat 4 spray on applicator [band length 6 mm, application rate 4 sec/µL (15 sec/µL for solutions containing water), 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). HPTLC-densitometry in the absorption-reflectance mode was performed using a CAMAG Scanner 3 (4.00 x 0.45 mm Micro slit dimensions, 20 mm/s scan rate). The mobile phases used for the five pharmaceutical products and drug R_f values are shown in Table 4. The fluorescence-quenching bands were scanned with 254 nm UV light, and the colored bands of gabapentin were scanned with 510 nm light. The Scanner 3 winCATS software automatically created calibration curves (linear or 2nd order polynomial) based on scan areas versus standard weights applied, interpolated

weights of drugs in bracketed samples based on scan areas, and tested peak purity and identity of the sample based on spectral comparison. Accuracy of the developed methods was validated by using standard addition with a 70-130% calibration curve as described earlier [3].

Table 4. Mobile phases selected in our methods for pharmaceutical products containing caffeine, fluoxetine HCl, gabapentin, metformin HCl, potassium clavulanate respectively

Pharmaceutical product	Mobile phase[a]	R_f
Caffeine	Ethyl acetate-methanol (85:15)	0.36
Fluoxetine HCl	Acetone-toluene-ammonia (6:3.5:0.5)	0.62
Gabapentin	Ethanol-deionized water-glacial acetic acid (3:3:2)	0.75
Metformin HCl	Methanol-water-1% ammonium chloride solution (5:4:1)	0.25
Potassium clavulanate	Ethyl acetate-glacial acetic acid-water (15:5:5)	0.43

[a] All solutions are shown in volume proportions

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