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Development of HPTLC-densitometry methods for quantifying naproxen sodium, loperamide hydrochloride and loratadine in pharmaceutical tablets using a model procedure reported earlier to transfer TLC screening methods for fake and substandard drugs

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ABSTRACT

An earlier reported model process for transfer of Global Pharma Health Fund E.V. Minilab® Manual and U.S. Food and Drug Administration (FDA) Compendium thin layer chromatography (TLC) screening methods for fake and substandard pharmaceuticals to quantitative high performance TLC-densitometry methods was used to develop and validate analyses of tablets containing naproxen sodium, loperamide hydrochloride and loratadine. Only readily available, inexpensive, and relatively nontoxic 'green' solvents specified in the model process were used for sample preparation and the mobile phases. The new methods were used to assay three tablets of each drug in triplicate within the calibration range of 70-130% of the label value, and results were validated for accuracy, precision, peak purity and peak identity according to the criteria of the model. TLC screening methods for these products are not available in either the Minilab® Manual or FDA Compendium, and we were able to develop, test and publish new methods for naproxen sodium and loratadine online in a Supplement to the Compendium.

KEYWORDS: naproxen sodium, loperamide hydrochloride, loratadine, thin layer chromatography, TLC, HPTLC, pharmaceutical product analysis, fake drugs

INTRODUCTION

Previously published papers [1-7] described a model process for transfer of qualitative/semiquantitative thin layer chromatography (TLC) methods used to identify pharmaceutical products with quality defects to high performance TLC (HPTLC)-densitometry methods that can be used to support regulatory sanctions. The TLC screening methods are contained in a U.S. Food and Drug Administration (FDA) Compendium [8] and the Minilab® manual published by the Global Pharma Health Fund E.V. (GPHF) [9], a charitable organization maintained by Merck KGaA. The methods transferred with the model were for the drugs acetaminophen, acetylsalicylic acid, ibuprofen, chlorpheniramine maleate, mebendazole, diphenhydramine HCl, amodiaquine, artesunate, diazepam, lumefantrine, artemether, albendazole, artesunate, amoxicillin, pyrazinamide, ethambutol, isoniazid, rifampicin, quinine sulfate, mefloquine, dihydroartemisinin and piperaquine phosphate. In addition, the model was used earlier to develop HPTLC-densitometry methods for two drugs, amitriptyline HCl [2] and aciclovir [5], for which there are no Minilab® or Compendium methods.

The model process comprises sample and standard solution 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 precision and accuracy

of the method using the standard addition method, and peak purity and peak identification tests. In this paper we report the use of the model to develop and validate HPTLC-densitometry methods for tablet formulations of three drugs namely the non-steroidal anti-inflammatory drug (NSAID) naproxen sodium (CAS No. 26159-34-2), the anti-diarrheal loperamide hydrochloride (HCl; CAS No. 34552-83-5), and the antihistamine loratadine (CAS No. 79794-75-5), for which there are no Minilab® or Compendium methods published. Based on the development of these methods, TLC screening methods were developed, tested and published for naproxen sodium and loratadine tablets in an open access online Supplement to the FDA Compendium [10] from which they could be easily transferred to Minilab® TLC screening methods, if desired, by taking into account the 2 uL rather than 3 uL manual spotting volumes and the use of an authentic drug product rather than a commercial standard to prepare the standard solutions in the Minilab®.

MATERIALS AND METHODS

Standard and sample solutions were prepared for the three drugs as described in the next section. Detailed general sample preparation methods for the preparation of these solutions as described earlier [1-4] were used unless otherwise noted. All tablet samples were ground with a mortar and pestle and dissolved with the aid of magnetic stirring and sonication for 10 min each, and a portion was syringe filtered to remove any undissolved excipients before further dilution or direct application to the HPTLC plate. All chemicals and solvents used were of analytical reagent grade. Solutions were placed in sealed vial wrapped in parafilm and stored in a refrigerator.

Standard and sample preparation

For the naproxen sodium method, 40.0 mg of standard (Sigma-Aldrich, Co., St. Louis, MO, USA; Catalog No. PHR1165) was dissolved in 100 mL of methanol in a 100 mL volumetric flask to prepare the 100% standard solution at a concentration of 4.00 µg/10.0 µL. A naproxen sodium tablet with a label declaration of 220 mg active pharmaceutical ingredient (API) (Bayer Healthcare, LLC, Whippany, NJ, USA) was dissolved in 100 mL of methanol, and 2.00 mL was diluted with 9.00 mL of methanol to prepare the 100% sample solution at a concentration of 4.00 µg/10.0 µL.

For the loperamide HCl method, the 100% standard solution was prepared by dissolving 20.0 mg of standard (Sigma-Aldrich No. PHR1162) in 100 mL methanol to give a concentration of 2.00 µg/10.0 µL. A loperamide HCl tablet with a label declaration of 2 mg API (Remedica Ltd., Limassol, Cyprus) was dissolved in 10 mL of methanol to give the 100% sample solution a theoretical concentration of 2.00 µg/10.0 µL.

For the loratadine method, the 100% standard solution was prepared by dissolving 5.0 mg standard (Sigma-Aldrich No. PHR1376) in 100 mL of methanol to give a concentration of 0.500 µg/10.0 µL. A loratadine tablet with a label declaration of 10 mg API (Hovid Bhd, Ipoh, Malaysia) was dissolved in 50 mL of methanol, and 5.00 mL of this solution was further diluted with 15.0 mL methanol in a 25 mL volumetric flask to give a 0.500 µg/10.0 µL 100% sample solution.

HPTLC

HPTLC-densitometry was carried out as described in detail earlier [1-4] using silica gel 60 F_{254} Premium Purity HPTLC glass plates (20 x 10 cm; EMD Millipore Corp., Billerica, MA, a division of Merck KGaA, Darmstadt, Germany; Part No. 1.05648.0001) without prewashing, a CAMAG (Wilmington, NC, USA) Linomat 4 spray-on applicator (band length 6 mm, application rate 4 s/µL, 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) and Scanner 3 (4.00 x 0.45 mm Micro slit dimensions, 20 mm/s scan rate). The only change was the application of a band of a visible pink dye solution on the first track after plate development to aid exact centering of the green illuminated light beam for subsequent scanning of all other tracks containing non-visible fluorescence quenching bands. The mobile phase was ethyl acetate-glacial acetic acid (95:5) for naproxen sodium (R_f 0.68), ethyl acetate-methanol-concentrated ammonium hydroxide (24:3:1) for loperamide HCl (R_f 0.73) and acetone-concentrated ammonium hydroxide (99:1) for loratadine. All drugs quenched fluorescence of the phosphor in the F-layers and were detected and scanned at 254 nm. The Scanner 3 winCATS software automatically created calibration curves and interpolated weights

of drugs in samples based on their scan areas. Peak purity and identity of the sample were tested using the respective scanner software options. Accuracy of the developed methods was validated by using standard addition with a 70-130% calibration curve as described earlier [3].

RESULTS

Naproxen sodium tablets

Tablet 1 gave a mean weight of 3.64 µg and a mean assay of 91.1% relative to the label value (4.00 µg theoretical) with RSD of 0.441%, Tablet 2 gave mean weight 4.01 µg and mean assay 100% with RSD 2.24%, and Tablet 3 gave mean weight 3.77 µg and mean assay 94.3% with RSD 0.362%. Accuracy estimation based on the standard addition recovery results was a mean of 105% with RSD 1.21% at the 50% spike level, 101% with RSD 0.663% at the 100% spike level, and 98.2% with RSD 2.21% at the 150% spike level for a fourth, unspiked tablet.

Loperamide HCl tablets

Tablet 1 gave a mean weight of 2.02 µg and a mean assay of 101% relative to the label value (2.00 µg theoretical) with RSD of 0.914%, Tablet 2 gave mean weight 2.17 µg and mean assay 109% with RSD 0.560%, and Tablet 3 gave mean weight 2.19 µg and mean assay 110% with RSD 0.406%. Accuracy estimation based on the standard addition recovery results was a mean of 105% with RSD 2.66% at the 50% spike level, 103% with RSD 2.30% at the 100% spike level, and 101% with RSD 2.72% at the 150% spike level for a fourth, unspiked tablet.

Loratadine tablets

Tablet 1 gave a mean weight of 0.486 µg and a mean assay of 97.2% relative to the label value (0.500 µg theoretical) with RSD of 0.267%, Tablet 2 gave mean weight 0.477 µg and mean assay 95.5% with RSD 1.13%, and Tablet 3 gave mean weight 0.472 µg and mean assay 94.3% with RSD 1.96%. Accuracy estimation based on the standard addition recovery results was a mean of 97.6% with RSD 0.840% at the 50% spike level, 97.5% with RSD 1.36% at the 100% spike level, and 103% with RSD 0.954% for a fourth, unspiked tablet.

DISCUSSION

Densitometry was carried out using polynomial regression for naproxen sodium and linear regression for loperamide HCl and loratadine because these modes gave better results in terms of calibration curve r-values, assay values closer to the label value, accuracy of the standard addition validation and lower RSDs for each drug. All calibration curve r-values in our assay and validation experiments were at least 0.99, all validation recoveries were within +/- 5%, RSDs for triplicate assays and validation analyses at 50, 100, and 150% spike levels were within 3%, and peak purity and peak identity r-values were 0.99 consistent with the model requirements. All assays were within 85-115% specifications limits of the label value as specified by the U.S. Pharmacopeia (USP) for individual tablets.

The naproxen sodium method was developed using the ethyl acetate-glacial acetic acid (95:5) mobile phase reported earlier by our laboratory [11], but that method is now more fully validated according to the model process.

The only HPTLC-densitometry method available in the literature [12] for assay of loperamide HCl in a pharmaceutical product specified the mobile phase 1-butanol-glacial acetic acid-water (7:1:2), but 1-butanol is not a solvent usable in the model procedure. This mobile phase was modified by substituting ethanol for butanol and changing the proportions to 42.5:5:2.5, but a dark line (secondary or beta front) was produced immediately above the drug bands and precluded their accurate scanning. The formation of a beta front that disturbs scanning of the drug bands upon mobile phase development on Merck HPTLC silica gel 60 F_{254} plates was discussed earlier [3], and it was shown that changing to Merck Premium Purity HPTLC plates reduced this front but did not eliminate it. In the case of loperamide HCl, it was found that by changing the mobile phase to ethyl acetate-methanol-concentrated ammonium hydroxide (24:3:1) [1], the bands and the beta front were baseline separated, and the bands could be measured accurately by scanning (see example densitogram in Figure 1). All of the mobile phases employed in this research formed beta fronts with R_f values ranging from 0.72 to 0.85; all fronts gave a scan peak at 254 nm, but not all were visible to the eye at this wavelength. In drug analysis with our model, it is important to choose a layer-mobile phase system that gives the maximum possible separation between the drug and beta front scan peaks and to choose consistent integration limits for the standard and sample drug scan peaks across

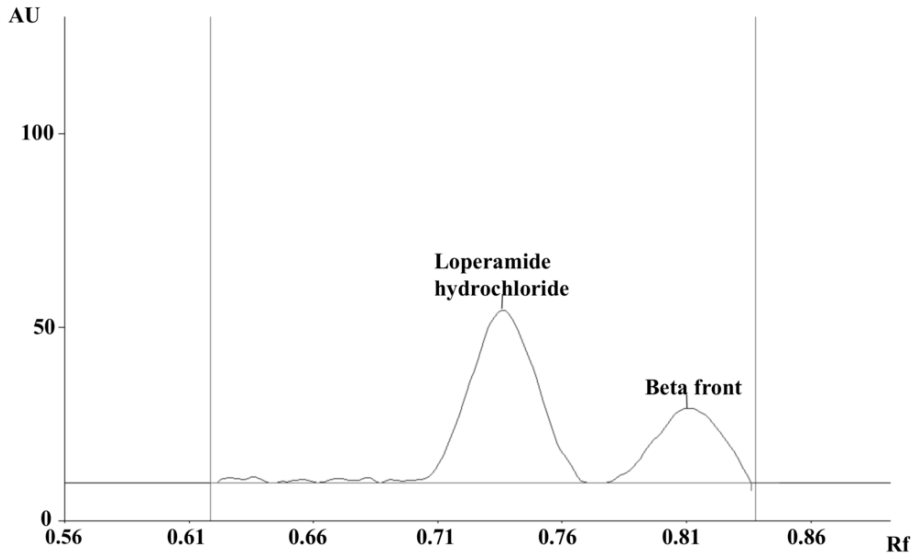

Figure 1. Densitogram of 10.0 µL of loperamide HCl sample solution representing 2.01 µg when interpolated from the calibration curve based on its area.

all tracks of a plate with the winCATS software to minimize the interference of the beta front. Beta fronts were not dark enough on the aluminum-backed TLC plates used for the Compendium methods discussed below to interfere with the visual comparison of standard and sample zones using any mobile phase.

Only one previous paper on TLC reported the use of a mobile phase with allowed solvents, i.e., methanol-concentrated ammonium hydroxide (10:0.3) [13] for assay of loratadine pharmaceutical products. This mobile phase did not separate the drug bands from the beta front on Premium Purity plates sufficiently for accurate scanning, but it was found that acetone-concentrated ammonium hydroxide (99:1) [2] gave adequate separation.

Supplemental Compendium TLC methods were developed and tested on 5 x 10 cm EMD Millipore Corp. aluminum-backed silica gel F_{254} plates (Part No. 1.16834.0001) rather than Premium Purity plates and posted online in a Supplement to the Compendium [10]. For naproxen sodium 220 mg tablet, the Compendium method was a direct transfer from the HPTLC-densitometry method described above because the same weights of drug were spotted in the same solvent, and the same mobile phase and detection methods were used. A Compendium method for naproxen 500 mg tablet (naprosyn) was also developed by simply changing the 100% sample solution preparation procedure compared to naproxen 220 mg [10]. The Compendium method [10] for loratadine uses methanol-concentrated ammonium hydroxide (10:0.3) [13], which did not form a significant interfering beta front for visual comparison of 85% and 115% standard and 100% sample spots on aluminum-backed silica gel TLC plates as it did on the Premium Purity plates, requiring a change of mobile phase to acetone-concentrated ammonium hydroxide (99:1) for the HPTLC-densitometry method described above. Direct transfer of Compendium methods [10] were also developed for the HPTLC-densitometry methods reported earlier for amitriptyline HCl 25 mg tablet [2] and aciclovir 200 mg tablet [5]. A Compendium method for loperamide HCl could not be worked out because the low, 2 mg drug content did not allow the convenient and accurate preparation of a 100% sample solution of a sufficient concentration from one tablet for visual detection and comparison using the required 3 uL application volumes.

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 guidelines such as those described by the International Conference on Harmonization [14] or subjected to an

interlaboratory study [15] to prove that they are suitable for their intended purpose.

CONCLUSION

HPTLC-densitometry procedures were developed and validated for assay of pharmaceutical tablets containing the drugs naproxen sodium, loperamide hydrochloride and loratadine as active ingredients based on a model process published earlier for transfer of TLC qualitative/semiquantitative screening methods for fake and substandard drugs to quantitative methods more suitable for support of regulatory compliance actions. Merck Premium Purity silica gel HPTLC plates, a CAMAG Linomat 4 for spray-on application of sample and standard bands, "green" mobile phases for development of chromatograms, and a CAMAG Scanner 3 for measurement of separated bands were used in each method.

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CONFLICT OF INTEREST STATEMENT

None of the authors has a conflict of interest related to this work.

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