

Use of a Model Process for Transfer of Minilab TLC Screening Methods for Quinine Sulfate, Mefloquine, and Dihydroartemisinin–Piperaquine Phosphate Tablets to Quantitative HPTLC–Densitometry Methods

J. STROCK, M. NGUYEN, AND J. SHERMA*

Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA

*E-mail: shermaj@lafayette.edu

Summary. Transfer of four thin-layer chromatography (TLC) Global Pharma Health Fund Minilab mobile kit protocols for detecting fake pharmaceutical products to quantitative high-performance TLC (HPTLC)–densitometry methods was carried out using a model process published earlier. The developed and validated methods were for the drugs quinine sulfate, mefloquine, dihydroartemisinin, and piperaquine phosphate. EMD Millipore Premium Purity silica gel 60 F₂₅₄ glass plates, automated standard and sample solution application with a CAMAG Linomat 4, and automated densitometry with a CAMAG Scanner 3 for detection, identification, and quantification were used. Sample peak identity and purity validation were carried out by spectral comparison checks available in the winCATS software, and accuracy was estimated by the standard addition approach. HPTLC gives better efficiency, selectivity, and resolution than TLC, and the new methods overcome the deficiencies in technology related to manual application and visual zone comparison that do not allow the Minilab TLC procedures to support regulatory compliance actions. These new methods should be fully validated according to International Conference on Harmonization guidelines or by interlaboratory studies if required by their applications. In addition, a previously reported transferred simultaneous HPTLC–densitometry method for lumefantrine and artemether was used to analyze a new combination tablet to demonstrate its applicability.

Key Words: quinine sulfate, mefloquine, dihydroartemisinin, piperaquine, lumefantrine, artemether, thin-layer chromatography, fake drugs, transfer of TLC methods to HPTLC–densitometry

Introduction

Previously published papers [1–6] described a model process for transfer of qualitative/semiquantitative thin-layer chromatography (TLC) methods used to identify pharmaceutical products with quality defects to quantitative high-performance TLC (HPTLC)–densitometry methods that can be

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used to support regulatory sanctions. The model process includes sample and standard solution preparation, establishment of the calibration curve, assay of pharmaceutical products for drug active ingredients relative to the label value, validation of accuracy and precision of the new method using the standard addition method, and sample peak purity and identity tests. The TLC screening methods are contained in an early U.S. Food and Drug Administration Compendium [7] and in manuals published by the Global Pharma Health Fund E.V. (GPHF) [8], a charitable organization maintained by Merck KGaA, to detect fake medicines. In this paper, we report the use of the model process to transfer methods for the anti-leg cramp drug quinine sulfate and the antimalaria drug mefloquine in individual tablets and the antimalaria drugs dihydroartemisinin and piperaquine phosphate in a combination tablet dosage form. In addition, a previously published combination method for the antimalaria drugs artemether and lumefantrine was applied successfully to a new tablet pharmaceutical product using a secondary standard.

Experimental

Analytical procedures described in detail in earlier papers [1–6] were used in this work unless otherwise noted. All tablet samples were ground with a mortar and pestle and dissolved with the aid of 10 min each of magnetic stirring and sonication, and a portion was syringe filtered to remove any undissolved inert ingredients before further dilution or direct application to the HPTLC plate. All chemicals and solvents used were analytical reagent grade.

Standard and Sample Preparation

The Minilab specifies use of reference tablets with known amounts of active pharmaceutical ingredients (APIs) to prepare the 100% standard solutions for their methods. In the absence of these, we used commercially available drug standards. Standard and sample solutions in volumetric flasks or vials were sealed with parafilm and stored in a refrigerator between analyses.

For the quinine sulfate method, 300 mg standard (Sigma-Aldrich, St. Louis, MO USA; Catalog No. Q0132) was dissolved in 20.0 mL of water followed by 180 mL of methanol in a 200-mL volumetric flask. One milliliter of this solution was further diluted with 5.00 mL of methanol to make the 100% standard solution at a concentration of 2.50 $\mu\text{g}/10.0 \mu\text{L}$. Quinine sulfate tablets from Remedica Ltd., Limassol, Cyprus, and Elys Chemical Ind.

Ltd., Nairobi, Kenya, with label declarations of 300 mg active pharmaceutical ingredient (API) were dissolved in water and methanol in a 200-mL volumetric flask as described above for the standard. One milliliter was diluted with 5.00 mL of methanol to prepare the 100% sample solution at a concentration of 2.50 $\mu\text{g}/10.0 \mu\text{L}$.

For the mefloquine method, the 100% standard solution was prepared by dissolving 10.0 mg of mefloquine base standard (Sigma-Aldrich No. M2319) in methanol in a 20-mL volumetric flask to give a concentration of 5.00 $\mu\text{g}/10.0 \mu\text{L}$. Mephaquine tablets from Mepha LLC, Aesch-Basel, Switzerland, with a label value of 250 mg mefloquine base were dissolved in a 25-mL volumetric flask, and then, 1.00 mL was diluted with 19.0 mL of methanol to give a 5.00 $\mu\text{g}/10.0 \mu\text{L}$ 100% sample solution.

For the dihydroartemisinin method, the 100% standard solution was prepared by dissolving 4.00 mg of dihydroartemisinin standard (Sigma-Aldrich No. D7439) in methanol in a 10-mL volumetric flask to give a concentration of 4.00 $\mu\text{g}/10.0 \mu\text{L}$. Duo-Cotecxin combination tablets from Zhejiang Holly Nanhu Pharmaceutical Co., Ltd., Jiaxing City, People's Republic of China, with a label value of 40 mg dihydroartemisinin + 320 mg piperaquine phosphate were dissolved in methanol in a 100-mL volumetric flask to give a 4.00 $\mu\text{g}/10.0 \mu\text{L}$ 100% sample solution.

For the piperaquine tetraphosphate method, the 100% standard solution was prepared by dissolving 2.16 mg of piperaquine tetraphosphate tetrahydrate (Sigma-Aldrich No. C7874) in methanol-concentrated (32%) HCl (99:1) to give a concentration of 2.00 $\mu\text{g}/10.0 \mu\text{L}$ piperaquine tetraphosphate. The 2.16 mg amount of tetraphosphate tetrahydrate (molecular weight [MW] 999.55) in the standard is equivalent to 2.00 mg of the tetrahydrate (MW 927.55), the form in the tablets. The Duo-Cotecxin combination tablets were dissolved in methanol-concentrated HCl (99:1) in a 100-mL volumetric flask. One milliliter of this solution was mixed in a vial with 15.0 mL of methanol-concentrated HCl (99:1) to give a 2.00/10.0 μL 100% sample solution. After refrigeration of the 100% standard solution overnight between analyses, it was necessary to bring it to room temperature and sonicate for 10 min in order to redissolve a small amount of white precipitate that appeared; alternatively, fresh standard solution can be prepared each day.

Standard and sample solution preparation for analysis of artemether-lumefantrine tablets is described in the Results section below.

HPTLC

HPTLC–densitometry for tablet formulation analysis was carried out as described in detail earlier [1–3] using EMD Millipore Corp. (Billerica, MA, USA, a division of Merck KGaA, Darmstadt, Germany) silica gel 60 F₂₅₄ Premium Purity for Pharmacopeia Applications HPTLC glass plates (20 × 10 cm, Part No. 1.05648.001) without prewashing. For each assay, the 100% standard solution was applied in 7.00, 9.00, 11.0, and 13.0 µL aliquots (representing 70–130% API content based on the label value), and the 100% sample solution was applied in 10.0 µL aliquots in triplicate ($n = 3$). Sample and standard solutions were applied on the plate in 6-mm length bands using a CAMAG (Wilmington, DE USA) Linomat 4 equipped with a 100-µL syringe. Plates were developed to a distance of 7 cm beyond the bottom of the plate in a mobile phase vapor saturated CAMAG HPTLC twin-trough chamber. The mobile phase was methanol–concentrated ammonium hydroxide (20:0.5) for quinine sulfate (R_f 0.51); ethyl acetate–methanol–concentrated ammonium hydroxide (16:4:3) for mefloquine (R_f 0.69), hydroartemisinin (R_f 0.78), and piperaquine tetraphosphate (R_f 0.64); and ethyl acetate–glacial acetic acid–toluene (4:2:18) for artemether and lumefantrine.

After drying the plates, quinine sulfate, mefloquine, lumefantrine, and piperaquine phosphate bands that quenched fluorescence of the F layers were scanned in the absorbance–reflectance mode using a CAMAG Scanner 3 at 254 nm with 4.00 mm slit length. Dark brown bands of dihydroartemisinins were scanned at 520 nm after spraying the plate with methanol–concentrated sulfuric acid (19:1) and heating for 5 min at 100 °C. The winCATS software automatically created calibration curves by linear or second order polynomial regression. Sample peak purity and identity were tested using the respective Scanner 3 winCATS software options. Accuracy of the developed methods was validated by standard addition using a 70–130% calibration curve as described earlier [3].

Results

Densitometry was carried out using linear regression for quinine sulfate, mefloquine, artemether, and piperaquine tetraphosphate and polynomial regression for lumefantrine and dihydroartemisinin because these modes gave the best results in terms of calibration curve r -values, assay values closer to the label value, accuracy of the standard addition validation, and lower RSDs; r -values of all calibration curves in our assay and validation

experiments were >0.99 . Standard weights applied represented 70–130% of the theoretical weight of the 100% sample solution applied (100% of the label value). All assay and validation analyses were performed in triplicate ($n = 3$). Peak purity and identity for all methods were confirmed by r -values of 0.99 for these tests. The model procedure acceptance criteria for validation of accuracy (recovery) 95–105% and $RSD \leq 3\%$ [3–7] were met in all cases. Almost all tablets assayed in the range 90–110% of the label value as set by the U.S. Pharmacopeia, and all were within the 70–130% test range of the model procedure. None of the pharmaceutical products had the inactive ingredients listed on their labels; no sample chromatogram contained visible zones other than those of the APIs.

Quinine Sulfate Tablets

Remedica Tablet 1 gave a mean weight of 2.49 μg and a mean assay of 99.8% relative to the label value (2.50 μg theoretical) with relative standard deviation (RSD) of 0.402%, Tablet 2 gave a mean weight 2.50 μg and a mean assay of 100% with RSD of 0.166%, and Tablet 3 gave a mean weight of 2.48 μg and a mean assay of 99.2% with RSD of 0.553%. Elys Tablet 1 gave a mean weight of 2.44 μg and a mean assay of 97.5% with RSD of 1.01%, Tablet 2 gave a mean weight of 2.32 μg and a mean assay of 92.8% with RSD of 1.17%, and Tablet 3 gave a mean weight of 2.22 μg and a mean assay of 88.7% with RSD of 2.77%. Accuracy estimation based on the standard addition recovery results was a mean of 104% with RSD of 1.16% at the 50% spike level, 101% with RSD of 1.25% at the 100% spike level, and 102% with RSD of 0.336% at the 150% level for a fourth, unspiked Remedica tablet that assayed at 105% of the label value and 103% with RSD 0.820% at the 50% spike level, 103% with RSD 1.15% at the 100% spike level, and 101% with RSD 0.557% at the 150% level for a fourth, unspiked Elys tablet that assayed at 76.4% of the label value.

Mefloquine Tablets

Tablet 1 gave a mean weight of 5.20 μg and a mean assay of 104% relative to the label value (5.00 μg theoretical) with RSD of 1.19%, Tablet 2 gave a mean weight of 5.15 μg and a mean assay of 103% with RSD of 2.59%, and Tablet 3 gave a mean weight of 5.15 μg and a mean assay of 103% with RSD of 1.44%. Accuracy estimation based on the standard addition recovery results

was a mean of 97.2% with RSD of 0.855% at the 50% spike level, 96.6% with RSD of 0.135% at the 100% spike level, and 95.3% with RSD of 2.52% at the 150% level for a fourth, unspiked tablet that assayed at 107% of the label value.

Dihydroartemisinin-Piperaquine Tetraphosphate Tablets

For dihydroartemisinin, Tablet 1 gave a mean weight of 4.36 μg and a mean assay of 109% relative to the label value (4.00 μg theoretical) with RSD of 1.42%, Tablet 2 gave a mean weight of 4.16 μg and a mean assay of 104% with RSD of 1.22%, and Tablet 3 gave a mean weight of 3.75 μg and a mean assay of 93.8% with RSD of 1.20%. Accuracy estimation based on the standard addition recovery results was a mean of 99.3% with percent difference of 0.303% at the 50% spike level, 103% with RSD of 2.43% at the 100% spike level, and 94.0% with RSD of 0.823% at the 150% level for a fourth, unspiked tablet that assayed at 116% of the label value. The 50% spike level results were based on $n = 2$ trials because the third trial result could be rejected by the statistical Q test ($Q = 0.94$ versus tabulated $Q = 1.0$ at $n = 3$).

For piperaquine tetraphosphate, Tablet 1 gave a mean weight of 1.66 μg and a mean assay of 83.2% relative to the label value (2.00 μg theoretical) with RSD of 2.28%, Tablet 2 gave a mean weight of 2.04 μg and a mean assay of 102% with RSD of 0.853%, and Tablet 3 gave a mean weight of 2.06 μg and a mean assay of 103% with RSD of 1.01%. Accuracy estimation based on the standard addition recovery results was a mean of 98.0% with RSD of 1.90% at the 50% spike level, 99.4% with RSD of 1.89% at the 100% spike level, and 96.9% with RSD of 0.802% at the 150% level for a fourth, unspiked tablet that assayed at 109% of the label value.

Artemether-Lumefantrine Tablets

A previously published [4] validated combination method for artemether 20 mg + lumefantrine 120 mg tablets was used to analyze a new product to illustrate its applicability. The exact procedure published earlier was followed except that instead of using primary standards of the two drugs from Sigma-Aldrich, tablets from Ipca Laboratories, Ltd., Mumbai, India, that were analyzed in the previous work [4] were used as a secondary standard for analysis of Artefan 20/120 tablets with the same formulation from Ajanta Pharma Ltd., Mumbai, India. The 100% standard solutions and 100%

sample solutions were prepared in acetone as in the earlier study at 0.113 $\mu\text{g}/\mu\text{L}$ for artemether and 0.800 $\mu\text{g}/\mu\text{L}$ for lumefantrine. For lumefantrine, Tablet 1 gave a mean weight of 8.08 μg and a mean assay of 101% relative to the label value (8.00 μg theoretical) with relative RSD of 2.21%, Tablet 2 gave a mean weight of 7.98 μg and a mean assay of 99.7% with RSD of 1.55%, and Tablet 3 gave a mean weight of 8.08 μg and a mean assay of 101% with RSD of 1.69%. For artemether, Tablet 1 gave a mean weight of 1.45 μg and a mean assay of 110% relative to the label value (1.33 μg theoretical) with relative RSD of 1.77%, Tablet 2 gave a mean weight of 1.46 μg and a mean assay of 110% with RSD of 0.860%, and Tablet 3 gave a mean weight of 1.43 μg and a mean assay of 108% with RSD of 1.60%.

Discussion

Both the FDA Compendium and Minilab have TLC methods with fluorescence quenching detection for quinine sulfate. Our use of water plus methanol as the standard and sample solvents, application of 2.50 μg of sample and standard, and methanol–water (20:0.5) mobile phase followed the Minilab method, which were better than water solvent, 1.5 μg applied, and ethanol–water–concentrated ammonium hydroxide (20:3:1) mobile phase from the Compendium.

The Minilab TLC method for mefloquine was transferred directly in terms of the use of methanol as the standard and sample solution solvent, 5.00 μg applied for the 100% standard and sample solutions, the mobile phase, and the detection method. A literature search on January 8, 2015, using the ISI Web of Science found no TLC–densitometry method for any mefloquine pharmaceutical product reported.

The dihydroartemisinin and piperazine tetraphosphate methods were direct transfers from the Minilab methods for these drugs in a combination tablet in terms of applied standard and sample weights, mobile phase, and detection reagent. A literature search on January 8, 2015, using the ISI Web of Science found no TLC–densitometry method for any individual or combination dihydroartemisinin and/or piperazine pharmaceutical products reported. As found earlier for the drugs artesunate [5] and artemether [4], heating of the plate for 5 min at 160 °C allowed dihydroartemisinin to be detected under 254 nm UV light (reagent-free thermochemical activation fluorescence quench detection). However, densitometric determination attempted at this wavelength was unsuccessful because the scans of the two drugs were not baseline separated at the levels applied within the calibra-

tion ranges. Therefore, the methanol-sulfuric acid detection reagent was used for quantification as specified in the Minilab manual; an example densitogram of a standard zone after application of the spray reagent is shown in Fig. 1. Piperaquine phosphate reacted slightly with methanol-sulfuric acid reagent giving a very light band that did not interfere with scanning of the dihydroartemisinin. Thermochemical activation would be adequate for qualitative/semiquantitative screening of both drugs in a combination formulation on one plate without need for the spray reagent. A simultaneous

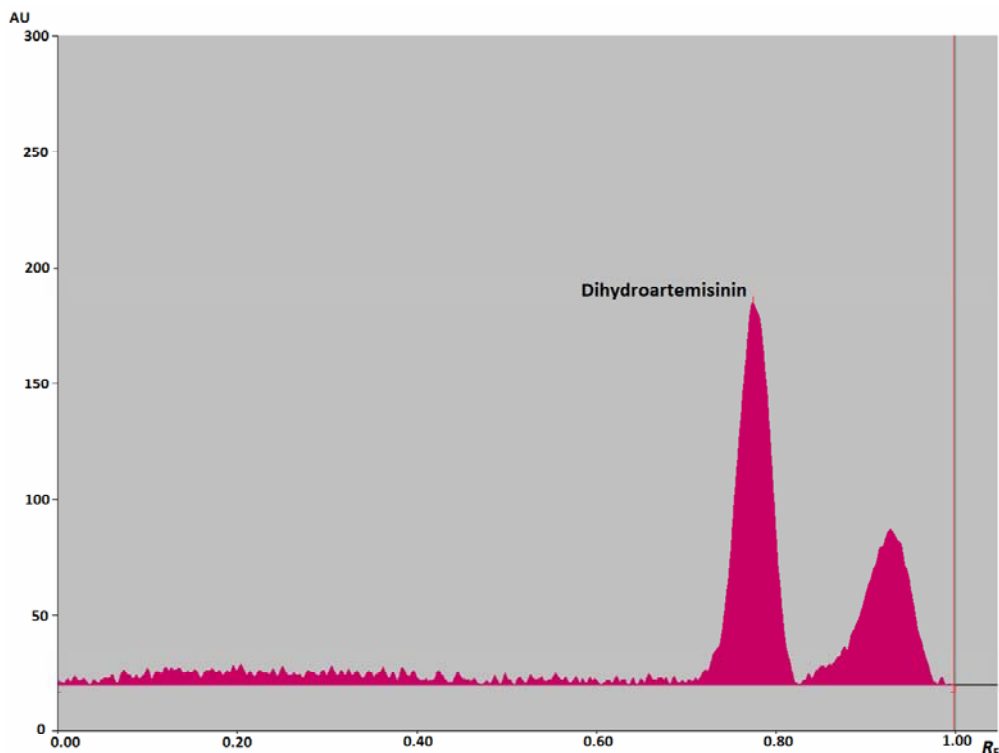


Fig. 1. Densitogram of dihydroartemisinin scanned at 520 nm after spraying with methanol-concentrated sulfuric acid (19:1) and heating. The peak after the drug peak is the result of a brown line that appeared across the whole plate just behind the mobile phase front

method for the two components could not be developed using one sample solution on one plate because of the large differences in API amounts in the combination tablet. The closest method would involve preparing the 100% sample solution of dihydroartemisinin in methanol-HCl rather than metha-

nol in the individual method described above, overspotting 7.00, 9.00, 11.0, and 13.0 μL of the 100% standard solutions from the individual methods on the first four lanes, applying triplicate 10.0 μL aliquots of the 100% sample solution for determination of dihydroartemisinin on the next three lanes, spotting triplicate aliquots of the 100% sample solution after 1.00 mL + 15.0 mL dilution with methanol-HCl to bracket scan areas within the piperazine tetraphosphate calibration curve on the next three lanes, developing the plate with the common mobile phase of the individual methods, scanning the piperazine tetraphosphate bands at 254 nm using the deuterium source of the Scanner 3 (dihydroartemisinin bands not detected), spraying with methanol-sulfuric acid reagent, and scanning the dihydroartemisinin bands at 520 nm using the tungsten source (piperazine tetraphosphate bands not significantly detected).

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 [9] or subjected to an interlaboratory study [10] to prove that they are suitable for their intended purpose.

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References

- [1] J. Sherma and C. O'Sullivan, *Acta Chromatogr.*, **24**, 241–252 (2012)
- [2] K. Lianza and J. Sherma, *J. Liq. Chromatogr. Relat. Technol.*, **36**, 2446–2452 (2013)
- [3] N. Popovic and J. Sherma, *Acta Chromatogr.*, **26**, 615–623 (2014)
- [4] M. Nguyen and J. Sherma, *Trends Chromatogr.*, **8**, 131–135 (2013)

- [5] M. Nguyen and J. Sherma, *J. Liq. Chromatogr. Relat. Technol.*, **37**, 2956–2970 (2014)
- [6] J. Strock, M. Nguyen, and J. Sherma, *J. Liq. Chromatogr. Relat. Technol.*, in press
- [7] K. Ferenczi-Fodor, Z. Vegh, A. Nagy-Turak, B. Renger, and M. Zeller, *J. AOAC Int.*, **84**, 1265–1276
- [8] A.S. Kenyon and T.P. Layloff, <http://www.pharmweb.net/pwmirror/library/tlc/tlcall.pdf>
- [9] <http://www.gphf.org>
- [10] E. Kaale, P. Risha, E. Reich, and T.P. Layloff, *J. AOAC Int.*, **93**, 1836–1843 (2010)