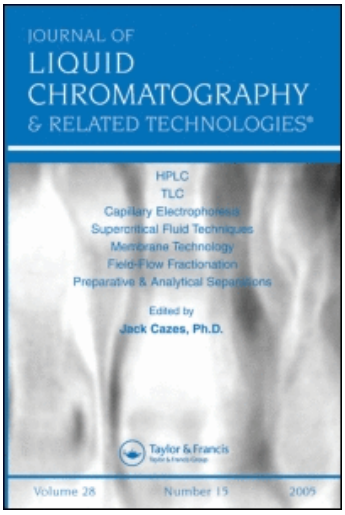

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Quantitative HPTLC Determination of Salicyclic Acid in Topical Acne Medications

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QUANTITATIVE HPTLC DETERMINATION OF SALICYCLIC ACID IN TOPICAL ACNE MEDICATIONS

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ABSTRACT

A method was developed for determination of salicylic acid in anti-acne pharmaceutical preparations involving separation on a preadsorbent high performance thin layer chromatography (HPTLC) silica gel plate with fluorescent phosphor, detection by fluorescence quenching, and quantification by densitometric scanning. Salicylic acid was directly detected and quantified on the plate at levels as low as 1 μg, and no interference was encountered from other ingredients in the medication formulations.

The method was applied to the analysis of a commercial gel preparation with a label value of 2.0% salicylic acid, and an average value of 99.2% of theoretical was obtained (n=16). Accuracy was validated by analysis of a 10% benzoyl peroxide anti-acne gel spiked with 2% salicylic acid, and recovery averaged 100.0% (n=10). The coefficients of variation for the analyses were 3.9 and 5.6%, respectively. Advantages of the new HPTLC method are discussed.

INTRODUCTION

In earlier studies, quantitative high performance thin layer chromatography (HPTLC) methods were developed for the active ingredients sulfur[1] and benzoyl peroxide[2] in a variety of topical ointment, cream, and liquid pharmaceutical preparations for control of acne. Other acne medications contain salicylic acid as the active ingredient, and in this paper we report an HPTLC method with scanning densitometry for their analysis. The official methods for determination of salicylic acid raw material and formulated pharmaceutical products involve titration with sodium hydroxide, ultraviolet spectrophotometry, or high performance column liquid chromatography.[3,4] The quantitative HPTLC method, which is based on separation of salicylic acid on preadsorbent silica gel plates containing fluorescent indicator, detection by fluorescence quenching, and *in situ* densitometric scanning at 244 nm, has advantages over the earlier methods and is shown to be sufficiently rapid, accurate, and precise for use in routine pharmaceutical analysis.

EXPERIMENTAL

Standard Solution

A standard solution (1.00 μg/μL) was prepared by dissolving 25.0 mg of reagent grade salicylic acid (Aldrich Chemical Co.) in reagent grade methanol in a 25 volumetric flask.

Thin Layer Chromatography

HPTLC was carried out on 10 x 20 cm Whatman LHPKDF high performance silica gel plates with 19 lanes, preadsorbent spotting area, and fluorescent phosphor (catalog no. 4806711). Standard and sample solutions were applied to the preadsorbent using a 10 μL Drummond digital microdispenser. Plates were developed with n-pentyl formate-chloroform-formic acid (2:7:1) for a distance of 7 cm beyond the silica gel preadsorbent interface in a paper-lined, solvent-saturated Camag twin trough chamber and then dried in a fume hood. Separated salicylic acid zones were detected by inspection under 254 nm UV light in a viewing cabinet and scanned using a Shimadzu CS-930 densitometer in the single-beam reflectance mode at 244 nm. This wavelength of maximum absorption was obtained by an *in situ*

spectral scan of a 6.00 μg standard zone between 200 and 370 nm.

Analysis of Samples

The medication analyzed to test the new method was a gel with a label declaration of 2% salicylic acid purchased in a local pharmacy without prescription. Approximately 500 mg of the sample was accurately weighed into a 15 ml vial, and 12.5 ml of methanol was added by pipet. The sample was dissolved by vigorous shaking.

A spiked acne gel medication having 10% benzoyl peroxide as the active ingredient and many other ingredients in common with the salicylic acid medication was used to test the accuracy of the new method. The spiked sample was prepared by weighing 500 mg of the gel and 10.0 mg of salicylic acid into a 15 ml vial and dissolving in 12.5 ml of methanol. A calibration curve was determined from the scan areas and spotted weights of 2.00, 4.00, and 6.00 μL aliquots of the 1.00 μg/μL standard using a Quattro curve-fitting program on an IBM-PC.

The percent salicyclic acid in the unknown was determined by spotting duplicate 4.00 μL aliquots of the standard and duplicate 5.00 μL aliquots of the sample, which would contain a theoretical value of 4.00 μg of salicylic acid according to the label value. The weight of salicylic acid in the sample was calculated by multiplying the weight of salicylic acid in the standard zones (4.00 μg) by the ratio of the average sample peak area to the average standard peak area. Percent salicylic acid was calculated by dividing the weight of salicylic acid by the weight of sample in the 5.00 μL aliquot and multiplying the quotient by 100.

The method was validated by analyzing the spiked sample as described above and calculating recovery by comparing the amount determined experimentally with the theoretical 2.00% fortification level. A blank containing 500 mg of sample without spike was also analyzed.

RESULTS AND DISCUSSION

Methanol completely dissolved the salicylic acid in the standard and

samples, but other insoluble sample ingredients settled to the bottom of the volumetric flasks and did not interfere with spotting. Salicylic acid produced a tight band with R_f 0.71 across the lane of the high performance preadsorbent silica gel plate when viewed under 254 nm UV light. Despite the presence of many ingredients in the medications analyzed, including Carbomer 940, dioctyl sodium sulfosuccinate, disodium EDTA, phenoxyethanol, triethanolamine, and polyglycerylmethacrylate, only one additional fluorescence-quenched zone, with an R_f of 0.61, appeared in sample chromatograms, and it did not interfere with scanning the salicylic acid zone. The benzoyl peroxide in the spiked sample migrated near the solvent front because of the high strength (polarity) of the mobile phase compared to methylene chloride-methanol (1:1), which gave an R_f value of 0.50 for benzoyl peroxide on the same layer.[2]

The calibration curve was repeated many times and found to have a typical linearity correlation coefficient (r value) of 0.93, with a range of 0.83-0.97, for 2-6 μg/spot of salicylic acid. In order to simplify and optimize the reliability of the analysis, quantification was performed by comparing the sample with a single standard having a similar zone area within the linear range, rather than constructing a new calibration curve on each plate. Standards and samples were always chromatographed together on each plate to correct for inevitable slight layer variations. The least intense zone that could be reproducibly scanned was 1.00 μL of standard (1.00 μg), which produced a scan area of about 1000 counts. The 244 nm maximum absorption determined from an *in situ* spectral measurement was quite different from the 270-280 nm detector setting used in HPLC.[4]

Four different samples of the salicylic acid medication with a 2.0% label value were analyzed four times each, for a total of 16 analyses. The overall average obtained was 1.98% salicylic acid, 99.2% of the label value. Standard deviation values for the replicate analyses averaged 3.9%. As a further measure of precision, the percent difference between the scan areas of the duplicate samples and standards spotted in each analysis was typically about 3%, with a range of 0.4 to 9%.

Four different portions of the benzoyl peroxide gel, spiked with 2.00% salicylic acid, were each was analyzed two or three times (total of 10 analyses). Recovery of the spike averaged 100.0% with a 5.6% standard deviation, which verifies the accuracy of the method and the value obtained for the analysis of the unknown. Blanks analyzed in the fortified sample assays did not show the presence of any interferences at the R_f of the analyte.

The quantitative HPTLC method described has high sample throughput because up to eight duplicate samples can be analyzed on a single plate along with the required duplicate standards, and it has adequate sensitivity, selectivity, accuracy, and precision for routine use in a pharmaceutical analytical laboratory. It is more selective than the earlier spectroscopic and titration methods because of the separation step. In contrast to HPLC, insoluble, inactive ingredients do not have to be filtered prior to TLC because the layer is not reused, and an internal standard is not required because samples and standards are separated under essentially identical conditions on the same plate. Other types of samples can be analyzed using the same basic procedure unless an interfering fluorescence-quenched zone migrates with the salicylic acid-zone. It is only required to dissolve the salicylic acid completely in methanol at a concentration that allows an aliquot of sample between 1 and 10 μL to produce a separated zone with a scan area similar to the 4 μL standard.

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