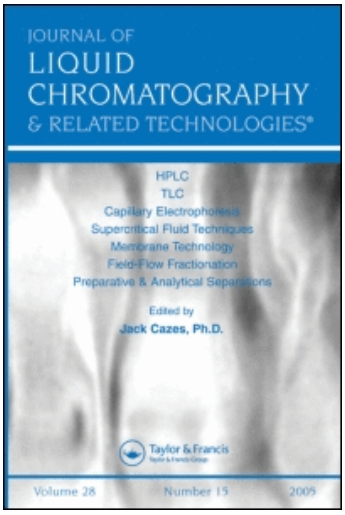

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D. Ruddy[a]; J. Sherma[a]
[a] Department of Chemistry, Lafayette College, Easton, PA, U.S.A.

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ASSAY OF THE ACTIVE INGREDIENT, POTASSIUM SALICYLATE, IN DIURETIC TABLETS AND CAPSULES BY HPTLC WITH ULTRAVIOLET ABSORPTION DENSITOMETRY

D. Ruddy and J. Sherma*

Department of Chemistry, Lafayette College, Easton, PA 18042, USA

ABSTRACT

A new quantitative method using preadsorbent, channeled silica gel HPTLC plates with fluorescent indicator, automated sample application, double development with ethyl acetate-methanol-concentrated ammonia (80 : 15 : 5) mobile phase, and ultraviolet absorption densitometry is reported for the determination of potassium salicylate in diuretic pharmaceutical preparations.

Tablet and capsule products containing potassium salicylate, acetaminophen, caffeine, and salicylamide as the active ingredients were analyzed to test the applicability of the new method, and precision and accuracy were validated. The milligrams of potassium salicylate in each tablet analyzed, ranged from 72.9 to 79.4, and the milligrams in each capsule from 26.5 to 28.3.

Precision was 1.11% and 1.45% relative standard deviation for samples analyzed with six replicates, and the errors from

*Corresponding author. E-mail: shermaj@Lafayette.edu

spiked blank and standard addition analyses averaged 1.01% and 1.65%, respectively, compared to the fortification levels. The limit of detection was 1.50 μg of potassium salicylate.

INTRODUCTION

In earlier papers, we described new quantitative high performance thin layer chromatography (HPTLC) methods for the analysis of different classes of pharmaceuticals, including analgesic, (1) antihistamine and decongestant, (2) sleep aid, (3) motion sickness, (4) stomach acid reduction, (5) alertness, (6) acne, (7) and diarrhea (8) medications. In this paper, we extend our method development studies to diuretic tablets and capsules containing potassium salicylate as the active ingredient. Diuretics are prescribed widely to increase the outflow of excess body water during the monthly menstrual period of women and for treatment of other medical conditions involving fluid retention, such as heart and kidney failure and cirrhosis. Tablets and capsules containing potassium salicylate as the active ingredient are among the most commonly used diuretics. The analgesics, acetaminophen and salicylamide and the anti-fatigue agent caffeine are additional active ingredients in these products.

The analysis of potassium salicylate is not covered in the latest USP. (9) A computer-based literature search of Chemical Abstracts located only one paper describing the high performance liquid chromatography (HPLC) determination of potassium salicylate. It reported analysis of tablets, capsules, and injection solution using a C-18 bonded silica gel column, aqueous methanol-pH 4 phosphate buffer mobile phase, and 300 nm UV detection. No previous TLC or HPTLC papers on the qualitative or quantitative determination of potassium salicylate were found. The new quantitative HPTLC method described below resolves all active ingredients in the pharmaceutical formulations, has excellent precision and accuracy and high sample throughput, and uses small amounts of solvent on a per-sample basis.

EXPERIMENTAL

Preparation of Standard Solutions

A standard of potassium salicylate (potassium 2-hydroxybenzoate, CAS #578-36-9) could not be obtained commercially. Therefore, the standard used was salicylic acid (2-hydroxybenzoic acid, catalog no. S-5922, Sigma, St. Louis, MO). A gravimetric factor, 1.275, the ratio of the molecular weight of potassium

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salicylate to that of salicylic acid, was used to convert the weight of salicylic acid to an equivalent weight of potassium salicylate in the standard solutions.

A stock solution containing the equivalent of 7.50 mg mL^{-1} of potassium salicylate was prepared by dissolving salicylic acid standard in absolute ethanol. A 0.750 mg mL^{-1} potassium salicylate TLC standard solution was prepared by 1:10 dilution of the stock solution with absolute ethanol.

Preparation of the Sample Solutions

Tablets containing potassium salicylate, caffeine, and salicylamide and capsules containing potassium salicylate, caffeine, and acetaminophen were obtained from a local pharmacy with no label specification of the active ingredients. Tablet sample solutions were prepared by grinding one tablet to a fine powder in a few milliliters of methanol-deionized water (7 : 3) using a mortar and pestle, and quantitatively transferring the powder through a funnel to a 100 mL volumetric flask by washing with ca. 75 mL of methanol-water (7 : 3).

Capsule sample solutions were prepared in exactly the same way after cutting open a capsule with a razor and placing the contents in a mortar. Each solution was magnetically stirred for 2 h, after which the stir bar was removed from the flask by use of a magnetic rod. The solution was diluted to the line with methanol-water (7 : 3) and shaken, and the undissolved material that remained was allowed to settle to the bottom of the flask. The clear supernatant liquid was used for HPTLC analysis.

HPTLC Analysis

Analyses were performed on Merck 10 cm × 20 cm HPTLC silica gel 60 CF_{254} plates (Art. 13 153, EM Science, Gibbstown, NJ, USA; an affiliate of Merck KGaA, Darmstadt, Germany) containing a preadsorbent or concentrating zone, nineteen channels, and fluorescent indicator. The plates were pre-washed by development to the top with dichloromethane-methanol (1 : 1) and dried in a fumehood.

Standard and sample solutions were applied onto the preadsorbent zone of the channels by means of a Linomat IV automated spray-on instrument (Camag, Wilmington, NC) equipped with a 100-μL syringe and operated with the following settings: band length 6 mm; application rate 4 s μL^{-1} for standard solutions, 10 s μL^{-1} for sample solutions; table speed 10 mm s^{-1}; and distances of 4 mm between bands, 6.5 cm from the plate edge, and 1.5 cm from the bottom of the plate. The volumes of TLC standard applied for each analysis were 2.00 μL, duplicate 4.00 μL, and 8.00 μL (equivalent to 1.50–6.00 μg of potassium

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salicylate). Preliminary experiments were performed for tablet and capsule samples to determine the volume of sample aliquots needed for the sample zone scan areas to be bracketed by the scan areas of the standard zones and as close as possible to the areas of the duplicate middle standard zones. As a result, duplicate 4.00 μL volumes were applied for tablet solutions and 12.0 μL for capsule solutions.

Plates were double-developed for a distance of 6 cm beyond the preadsorbent-layer interface, using a mobile phase consisting of ethyl acetate-methanol-concentrated ammonia (80 : 15 : 5) in a vapor-equilibrated Camag twin-trough HPTLC chamber containing an Analtech (Newark, DE, USA) HPTLC saturation pad. Approximately 30 mL of solvent was used for each development, which required 10–15 min. After each development, the plates were allowed to dry in a fumehood for 15 min and were then blown dry by means of a hair dryer on a low setting for 5 additional min.

The wavelength of maximum absorption was determined to be 302 nm by measurement of the in-situ UV absorption spectrum of a standard potassium salicylate zone by use of the spectral mode of the Camag TLC Scanner II. Sample and standard zones were quantified by linear scanning at 302 nm with the deuterium source, slit length 4, slit width 4, and scanning rate 4.0 mm s^{-1}. The CATS-3 software controlling the densitometer produced a calibration curve by polynomial regression relating the standard zone weights to their scan areas. By using the gravimetric factor to convert the standard weights, sample weights in μg of potassium salicylate were automatically determined from their scan areas by interpolation from the calibration curve. The number of milligrams per tablet, or milligrams per capsule, was calculated for each sample analysis by computing the concentration of the sample solution from the sample masses, determined by the densitometer (sample mass divided by volume spotted) and multiplying the concentration by the volume of the solution (concentration times 100 mL).

The limit of detection, the amount of potassium salicylate yielding a zone that is barely visible, was found by preparing 0.600 mg mL^{-1} and 0.750 mg mL^{-1} potassium salicylate standard solutions, applying each solution to a silica gel plate in volume increments of 2.00 to 8.00 μL, and visually examining the plates under 254 nm UV light after development.

The accuracy of the method was validated by standard addition analysis in which a sample solution was prepared and analyzed, as described above. An aliquot of the 7.50 mg mL^{-1} stock standard solution was added to 1.00 mL of the preanalyzed sample solution to double the concentration of potassium salicylate. The spiked solution was reanalyzed on a separate plate, and recovery was calculated by comparing the weight obtained in the analysis to the theoretical weight added.

The accuracy of the method was also validated by determining the recovery from two blank solutions spiked with an aliquot from the 7.50 mg mL^{-1} stock

standard solution. An alertness tablet containing caffeine (200 mg) active ingredient and an extra-strength non-aspirin pain reliever caplet containing acetaminophen (500 mg) active ingredient, were used as blanks. These were appropriate blanks because they contained five of the ten inactive ingredients and two of the three other active ingredients listed on the tablet and capsule labels. Additional inactive ingredients, including fillers, coloring, and coating, were present in the blank solutions that may have been among the unspecified inactive ingredients referred to on the tablet and capsule labels. To prepare the spiked samples, one caffeine tablet blank stock solution and one acetaminophen caplet blank stock solution was prepared as described above. Four spiked blank solutions were made by combining an aliquot from each blank solution with an aliquot from the 7.50 mg mL^{-1} standard stock solution in a 4-mL screw-cap vial, using 100-µL and 1000-µL Drummond (Broomall, PA, USA) microdispensers to total 1.00 mL in volume. Two spiked blank solutions, one caffeine and one acetaminophen, had the concentration of a tablet sample solution (ca. 0.750 mg mL^{-1}) and two more spiked blank solutions, one caffeine and one acetaminophen, had the concentration of a capsule sample solution (ca. 0.250 mg mL^{-1}). These concentrations were determined from preliminary analyses of tablets and capsules. The TLC standard, unspiked blank solutions, and duplicate spots of the spiked blank solutions were applied to one plate and analyzed as described above. Recovery was calculated by comparing the analytical result for the spiked blank solution with the theoretical value based on the weight of potassium salicylate added.

The precision of the method was validated by analyzing one capsule sample and one tablet sample six times on one plate, and calculating the relative standard deviation (RSD) of the determined potassium salicylate weights.

RESULTS AND DISCUSSION

The Merck Index states that potassium salicylate is "very soluble in water and alcohol," and stirring of crushed samples for 2 h with ethanol and methanol was originally tested as the extraction method, because these solvents are known to be excellent for sample application using the Linomat IV instrument. Later experiments proved that extraction with methanol-water (7 : 3) provided increased recovery efficiency. Different application parameters, as described above, were used so that standards applied in ethanol and samples in methanol-water (7 : 3) would have equally compact zones. Any minor difference in zone size, caused by the change in solvent, was eliminated by the concentrating effect of the preadsorbent zone on the plate. The channels on the plate facilitated initial line-up of the scanner light beam for automated scanning.

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Potassium salicylate appeared as flat, compact, light blue zones on a bright green background when viewed under 254 nm UV light. Fig. 1 shows the zones of potassium salicylate (R_f 0.30) and of acetaminophen, caffeine, and salicylamide (all R_f 0.78). Neither this zone, nor the dye zone (R_f 0.20) seen in the capsule chromatograms, interfered with scanning the potassium salicylate zone. Double development, a technique that is known to improve resolution of low R_f zones, was needed to fully separate the potassium salicylate from the dye in capsule samples. The correlation coefficient (R) values of the calibration curves produced for each plate by polynomial regression of the four standards (scan area count against µg spotted) was consistently 0.999.

The limit of detection was determined by viewing, under 254 nm UV light, the developed plates containing 1.20 to 6.00 µg standard zones of potassium salicylate. The zone from the 2.00 µL spot of the 0.600 mg mL^{-1} solution (1.20 µg) was not visible, but the zone from the 2.00 µL spot of the 0.750 mg mL^{-1} (1.50 µg) was barely visible. From these results, the limit of detection was determined to be 1.50 µg.

One brand of potassium salicylate diuretics is available over-the-counter in pharmacies in tablet and time-release capsule form. Five analyzed tablets gave a mean of 75.3 mg $tablet^{-1}$, and five capsules a mean of 27.2 mg $capsule^{-1}$. The individual results are shown in Table 1. Unlike most pharmaceutical preparations,

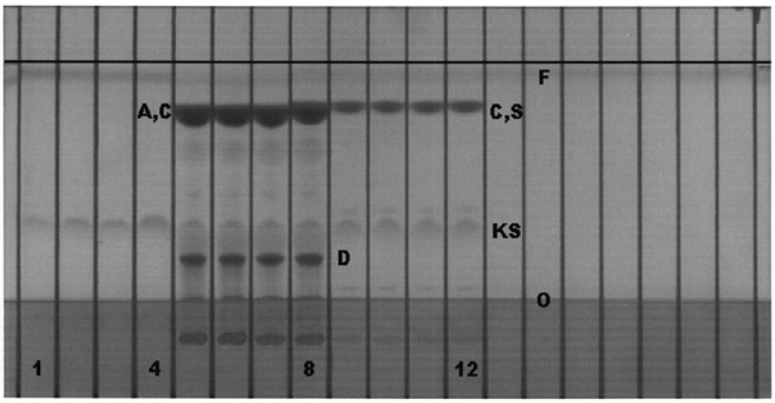

Figure 1. Appearance of chromatograms obtained in analysis of potassium salicylate tablets and capsules by the described HPTLC-densitometry method. The plate was photographed under 254 nm UV light with a Camag VideoStore Image Documentation System. F, mobile phase front; O, origin: preadsorbent-layer interface; A, acetaminophen, C, caffeine; S, salicylamide; D, dye; lanes 1–4, potassium salicylate (KS) standards; lanes 5–6, duplicates of capsule Sample 1 (Table 1); lanes 7–8, duplicates of capsule Sample 2; lanes 9–10, duplicates of tablet Sample 1; lanes 11–12, duplicates of tablet Sample 2.

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Table 1. Results of Analyses of Tablet and Capsule Dosage Forms of Potassium Salicylate

	Tablet		Capsule	
Sample	mg Tablet^{-1}	Average	mg Capsule^{-1}	Average
1	79.4	75.3	26.5	27.2
2	72.9		28.3	
3	73.3		27.6	
4	74.6		27.3	
5	76.3		26.5	

the amounts of the active ingredients were not specified on the labels of the tablets or capsules. Therefore, comparison of our results to expected values could not be made.

One tablet was analyzed six times on a single plate, and the RSD was 1.11%. Analysis of one capsule six times on a single plate yielded an RSD of 1.45%. For a further assessment of precision, differences between the scan areas of duplicate sample aliquots spotted in each analysis ranged from 0.00530 to 1.88%, with an average of 0.590%. These results prove the excellent reproducibility of the Merck HPTLC plates and the Camag automated Linomat and scanner.

Accuracy of the new method was validated by determining recoveries from the spiked blank solutions. No zone was present at the R_f value of potassium salicylate in the unspiked blank chromatograms, and no area counts were obtained when this region of the layer was scanned. Therefore, correction of the spiked blank chromatogram scans was not required. Comparison of the amount of potassium salicylate determined by analysis with the amount of potassium salicylate added as described above yielded recoveries of 102 and 101% from the acetaminophen caplet, and 102 and 99.3% from the caffeine tablet. These results represented errors of 1.86, 0.490, 2.39, and 0.710%, respectively. The accuracy of the method was further validated by standard addition of potassium salicylate to a preanalyzed sample to double the concentration, followed by reanalysis as described above. The recovery of the added capsule weight was 97.3%, and that of the tablet was 101%, representing errors of 2.67% and 0.618%, respectively.

It is shown, above that the new HPTLC method achieved standard deviations for replicate analyses and recoveries of analyte from spiked blank and standard addition samples that compare favorably with those reported regularly in the literature for HPTLC and HPLC pharmaceutical dosage forms. The results also meet the TLC requirements proposed to meet the International Conference on Harmonization (ICH) guidelines for validation of pharmaceutical assay

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analyses, which are a precision of 2–3% RSD and recovery (accuracy) or 95–105%.(10) The method is relatively cheaper, simpler, and faster compared to HPLC, and it is suitable for routine analysis of similar products containing potassium salicylate or other metal salicylates in pharmaceutical quality control laboratories. Previous papers describe the overall advantages of quantitative HPTLC relative to HPLC and other methods for assay of pharmaceutical dosage forms (1,2,11).

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