

**DETERMINATION OF SALICYLAMIDE
IN PHARMACEUTICAL TABLETS
BY HIGH-PERFORMANCE THIN-LAYER
CHROMATOGRAPHY
WITH ULTRAVIOLET ABSORPTION DENSITOMETRY**

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SUMMARY

A new quantitative method using silica gel high-performance thin-layer chromatography (HPTLC) plates with fluorescent indicator, channels, and a concentration zone; instrumental bandwise application of standards and samples; development with dichloromethane–acetone, 4:1, as mobile phase; and ultraviolet absorption densitometry of fluorescence quenched zones is reported for determination of salicylamide in pharmaceutical formulations. The products analyzed to test the applicability of the method were diuretic tablets containing salicylamide, potassium salicylate, and caffeine as active ingredients in unspecified amounts, and extra-strength pain-relief tablets containing salicylamide (152 mg), acetaminophen (110 mg), aspirin (162 mg), and caffeine (32.4 mg). Precision, accuracy, linearity, limits of detection and quantitation, and selectivity were validated. Amounts of salicylamide ranged from 2.67 to 2.89 mg in the five diuretic tablets analyzed, and recoveries from five pain-relief tablets ranged from 104.7 to 107.1%. Within-day precision was 0.743% and 0.863% ($n = 6$) for analysis of the fifth diuretic and fifth pain-relief tablets, respectively. The interday precision was 1.35% and 1.33% ($n = 5$) for analysis of the fourth diuretic and fourth pain-relief tablets, respectively. Validation of the accuracy of the diuretic tablet results showed the error to be 1.10% for spiked blank analysis, and standard addition analyses gave errors of 1.75% and 0.40% for diuretic and pain-relief tablets, respectively, compared with fortification levels. The limits of detection and quantification were both 2.00 μg , and the correlation coefficient for the salicylamide calibration plot was 0.997 over the range 2.00 to 8.00 μg . The method is suitable for routine quality-control analysis of pharmaceuticals containing salicylamide.

INTRODUCTION

Salicylamide has analgesic and antipyretic effects similar to those of salicylates and is an active ingredient in some pharmaceutical analgesic formulations. Methods have been reported for the determination of salicylamide in such formulations by column high-performance liquid chromatography (HPLC) [1], Kalman filter spectrophotometry [2], polarography [3], first-derivative spectrofluorimetry [4], and ultraviolet (UV) spectrophotometry with multivariate calibration methods [5].

Thin layer chromatography (TLC) has well recognized advantages over other analytical methods for assay of active drug ingredients and impurities in pharmaceutical product analysis [6–8]. Use of TLC has been reported for qualitative purity testing of USP salicylamide drug material [9], semiquantitative detection of free salicylamide in *o*-ethoxybenzamide [10], and quantitative determination of salicylamide, ethoxybenzamide, salicylic acid, and paracetamol in saliva [11], but no TLC or HPTLC method for quantitative determination of salicylamide in pharmaceutical dosage forms was found by a computer-assisted literature search through Chemical Abstracts, the ISI Web of Science, and Camag Bibliography Service (CBS). The purpose of this research was to develop and validate, according to the guidelines of the International Conference on Harmonization (ICH) [12], the first HPTLC–densitometry method for quantification of salicylamide active drug ingredient and apply it to the analysis of commercial multi-component diuretic and analgesic tablet formulations.

EXPERIMENTAL

Preparation of Standard and Sample Solutions

A standard solution of salicylamide (#S-0750; Sigma, St Louis, MO, USA; CAS registry no. 65-45-2; 99.0% purity) was prepared at a concentration of 1.00 mg mL^{-1} in absolute ethanol for use in HPTLC analysis. Magnetic stirring for 30 min was required to achieve complete dissolution. For standard addition validation, a 100.0 mg mL^{-1} salicylamide stock standard solution in absolute ethanol was prepared; magnetic stirring for 1 h was required for complete dissolution.

Two different salicylamide-containing products were purchased from a local pharmacy. Product 1 was a diuretic pharmaceutical tablet formulation with the active ingredients potassium salicylate, caffeine, and salicylamide. Inert ingredients included dicalcium phosphate, microcrystalline

cellulose, starch, calcium sulfate, magnesium trisilicate, magnesium stearate, and stearic acid. Amounts present were not specified. Product 2 was an extra-strength pain-relief tablet formulation with the active ingredients acetaminophen (110 mg), aspirin (162 mg), caffeine (32.4 mg), and salicylamide (152 mg). The inactive ingredients were cellulose, cornstarch, croscarmellose sodium, FD&C yellow #6, aluminum lake, hydroxypropyl cellulose, magnesium stearate, polyvinylpyrrolidone, silicon dioxide, sodium carboxymethyl cellulose, and stearic acid.

Sample stock solutions for both products were prepared by grinding 1 tablet using a mortar and pestle and completely transferring the powder into a 10.0-mL volumetric flask for Product 1 and a 100.0-mL volumetric flask for Product 2, by washing with ca 5 mL and 75 mL ethanol, respectively. The solutions were stirred magnetically for 2 h, and the stir bar was removed with a magnetic rod. Both solutions were diluted to volume with ethanol and mixed by shaking. Before application to HPTLC plates, undissolved inert ingredients were removed from Product 1 by filtering ca 5 mL of solution through a Pall Gelman (Ann Arbor, MI, USA) Acrodisc LC 13 mm syringe filter with 0.45 μm PVDF membrane into a capped vial.

The theoretical concentration of Product 2 solutions was 1.52 mg mL⁻¹ salicylamide; the theoretical concentration of Product 1 solutions could not be calculated because the amount present in the tablet was not specified on the label.

Thin-Layer Chromatographic Analysis

Analyses were performed on 20 cm \times 10 cm high-performance silica gel 60 CF₂₅₄ channeled plates with concentration zone (EMD Chemicals, Gibbstown, NJ, USA, an affiliate of Merck, Darmstadt, Germany; #13 153). The plates were precleaned by development with dichloromethane–methanol, 1:1, and dried in a fumehood before use. Standard and sample solutions were applied to the plate by means of a Camag (Wilmington, NC, USA) Linomat IV automated spray-on band applicator equipped with a 100- μL syringe and operated with the settings: band length 6 mm, application rate 4 s μL^{-1} , table speed 10 mm s⁻¹, distance between bands 4 mm, distance from the plate edge 6.5 mm, and distance from the bottom of the plate 1.0 cm. The volumes applied for each analysis were 2.00 μL , duplicate 4.00 μL , and 8.00 μL of the standard (2.00–8.00 μg). Preliminary experiments were performed to determine the volume of sample aliquots for Product 1 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 average areas of the duplicate middle standard zones. As a result, duplicate 15.0 μL volumes were applied for Product 1 solutions. On the basis of the label declaration, 3.0- μL aliquots of Product 2 tablet solutions were applied (4.56 mg per zone theoretical).

Plates were developed 6 cm beyond the origin with dichloromethane–acetone, 4:1, as mobile phase in a vapor-equilibrated Camag HPTLC twin-trough chamber containing a saturation pad (Analtech, Newark, DE, USA). The development time was ca 15 min. After development, the mobile phase was evaporated from the plate by drying in a fumehood for 10 min.

The sample and standard zone areas were measured by use of a Camag TLC Scanner II in single-beam reflectance mode with the deuterium source set at 254 nm, slit length 4, slit width 4, and scanning rate 4.0 mm s^{-1} . The CATS-3 software controlling the densitometer produced a linear regression calibration graph relating the standard zone weights to their scan areas and interpolated the sample zone weights from the graph. For Product 1, active ingredient content values were not given on the product labels, so recovery could not be calculated. Results were instead calculated on a mg per tablet basis. For Product 2 tablets, recovery of salicylamide was calculated on the basis of the label declaration.

Validation

The accuracy of the new method was validated by spiked blank and standard addition analyses. An allergy/congestion tablet containing diphenhydramine hydrochloride (25 mg) and pseudoephedrine hydrochloride (60 mg) as active ingredients and a nasal decongestant antihistamine tablet containing brompheniramine maleate (12 mg) and phenylpropanolamine hydrochloride (75 mg) as active ingredients were used as blanks for Product 1 tablet analysis. These were appropriate blanks because they contained all seven of the inactive ingredients present in the diuretic tablets analyzed. Also, all of the active ingredients in the blanks separated from salicylamide on the layer. A blank solution containing both tablets was prepared as described above for a diuretic tablet, and 25.0 μL of the 100.0 mg mL^{-1} salicylamide stock standard was added to the 10-mL volumetric flask before dilution to volume to simulate a 2.50-mg salicylamide diuretic tablet solution. An unspiked solution of the blank tablets was prepared identically. Recovery was calculated by comparing the mean analy-

tical result for the spiked blank solution with the theoretical value of the added weight of salicylamide.

Validations by standard addition analyses were also performed. For Product 1, a diuretic tablet solution was prepared in a 10-mL volumetric flask as described above, and 1.00 mL of this solution was placed in a 6 mL vial and mixed with 2.50 μL of the 100.0 mg mL^{-1} salicylamide stock standard solution. The unspiked solution was also prepared in the usual way. The solutions were analyzed on the same plate by applying the usual four standard amounts, duplicate 7.50 μL aliquots of the spiked solution, and duplicate 15.0 μL aliquots of the unspiked solution. The mean result for the spiked sample was compared with the theoretical result (the mean result for the unspiked sample plus the weight added) to calculate the recovery.

For standard addition validation analysis for Product 2 a tablet solution was prepared in a 100-mL volumetric flask, as described above, and 1.00 mL was placed in a 6-mL vial and mixed with 15.2 μL 100.0 mg mL^{-1} salicylamide stock standard solution. The unspiked solution was prepared in the usual way. The solutions were analyzed on the same plate by applying the usual four standard aliquots, duplicate 1.50 μL aliquots of the spiked solution, and duplicate 3.00 μL aliquots of the unspiked solution. The recovery was calculated as described above for Product 1.

The precision (repeatability) of the method was evaluated by analyzing a Product 1 test solution and a Product 2 test solution six times on the same plate and calculating the relative standard deviation (RSD) of the recovery. Intermediate precision was determined by analyzing one sample of each product on different plates on five days over a seven-day period. As another evaluation of precision, the difference (%) between the scan areas of the duplicate sample aliquots applied in each analysis was calculated.

Digital Drummond (Broomall, PA, USA) microdispensers (10, 25, and 100 μL) were used to measure volumes for preparation of standard, sample, and validation solutions throughout this research.

RESULTS AND DISCUSSION

In preliminary studies, it was found that absolute ethanol was an excellent solvent for salicylamide standard compound. After initial dissolution of the products in 10.0-mL and 100.0-mL volumetric flasks for Products 1 and 2, respectively, and, for Product 1, removal of undissolved

excipients by filtration, the sample zone concentrations were within the calibration range of the standards. Development on silica gel HPTLC layers containing fluorescent indicator using dichloromethane–acetone, 4:1, as mobile phase produced flat, compact bands for salicylamide, R_F 0.55, on a bright green background, under 254 nm UV light (Fig. 1). The development time was 15 min. The other active ingredient zones appearing on chromatograms were potassium salicylate (R_F 0.42) and caffeine (R_F 0.25) for Product 1 and caffeine (R_F 0.25) and acetaminophen (R_F 0.35) for Product 2. None of the inactive ingredients was detected.

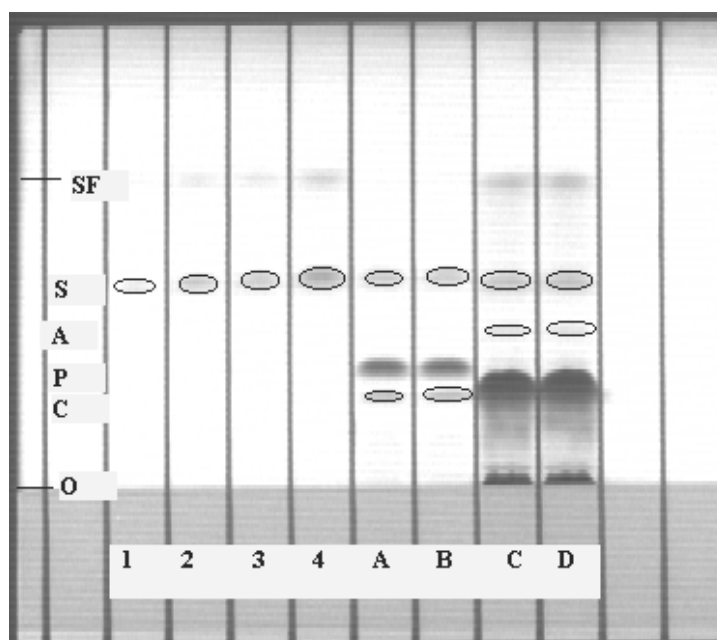


Fig. 1

Chromatograms obtained from Tablet 1 (Product 1) and Tablet 5 (Product 2) analyzed on a high-performance silica gel 60CF₂₅₄ channeled plate, with concentration zone, by the method described. The plate was photographed under UV 254 nm light using a Camag Videostore Image-Documentation System. SF, mobile phase front; O, origin; S, salicylamide; A, acetaminophen; P, potassium salicylate; C, caffeine; lanes 1–4, salicylamide standards (2, 4, 4, and 8 μ L, respectively); lanes A and B, duplicate 15.0- μ L samples of Product 1 tablet solution; lanes C and D, duplicate 3.00- μ L samples of Product 2 tablet solution. The lighter zones were outlined to make them readily visible.

Tablets of Product 1 were dissolved in a volumetric flask of only 10-mL volume and a high sample volume of 15 μ L had to be applied to be

bracketed within the calibration plot, because a relatively small amount of salicylamide was present in each tablet (ca 2.79 mg, Table I); the 15.0 μL spotted contained ca 4 μg analyte. Also, salicylamide does not absorb UV light strongly (i.e. the molar absorption coefficient term, a , in the Kubelka–Munk equation has a low value). The potassium salicylate zones seen in Fig. 1 are darker than the salicylamide zones because much more of this ingredient was present in each tablet (ca 112 μg per zone, as reported elsewhere [13]), and the caffeine zones were much darker because ca 75 mg per zone was present [14] and its molar absorption coefficient is higher. Tablets of Product 2 were dissolved in a larger volumetric flask and a smaller sample volume was used for analysis using the same standard weight range for calibration, because the salicylamide content was much higher. The sample zones in Fig. 1 for Product 2 represent ca 3 to 5 μg of each ingredient according to the declared label values; the acetaminophen zone is very dark because this compound has a very high molar absorption coefficient.

Table I

Results from analysis of Products 1 and 2

Sample	Average amount (mg) per tablet	n^a	RSD (%) ^b
Product 1			
Tablet 1	2.75	2	
Tablet 2	2.85	2	
Tablet 3	2.89	2	
Tablet 4 ^c	2.67	5	1.35
Tablet 5 ^d	2.79	6	0.743
Sample	Average recovery (%)	n^a	RSD (%) ^b
Product 2			
Tablet 1	106	2	
Tablet 2	107	2	
Tablet 3	106	2	
Tablet 4 ^c	107	5	1.33
Tablet 5 ^d	105	6	0.813

^a n = number of sample zones applied to the layer

^bRSD = relative standard deviation

^cInter-day precision (intermediate precision)

^dIntra-day precision (repeatability)

During the validation experiments preparation of calibration graphs by linear regression led to superior analytical results compared with polynomial regression. As recommended by International Conference on Harmonization (ICH) guidelines [12], a calibration graph was established for salicylamide using five concentrations (2.00, 3.00, 4.00, 6.00, and 8.00 μg per zone) of the standard. The r value (correlation coefficient) of this graph (scan areas versus weights) was 0.997. For routine analysis a three-concentration calibration graph within this range was used, produced by applying 2.00, duplicate 4.00, and 8.00 μL of the salicylamide standard on each plate. The r value for polynomial regression of these calibration graphs was always 0.999.

The limit of detection for salicylamide was determined by viewing developed plates containing 1.00 and 2.00 μg standard zones under 254 nm UV light. The zone from 2.00 μL of the 1.00 mg mL^{-1} standard solution (2.00 μg) was barely visible, and the zone from 2.00 μL of a 0.500 mg mL^{-1} solution (1.00 μg) was not visible. From these results the limit of detection and quantification were taken as 2.00 μg , and this amount was the lowest weight used for the calibration graph.

Recoveries for five tablets of Product 2 were determined by analysis with the described procedure using duplicate sample aliquots ($n = 2$). Label values were available for this product, so recoveries could be calculated. Values for all tablets of Product 2 were within 90 and 110% of the label value, which is the usual range specified for pharmaceutical dosage forms in the USP; salicylamide tablets are not specifically covered in the USP [9]. Five tablets of Product 1 were analyzed on a mg tablet^{-1} basis because content was not listed on the label. The average was 2.79 mg tablet^{-1} , and all results were within $\pm 10\%$ of this value. Recoveries compared with the label value for Product 1 and the mg tablet^{-1} values for Product 2 are shown in Table I.

Accuracy was validated by preparing and analyzing a spiked blank sample for Product 1 as described above. The chosen spike level of 2.50 mg is similar to the average content of the tablets analyzed (Table I). Recovery of added salicylamide from the spiked tablet solution was 98.9% (1.1% error). Analysis of the unspiked tablet solution showed that no interference occurred at the R_F value of salicylamide and, therefore, no correction of the scan areas of the spiked blank solutions was required.

Accuracy was also validated by standard addition analysis of a sixth tablet of Products 1 and 2. Spiked and unspiked solutions of each sample were analyzed on the same plate as described above. For Product 1 the

tablet solution initially assayed at 3.70 mg per tablet and recovery was 98.3% of the amount of salicylamide added to this solution (1.74% error). For Product 2 the tablet solution initially assayed at 98.7% recovery compared with the label value, and recovery was 100.4% of the amount of salicylamide added to this solution (0.4% error). Successful analyses of spiked blank and standard addition samples, which contained all of the matrix components in the formulations, revealed the accuracy of the method, its selectivity, and the absence of interferences.

Repeatability was determined by spotting six 15.0- μ L aliquots and 3.0 μ L ($n = 6$) of Products 1 and 2 sample solutions, respectively, instead of the usual duplicate sample aliquots ($n = 2$). As shown in Table I, the RSD values were 0.743% and 0.813%, respectively. As a second measure of precision, differences between scan areas of duplicate sample aliquots ranged from 0.750 to 2.15% and from 0.124 to 2.01%, respectively, for Products 1 and 2. Intermediate precision was evaluated by performing five replicate analyses of the fourth tablet of Products 1 and 2 on different plates on the day of sample preparation, the next day, and the fifth, sixth, and seventh day after preparation. The average amount of salicylamide found for Product 1 was 2.67 mg, and the RSD was 1.35%. For Product 2, the average amount was 4.88 mg, 107% of the label value, and the RSD was 1.33%.

It is shown above that the new HPTLC–densitometry method achieved accuracy, reproducibility (repeatability and intermediate precision), linearity, sensitivity, and selectivity that compare favorably with those of analyses reported regularly in the literature for HPTLC, HPLC, spectrophotometry, and other methods; the results also meet the ICH guidelines for validation of pharmaceutical TLC methods. The method is less expensive, simpler, more rapid, and more flexible than HPLC. Up to 16 individual samples, or eight samples applied in duplicate, can be analyzed on a single plate with the minimum three standards required to produce the calibration plot, leading to high sample throughput and low cost for solvent use and disposal. Application of samples and standards on the same plate (in-system calibration), all developed under identical conditions, can provide increased accuracy and precision. Selectivity was proven by the successful determination and validation of salicylamide in the presence of the other four active ingredients (potassium salicylate, acetaminophen, aspirin, and caffeine) and the sixteen inactive ingredients listed above that were in the tablet, blank, and standard addition samples.

In previous publications we have reported validated HPTLC–densitometry methods for all of the other active ingredients present in the two salicylamide products:

- caffeine [14] and potassium salicylate [13] in diuretic tablets using the same layers as in this study and ethyl acetate–methanol, 85:15, and ethyl acetate–methanol–concentrated ammonia, 80:15:5, respectively, as mobile phases;
- caffeine and acetaminophen in extra-strength pain-relief tablets using EMD Chemicals silica gel 60F₂₅₄ GLP plates and ethyl acetate–glacial acetic acid, 95:5, as mobile phase [15]; and
- aspirin in extra-strength pain-relief tablets using Whatman (Clifton, NJ, USA) channeled, preadsorbent LHPKDF high-performance silica gel plates with ethyl acetate–glacial acetic acid, 95:5, as mobile phase [16].

All of these methods are suitable for routine analysis of these products and for similar products containing salicylamide, caffeine, aspirin, and acetaminophen, either alone or in various combinations in pharmaceutical company quality-control and governmental regulatory laboratories.

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