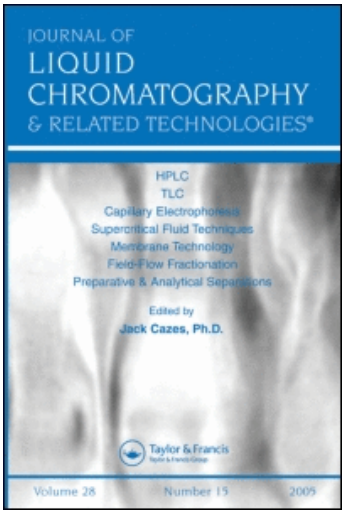

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LIQUID CHROMATOGRAPHIC ASSAY FOR SALICYLIC ACID IN LIQUID AND SEMISOLID FORMULATIONS

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ABSTRACT

A high performance liquid chromatographic assay for quantitating salicylic acid (S) in lotion, collodion, ointment and cream formulations is described. The method involved direct determination of S in the liquid dosage forms after appropriate dilutions with the mobile phase. Prior extraction of S from ointments and creams was carried by chloroform. Metronidazole was used as the internal standard and a μ-Bondapak phenyl column with a mobile phase made of 30% methanol in 5 mM solution of tetrabutylammonium phosphate (pH 7.5) led to an efficient separation. Retention times of about 3 and 7.5 min were obtained for the internal standard and S respectively. The recovery of S from the dosage forms was tested by adding known amounts of S to each preparation and mixing before determination. The mean percentage recovery was in the range of 98-101.1%. The method was found to be accurate, simple and reproducible.

INTRODUCTION

Salicylic acid is used externally in lotions, ointments, creams or powders for its keratolytic, bacteriostatic or fungicidal actions (1). To destroy warts or corns, it is applied in the form of a paint in a collodion base (2). In many of these preparations, S is used in conjunction with sulfur, resorcinol, coal tar or lactic acid. Several

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methods for S determination in various dosage forms are reported in the literature. These methods vary from titrimetry (3-5), colorimetry (6-8), spectrofluorimetry (9), UV spectrophotometry (10) and titration in non-aqueous solvents (11). Most of these methods were developed for S determination in a single dosage form. They involved many steps in the assay procedure and possessed limited sensitivity. A high pressure liquid chromatographic method (12) was also developed for S determination in ointment preparations. The method was simple and accurate, however, it was only limited to one kind of dosage form.

This communication describes a HPLC method for S determination in five different formulations; a lotion, a collodion, an ointment and two creams. Those formulations contained sulfur, resorcinol, lactic acid and some other active and inactive ingredients.

MATERIALS

Instrumentation:

A Waters Associates (Milford, MA) liquid chromatograph was used for analysis. The instrument was equipped with a 6000 A pump, U6K injector, Lambda Max 481 detector and a M730 data module. A micro BONDAPAK-Phenyl column (3.9 mm X 30 cm, Waters Associates) was used for separation.

Chemicals:

A lotion, a collodion, an ointment and two creams were obtained from the local market. A list of the active ingredients present in each is shown in Table 1. The chemicals used for drug extraction and in the mobile phase were chloroform and hydrochloric acid, B.D.H. Chemicals Ltd. (Poole, England); methanol, Merk (Darmstadt, Germany) and tetrabutylammonium phosphate, PIC Reagent A, Waters Associate (Milford, MA). The mobile phase

TABLE 1

Active Ingredients Present in the Pharmaceutical Preparations

Pharmaceutical Preparation	Active Ingredients
Lotion	Salicylic acid, quinine hydrochloride, pilocarpine nitrate.
Collodion	Salicylic acid, lactic acid.
Ointment	Salicylic acid, buclosamide.
Cream I	Salicylic acid, resorcinol.
Cream II	Salicylic acid, precipitated sulfur.

was prepared by adding 300 ml methanol to 700 ml water containing enough PIC Reagent A to form 5 mM solution. The mixture was then filtered through a 0.5 μm pore size membrane filter obtained from Millipore Corporation (Bedford, Massachusetts) before degassing. A pure sample of salicylic acid, B.D.H. Chemicals Ltd. (Poole, England) was used for standardization.

METHODS

Development of HPLC conditions:

Preliminary runs on extracted samples based upon literature review (12) using the μ Bondapak-C18 column and 0.02 M potassium dihydrogen phosphate in methanol-water mixture as the mobile phase were unsatisfactory. Broad peaks were obtained and a need for adjustment in the mobile phase was indicated for each of the pharmaceutical preparations tested. Upon using the μ Bondapak-phenyl column and a mobile phase made of 30% methanol in a 5 mM solution of tetrabutylammonium phosphate (pH 7.5), sharp peaks and good separation of S were obtained with all preparations tested. A retention time for S of about 7.5 min was

observed. Examination of the integrated areas under the peaks for three different drug concentrations indicated proper drug resolution. A pure sample of metronidazole obtained by alcoholic extraction and thrice recrystallization from commercial tablets, was used as the internal standard. It was found to have a well defined peak separated from that of the drug with a retention time of about 3 min. Ultraviolet absorption at a wave length of 313 nm was used for detection.

Assay calibration:

A number of standard solutions of salicylic acid were prepared by proper dilutions from a stock solution containing 1 mg/ml of S and 5 µg/ml internal standard in the mobile phase. Dilutions were made by 5 µg/ml internal standard solution in the mobile phase and concentrations of S in the range of 5-50 µg/ml were obtained. Exactly 20 µl of each solution were injected in the HPLC and each measurement was carried in duplicate.

Determination of Salicylic Acid in Lotion and Collodion:

Aliquots of the lotion and the collodion were diluted directly with the mobile phase containing the internal standard. Two other sets of determinations were also carried with the preparations spiked with a solution of two different concentrations of salicylic acid in the mobile phase. Exactly, 20 µl of the product were injected in the HPLC for S determination. A second injection of different volume was carried for confirmation.

Determination of Salicylic Acid in Ointment:

Ointment containing 2 mg of labelled salicylic acid was accurately weighed in a glass centrifuge tube. Exactly, 10 ml of the internal

standard solution in the mobile phase were added and the product was mixed in a vortex mixer for 30 min. The tube was then centrifuged at 2000 rpm for 15 min. The top layer was aspirated in a clean tube, cooled in a refregirator and filtered through a 0.45 µm filter, Millipore Corporation (Bedford, Massachusetts). An aliquot was then diluted 4-fold with the internal standard solution before injection. A second aliquot was diluted 8-fold with the same solution and injected for confirmation.

Recovery studies were performed by mixing 40% and 80% of the S labelled content with two ointment samples immediately before extraction. Determination of S was then carried out in same way.

Determination of Salicylic Acid in Creams:

Direct extraction of salicylic acid from creams with the mobile phase was unsuccessful as the mobile phase mixed intimately with the components of the creams. Three other methods were then tried. In the first method, cream containing 4 mg of labelled salicylic acid was accurately weighed in a 35 ml glass tube, 20 ml chloroform were added and the product was mixed in a vortex mixer for 10 min. The chloroform layer was then aspirated in a centrifuge tube, centrifuged for 15 min. at 2000 rpm to separate cream residues. Exactly 10 ml of the pure chloroform solution were evaporated and the residue dissolved in 20 ml mobile phase to produce a theoretical S concentration of 100 µg/ml. Two different dilutions were then injected in the HPLC for S determination. In the second and third methods, the same procedure was followed except that 0.5 ml and 5 ml of 1N hydrochloric acid were mixed with the cream sample respectively before chloroform extraction. Recovery studies on S extraction from creams were performed by mixing 50% and

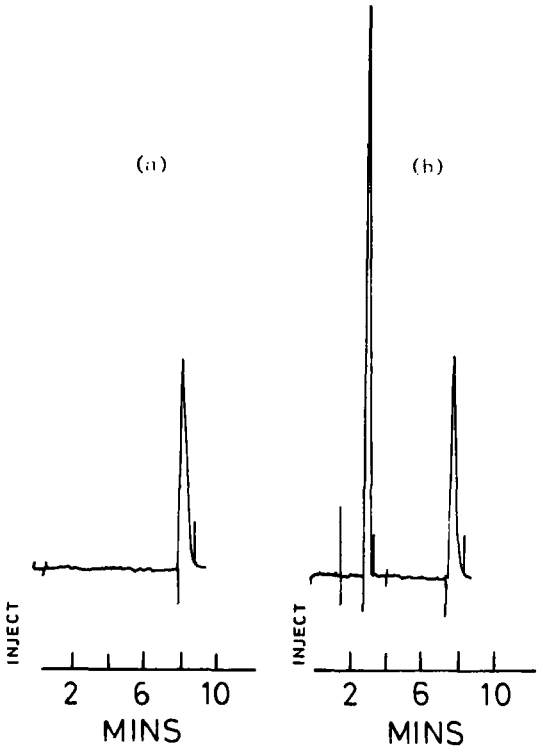

Figure 1: Typical saliyclic acid chromatogram
a, salicylic acid solution in mobile phase;
b, salicylic acid and internal standard solution in mobile phase.

100% of the S labelled content with two different samples of each cream. Extraction was carried by the second method described above and S content was then determined as before.

RESULTS AND DISCUSSION

Figure 1 shows representative chromatograms for S and S with the internal standard as determined in the assay calibration. As can be

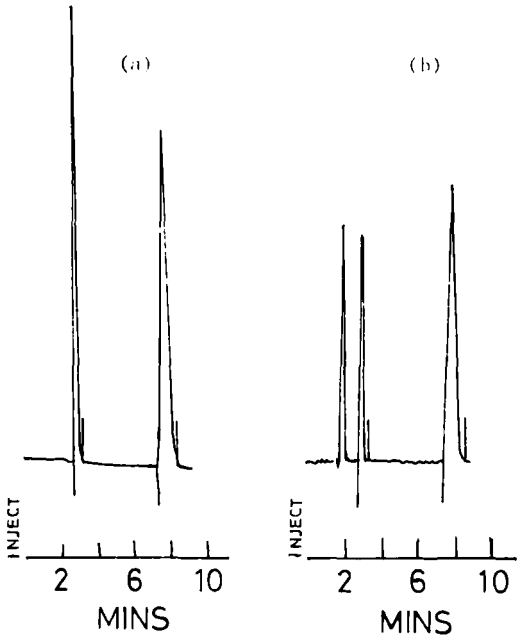

Figure 2: Chromatograms for salicylic acid in, a, lotion; b, collodion.

seen, well identified peaks with good separation between S and the internal standard were obtained. Figures 2 and 3 show representative chromatograms for S as determined in the different dosage forms. No interference from other components was detected.

Quantitative analysis of the results based upon the areas under the curve (AUC) and the ratios of S to the internal standard peak heights were found to be superimposed. Standard curves for AUC versus S concentration showed good linearity over the concentration range tested with zero Intercepts.

Table 2 summarizes the results obtained for S determination in cream I after extraction by three different methods. As can be seen,

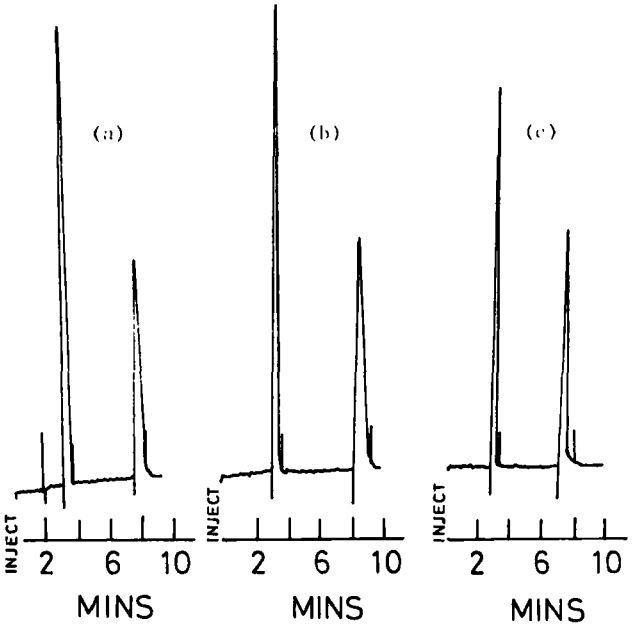

Figure 3: Chromatograms for salicylic acid after extraction from, a, cream I; b, cream II; c, ointment.

TABLE 2.

Comparison of the Extraction Methods used in Determining Salicylic Acid in Cream I

Method of Extraction	% S Recovered (Mean of 4, S.D.)
direct extraction with chloroform	84.30, 8.152
chloroform extraction in presence of 0.5ml 1N HCl.	105.28, 6.303
chloroform extraction in presence of 5ml 1N HCl.	93.12, 8.022

better recovery was obtained when the cream was acidified with 0.5ml of 1N hydrochloric acid solution before extraction. Incomplete recovery with the two other methods is probably due to losses by partitioning in the aqueous phase. Such losses were minimized when the aqueous phase volume was kept to minimum and after acidification. Further determinations of S in creams were performed according to the second method.

Table 3 presents the summary of four determinations of S content in each of the preparations tested and their spiked samples. Recovery was calculated by substracting the average experimental S content of each preparation from its corresponding spiked samples and percentage of recovered amount to added amount was then determined. Average percentage and standard deviations are presented in the Table. As can be seen, 98-101% recovery was obtained which indicate good sensitivity and linearity of the assay method and high percision of the extraction methods performed on the ointment and the creams.

TABLE 3.

Salicylic Acid Recovery from Spiked Forms

Dosage Form	Percent S spiked to S label (%)	Percent Recovered (%) (Mean of 4, S.D.)
Lotion	16.67	98.4, 3.724
	33.33	99.4, 5.865
Collodion	12.5	98.9, 5.290
	25	98.4, 6.844
Ointment	40	100.7, 2.998
	80	101.1, 4.305
Cream I	50	97.4, 6.034
	100	98.9, 4.212
Cream II	50	98.0, 3.006
	100	98.8, 3.842

TABLE 4.

Salicylic Acid Content in the Dosage Forms

Dosage Form	Label S content (% W/W)	Experimental S content (% W/W) (Mean of 4, S.D.)	Percent of label (Mean of 4, S.D.)
Lotion	1.5	1.445, 0.014	96.33, 0.947
Collodion	20	22.029, 1.567	110.15, 7.84
Ointment	2	1.902, 0.039	95.10, 1.974
Cream I	2	2.105, 0.126	105.28, 6.303
Cream II	2	2.050, 0.124	102.49, 6.193

Table 4 compares the experimental S content to the label values for each of the preparations. The range of the results obtained was reproducible for other samples from the same lot of each preparation. While the ointment and the lotion showed S content slightly lower than the label value within the permissible range, the two creams and the collodion showed overdosage of S which reached up to 10%.

The chromatographic method described in this communication is accurate and precise for determination of salicylic acid in various dosage forms and in presence of a number of active and inactive ingredients. The method was found reproducible and simple, making it suitable for routine assay. The separation of salicylic acid achieved from its products makes the method useful in monitoring drug content conformity with requirements as well as product stability.

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