Drug Development and Industrial Pharmacy, 26(5), 539–547 (2000)

RESEARCH PAPER

Comparing HPLC and UV Spectrophotometric Analysis Methods for Determining the Stability of Sorbic Acid in Nonionic Creams Containing Lactic Acid

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ABSTRACT

This paper describes a comparison between ultraviolet (UV) spectrophotometric and high-performance liquid chromatographic (HPLC) methods of analysis for the determination of sorbic acid in nonionic creams containing lactic acid. Sorbic acid is an antimycotic agent and is used as a preservative in pharmaceuticals, cosmetics, and food products. UV spectrophotometric analysis was done by calculating the concentration of remaining sorbic acid from the absorbance values and the molar extinction coefficient $E_M^{258} = 24{,}080$*. A decrease in absorbance at 258 nm was accompanied by a simultaneous increase in total carbonyls and monoaldehyde content and the appearance of a very weak absorption maximum between 215 and 225 nm. HPLC analysis was done with a Hypersil BDS* C_8 *column with detection at 254 nm and employing a mobile phase consisting of a mixture of buffer and methanol (7:3 v/v) at a pH of 2.25. The buffer consisted of 0.85%* H_2SO_4 *in 17.5 mM* KH_2PO_4*. The validation results, together with statistical treatment of the data, demonstrated the reliability of both procedures. A drawback of the UV methods was, however, its lack of adequate measurement of sorbic acid stability at higher temperatures. For these assays, the HPLC method was found to be adequate, and it should therefore be used to obtain accurate stability data for sorbic acid in creams.*

Key Words: *Analysis; HPLC; Sorbic acid; Spectrophotometric; Stability.*

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INTRODUCTION

One of the basic requirements for the effective quality control of pharmaceuticals is adequate and validated test procedures. The work presented here is part of a study intended to develop practical approaches for selecting the best test procedures for analyzing sorbic acid (2,4-hexadienoic acid) and/or its related impurities in nonionic creams containing lactic acid. Creams containing lactic acid form an important part of the so-called cosmeceutical market for antiaging products. The paper describes a comparison between ultraviolet (UV) spectrophotometric and high-performance liquid chromatographic (HPLC) methods of analysis for the determination of sorbic acid in nonionic creams containing lactic acid, an alpha hydroxy acid.

Sorbic acid has been advocated as a preservative of choice in many pharmaceutical preparations (1). Sorbic acid is an antimycotic agent and is used as a preservative in pharmaceuticals, cosmetics, and food products (2). Sorbic acid was first described by Hofmann in 1859 and introduced for preservation by Müller in 1939. While the pharmacopoeias (for example, the USP and BP) do not include positive lists of antimicrobial preservatives, several unofficial lists have been compiled. Sorbic acid is one of just six compounds or classes of compounds that appears on all these lists (3).

Up to 6% sorbic acid is allowed in products, and it is effective against gram-positive (minimum inhibitory concentration [MIC] = 100 PPM) bacteria, gramnegative (MIC = 300 PPM) bacteria, fungi and yeast (MIC = 500 PPM), and molds (MIC = 300 PPM). It is optimal at a pH of 4 to 6 (4) because, to ensure inhibition, it is necessary that sorbic acid traverses the cell membrane of the microorganisms. This is done by the undissociated part independent of the pH. At pH 3.15, the undissociated quantity is about 40%, and at pH 7.0, less than 10% is undissociated (4), which is ideal for creams containing alpha hydroxy acid, which are prepared to be at a pH below 4.

Numerous assay methods have been reported for the quantification of sorbic acid. These include UV spectrophotometry (5,6) and HPLC (7,8) methods. Most of these methods were developed for the determination of sorbic acid in food products. This study reports the results obtained from the validation and comparison of UV spectrophotometric and HPLC methods for the determination of sorbic acid in nonionic creams at a low pH. Although sorbic acid losses in nonaqueous products or even creams appear to be minimal when assayed chemically (9), the study also looked at the applicability of the two assay methods for stability assessment of sorbic acid in the creams, especially since the unsaturated nature of the sorbic acid molecule suggests that oxidation would result in decomposition of sorbic acid to small aldehyde moieties.

ANALYTICAL METHODS

Materials

Sorbic acid was obtained from Sigma Chemical Company (St. Louis, MO). The aqueous cream (BP) and hydrophilic ointment (USP) were prepared as described in the USP (10) and BP (11). These creams represent oil-in-water emulsion bases that, although insoluble in water, are water washable, nonocclusive, and nongreasy. The creams were modified by the addition of 10% lactic acid partially neutralized with sodium lactate so that the pH of the creams was 3.5. In both creams, the preservatives were replaced with 0.2% sorbic acid. Three batches were prepared of each cream. All chemicals, reagents, and sol-

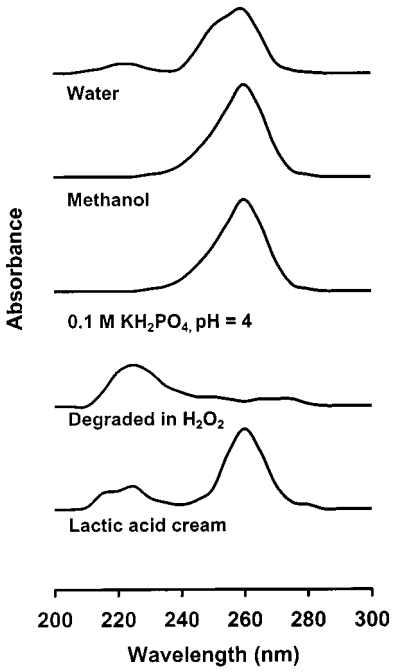

Figure 1. The UV spectra of sorbic acid in water, methanol, and 0.01 M KH_2PO_4 at pH 4.0. The spectrum of a decomposed sample treated with hydrogen peroxide and sorbic acid extracted from a nonionic cream containing lactic acid is also shown.

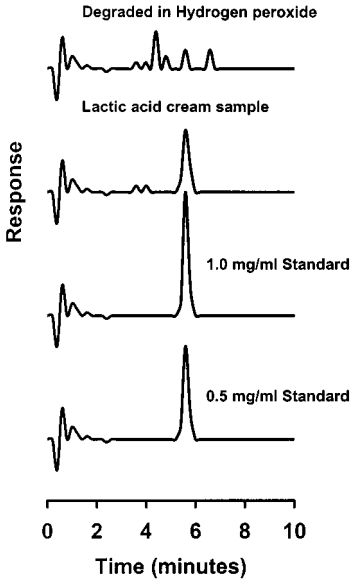

Figure 2. Typical chromatograms of sorbic acid in standard and test solutions and after degradation with hydrogen peroxide (Hypersil BDS C_8, 250 × 4.6 mm id column; mobile phase of 0.01 M KH_2PO_4 adjusted to pH 2.25 with H_3PO_4; flow rate of 1 ml · min^{-1}; ambient temperature).

vents used were either analytical grade or complied with USP or BP standards.

Spectrophotometric Analysis

Sorbic acid in aqueous solution has a strong absorption maximum at 258 nm ($E_{1\%}^{258}$ 2150) due to the conjugated double-bond carbonyl system in its molecule (5). During storage, the intensity of the maximum decreases considerably, and a new, very weak, absorption maximum appears between 215 and 225 nm (Fig. 1). In this study, sorbic acid was analyzed, and the concentration of remaining sorbic acid was calculated from the absorbance values and the molar extinction coefficient E_M^{258} = 24,080. A decrease in absorbance at 258 nm is accompanied by a simultaneous increase in total carbonyls and monoaldehyde content (5,12). A Shimadzu UV-160 spectrophotometer (Shimadzu, Kyoto, Japan) was used for all measurements.

High-Performance Liquid Chromatographic Analysis

The isocratic LC system consisted of a Shimadzu LC-6A pump, a Shimadzu SIL-6B autoinjector equipped with a 100-µl loop, a Hypersil BDS C_8 column (250 × 4.6 mm id) packed with 3.5-µm particles with a 130 Å, a Shimadzu SPD-6A detector set at 254 nm, and a Shimadzu C-R6A integrator. A Shimadzu SCL-6B system controller controlled the whole system. The column temperature was ambient, and 20-µl samples were injected onto the chromatograph. The mobile phase used was a mixture of buffer and methanol (7:3 v/v) at a pH of 2.25. The buffer consisted of 0.85% H_2SO_4 in 17.5 mM KH_2PO_4.

Test and Standard Solutions

Standard solutions were prepared by dissolving sorbic acid in methanol to obtain a series of five solutions ranging from 0.5 to 1.5 mg·ml^{-1}. Cream formulations (1.0 g) were dissolved in 50 ml hexane and extracted twice with 10 ml of 0.1 M NaOH solution in a separating funnel. The basic aqueous extracts were acidified with 1 ml of 5 M H_2SO_4 and diluted to volume with methanol. These solutions were then analyzed by UV spectrophotometry and HPLC.

Validation of Test Procedures

Specificity was tested by means of testing standard solutions and test solutions of sorbic acid after treatment with a solution of concentrated hydrogen peroxide, concentrated hydrochloride acid, or 10 M sodium hydroxide (7). The linearity of response of the standard solutions was assessed using five solutions by varying the analyte concentration over a range of 50% to 150% of the nomi-

Table 1

Absorption Maximum of a 0.5 mg · ml^{-1} Sorbic Acid Depending on pH

pH	4.0	7.1	7.6	8.6	9.7
$\lambda_{max\ (nm)}$	258	258	256	254	254
Absorbance	0.235	0.236	0.233	0.212	0.212

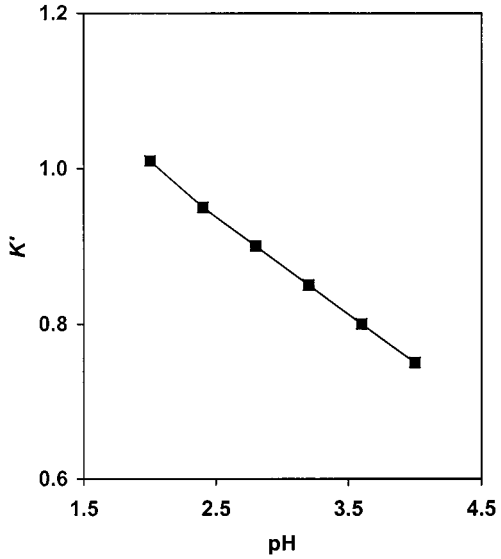

Figure 3. Effect of eluent pH on the capacity factor of sorbic acid. Column and chromatographic conditions except pH as in Fig. 2.

nal working concentration. The linearity and accuracy of the two test procedures were assessed by recovery studies in this range. Three independent determinations (three linearity experiments) were performed, each carried out separately on different days.

Repeatability and reproducibility were tested by spiking a matrix (creams) with an amount of sorbic acid corresponding to 100% of the theoretical content (0.2%). On three different days, six independent determinations were performed on each matrix.

The stability of the standard solutions was determined by calculating the response factors (concentration/absorbance or area). The response factors for freshly prepared solutions and solutions stored for 24 hr at ambient temperature were determined. To determine the stability of test solutions, the percentage of sorbic acid in cream extracts freshly prepared and then allowed to age for 24 hr was calculated by reference to a standard solution, each time freshly prepared. Three duplicate determinations were carried out.

Statistical Evaluation and Comparison of Results

Mean HPLC and UV spectrophotometric results were compared. Multivariate analysis of variance (MANOVA) was used to test for significant differences between means. Post hoc comparison using the Newman-Keuls test was performed on the marginal means to look for significant differences. This test is based on the Studentized range statistic. It computes a matrix of p values for pairs of means showing the critical ranges between ordered means for the particular alpha level ($p < .05$). For this study, a 95% confidence level ($p \leq .05$) was considered satisfactory for indicating significant differences. Statistical evaluations were done with the program Statistica for Windows, version 5.1 (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

In Fig. 1, the spectrophotometric separation of sorbic acid and its degradation products are shown. Figure 2 shows the separation obtained by HPLC. The UV spectrum of sorbic acid was recorded in methanol, water, and 0.01 M KH_2PO_4 at various pH values. Sorbic acid has one absorption maximum at 258 nm in water and methanol. The UV spectrum at pH 4.0, 7.1, 7.6, 8.6, and 9.7 were the same as in water and methanol. The absorption

Table 2

A Summary of Precision and Validation Data for Sorbic Acid

	HPLC	UV
Concentration range x (mg · ml^{-1})	0.25–1.50	0.25–1.50
Signal range (arbitrary units)	28,791–177,238	0.121–0.703
Intercept y (arbitrary units)	−481	0.0017
SD (y)	200	0.0008
Slope a (ml · mg^{-1}	118,372	0.4681
SD (x) 9mg · ml^{-1}	222	0.0009
Correlation coefficient (mg · ml^{-1})	0.9999	0.999
n (calibration data)	18	18
LOD (mg · ml^{-1})	0.0082	0.015

Table 3

Recovery from Standard Solutions and Extraction Recovery from Blank Matrices Spiked with Known Amounts of Sorbic Acid

				HPLC			UV		
Sample	Amount in Sample ($mg \cdot ml^{-1}$)	Amount Added ($mg \cdot ml^{-1}$)	Calculated ($mg \cdot ml^{-1}$)	Found ($mg \cdot ml^{-1}$)	Recovery ($mg \cdot ml^{-1}$)	Bias (%)	Found ($mg \cdot ml^{-1}$)	Recovery (%)	Bias (%)
Standard 1	0.251	0.106	0.357	0.361	101.1	1.1	0.352	98.7	−1.3
		0.202	0.453	0.451	99.6	−0.4	0.450	99.3	−0.7
		0.412	0.663	0.659	99.4	−0.6	0.655	98.8	−1.2
Standard 2	0.501	0.106	0.607	0.605	99.6	−0.4	0.597	98.4	−1.6
		0.202	0.703	0.695	98.9	−1.1	0.697	99.1	−0.9
		0.412	0.913	0.915	100.2	0.2	0.915	100.2	0.2
Standard 3	1.012	0.106	1.118	1.119	100.1	0.1	1.103	98.7	−1.3
		0.202	1.214	1.206	99.3	−0.7	1.208	99.5	−0.5
		0.412	1.424	1.415	99.4	−0.6	1.414	99.3	−0.7
BP cream	1.011	0.106	1.117	1.100	98.5	−1.5	1.100	98.5	−1.5
		0.202	1.213	1.209	99.7	−0.3	1.208	99.6	−0.4
		0.412	1.423	1.416	99.5	−0.5	1.412	99.2	−0.8
USP cream	0.992	0.106	1.098	1.089	99.2	−0.8	1.088	99.1	−0.9
		0.202	1.194	1.189	99.6	−0.4	1.175	98.4	−1.6
		0.412	1.404	1.387	98.8	−1.2	1.386	98.7	−1.3

maximum of sorbic acid at chosen pH values is shown in Table 1.

A HPLC method in reverse-phase mode was used to investigate the behavior of sorbic acid depending on pH of the mobile phase (Fig. 3). At lower pH values, sorbic acid is essentially in the nonionic form, and retention of the acid is favored. Because sorbic acid retention decreased as the pH was increased (due to dissociation), an optimum pH of 2.25 was selected for all analyses by HPLC. The effect of ionic strength of the eluent on the retention time of sorbic acid was slight, but it was observed that, when the ionic strength was decreased, the width of the sorbic acid peak increased, and the chromatograms lost some resolution. Based on these results, reverse-phase chromatography, using acid or buffer in the mobile phase to suppress the ionization of sorbic acid, proved a suitable and simple separation method.

Examples of the practical application of the described HPLC and spectrophotometric procedures for the separation and determination of sorbic acid in samples of nonionic creams containing lactic acid are also shown in Figs. 1 and 2. The quantification of sorbic acid was achieved using the external standard method. For HPLC determination, calibration plots were generated by repeated injections of a fixed volume (20 μl) of standard solutions of different concentrations. The UV absorbance of the same solutions at 258 nm was also measured and used to construct calibration plots.

The linearity of the calibration curves (absorbance or area versus sorbic acid concentration) was first determined. Calibration curves were determined by replicate analysis ($n = 3$) of five sorbic acid solutions corresponding to 25%, 50%, 75%, 100%, 125%, and 150% of the amount present in the test samples. The response was found to be linear from 0.5 to 1.5 mg · ml^{-1}. A summary of precision and validation data for sorbic acid, obtained with HPLC and UV calibration, is given in Table 2.

As the use of correlation coefficients is not always sufficient to assess linearity, an analysis of variance (ANOVA) was carried out to confirm the linearity. The linearity was assessed with $F_{calc} = 258{,}799$ ($p \ll .01$) for HPLC and $F_{calc} = 283{,}959$ ($p \ll .01$) for UV analysis. The regression lines for both HPLC and UV analyses almost passed through the origin, with $t_{calc} = 2.083$ ($p = .0917$) and $t_{calc} = -2.40$ ($p = .06147$). These results allow the possibility of one concentration only to be used for the standard solution when doing assay tests.

Another way to assess the linearity is to calculate response factors (RF = concentration/absorbance or area) for each experiment and the average and relative standard deviation (RSD). The following results were obtained for the standard solutions: RF = 8.52×10^{-6} (RSD = 0.97%), $n = 6$ concentrations for HPLC; RF = 2.12 (RSD = 1.09%), $n = 6$ concentrations for UV spectrophotometry. The graph is considered linear if the RSD is lower than 2%. The average for the two RF values of the standard solutions measured on 3 days were 8.52×10^{-6}, 8.14×10^{-6}, 8.26×10^{-6} for HPLC and 2.12, 2.09, and 2.14 for UV spectrophotometry. From the individual RF values for each of the five standard solutions, it is easy to calculate the bias of each method at each concentration by reference to the corresponding standards.

Recovery studies were performed for the two creams and for standard solutions. The blank creams and standard solutions were spiked with three different concentrations of sorbic acid; typical recoveries using HPLC and

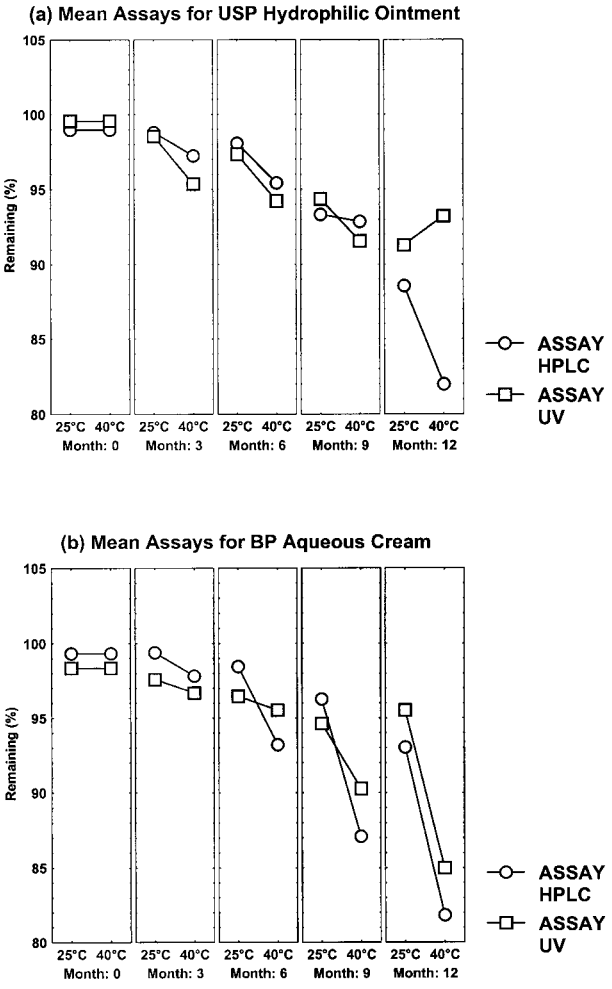

Figure 4. Comparison of assay results of sorbic acid products by UV and HPLC methods during stability testing: (a) USP hydrophilic ointment and (b) BP aqueous cream.

Table 4

Stability of Sorbic Acid in BP Aqueous Cream and USP Hydrophilic Ointment Containing 10% Partially Neutralized Lactic Acid After Storage at 25°C and 40°C

	USP Hydrophilic Ointment (%)					BP Aqueous Cream (%)				
		HPLC		UV			HPLC		UV	
Storage Period	pH	25°C	40°C	25°C	40°C	pH	25°C	40°C	25°C	40°C
Initial	3.5	99.0 ± 0.3		99.6 ± 1.5		3.5	99.3 ± 1.5		98.4 ± 1.0	
3 months	3.6	98.8 ± 0.5	97.2 ± 0.1	98.5 ± 0.5	95.4 ± 1.1	3.7	99.4 ± 1.2	97.8 ± 0.6	97.6 ± 0.6	96.7 ± 0.5
6 months	4.1	98.1 ± 0.7	95.4 ± 0.8	97.3 ± 0.9	94.2 ± 2.1	3.9	98.5 ± 1.1	93.2 ± 0.4	96.5 ± 0.5	95.5 ± 1.0
9 months	4.4	93.3 ± 0.8	93.8 ± 1.5	94.4 ± 1.2	91.6 ± 1.5	4.0	96.3 ± 1.0	87.1 ± 0.3	94.6 ± 0.9	90.3 ± 1.8
12 months	4.8	88.6 ± 1.5	82.0 ± 0.7	91.3 ± 1.0	93.2 ± 2.1	4.2	93.0 ± 0.8	81.8 ± 0.4	95.5 ± 0.9	85.0 ± 3.3

UV spectrophotometry are listed in Table 3. The applicability of the methods for the determination of sorbic acid in the nonionic creams was demonstrated by the recoveries (Table 3) from the blank creams. Mean recoveries of 99.5% were obtained with a precision of 0.63% for the HPLC analysis and 99.0% with a precision of 0.50% for the UV analysis. These methods were shown to be free from background interference of matrix components by analysis of blank matrices. For cream samples with known concentrations of sorbic acid after extraction, 99.2% ± 0.48% of the sorbic acid were recovered using HPLC and 98.9% ± 0.46% were recovered using the spectrophotometric method.

The precision of the methods expressed as %RSD was 0.5 for $n = 10$ (number of injections) for the HPLC method and 0.8 for the spectrophotometric analysis of a standard solution containing 0.25 mg · ml^{-1}. For extracted cream samples, the relative standard deviations (%RSD) in the case of HPLC determination on the basis of 10 determinations were 0.4% and 1.3% for spectrophotometry. When the same samples were analyzed after 24 hr, the amount of sorbic acid in the creams was found to be 99.08% of the label claim with an RSD of 0.83% ($n = 6$) for HPLC and 98.23% of the label claim with an RSD of 1.06% for UV analysis.

The specificity of the methods was tested by means of injecting solutions of sorbic acid after treatment with a solution of concentrated hydrogen peroxide, concentrated hydrochloride acid, or 10 M sodium hydroxide (7). The treatment with acid or base gave no changes in the chromatogram or UV spectrum of sorbic acid. Figures 1 and 2 show the results for sorbic acid after oxidation with hydrogen peroxide. The sorbic acid peak has almost disappeared after oxidation. No breakdown products with retention times equal to that of sorbic acid were present.

It may be observed that degradation of sorbic acid in aqueous solution follows first-order or pseudo-first-order reaction kinetics, and the rate of reaction is very much dependent on the temperature and hydrogen concentration (5). The rate of reaction decreases with an increase in pH and becomes negligible above a pH of 5. Since the pK_a of sorbic acid is 4.75, it would suggest that only undissociated sorbic acid molecules are susceptible to oxidative degradation in aqueous solutions: Ionized molecules are degraded to a negligible extent (5). Light, oxygen, container plastic, pH, and salts also influence the stability of sorbic acid.

To verify whether the low results obtained for some creams after storage were due to instability of sorbic acid in the creams, they were stored for 12 months at 25°C and 40°C and analyzed periodically. The results are shown in Fig. 4 and summarized in Table 4. Statistical comparison clearly indicated the decrease in sorbic acid content of the creams with time at both 25°C ($p = .0341$) and 40°C ($p = .0075$). It also showed that temperature had the biggest effect on sorbic acid degradation ($p \lll .05$). There were also significant differences between the assay results obtained by HPLC and those obtained by UV methods ($p = .04189$). This difference became more pronounced after 6 months ($p = .0004$). These results indicated that the UV method may predict up to 10% higher assay values for partially degraded samples (Fig. 4). The most pronounced difference occurred in lots of the USP hydrophilic cream kept at 40°C (Fig. 4). An increase in pH could have caused these differences.

CONCLUSIONS

Sorbic acid has been used in foods for many years because of its low toxicity, and although sorbic acid is often reported to be ineffective in the presence of nonionic compounds, it is used as a preservative in creams. The intent of this study was to provide basic experimental results for two simple test procedures for the assay of sorbic acid. It was shown that both the HPLC and UV methods could accurately determine sorbic acid in nonionic creams containing lactic acid. The validation results, together with the statistical treatment of the data, demonstrated the reliability of both procedures. Particularly, the determination of sorbic acid using UV spectrophotometry should make analysis easier. A drawback of the UV method is, however, its lack of adequate measurement of sorbic acid stability at higher temperatures. For these assays, the HPLC method was found to be adequate, and it should therefore be used to obtain accurate stability data for sorbic acid in creams.

ACKNOWLEDGMENT

We thank the National Research Foundation of South Africa for financial support.

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