Simultaneous Determination of Ascorbic Acid and Acetylsalicylic Acid in Pharmaceutical Formulations

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A direct, simple, and practical first-derivative spectrophotometric method is described for simultaneous determination of ascorbic acid and acetylsalicylic acid. The effects of the solvent, excipients, and spectral variables on the analytical signal were investigated. The drugs were determined simultaneously with a 0.01M methanolic hydrochloric acid solution as the solvent, and the signals were evaluated directly by using the zero-crossing method at 245.0 and 256.0 nm for acetylsalicylic acid and ascorbic acid, respectively. The method allows the simultaneous determinations of acetylsalicylic acid and ascorbic acid in the ranges of 6.6×10^{-6} to 1.5×10^{-4}M and 3.4×10^{-6} to 2.0×10^{-4}M, respectively, with standard deviation of <2.0%. The proposed method was applied to determinations of these drugs in tablets.

Ascorbic acid, 3-ketothreohexuronic acid lactone (I), and acetylsalicylic acid, 2-acetyloxybenzoic acid (II), are recommended and widely used to relieve cold and influenza symptoms and to prevent the effects of these infections, respectively. For this reason, these compounds are both prescribed in the treatment of colds and influenza, even if they have been included together in pharmaceutical formulations. Nevertheless, few methods have been reported for the simultaneous determination of these drugs. The *United States Pharmacopoeia* (1) reports methods only for the individual determination of each compound, but not for their simultaneous determination.

Ascorbic acid has been determined in pharmaceuticals by titrimetry (2), spectrophotometry (3, 4), electrochemistry (5, 6), and liquid chromatography (LC; 7, 8), and also in combination with other analgesics by LC (9, 10). On the other hand, acetylsalicylic acid has been determined by spectrophotometry (11) and thin-layer chromatography (12), and in combination with other drugs by spectrophotometry (13) and LC (14, 15).

Both compounds have been determined simultaneously only by LC (16, 17).

Derivative spectrophotometry is useful for improving the resolution of mixtures, without previous chemical separation, because this technique enhances the detectability of minor spectral features (18, 19). Taking into account this important feature, we selected this technique to develop the proposed method.

This paper describes a direct, simple, and practical first-derivative spectrophotometric method for simultaneous determination of acetylsalicylic acid and ascorbic acid. The method was applied to the determination of both compounds in pharmaceutical formulations.

Experimental

Instrumentation

A Shimadzu (Kyoto, Japan) UV-160 spectrophotometer with 10 mm cells was used for measurement of the absorbance and derivative absorption spectra. For all solutions, first-derivative spectra were recorded over a range of 320–200 nm versus solvent, at a scan speed of 480 nm/min with a $\Delta\lambda$ of 6.4 nm. The derivative spectra were obtained digitally with the Shimadzu UV-160 spectrophotometer. The derivative units (DU) were given directly by the spectrophotometer.

Materials and Reagents

All reagents were analytical reagent grade. Ascorbic acid and acetylsalicylic acid were kindly provided by Laboratorio Chile (Santiago, Chile).

Solvent Selection

Stock solutions of I and II were prepared daily by dissolving 17.61 ± 0.01 and 18.01 ± 0.01 mg, respectively, in 100 mL of different solvents to obtain 1.0×10^{-3}M solutions. Concentrations between 2×10^{-5} and 1×10^{-4}M were prepared by appropriate dilution with the respective solvent. The ascorbic acid solutions were prepared daily and protected from light.

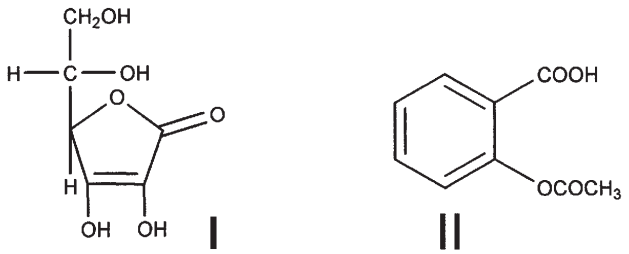

Figure 1. Structures of ascorbic acid and acetylsalicylic acid: (I) ascorbic acid, 3-ketothreohexuronic acid lactone; (II) acetylsalicylic acid, 2-acetyloxybenzoic acid.

Spectral Study

Stock solutions of 1.0×10^{-3}M ascorbic acid and 1.0×10^{-3}M acetylsalicylic acid were prepared by adding 17.61 ± 0.01 and 18.01 ± 0.01 mg of each compound, respectively, to a 100 mL flask and diluting to volume with a 0.01M methanolic HCl solution. Other concentrations were obtained by appropriate dilution with the same solvent. The classic and derivative spectra were obtained between 200 and 320 nm. The DU were given directly by the spectrophotometer software.

Calibration Procedure for Determination of Acetylsalicylic Acid and Ascorbic Acid in Mixtures

Aliquots of the stock solutions of acetylsalicylic acid and ascorbic acid were simultaneously diluted with 0.01M methanolic HCl solution over the concentration range 2.0×10^{-6} to 3.0×10^{-4}M. The calibration procedure was performed for each compound in the presence of a 6.0×10^{-5}M solution of the other. In all cases, the corresponding absolute values of the first-derivative spectra at 245.0 and 256.0 nm for acetylsalicylic acid and ascorbic acid, respectively, were obtained and plotted vs the corresponding concentrations.

Procedure for Determination of Ascorbic Acid and Acetylsalicylic Acid in Synthetic Pharmaceutical Formulations

A synthetic sample containing a mixture of 200.0 ± 0.01 mg ascorbic acid, 500.0 ± 0.01 mg acetylsalicylic acid, 35.0 mg magnesium stearate, and 665.0 mg gelatin was prepared.

Portions of the powder equivalent to 23–32 mg of the mixture were accurately weighed and dissolved in a 0.01M methanolic HCl solution; each solution was transferred to a separate 100 mL calibrated flask and diluted to volume. The contents of the flasks were shaken for 20 min, and then the solutions were centrifuged. A fraction of each solution was evaluated by first-derivative spectrophotometry between 200 and 320 nm, with $\Delta\lambda$ = 6.4 nm at 245.0 and 256.0 nm.

Procedure for Determination of Ascorbic Acid and Acetylsalicylic Acid in Tablets

Twenty tablets of PirinaCe Lasifarma S.A. Argentina, with a nominal content of 500 mg acetylsalicylic and 200 mg ascorbic acid, were weighed and powdered. A portion of powder equivalent to 23–32 mg was accurately weighed and dissolved in a 0.01M methanolic HCl solution. The procedure for synthetic pharmaceutical formulations was followed next.

Results and Discussion

The structures of ascorbic acid and acetylsalicylic acid are shown in Figure 1. Because the structures of these drugs are quite different, differences in the spectral behavior of both compounds may be expected. On the other hand, ascorbic acid is unstable, with degradation in solution occurring under both aerobic and anaerobic conditions and producing different degradation products. In this context, the spectral behavior of

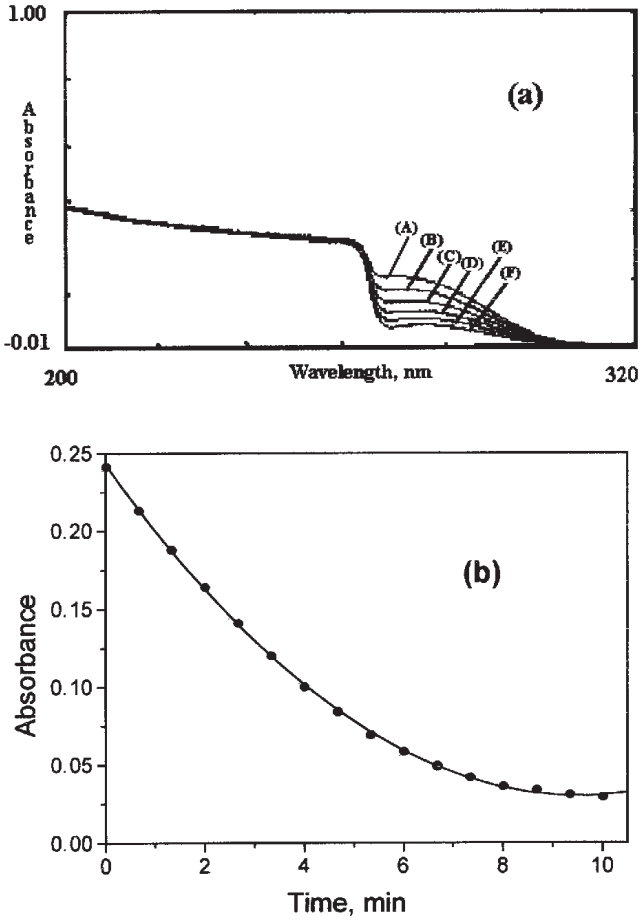

Figure 2. (a) Classic zero-order spectra of ascorbic acid in dimethylformamide as the solvent at different times: (A) 30 s, (B) 60 s, (C) 90 s, (D) 120 s, (E) 150 s, and (F) 180 s; (b) effect of time on the decomposition of ascorbic acid, 1.0×10^{-4} mol/L, in dimethylformamide as the solvent.

Table 1. Stability of acetylsalicylic acid and ascorbic acid in different solvents and the effect of concentration on the spectral bands

	Stability		Concentration effect on spectral bands	
Solvent	Acetylsalicylic acid	Ascorbic acid	Acetylsalicylic acid	Ascorbic acid
Dimethylformamide	Stable[a]	1 min	Altered[b]	Severe
Acetonitrile	Stable	20 min	Altered[b]	Severe
Ethanol	Stable	50 min	Altered[b]	Not altered
0.01M NaOH	Stable	30 min	Altered[b]	Not altered
Methanol	Stable	40 min	Not altered	Not altered
0.01M HCl	Stable	40 min	Not altered	Not altered
0.01M HCl in ethanol	Stable	180 min	Altered[b]	Not altered
0.01M HCl in methanol	Stable	180 min	Not altered	Not altered

[a] Stable indicates the derivative units varied by <2%. Maximum testing time was 180 min.
[b] Range was 200–250 nm.

these drugs and the stability of ascorbic acid in different solvents were studied.

The stability of ascorbic acid in dimethylformamide was very low. The spectral band between 270.0 and 290.0 nm decreased quickly—in a few minutes (Figure 2). The same effect was detected when solvents like acetonitrile, methanol, ethanol, NaOH aqueous solutions, and 0.01M methanolic or ethanolic HCl were used, but the decomposition in such cases was progressive over time (Table 1).

The concentration effect of both compounds on the classic spectra in some solvents was studied. The acetylsalicylic acid bands in the range of 200–250 nm were altered in some cases (Table 1). An example of band distortion in acetonitrile is shown in Figure 3.

In 0.01M ethanolic HCl solution, ascorbic acid was stable for ≥180 min, but the concentration altered the signal bands of acetylsalicylic acid. However, in 0.01M methanolic HCl solution, ascorbic acid was also stable for 180 min, but the signal bands were not altered when the acetylsalicylic acid concentration was increased. In this context, a 0.01M methanolic HCl solution was selected, because in this solvent the simultaneous determination was effective; both drugs were stable for ≥3 h, and the bands were not altered.

Spectral Features

It was found that the zero-order spectrum of ascorbic acid, with 0.01M methanolic HCl solution as the solvent, exhibited 1 band centered at 250 nm (Figure 4, I). On the other hand, the zero-order spectrum of the acetylsalicylic acid showed 3 bands centered at 208.0, 224.0, and 278.0 nm, respectively (Figure 4, II).

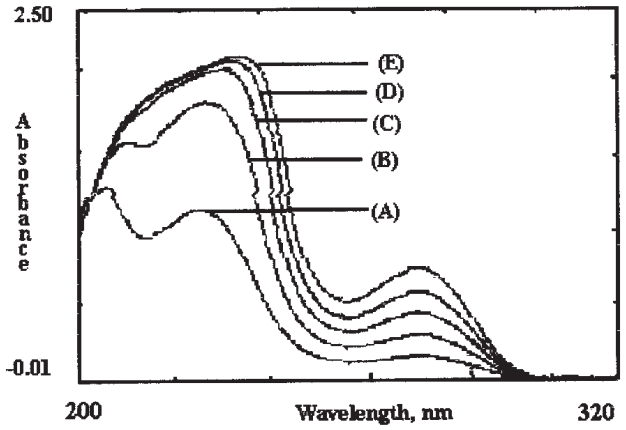

Figure 3. Effect of acetylsalicylic acid concentration on the classic spectra with acetonitrile as the solvent: (A) 0.2×10^{-3} mol/L; (B) 0.4×10^{-3} mol/L; (C) 0.6×10^{-3} mol/L; (D) 0.8×10^{-3} mol/L; (E) 1.0×10^{-3} mol/L.

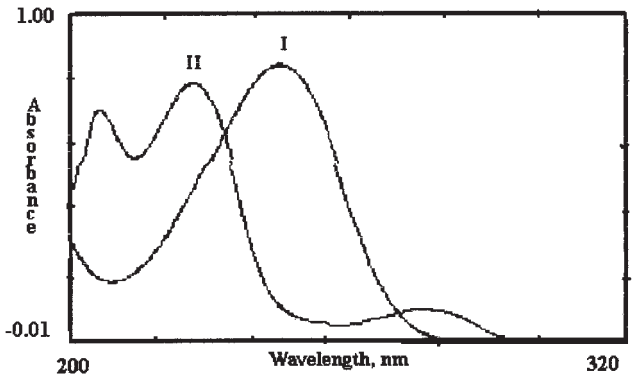

Figure 4. Spectra of acetylsalicylic acid and ascorbic acid in 0.01M methanolic hydrochloric acid solution measured versus 0.01M methanolic hydrochloric acid solutions: (I) ascorbic acid, 1.0×10^{-4} mol/L; (II) acetylsalicylic acid, 1.0×10^{-4} mol/L.

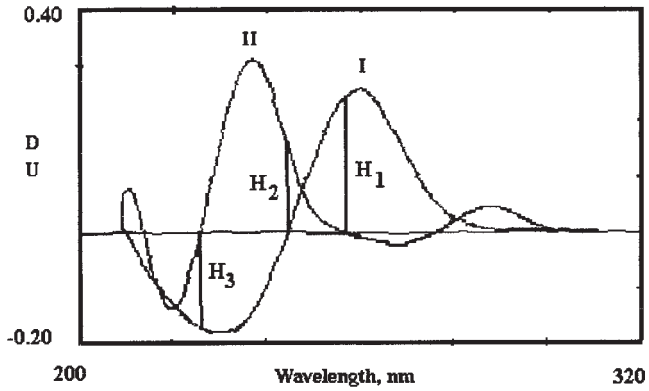

Figure 5. First-derivative spectra of acetylsalicylic acid and ascorbic acid in 0.01M methanolic hydrochloric acid solution measured versus 0.01M methanolic hydrochloric acid solution: (I) ascorbic acid, 1.0×10^{-4} mol/L; (II) acetylsalicylic acid, 1.0×10^{-4} mol/L.

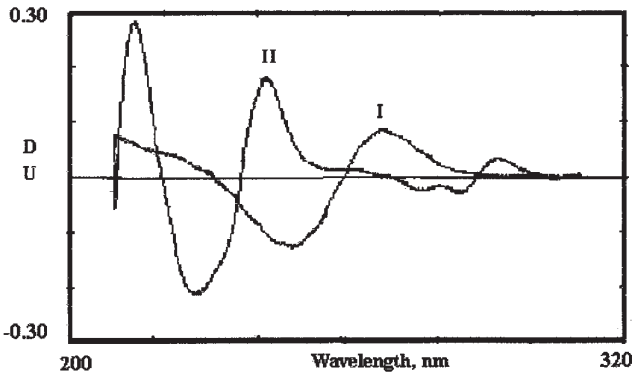

Figure 6. Second-derivative spectra of acetylsalicylic acid and ascorbic acid in 0.01M methanolic hydrochloric acid solution measured versus 0.01M methanolic hydrochloric acid solution: (I) ascorbic acid, 1.0×10^{-4} mol/L; (II) acetylsalicylic acid 1.0×10^{-4} mol/L.

The zero-order spectra of both analytes do not contain prominent peaks for reliable, direct measurement of absorbance for simultaneous determinations in mixtures because the spectra almost totally overlap (Figure 4). One possibility for the simultaneous determination of these compounds is to solve a system of equations, which do not produce very reliable results. Another possibility for the simultaneous determinations of multicomponents is to apply a chemometric approach to the spectrophotometric signal. Multiwavelength evaluation (20) and derivative spectrophotometry (21) are well-known examples of these types of approaches. In this work, we adopted derivative spectrophotometry for resolution of the analyte spectral bands because this approach is simple and does not require further mathematical treatment of the data. The order of the derivative, the analytical wavelengths, and the $\Delta\lambda$ value were optimized to obtain maximum resolution and reproducibility.

Selection of Spectral Variables

Derivative order.—To choose the optimum derivative order, the first- and second-derivative spectra of the 0.01M methanolic HCl solutions containing each compound were recorded. As shown in Figures 5 and 6, the first-derivative spectra have good resolution and also a high signal-to-noise ratio (S/N). With the second-derivative spectra, the noise increases considerably and the S/N ratio decreases. Because the first-derivative spectra offer a more valuable means for simultaneous determination of both analytes, this approach was selected. Higher-derivative orders were also examined; however, they produced very high but nonreproducible signals. On the other hand, it was observed that the noise increased proportionally with the derivative order.

Selection of analytical wavelengths.—Analytical wavelengths were selected by taking into account the first-derivative spectra of both analytes separately. Figure 5

Table 2. Analytical parameters[a] of calibration graphs with concentration of one analyte variable and that of the other constant

	Acetylsalicylic acid, 1.0×10^{-5} to 10×10^{-5}M[b]			Ascorbic acid, 1.0×10^{-5} to 10×10^{-5}M[c]					
				226.0 nm			256.0 nm		
$\Delta\lambda$/nm	Sensitivity	Intercept	*r*	Sensitivity	Intercept	*r*	Sensitivity	Intercept	*r*
1.6	320	-4×10^{-4}	0.998	510	-2.2×10^{-3}	0.998	760	-2.4×10^{-3}	0.999
3.2	685	-2.3×10^{-3}	0.999	1070	-7.8×10^{-3}	0.999	1535	-3.7×10^{-3}	0.999
4.8	1050	-2×10^{-4}	0.998	1590	-1.22×10^{-2}	0.999	2285	-6.3×10^{-3}	0.999
6.4	1515	-5×10^{-4}	0.999	2090	-1.74×10^{-2}	0.999	2925	-6.7×10^{-3}	0.999

[a] Sensitivity = slope of calibration graph; *r* = correlation coefficient.

[b] The ascorbic acid concentration was 6.0×10^{-5}M, and the signal was constant at 256.0 nm, i.e., the DU varied by ≤2%.

[c] The acetylsalicylic acid concentration was 6.0×10^{-5}M, and the signal was constant at 245.0 nm, i.e., the DU varied by ≤2%.

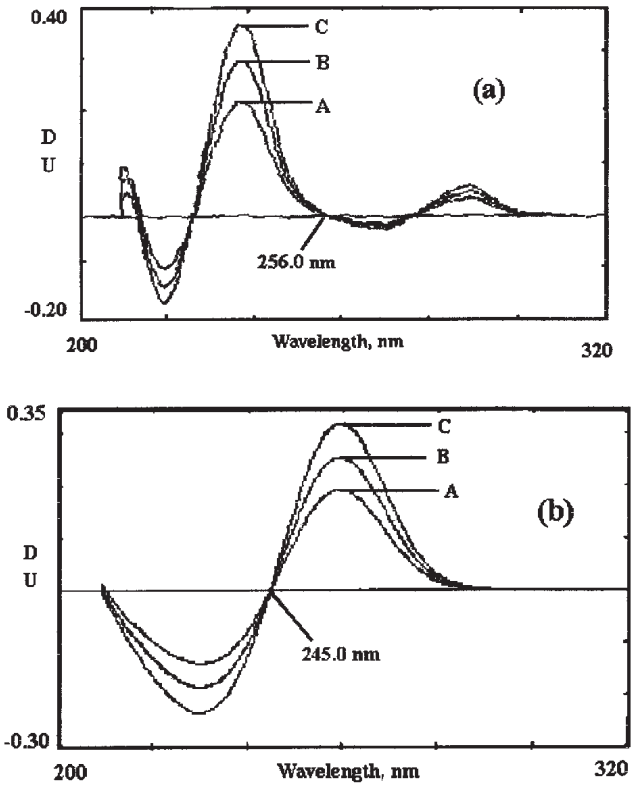

Figure 7. (a) Effect of concentration of acetylsalicylic acid on the analytical wavelength when first-derivative spectrophotometry and $\Delta\lambda$ = 6.4 nm are used: A = 0.6×10^{-4} mol/L; B = 0.8×10^{-4} mol/L; C = 1.0×10^{-4} mol/L. (b) Effect of concentration of ascorbic acid on the analytical wavelength when first-derivative spectrophotometry and $\Delta\lambda$ = 6.4 nm are used: A = 0.6×10^{-4} mol/L; B = 0.8×10^{-4} mol/L; C = 1.0×10^{-4} mol/L.

shows that the zero-crossing method at 226.0 (H_3) and 256.0 nm (H_1) can be used for determination of ascorbic acid. As shown in Table 2, a wavelength of 256.0 nm was selected because the sensitivities were higher than those observed at 226.0 nm, favoring the detection and determination limits. In addition, the acetylsalicylic acid band did not present the possibility of overlapping with the ascorbic acid band. On the other hand, acetylsalicylic acid can be determined by both the zero-crossing method at 245.0 nm (H_2) and the graphic method between 290.0 and 300.0 nm. In general, when it is possible to measure signals for both the graphic and the zero-crossing methods, the former is normally selected because the accuracy is higher. Nevertheless, the graphic method was not selected because the sensitivity was considerably lower than that obtained with the zero-crossing method. Furthermore, in this case, the shape of the bands did not suggest possible overlapping. For these reasons, 245.0 nm was selected for determination of acetylsalicylic acid. As shown in Figure 7, in all cases, the concentration of either compound did not affect the analytical wavelength selected.

Selection of $\Delta\lambda$ value.—The selection of the $\Delta\lambda$ value for differentiation of the spectra was based on the sensitivity, precision, and lack of interference between the analytes. In this context, as shown in Table 2, when a $\Delta\lambda$ of 6.4 nm was used, the sensitivities were higher and the signal of the analyte having a constant concentration was not altered. For this reason, a value of 6.4 nm was used for differentiation of the spectra.

In summary, the spectral conditions selected for the determination were first-derivative spectra, $\Delta\lambda$ = 6.4 nm, and 245.0 and 256.0 nm for the determination of acetylsalicylic acid and ascorbic acid, respectively.

Effect of Excipients on the Spectra

The effect of excipients such as magnesium stearate, gelatin, lactose, and starch was examined. Approximately 0.5 mg magnesium stearate + gelatin and 10 mg lactose–starch were added to 6.0×10^{-5}M solutions of acetylsalicylic acid and ascorbic acid. The spectra of these solutions were not changed, indicating that these excipients do not interfere.

Analytical Features

Linear calibration graphs of DU (H) versus concentration of ascorbic acid and acetylsalicylic acid were obtained over the ranges of 2.9×10^{-7} to 2.0×10^{-4}M for I and 4.8×10^{-7} to 1.5×10^{-4}M for II. The equations of the regression line obtained were $H_1 = 2925 \times C$ (mol/L) $- 7 \times 10^{-3}$ (r = 0.999) for ascorbic acid and $H_2 = 1515 \times C$ (mol/L) $- 5 \times 10^{-4}$ (r = 0.999) for acetylsalicylic acid, where H is in DU and C corresponds to the concentration in mol/L.

The detection (3δ) and determination (10δ) limits together with the determination ranges are shown in Table 3. Under the conditions selected, the standard deviation (δ) of a blank, consisting of 0.01M methanolic HCl solution, at 245.0 and 256.0 nm was in both cases 1×10^{-3} DU (n = 11).

Table 3. Analytical parameters for acetylsalicylic acid and ascorbic acid

Analyte	Detection limit[a]	Determination limit[a]	Determination range
Acetylsalicylic acid	2.0×10^{-6}M	6.6×10^{-6}M	6.6×10^{-6} to 1.5×10^{-4}M
Ascorbic acid	1.0×10^{-6}M	3.4×10^{-6}M	3.4×10^{-6} to 2.0×10^{-4}M

[a] Detection limit = 3δ/s and determination limit = 10δ/s, where δ = standard deviation of the solvent at the analytical wavelength, and s = sensitivity (slope of the calibration graph).

Table 4. Determination of ascorbic acid and acetylsalicylic acid in different standard mixtures

	Concentration stated, $\times 10^{-5}$M		Concentration found, $\times 10^{-5}$M[b] (recovery, %)	
Ratio of analytes, I:II[a]	I	II	I	II
1:1	6.00	6.00	5.98 ± 0.04 (99.7)	6.11 ± 0.10 (101.8)
1:2	4.00	8.00	3.99 ± 0.07 (99.8)	7.85 ± 0.13 (98.1)
1:3	2.00	6.00	2.05 ± 0.04 (102.5)	5.89 ± 0.06 (98.2)
1:4	2.00	8.00	2.04 ± 0.03 (102.0)	7.84 ± 0.11 (98.0)
1:5	2.00	10.00	2.02 ± 0.03 (101.0)	9.85 ± 0.12 (98.5)

[a] I = ascorbic acid; II = acetylsalicylic acid.
[b] Mean of 5 determinations.

The values for repeatability of the determination, expressed as the relative standard deviation of 11 replicates containing 2×10^{-5}M ascorbic acid and 2×10^{-5}M acetylsalicylic acid, were 1.5 and 1.6%, respectively.

To assess the accuracy of the proposed method, recovery experiments were performed with standard mixtures containing different known amounts of both analytes. The concentration of each compound was determined by the use of the equations given above. The results obtained are shown in Table 4.

The results show that the concentration of each compound can be reliably determined in mixtures containing different molar ratios of the analytes.

Practical Application

Synthetic samples containing 200 mg ascorbic acid, 500 mg acetylsalicylic acid, and approximately 3–5% magnesium stearate, gelatin, and 95% lactose–starch were analyzed. The recoveries were 98.79 ± 0.57 and 97.39 ± 1.10% for ascorbic acid and acetylsalicylic acid, respectively. The method was applied to formulations prepared by PirinaCe Lasifarma S.A. Argentina, in which the nominal contents were 200 mg ascorbic acid and 500 mg acetylsalicylic acid per tablet. The amounts found were 194.5 ± 2.1 and 507 ± 8.3 mg per tablet for ascorbic acid and acetylsalicylic acid, respectively.

Acknowledgments

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