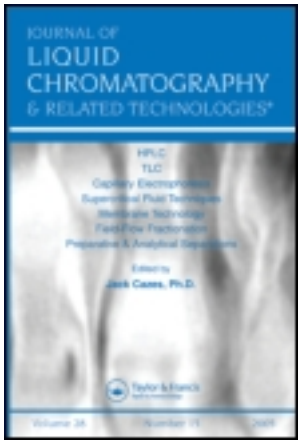

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SIMULTANEOUS ANALYSIS OF VITAMIN C AND ASPIRIN IN ASPIRIN C EFFERVESCENT TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-PHOTODIODE ARRAY DETECTOR

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□ *High performance liquid chromatography–photodiode array (HPLC–PDA) method has been employed for the simultaneous determination of vitamin C (ascorbic acid) and aspirin (acetylsalicylic acid) in aspirin C effervescent tablets. Chromatographic separation of ascorbic acid (AA) and acetylsalicylic acid (ASA) was performed by reversed–phase using a Betasil C_{18} column with particle size of 3 μm, 150 mm × 4.6 mm I.D. Optimum separation was achieved in isocratic mode with a binary mobile phase (mixture of water and acetonitrile with 0.1% of formic acid, 75:25, v/v) at a flow rate of 1.0 mL min^{-1}. Recovery of the target compounds was obtained more than 98% with good quality parameters: linearity ($r^2 > 0.997$), limit of detection (LOD) and limit of quantification (LOQ) values between 1.4×10^{-3} and 5.1×10^{-4} mg mL^{-1}, and intra-day and interday precisions with relative standard deviation (RSD) lower than 2.1%. The content of the analyzed samples ascorbic and acetylsalicylic acid was found 238.23 and 393.49 mg, respectively, which are very close to the label claimed by the manufacturer in aspirin C effervescent tablets. Therefore, this method can be proposed for routine analysis of these compounds in aspirin C effervescent tablets.*

Keywords acetylsalicylic acid, ascorbic acid, aspirin C effervescent tablets, column, HPLC–PDA, mobile phase

INTRODUCTION

Acetylsalicylic acid is a salicylate drug that is usually known as aspirin; it is often used as an analgesic, antipyretic, and anti–inflammatory medication.[1] Once swallowed, ASA is rapidly hydrolyzed in the human body, which is primarily responsible for the pharmacological activity of this compound.[2] ASA has been shown to be effective in reducing cardiovascular

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morbidity and mortality in high–risk patients with myocardial infarction or stroke.[3] The over dosage of aspirin include dizziness, sweating, nausea, vomiting, altered glucose metabolism, hyperventilation, respiratory alkalosis, metabolic acidosis, and so forth.[4] Therefore, in order to achieve a better curative effect and a lower toxicity, it is important to rapidly control the content of aspirin and its impurities in pharmaceutical formulations.

Relating to ascorbic acid, it is a water-soluble vitamin, known as an anti-scorbutic food factor.[5] It is essential for the formation of intercellular material, collagen, and for the healing of wounds.[6] It also facilitates the softness of skin, helps the absorption of iron, and recovers resistance to infection. Due to its anti-oxidative effect, it is also used in the treatment of scurvy and to prevent the occurrence and development of cancer.[7,8] The deficiency of this vitamin may increase the level of oxidative stress, hemorrhage, anemia, and ecchymosis.[9–11] Nevertheless, the overdoses of vitamin C may cause diarrhea and other gastrointestinal disturbances.[12]

On the other hand, a combination of AA and ASA have been widely employed in pharmaceutical formulations for pain and fever relief including fevers associated with colds and flu and are, perhaps, some of the most consumed drugs in the world.[13,14] Various pharmaceutical companies have started to commercialize these drugs together in pharmaceutical formulations due to the combined action of AA and ASA. The AA increases the organism's resistance against microorganisms as it is involved in the antibody formation; however, the ASA helps to relieve headaches and fever.[15] As a result, with the increase of the manufacture and consumption of drugs that employ AA and ASA together, it becomes important to develop new analytical methods for their simultaneous determination. Several works have appeared in the literature for the analysis of these drugs. The content of AA and ASA drugs has been analyzed simultaneously by high performance liquid chromatography equipped with different detection systems, excluding the PDA detector.[15–17] These drugs, in combination with other active compounds, were also determined by different analytical techniques such as differential spectrophotometry, spectrofluorimetry, voltammetry, potentiometry, derivative spectrophotometry, and ratio-spectra derivative methods.[18–24] However, the developed spectrophotometric methods have some well-known limitations in analytical applications. The direct spectrophotometric methods have shown the spectral overlap of the main maxima during the simultaneous determination of these drugs.[25]

Considering the properties of the investigated compounds, such as low volatility, mid- polarity, as well as thermolability, HPLC methods have been the most frequently explored.[15,16,18,26] Therefore, in this work a new method based on HPLC–PDA has been proposed to quantitate the amount of active components such as AA and ASA in commercial pharmaceutical effervescent tablet formulations. The proposed method is simple, rapid, less

expensive, and highly sensitive, in addition to an additional advantage that the quantitation may be accurately carried out without the use of an internal standard.

EXPERIMENTAL

Chemicals and Materials

Gradient-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and formic acid was supplied by Panreac (Bercelona, Spain). Ascorbic and acetylsalicylic acid were provided by Fluka (Buchs, Switzerland). Water was purified through a Milli-Q water purification system from Millipore (Bedford, MA, USA). Aspirin C effervescent tablets were obtained from Bayer pharmaceutical company (AG, Germany), containing 240 mg AA and 400 mg ASA per tablet. The chemical structures of the AA and ASA are shown in Figure 1.

Individual stock standard solutions of AA and ASA at 500 $\mu g\,mL^{-1}$ were prepared in Milli-Q water, and used for further dilution. Standard mixture of AA and ASA at different concentration levels (10–200 $\mu g\,mL^{-1}$) were prepared for calibration purpose. The standards and samples were freshly prepared and filtered before being injected into the HPLC–PDA system.

Sample Preparation

Ten effervescent tablets were weighed using analytical balance and powdered in a mortar. A tablet amount was transferred to a 100 mL-calibrated flask and dissolved with Milli-Q water and then stirred and sonicated simultaneously until the effervescence tablet particles dissolved completely. The final solution was diluted to the working range for application of the proposed methods. After dilution, the sample were filtered through a 0.22-μm disposable nylon filter (Bellefonte, PA, USA) and finally injected into the HPLC–PDA system.

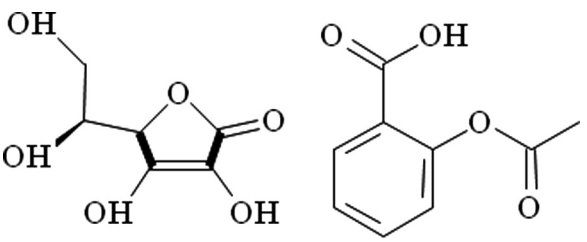

FIGURE 1 Chemical structures of ascorbic acid (I) and acetylsalicylic acid (II) used in this study.

Instrumentation

The samples were analyzed using a quaternary pump system from Thermo Scientific model Finnigan Surveyor Plus equipped with PDA detector (FL, USA). Chromatographic separation of effervescent tablets was performed by reverse-phase using a Betasil C_{18} column (Thermo Scientific, FL, USA) with particle size of 3 μm, 150 mm × 4.6 mm I.D. Optimum separation was achieved in isocratic mode using a binary mobile phase mixture of water and acetonitrile with 0.1% of formic acid, 75:25, v/v) at flow rate of 1 mL min^{-1}. The injection volume was 5 μL.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The optimization of chromatographic conditions was performed to obtain good peak resolution and to avoid peak tailing of the target compounds. The preliminary studies were carried out on standard solution of AA and ASA. To find the optimum conditions for AA and ASA analysis, the different chromatographic parameters such as mobile phase compositions, pH, flow rate and PDA detection wavelengths have been investigated. The mobile phases of various compositions of water and acetonitrile with 0.1% of formic acid were tested at different pH range (2–7) and flow rate (0.2–1 mL min^{-1}). The best results were obtained using mobile phase water:acetonitrile 75:25, v/v with 0.1% of formic acid (pH 3.5) at flow rate of 1 mL min^{-1}. The PDA detection wavelengths were studied in the range between 200 and 600 nm. The maximum intensity for AA and ASA was obtained 254 nm and 278 nm, respectively. Figure 2a and b displays the obtained chromatogram and absorption spectra of the analyzed compounds.

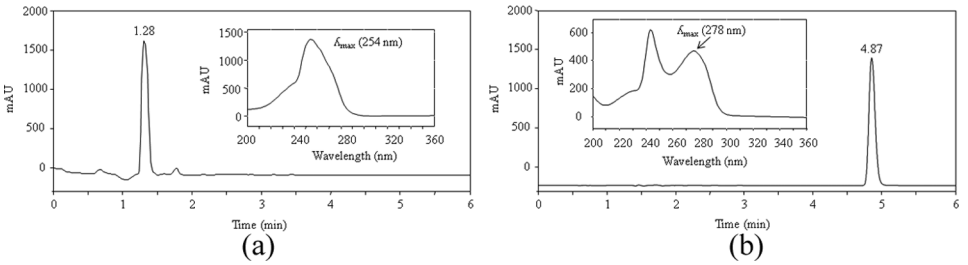

FIGURE 2 (a) The chromatogram and absorption spectra (inset) of ascorbic acid and (b) the chromatogram and absorption spectra (inset) of acetylsalicylic acid, obtained by HPLC–PDA system.

TABLE 1 Quality Parameters of the Proposed Method (HPLC–PDA)

Analyte	LOD[a] ($mg\,mL^{-1}$)	LOQ[b] ($mg\,mL^{-1}$)	Intra-day Precision [RSD (%)][c]	Inter-day Precision [RSD (%)][c]
Ascorbic acid	4.3×10^{-4}	1.4×10^{-3}	1.7	2.1
Acetylsalicylic acid	5.1×10^{-4}	1.7×10^{-3}	1.3	1.8

[a]Limit of detection was estimated at a signal-to-noise ratio of 3.
[b]Limit of quantification was estimated at a signal-to-noise ratio of 10.
[c]Relative standard deviation ($n = 5$).

Quality Parameters

Quality parameters were studied to assess the performance of the method, these were: linearity, LOD, LOQ, repeatability (intra-day precision), and reproducibility (inter-day precision). Table 1 shows the quality parameters of the proposed analytical method. Limit of detection and limit of quantification were estimated at a signal-to-noise ratio of 3 and 10, respectively. The LOD ranged between 4.3×10^{-4} and $5.1 \times 10^{-4}\,mg\,mL^{-1}$ and LOQ ranged between 1.4×10^{-3} and $1.7 \times 10^{-3}\,mg\,mL^{-1}$ was obtained for AA and ASA, respectively. Calibration curves based on the peak area were constructed. They were linear across the concentration range studied (10–200 $\mu g\,mL^{-1}$) and the correlation coefficients were higher than 0.997 for both the analytes. For repeatability, five replicates were carried out on standard solution of ascorbic and acetylsalicylic acid on the same day at concentration level $2\,\mu g\,mL^{-1}$. To assess the reproducibility, 15 replicates were performed with freshly prepared solutions of same concentrations over three consecutive days (five replicates each day). High repeatability and reproducibility was achieved with relative standard deviations lower than 2.1% in both the cases. This confirms that the proposed analytical method is successful in providing acceptable values of repeatability and reproducibility required for an accurate ascorbic and acetylsalicylic acid analysis. From these results, it can be concluded that the HPLC–PDA method can be used

TABLE 2 Level and Recovery of Ascorbic and Acetylsalicyclic Acid Obtained in Aspirin C Effervescent Tablets

Analyte	Conc. Found ±SD[a] (mg)	Conc. Claimed[b] (mg)	Recovery ±SD[c] (%)
Ascorbic acid	238.23 ± 0.03	240	99.26 ± 0.01
Acetylsalicylic acid	393.49 ± 0.05	400	98.37 ± 0.03

Conc. = Concentration.
[a]Standard deviation ($n = 5$).
[b]Amount claimed by the manufacturer (Bayer) in aspirin C effervescent tablet.
[c]Standard deviation ($n = 5$).

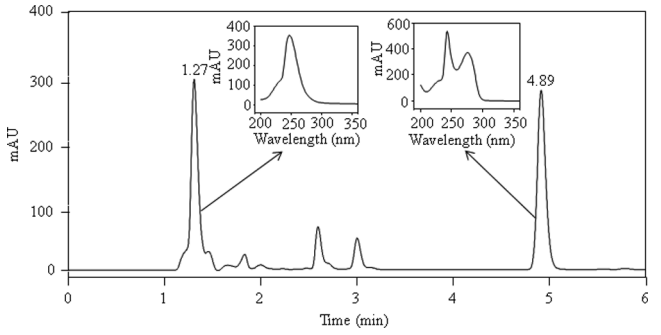

FIGURE 3 The chromatograms and absorption spectra (inset) of ascorbic acid (t_R–1.27) and acetylsalicylic acid (t_R–4.89), in aspirin C effervescent tablets obtained by HPLC–PDA system.

in the routine analysis of ascorbic and acetylsalicylic acid in aspirin C effervescent tablet samples.

Application: Analysis of Aspirin C Effervescent Tablets

In order to evaluate the applicability of the HPLC–PDA method for the simultaneous determination of AA and ASA in aspirin C effervescent tablets, five single tablets of the same batch were analyzed. The amount of ascorbic and acetylsalicylic acid were found 238.23 mg and 393.49 mg, respectively. The obtained experimental results and label claim (AA = 240 mg and ASA = 400 mg) of the commercial effervescent preparation evidence good concurrence. Table 2 shows the level of AA and ASA found in aspirin C effervescent tablets. The recovery of these compounds was estimated in laboratory-prepared prescribed drug amounts with formulations containing 60, 80, 100, 120, and 140% of active compounds. The recovery was obtained from 98.37% to 99.26%, depending on the sample (see Table 2).

Figure 3 shows, as an example, the HPLC–PDA chromatograms and absorption spectra of ascorbic and acetylsalicylic acid in aspirin C effervescent tablets. The chromatograms show good resolution and did not show any interference, as no detectable matrix peak was eluted in the retention time of the target analyte. Nevertheless, there are slight changes in the retention time of the analyzed compounds; this might be due to the matrix effect of the column. The absorption spectra of the corresponding analyte is similar and found at the same wavelength as obtained from the individual analysis of standard solution (Figure 2a and b).

CONCLUSIONS

The aim of this study was to propose a simple method based on HPLC–PDA to the simultaneous determination of AA and ASA drugs in aspirin C

effervescent tablets. The results of the quality parameters such as linearity ($r^2 > 0.997$), LOD, and LOQ (between 1.4×10^{-3} and $5.1 \times 10^{-}4\,\mathrm{mg\,mL^{-1}}$), and intra-day and inter-day precisions (<2.1%) achieved with this system, as well as the results obtained in the analysis of aspirin C effervescent tablets samples, confirmed that they are sufficient to propose it as a new methodology for the sensitive, rapid, and less expensive analysis of ascorbic and acetylsalicylic acid in aspirin C effervescent tablets. Nevertheless, previously developed methods based on different detection systems for the analysis of aspirin C effervescent tablets samples are less sensitive, laborious, and very expansive compared to our proposed method.[15,16,18] Consequently, we consider that the ability of this method for drug analysis will gain significance in the field of analytical chemistry as an alternative method.

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