ORIGINAL PAPER

Simultaneous Determination of Acetylsalicylic Acid and Caffeine in Pharmaceutical Formulation by First Derivative Synchronous Fluorimetric Method

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Abstract A sensitive, rapid, and specific assay has been developed for the simultaneous determination of acetylsalicylic acid and caffeine in commercial tablets based on their natural fluorescence. The mixture of these drugs was resolved by first derivative synchronous fluorimetric technique using two scans. At $\Delta\lambda = 106$ nm, using first derivative synchronous scanning, only acetylsalicylic acid yields a detectable signal at 316 nm (peak to zero method) which is unaffected by caffeine. At $\Delta\lambda = 30$ nm, the signal of caffeine at 288 nm (peak to zero method) is not affected by acetylsalicylic acid. The range of application is between 0.021 and 41.62 μg ml^{-1} (correlation coefficient, $R = 0.9995$) for acetylsalicylic acid and between 0.4486 and 44.86 μg ml^{-1} (correlation coefficient, $R = 0.99786$) for caffeine. The recovery range of 98.40–102% for acetylsalicylic acid and 90–100.5% for caffeine from their synthetic mixture was reported. Overall recovery of both compounds about 97–99% for acetylsalicylic acid and 97–98% for caffeine was obtained from real sample analysis. The detection limits are 0.0013 μg ml^{-1} and 0.0306 μg ml^{-1} for acetylsalicylic acid and caffeine, respectively. The relative standard deviation ($n = 10$) for 20 μg ml^{-1} of acetylsalicylic acid is 2.75% and for 2.2 μg ml^{-1} of caffeine is 1.7%.

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Introduction

Pain is an uncomforting feeling and sensation that we would quickly want to get rid of. When a splitting headache arises, the pain receptors will repeatedly remind us the headache is still present. This pain needs to be eased by taking a form of pain medication. For this pain, aspirin is the remedy. It is a synthetic chemical compound bearing the chemical name acetylsalicylic acid (ASA). It belongs to the non-steroidal anti-inflammatory drugs (NSAIDs). NSAID such as acetylsalicylic acid is valuable drug for the alleviation of pain, inflammation and fever. Of all the NSAIDs, acetylsalicylic acid is the most widely used since it is inexpensive, easily available and is indicated in many conditions such as headache and the common cold.

Caffeine (CAF) in combination with acetylsalicylic acid is used as an analgesic adjunct to enhance pain relief, although it has no analgesic activity of its own. Acute consumption of caffeine in combination with over-the-counter (OTC) analgesics such as acetylsalicylic acid or acetaminophen increases their activity by as much as 40% depending on the specific type of pain involved. It is apparently due to the ability of caffeine to cause constriction

of the cerebral blood vessels and possibly to facilitate the absorption of other drugs. The extensive use of these compounds in combined form and the need for clinical and pharmacological study require fast and sensitive analytical techniques for determination of their presence in biological fluids and pharmaceutical formulations.

For the simultaneous determination of acetylsalicylic acid and caffeine in the mixture, the different methods have been reported in the literature, including sequential injection chromatography [1], reversed-phase capillary electro chromatography [2], capillary zone electrophoresis based on the drug interactions with β-cyclodextrin [3], UV spectrophotometry with multivariate calibration [4], flow-through sensing method with UV detection [5], HPLC [6], PLS-UV spectrophotometric method [7].

The sensitivity of fluorescence method has largely justified the use of fluorescence for many determinations. The selectivity of fluorescence analysis has been good; variation in excitation and/or analytical wavelengths has allowed simultaneous determinations of compounds in many mixtures. However problems of selectivity can arise from the multi component analysis due to the overlapping of the broad-band spectra of structurally identical components. Specificity is a particular problem in the determination of fluorescent drugs. Generally, these compounds are determined by using a prior separation step, which is rather time-consuming for routine analysis and in some cases requires special and expensive instrumentation. But prior separation or derivatization step can be avoided by PLS technique [8] for quantitative analysis of multi component mixtures that can not be easily solved by univariate spectrofluorimetry [9, 10].

Fluorescence spectra of acetylsalicylic acid and caffeine overlap considerably according to our report and the report of Moreira et al. [8] who obtained the fluorescence spectra of these compounds by solid-phase fluorimetric method so that the conventional fluorescence does not allow the simultaneous determination of acetylsalicylic acid and caffeine. Some reports [11–17] demonstrated the emission overlapping spectra have been resolved by adapting synchronous fluorescence spectroscopy (SFS). Synchronous fluorescence spectroscopy is a recently developed methodology which was first suggested by Lloyd [18]. In conventional luminescence spectroscopy, two types of spectra can be obtained. To obtain a conventional emission spectrum, the emission wavelength λ_{em} is scanned while the excitation wavelength λ_{ex} is held at a fixed position, generally at the wavelength where the analyte absorbs most strongly. On the other hand, to obtain an excitation spectrum, the wavelength is scanned while the emission is observed at fixed λ_{em}. It was suggested that a third possibility consists of varying simultaneously or synchronously both λ_{ex} and λ_{em} while keeping a constant wavelength interval $\Delta\lambda$ between them. The spectral distribution is a function of the difference between the excitation and emission wavelengths. The maximum fluorescence intensity of a particular component occurs when $\Delta\lambda$ corresponds to the difference between the wavelengths of the excitation and emission maxima.

Although direct synchronous spectra are often sufficiently resolved for analytical purposes, the first derivative or second derivative technique shares the advantages of enhancement of spectroscopic quantitative analysis (1 to 3 orders of magnitude more sensitive), elimination of unwanted background and resolution of mixtures of components with spectra that are strongly overlapped [19]. The combination of synchronous and derivative technique results in high sensitivity and increased SNR values that are obtained by applying differentiation of the conventional spectrum because of narrow spectral band width in relation to emission spectrum and amplitude of the derivative signal which is inversely proportional to the band width of the original spectrum.

To the best of our knowledge, first derivative synchronous fluorimetric determination of acetylsalicylic acid and caffeine in pharmaceutical formulation has not yet been reported. In the method mentioned here, acetylsalicylic acid and caffeine were determined simultaneously in DPC surfactant media by their natural fluorescence. However, as the conventional and synchronous spectra of both compounds partially overlap the determination is performed by applying peak to zero technique to the first-derivative synchronous of the mixture. By using two scans at $\Delta\lambda = 30$ nm and $\Delta\lambda = 106$ nm to the mixture, the derivative peaks at 288 nm and 316 nm correspond to the identification of caffeine and acetylsalicylic acid respectively. The method was successfully applied to the simultaneous determination of caffeine and acetylsalicylic acid in pharmaceutical formulation.

Experimental

Reagents

All experiments were performed with analytical-reagent grade chemicals and pure solvents. Doubly distilled and dematerialized water was used throughout. Acetylsalicylic acid was obtained from Aldrich (USA) and caffeine was purchased from Sigma (USA). Standard solutions of 1×10^{-3} M acetylsalicylic acid and caffeine were made by direct weighing of the required amount of them and then dissolving in 0.1 M HCl. Acetylsalicylic acid solution was stored at 4°C. To prevent the possible decomposition of ASA to SA, all solutions were freshly prepared prior to experiments. CH_3COOH-NaOAc buffer solution (pH 5.0) was prepared as follows: 6.95 ml of 2 M NaOAc and 3.05 ml of 2 M CH_3COOH was transferred into a 100 ml standard flask and diluted to the mark with water. N-Dodecyl- pyridiniumchlorid (Merck), 1×10^{-3} M was

prepared by dissolving 0.0283 g of it in 100 ml deionized water. CAFIASPIRINA[®] (50 mg of caffeine + 500 mg of aspirin + excipient) and ACYLCOFFIN[®] (50 mg of caffeine + 450 mg of aspirin + excipient) were obtained from local pharmacy.

Apparatus

All the spectrofluorimetric measurements were conducted with a SPEX Fluorolog-2 spectrofluorometer. The spectrometer used a 450-W xenon lamp as the excitation as the excitation light source and a R 928 photomultiplier tube powered at 950V (Hamamatsu Co.) as the detector. For synchronous excitation measurements, both excitation and emission monochromators were locked together and scanned simultaneously with a constant difference $\Delta\lambda = \lambda_{em} - \lambda_{ex}$. Excitation and emission monochromator slit, increment, and integration time were set at 1 mm, 1 nm and 1 second, respectively. All spectral data and derivative spectra were obtained by SPEX DM 3000F spectroscopy computer. A pH meter (Model Orion 520A USA) was used for pH adjustment.

Basic procedure

To a 10 ml volumetric flask, a 2.31 ml of 1×10^{-3} M acetylsalicylic acid and caffeine each was added to give final concentrations in the range 0.021–41.62 μg ml^{-1} acetylsalicylic acid and 0.4486–44.86 μg ml^{-1} caffeine. Then 1.54 ml of 1×10^{-3} M DPC and 1.54 ml buffer solution (pH = 5.0) were added and the mixture was diluted to the mark with distilled water. For each sample two synchronous spectra were obtained by scanning both the monochromators simultaneously at constant wavelength differences of $\Delta\lambda = 30$ nm and $\Delta\lambda = 106$ nm for caffeine and acetylsalicylic acid, respectively. Hereafter, all wavelengths referring to synchronous spectra were taken to be equal to those of the corresponding excitation wavelengths. Cell concentration calculated for each acetylsalicylic acid and caffeine was 1.54×10^{-4} M and for DPC was 2.31×10^{-4} M. First derivative synchronous spectra were recorded by scanning the both monochromators simultaneously first with constant 30 nm and second with 106 nm difference between them. Acetylsalicylic acid and caffeine were determined through the derivative signals obtained by measuring the vertical distances to the zero line at 316 nm and 288 nm, respectively.

Treatment of samples (tablet formulations)

Two commercial pharmaceutical formulations were assayed such as CAFIASPIRINA[®] tablet (containing 50 mg of caffeine and 500 mg of aspirin) and ACYLCOFFIN[®] tablet (50 mg of caffeine and 450 mg of aspirin). Assay of the active ingredients in the tablets was performed by weighing

10 tablets (calculating average weight of one tablet), grinding the tablet mass, using an average weight of one tablet and dissolving it in 0.1M HCl with shaking for 10 min in an ultrasonic bath. The solution was filtered through a 0.45-μm pore size Millipore membrane filter, and the filtrate plus washings were diluted to the mark in a 100 ml flask. Appropriate dilutions were made from this solution to meet the linear range.
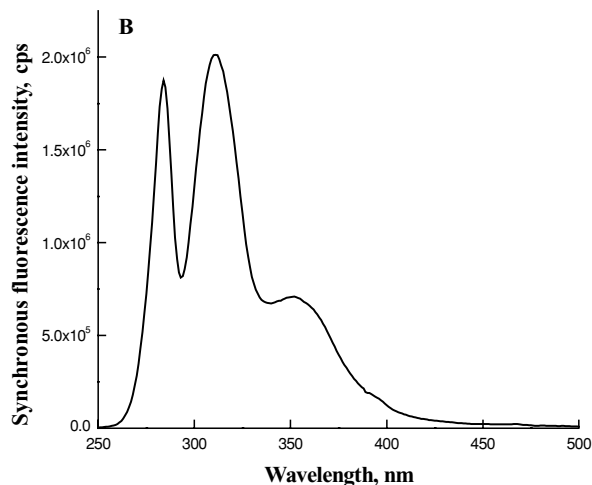
Results and discussion

Spectral characteristics

The quantification of acetylsalicylic acid and caffeine in pharmaceutical formulation is possible by various methods. Among these, fluorimetry would be expected to be attractive; the method in its conventional implementation suffers from the overlapping of peaks of interest. The excitation, emission and synchronous excitation spectra of caffeine and acetylsalicylic acid are shown in the Fig. 1 and Fig. 2. From Fig. 1(A), caffeine shows an excitation maximum at 311 nm and emission spectrum shows maxima at 363 nm. Synchronous excitation spectrum of caffeine was obtained by maintaining a constant interval ($\Delta\lambda = 52$ nm) between emission and excitation wavelengths at 363 nm and 311 nm, respectively (Hereafter, all wavelengths referring to synchronous spectra are taken to be equal to those of the corresponding excitation wavelengths). The intense peak at 311 nm is enhanced more strongly than the weak peaks at 283 nm and 350 nm [Fig. 1(B)] because $\Delta\lambda$ was chosen to match the two strong bands at emission and excitation spectra. From Fig. 2(A), the maximum excitation and emission wavelengths of acetylsalicylic acid were at 305 nm and 411 nm respectively. When synchronous technique was applied using a 106 nm value for $\Delta\lambda$, only one single excitation band at 305 nm was obtained [Fig. 2(B)] because the interval $\Delta\lambda$ can be found to match solely one pair of excitation and emission bands. When excitation and emission spectra consist of bands which are not separated by similar wavelength intervals ($= \delta\lambda$) the same situation also occurs (single peak). The width of the synchronous spectra of caffeine and acetylsalicylic acid is significantly smaller than that of their fluorescence emission spectra due to band-narrowing effect which is the consequence of multiplication of two functions [$E_M (\lambda)$ and $E_X (\lambda')$] increasing and/or decreasing simultaneously [20]. Obviously, the emission spectra of caffeine and acetylsalicylic acid were seriously overlapped. In addition, 305 nm was selected as the co-excitation wavelength for the simultaneous determination of these compounds but their emission spectra were overlapped as shown in the Fig. 3(A); so conventional fluorimetry could not permit the simultaneous determination of
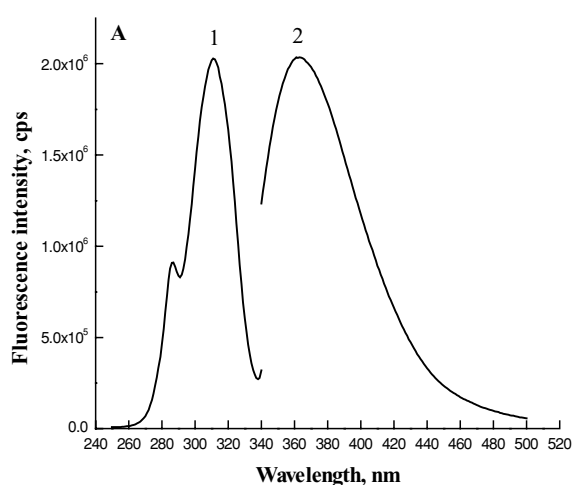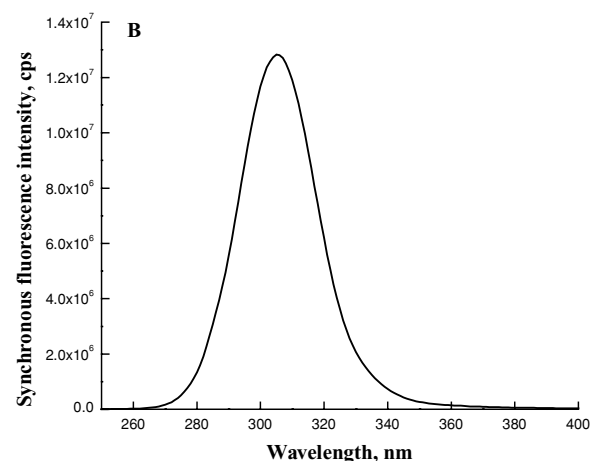
Fig. 1 (A) Fluorescence (1) excitation, (2) emission, and (B) synchronous excitation spectra of caffeine. Conditions: caffeine, 2.31×10^{-4} M; DPC, $1.54 \times 1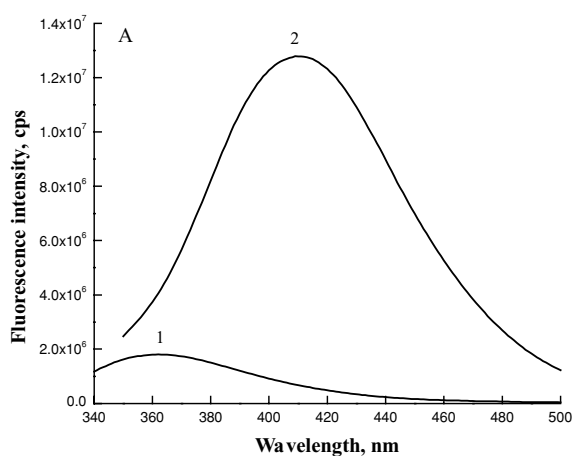0^{-4}$ M and CH$_3$COOH-NaOAc, 2 M (pH = 5.0). Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s
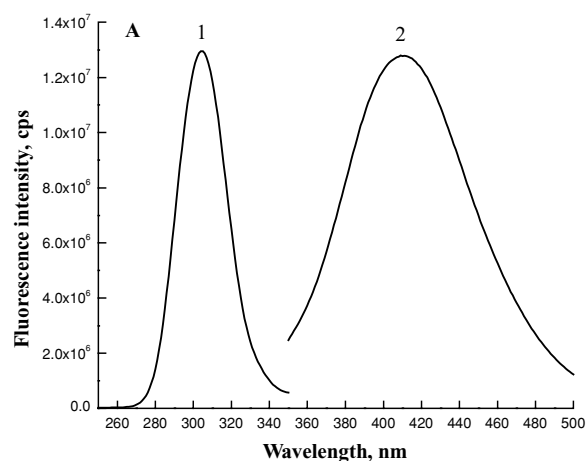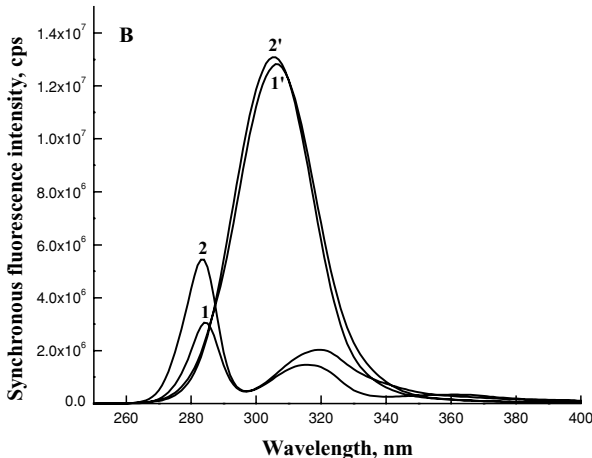

Fig. 2 (A) Fluorescence (1) excitation, (2) emission, and (B) synchronous excitation spectra of acetylsalicylic acid. Conditions: acetylsalicylic acid, 2.31×10^{-4} M; DPC, 1.54×10^{-4} M and CH$_3$COOH-NaOAc, 2 M (pH = 5.0). Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s

Fig. 3 (A) Fluorescence emission spectra of (1) caffeine and (2) acetylsalicylic acid at co-excitation of λ_{ex} = 305. (B) Synchronous excitation spectra of (1) caffeine, and (2) mixture at constant wavelength difference of $\Delta\lambda = 30$ nm; (1′) acetylsalicylic acid and (2′) mixture at constant wavelength difference of $\Delta\lambda = 106$ nm. Conditions: acetylsalicylic acid, 2.31×10^{-4} M; caffeine, 2.31×10^{-4} M; DPC, 1.54×10^{-4} M and CH$_3$COOH-NaOAc, 2 M (pH = 5.0). Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s

these analytes. We investigated the synchronous signals for caffeine using various wavelength intervals such as $\Delta\lambda = 52, 55, 60, 65, 75, 85, 95, 105, 45, 40, 35, 30, 25, 20$ nm. But the width of synchronous spectrum of caffeine was directly compressed or expanded just by decreasing or increasing the experimental parameter $\Delta\lambda$. Intensity was also decreased by increasing $\Delta\lambda$ value. We selected the optimum $\Delta\lambda = 30$ considering the decrease of the band width and a considerable reduction of spectral overlap. A $\Delta\lambda$ of 106 nm seems the optimum to pass around the maximum of acetylsalicylic acid without considerable loss of sensitivity and selectivity. Fig. 3(B) shows synchronous spectra of (1) caffeine, and (2) mixture of both compounds, maintaining a constant interval between the emission and excitation wavelengths $\Delta\lambda = \lambda_{em} - \lambda_{ex} = 30$ nm. It is evident that the maximum signal of caffeine in the presence of acetylsalicylic acid is obtained at 283 nm. Similarly, at optimum $\Delta\lambda = \lambda_{em} - \lambda_{ex} = 106$ nm the mixture of caffeine and acetylsalicylic acid yields the maximum signal at 305 nm corresponding to the individual peak of acetylsalicylic acid as shown in the Fig. 3(B).

First derivative spectral characteristics

For simultaneous determination of caffeine and acetylsalicylic acid in a binary mixture, the mutual independence of the analytical signals for caffeine and acetylsalicylic acid, i.e., the synchronous signal produced by one is independent of the concentration of the other is important. From an examination of Fig. 3, the assay of caffeine and acetylsalicylic acid

by synchronous fluorescence spectroscopy is still not feasible. Because of the closeness of the two partially overlapping spectra of both compounds, they are not sufficiently well-resolved to generate two distinct peaks in the synchronous fluorescence spectra of the mixture of both complexes. The combination of synchronous scanning and derivatives is an excellent improvement in terms of sensitivity and selectivity comparing to conventional fluorimetry, especially suitable for overlapping spectral bands [21], because the amplitude of the derivative signal is inversely proportional to the band width of the original spectrum [22, 23]. Fig. 4 shows the first-derivative synchronous fluorescence spectra of caffeine, acetylsalicylic acid and their mixture at the values of $\Delta\lambda = 30$ nm and $\Delta\lambda = 106$ nm. By applying the peak to zero techniques to the first derivative synchronous spectra of the mixture, both analytes can be determined by measuring the vertical distance to the zero line at 288 ($\Delta\lambda = 30$ nm) and 316 nm ($\Delta\lambda = 106$ nm), which are proportional to the concentration of caffeine and acetylsalicylic acid, respectively.
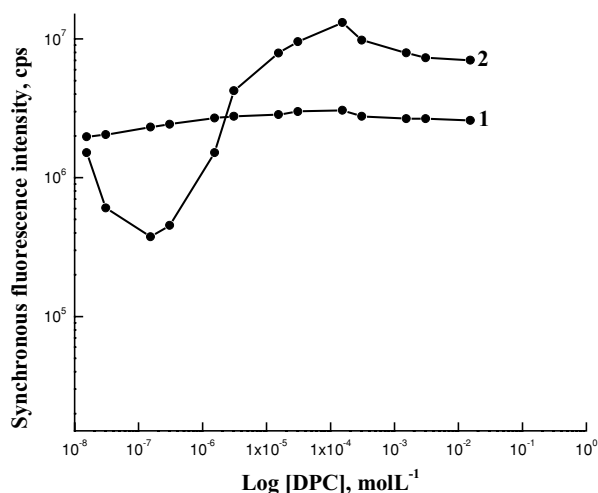
Effect of surfactant on the synchronous fluorescence intensity

The effects of different surfactants, for example Triton X-100 (non-ionic), sodium dodecyl sulfate (SDS, anionic), and N- dodecyl pridinium chloride (DPC, cationic) were investigated under the optimized conditions but significant effect was observed when DPC was used. Non-ionic, cationic, and anionic surfactants affect luminescence, depending on the nature of the surfactant and the species being analyzed
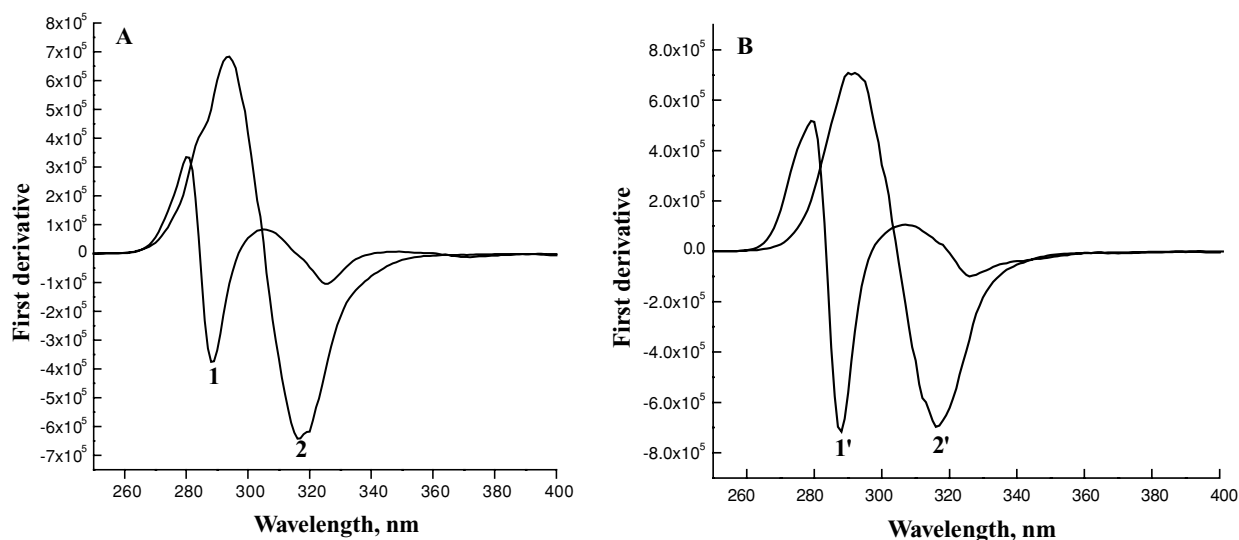
Fig. 4 (A) First derivative synchronous excitation spectra of (1) caffeine ($\Delta\lambda = 30$ nm) and (2) acetylsalicylic acid ($\Delta\lambda = 106$ nm). (B) First derivative synchronous excitation spectra of (1′) mixture at $\Delta\lambda = 30$ nm, and (2′) mixture at $\Delta\lambda = 106$ nm. By applying the peak to zero technique to the first derivative synchronous spectra of the mixture, caffeine and acetylsalicylic acid can be determined by measuring the vertical distances to the zero at 288 nm and 316 nm which correspond to the individual peaks of caffeine and acetylsalicylic acid, respectively. Conditions: acetylsalicylic acid, 2.31×10^{-4} M; caffeine, 2.31×10^{-4} M; DPC, 1.54×10^{-4} M and CH$_3$COOH-NaOAc, 2 M (pH = 5.0). Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s

Fig. 5 Effect of DPC on the synchronous fluorescence intensity of (1) caffeine, and (2) acetylsalicylic acid. Conditions: acetylsalicylic acid, 2.31×10^{-4} M; caffeine, 2.31×10^{-4} M and CH_3COOH-NaOAc, 2 M (pH = 5.0). Each point is the average of five measurements

[24]. The effect of N- dodecyl pridinium chloride (DPC) concentration on the synchronous fluorescence intensity of caffeine and acetylsalicylic acid is shown in Fig. 5. In the presence of DPC, the synchronous fluorescence intensity of caffeine remained almost constant over the entire concentration range 1.54×10^{-8} M to 0.0154 M DPC. But it was observed that DPC has complicated effect on the synchronous fluorescent intensity of acetylsalicylic acid. The intensity of acetylsalicylic acid experienced two stages of decreasing and increasing as the DPC was added to the system. Repetition of the experiment gains the identical results. The intensity was first decreased when the concentration of DPC was increased up to 1.54×10^{-7} M.

Acetylsalicylic acid can exist in ionic form. When the concentration of DPC was lower than its critical micelle concentration, DPC can exist as mono-cation of DPC, which has a strong electrostatic interaction with anionic form of acetylsalicylic acid. The interaction between cation and anion facilitates the non-radiative deactivation which results in decreasing the synchronous fluorescence intensity of acetylsalicylic acid. When the concentration was increased, the intensity began to increase and maximum intensity found at 1.54×10^{-4} M DPC. Because when the concentration was close to critical micelle concentration, the anionic analyte may prefer to be incorporated into the micelles due to the relatively high concentration of surface charge. Therefore, non-radiative deactivation process was weakened and intensity was increased in turn. At concentration higher than 1.54×10^{-4} M DPC, decrease of the acetylsalicylic fluorescence response was observed and the intensity was nearly independent of concentration up to 0.0154 M. Higher concentration was unfavorable due to bubble formation. There-

fore, the co-concentration of 1.54×10^{-4} M DPC for caffeine and acetylsalicylic acid was selected to run the assay.

Effect of pH on the synchronous fluorescence intensity

The influence of pH on the synchronous fluorescence intensity for each drug was studied over the range 1–12. The synchronous spectrum for caffeine exhibited no significant changes at any pH value. But the spectrum of acetylsalicylic acid was markedly affected by the pH of the medium. When pH of solution was increased the synchronous fluorescence intensity increased and reached a maximum value at pH 5.0. Spectrum shape was not affected up to pH 5.0. Gradual decrease in acidity (pH > 5.0) results in an appearance of two bands at 275 nm and 321 nm in the synchronous spectrum. The synchronous response of acetylsalicylic acid in alkaline media is independent of pH. The phenomenon seems that in alkaline medium acetylsalicylic acid tends to hydrolyze to salicylic acid. We experimentally examined the possibility that the synchronous spectrum did not originate from the acetylsalicylic acid but from the hydrolysis product of acetylsalicylic acid. First we obtained the fluorescence excitation, emission and synchronous excitation spectra (optimized $\Delta\lambda = 106$ nm) of salicylic acid (Fig. 6) in the same solvent used for the acetylsalicylic acid and spectra were compared with those obtained from acetylsalicylic acid. Such a comparison reveals distinct differences. The maximum excitation band for SA is at 321 nm not at 305 nm, maximum emission at 408 nm not at 411 nm and synchronous excitation at 321 nm not at 283 nm. Therefore, in order to exclude the possibility that the fluorescence was in fact originated from the hydrolysis product of acetylsalicylic acid, pH 5.0 was adopted as optimal for subsequent experiments. In order to determine the optimal buffer concentration, a series of experiments was conducted in the range of 0.05-5 M CH_3COOH-NaOAc. Such concentrations were found to have no effect on the spectra for caffeine so a buffer concentration, 2 M was selected.

Analytical features

The determination of caffeine and acetylsalicylic acid in binary mixture is carried out by first derivative synchronous fluorescence spectroscopy using two scans. The method involves the construction of independent calibration graphs (Fig. 7) for each component at $\Delta\lambda$ values corresponding to the difference between main excitation and emission maxima of both compounds. When both monochromators are scanned together with a 30 nm constant difference between them, the concentration of caffeine and the vertical distance to the zero line at 288 nm obtained from the first derivative synchronous spectrum was linearly related over a sample concentration range of
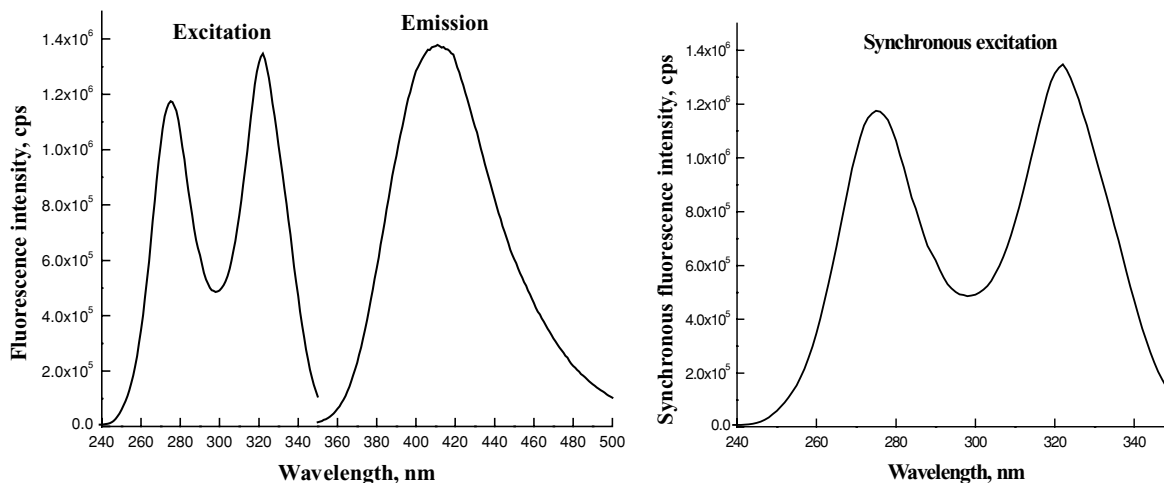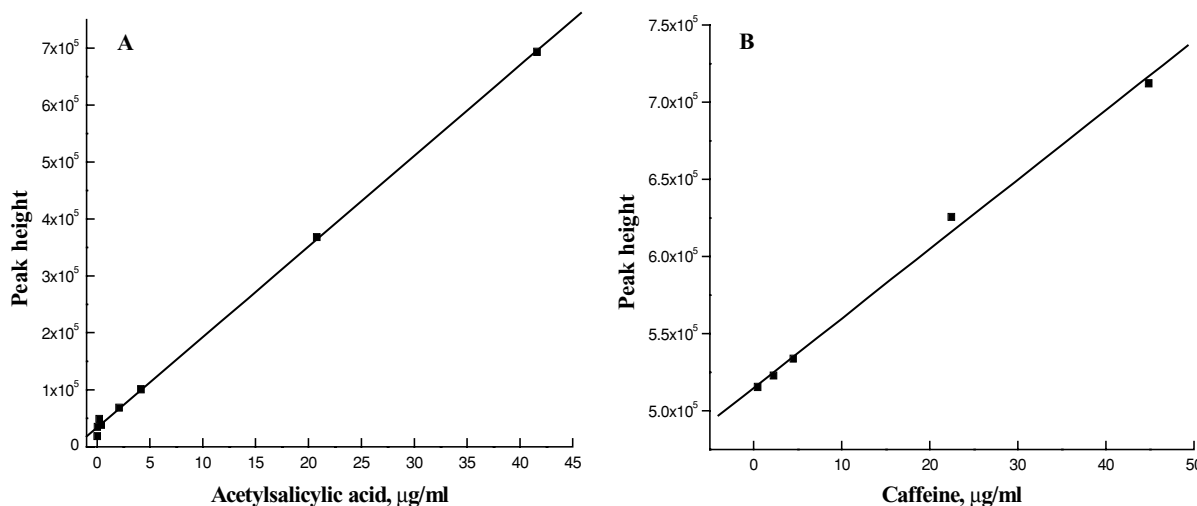
Fig. 6 Excitation, emission and synchronous excitation spectra of 2.31×10^{-4} M salicylic acid. Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s

0.4486–44.86 μg ml^{-1} ($R = 0.99786$). The variation of the signal at 316 nm ($\Delta\lambda = 106$ nm) with acetylsalicylic acid concentration is linear in the range 0.021–41.62 μg ml^{-1} ($R = 0.9995$). The regression equation for caffeine is $Y = 514857.29764 + 4502.13258X$ and for acetylsalicylic acid is $Y = 33318.03681 + 15914.35835X$. The detection limits are 0.0013 μg ml^{-1} and 0.0306 μg ml^{-1} for acetylsalicylic acid and caffeine, respectively. The relative standard deviation ($n = 10$) for 20 μg ml^{-1} of acetylsalicylic acid is 2.75% and for 2.2 μg ml^{-1} of caffeine is 1.7%.

Interferences

In a real sample, the analyte under investigation will be in the presence of interferents. They may suppress or enhance the synchronous signal, although they have no significant effect on the intensity. A systematic study of the effect of foreign species on the determination of 20 μg ml^{-1} acetylsalicylic acid and 2.2 μg ml^{-1} caffeine was made. If interference occurred, the concentration was progressively reduced until interference disappeared. The tolerance level was defined

Fig. 7 Calibration curves for (A) acetylsalicylic acid and (B) caffeine obtained independently by peak height of the first-order derivative curve as function of concentrations of acetylsalicylic acid and caffeine. The signal for the first derivative synchronous spectrum at 316 nm ($\Delta\lambda = 106$ nm) is linear with acetylsalicylic acid concentration and variation of the signal at 288 nm ($\Delta\lambda = 30$ nm) is linear with the caffeine concentration. Conditions: DPC, 1.54×10^{-4} M and CH$_3$COOH-NaOAc, 2 M (pH = 5.0). Excitation and emission monochromator slit, 1 mm; Increment, 1 nm and Integration time, 1 s

Table 1 Effect of foreign species on the simultaneous determination of ASA and CAF

Foreign species	(μg ml^{-1} interference/μg ml^{-1} analyte)	
	ASA	CAF
Saccharose, glucose, boric acid, codeine, nicotinamide, Zn(II), Cu(II)	>100	> 909.1
Ephedrine	10	90.9
Paracetamol	5	45.45
Ascorbic acid, saccharin	25	227.27

as the amount of foreign species that produce an error not exceeding ± 5 in the determination of the analytes. Salicylic acid is the major degradation product of acetylsalicylic acid, probably as a result of acetylsalicylic acid hydrolysis into salicylic acid and acetic acid but the effect is observed 15 hours after the acetylsalicylic acid solution was prepared. In order to prevent the hydrolysis of acetylsalicylic acid all the experiments were conducted with freshly prepared solutions. The results are summarized in the Table 1.

Application of the method

In order to test the proposed method, two sets of synthetic mixtures containing the two analytes in variable proportions (as per ASA and CAF ratio in tablets) were prepared and analyzed. Table 2 shows the results using first derivative synchronous fluorescence spectroscopy at the $\Delta\lambda$ values of 30 nm and 106 nm for caffeine and acetylsalicylic acid, respectively. As can be seen, the errors made never exceeded 2% in case of ASA but the largest error resulting from caffeine was 10%, possibly it was due to the low signal of caffeine in the mixture. But this highest error complied with the tolerance level established in the USP Pharmacopoeia [25]. The recovery study showed average values between

98.40% and 102% for acetylsalicylic acid and between 90% and 100.50% for caffeine so indicating the utility of the proposed method for routine analytical control in pharmaceuticals.

The proposed method allows the determination of ASA and CAF in binary mixture in spite of the much lower concentration from CAF in the real samples as compared with the other. Accuracy and utility were checked by analyzing commercial tablet formulations containing two analytes. The results obtained and the percentages of recovery are summarized in the Table 3. Overall recovery of both compounds was about 97–99% for acetylsalicylic acid and 97–98% for caffeine. So it could be concluded that satisfactory results were obtained for each compound and were found to be in agreement with label claims.

Conclusion

The simultaneous determination of acetylsalicylic acid and caffeine in pharmaceutical formulations has been accomplished by first derivative synchronous fluorescence spectroscopy using two scans at $\Delta\lambda = 106$ nm and $\Delta\lambda = 30$ nm for acetylsalicylic acid and caffeine respectively. All

Table 2 Determination of results of recovery of ASA and CAF in synthetic mixture

Mixture Composition (μg ml^{-1})			Found (μg ml^{-1})^c		Recovery (%)		Error (%)	
Mixture No	ASA	CAF	ASA	CAF	ASA	CAF	ASA	CAF
1^a	40	4.5	39.36	4.34	98.40	96.44	−1.6	−3.5
2^a	30	3.3	29.88	3.26	99.60	98.78	−0.4	−1.2
3^a	20	2.2	20.06	2.14	100.30	97.27	+0.3	−2.72
4^a	10	1.1	9.95	1.06	99.50	96.36	−0.5	−3.63
5^a	1	0.1	0.99	0.09	99.00	90.00	−1.0	−10.0
6^b	40	4.0	39.88	3.95	99.70	98.75	−0.3	−1.25
7^b	30	3.0	29.64	2.88	98.80	96.00	−1.2	−4.0
8^b	20	2.0	19.86	2.01	99.30	100.50	−0.7	+0.5
9^b	10	1.0	9.95	0.97	99.50	97.00	−0.5	−3.0
10^b	1	0.1	1.02	0.09	102.00	90.00	+2.0	−10.0

^{a}Mixture composition based on the ratio of ASA to CAF in ACYLCOFFIN.

^{b}Mixture composition based on the ratio of ASA to CAF in CAFIASPIRINA.

^{c}Average of five measurements.

Table 3 Determination of ASA and CAF in commercial tablets

Sample	ASA			CAF		
	Active (mg)	Found^a (mg)	Recovery (%) $\pm \sigma$	Active (mg)	Found^a (mg)	Recovery (%) $\pm \sigma$
Acylcoffin	450	435.40	97 ± 2	50	48.5	97 ± 1
Cafiaspirina	500	495	99 ± 1	50	49.05	98 ± 2

^{a}Average of five measurements.

conditions such as surfactant concentration, pH were optimized. The remarkable advantage of this method is the simplicity of real sample treatment without organic solvent. The range of application is between 0.021 and 41.62 μg ml^{-1} (correlation coefficient, $R = 0.9995$) for acetylsalicylic acid and between 0.4486 and 44.86 μg ml^{-1} (correlation coefficient, $R = 0.99786$) for caffeine. The results obtained by carrying out the experiment, indicate that derivative synchronous fluorescence spectroscopy is applicable wherever simplicity, speed and cost effectiveness are sought.

References

1. Dalibor S, Isabel N, Petr S, Hana S, Conce M, Montenegro BSM, Araujo AN (2004) Sequential injection chromatographic determination of paracetamol, caffeine, and acetylsalicylic acid in pharmaceutical tablets. J Sep Sci 27:529–536
2. Vincenzo P, Roberto M, Maria Augusta R, Salvatore F (2004) Reversed-phase capillary electro chromatography for the simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine in analgesic tablets. Electrophoresis 25:615–621
3. Wei W, Xiaodong Y, Huangxin J (2004) Simultaneous determination of several antalgic drugs based on their interactions with β-cyclodextrin by capillary zone electrophoresis. J Chromatogr Sci 42:155–160
4. Sena MM, Poppi RJ (2004) N-way PLS applied to simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol, and caffeine. J Pharm Biomed Anal 34:27–34
5. Dominguez Vidal A, Garcia Reyes JF, Ortega Barrales P, Molina Diaz A (2002) UV spectrophotometric flow-through multi parameter sensor for the simultaneous determination of acetaminophen, acetylsalicylic acid, and caffeine. Anal Lett 35:2433–2447
6. Franeta JT, Agbaba D, Eric S, Pavkov S, Aleksic M, Vladimirov S (2002) HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. Farmaco 57:709–713
7. Bouhsain Z, Garrigues S, De la Guardia M (1997) PLS-UV spectrophotometric method for the simultaneous determination of paracetamol, acetylsalicylic acid, and caffeine in pharmaceutical formulations. Fresenius J Anal Chem 357:973–976
8. Moreira AB, Dias ILT, Neto GO, Zagatto EAG, Ferreira MMC, Kubota LT (2005) Solid-phase spectrofluorimetric determination of acetylsalicylic acid and caffeine in pharmaceutical preparations using partial least-squares multivariate calibration. Talanta 67:65–69
9. Navalon A, Blanc R, del Olmo M, Vilchez JL (1999) Simultaneous determination of naproxen, salicylic acid and acetylsalicylic acid by spectrofluorimetry using partial least-squares (PLS) multivariate calibration. Talanta 48:469–475
10. Olmo M, Dıez C, Molina A, Orbe I, Vılchez JL (1996) Resolution of phenol, o-cresol, m-cresol and p-cresol mixtures by excitation fluorescence using partial least-squares (PLS) multivariate calibration. Anal Chim Acta 335:23–33
11. Pistonesi M, Centurion ME, Pereyra M, Lista AG, Fernandez BBS (2004) Synchronous fluorescence for simultaneous determination of hydroquinone and resorcinol in air samples. Anal Bioanal Chem 378:1648–1651
12. Chongqiu J, Jixiang H (2002) Simultaneous determination of Aloe-emodin and Rhein by synchronous fluorescence spectroscopy. J Pharm Biomed Anal 29:737–742
13. Perez-Ruiz T, Martinez Lozano C, Tomas V, Carpena J (1998) Sensitive synchronous spectrofluorimetric methods for the determination of naproxen and diflunisal in serum. Fresenius' J Anal Chem 361:492–495
14. Perez-Ruiz T, Martinez-Lozano C, Tomas V, Carpena J (1998) Simultaneous determination of propranolol and pindolol by synchronous spectrofluorimetry. Talanta 45:969–976
15. Shuyan J, Funing Q (1997) Simultaneous determination of ascorbic acid and pyruvic acid by synchronous scanning-dual wavelength fluorimetry. Fenxi Huaxue 25:1064–1067
16. Vilchez JL, Navalon A, Rohand J, Avidad R, Capitan-Vallvey LF (1995) Simultaneous determination of benomyl and morestan residues in waters by synchronous solid-phase spectrofluorometry. J Fluoresc 5:225–229
17. Martin-Lazaro AB, Hernandez Garcia J, Santana Rodriguez JJ (1992) Simultaneous determination of perylene and benzo [g,h,i] perylene by synchronous fluorescence using a micellar system. Fresenius' J Anal Chem 343:509–512
18. Lloyd JBF (1971) Synchronized excitation of fluorescence emission spectra. Nature 231:64–65
19. Talsky G (1994) Derivative spectrophotometry, VCH, Germany
20. Vo-Dinh T (1978) Multi component analysis by synchronous luminescence spectroscopy. Anal Chem 50:396–401
21. Rubio S, Gomez-Hens A, Valcarcel M (1986) Analytical applications of synchronous fluorescence spectroscopy. Talanta 33:633–640
22. Green GL, O'Haver TC (1974) Derivative luminescence spectrometry. Anal Chem 46:2191–2196
23. John P, Soutar L (1976) Identification of crude oils by synchronous excitation spectrofluorimetry. Anal Chem 48:520–524
24. Sanz-Medel A, Alonso JIG, Gonzalez EB (1985) Metal chelate fluorescence enhancement in micellar media and its applications to niobium and tantalum ultratrace determinations. Anal Chem 57:1681–1687
25. United States Pharmacopoeia XXIII revision, USP Convention Inc., Rockville, MD, 1990