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# The Spectrophotometric Multicomponent Analysis of a Ternary Mixture of Ibuprofen, Caffeine and Paracetamol by the Combination of Double Divisor-Ratio Spectra Derivative and H-Point Standard Addition Method

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**Abstract:** A new spectrophotometric method was developed for the simultaneous analysis of a ternary mixture containing paracetamol (PAR), ibuprofen (IBU) and caffeine (CAF) without prior separation. H-point standard addition method (HPSAM) was used for the first time in the analysis of a component (IBU) in a ternary mixture (paracetamol, ibuprofen and caffeine). In contrast, PAR and CAF determined using double divisor ratio spectra derivative method. This method is based on the use of derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the title mixture. The concentrations of PAR and CAF compounds in their mixture are determined by using their respective standard addition graphs which are obtained by measuring the amplitude at either the maximum or minimum wavelengths selected. The mathematical explanation of the procedure is illustrated. It was shown that at wavelengths 226 and 260 nm, the coordinate of H-point is only dependent on the concentration of IBU without any interference by PAR and CAF. This method was successfully applied for the analysis of Novafen capsule, with no interference from excipients as indicated by the recovery study results. The proposed method is simple and rapid and can be easily used in the quality control of drugs as an alternative analysis tools.

**Keywords:** Ibuprofen, Caffeine, Paracetamol, HPSAM, Double divisor-ratio spectra derivative, Novafen.

## Introduction

Paracetamol (Fig. 1A), ibuprofen (Fig. 1B) and caffeine (Fig. 1C) are active principles widely used and frequently combined in pharmaceutical preparations. PAR is a popular

antipyretic and analgesic agent.[1] In several countries, it is one of the most used medicines as an alternative to aspirin (acetylsalicylic acid). IBU is a non-steroidal anti-inflammatory drug with good analgesic, anti-inflammatory and antipyretic effects.[2] CAF, a methylated xanthene and potent stimulant of the central nervous system, has been added to PRT and IB in various combinations. This addition seems to be aimed at improving the analgesic efficacy.[3,4]

Various methods, including official methods,[5-7] spectrophotometry,[8-13] spectrofluorometry[14] and chromatography[15-20] are available for the determination of above compounds, whether alone or in combination with other drugs. The quality control of dosage form preparations of drug requires reliable and quick analytical methods. UV/vis spectrophotometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity and often demanding low cost equipment. Simultaneous quantitative analysis of pharmaceuticals containing multi-active compounds is difficult to perform by classical spectrophotometric method due to overlapping spectra.

Salinas[21] and Berzas Nevado et al.[22] developed two methods for the resolution of two or more compounds in mixtures by ratio spectra derivative spectrophotometry and the derivative ratio spectra-zero crossing method. Salinas' method is based on the use of the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentrations of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths. In Berzas Nevado's method, the simultaneous determinations of three compounds in ternary mixtures are based on the measurements of the amplitude at the zero crossing points in the derivative spectrum of the ratio spectra.

H-point standard addition method (HPSAM), which is a modification of the standard addition method, is a simple two variable chemometric technique. The fundamentals of HPSAM were outlined by P. Campins-Falcó et al. in 1988.[23] This method permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. HPSAM also makes it possible to determine the concentration of analyte in the presence of a direct interferent and even the concentration of interferent can be determined.[23] The basis of this new method was explained for the spectrophotometric determination of two analytes with extensively overlapped spectra.[23] The requirements for the application of HPSAM is that only to work at two wavelengths where the analytical signals due to the interferents are constant and for another one (analyte) to be different as possible as. By plotting the analytical signals versus added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, $S_H$). Where $-C$H is the unknown analyte concentration and $S_H$ is the analytical signal due to the interferent species. This method has been frequently applied to improve results in different analytical techniques, including spectrophotometry,[24-29] spectrofluorometry,[30] kinetic spectrophotometry[31-33] and chromatographic techniques.[34] However, there is no report on the application of HPSAM for analysis one component in ternary mixtures.

In this work, a sensitive, selective, accurate and inexpensive procedure was applied for simultaneous determination of ibuprofen, caffeine and paracetamol by combination of double divisor-ratio spectra derivative and HPSAM with simultaneous addition of three analytes. This method is a novel standard addition method based on the derivative of the ratio spectra concept. In this paper, an attempt was made to calculate derivative of the

double divisor-ratio spectra and attribute them to the analyte concentration using UV-visible spectrophotometry technique.

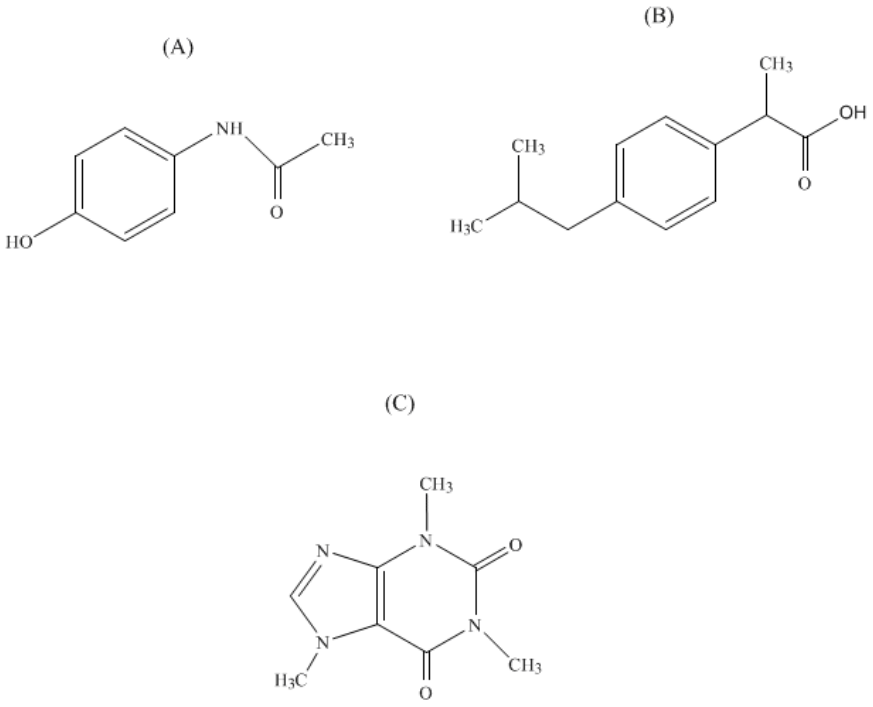


**Figure 1:** Chemical structures of paracetamol (A), ibuprofen (B) and caffeine (C).

*Double divisor ratio spectra derivative method*

If a mixture of three compounds (PAR, IBU and CAF) is considered, if Beer's law is obeyed for all compounds over the whole wavelength range used and if the path length is 1 cm, the absorption spectrum of the ternary mixture can be written in the form of the equation:

$$A_{m,\lambda_i} = \varepsilon_{PAR,\lambda_i} C_{PAR} + \varepsilon_{IBU,\lambda_i} C_{IBU} + \varepsilon_{CAF,\lambda_i} C_{CAF} \qquad (1)$$

where $A_{m,\lambda_i}$ is the absorbance of the mixture at wavelength $\lambda_i$, $\varepsilon_{PAR}$, $\varepsilon_{IBU}$ and $\varepsilon_{CAF}$ are the absorptivities of PAR, IBU and CAF, respectively.

A similar equation for two compounds in the same ternary mixture as in a standard binary mixture can be written as:

$$A_{m,\lambda_i} = \varepsilon_{PAR,\lambda_i} C^0_{PAR} + \varepsilon_{IBU,\lambda_i} C^0_{IBU} \qquad (2)$$

If Eq. (1) is divided by Eq. (2) corresponding to the spectrum of a standard solution of two of the components in the ternary mixture, the ratio spectrum is obtained in the form of Eq. (3):

$$\frac{A_{m,\lambda_i}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}}=\frac{\varepsilon_{PAR,\lambda_i}C_{PAR}+\varepsilon_{IBU,\lambda_i}C_{IBU}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}}+\frac{\varepsilon_{CAF_i}C_{CAF}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}} \quad (3)$$

The ratio of the sum of $\varepsilon_{PAR,\lambda_i}C_{PAR}$ and $\varepsilon_{IBU,\lambda_i}C_{IBU}$ to the sum of $\varepsilon_{PAR,\lambda_i}C^0_{PAR}$ and $\varepsilon_{IBU,\lambda_i}C^0_{IBU}$ is equal to a constant (k) with respect to □, in a certain region or point of wavelength and if the above constant is replaced in Eq. (3), we obtain Eq. (4):

$$\frac{A_{m,\lambda_i}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}}=k+\frac{\varepsilon_{CAF_i}C_{CAF}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}} \quad (4)$$

However, if the standard concentrations of $C^0_{PAR}$ and $C^0_{IBU}$ in Eq. (2) are equal or very close to each other, we could write:

$$\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}=C^0_{PAR}[\varepsilon_{PAR,\lambda_i}+\varepsilon_{IBU,\lambda_i}] \quad (5)$$

When Eq. (5) is substituted into Eq. (4), Eq. (6) is obtained:

$$\frac{A_{m,\lambda_i}}{\varepsilon_{PAR,\lambda_i}C^0_{PAR}+\varepsilon_{IBU,\lambda_i}C^0_{IBU}}=k+\frac{\varepsilon_{CAF_i}C_{CAF}}{C^0_{PAR}[\varepsilon_{PAR,\lambda_i}+\varepsilon_{IBU,\lambda_i}]} \quad (6)$$

If the first derivative of Eq. (6) is taken, since the derivative of a constant is zero, Eq. (7) would be obtained:

$$\frac{d}{d\lambda}\left[\frac{A_{m,\lambda_i}}{C^0_{PAR}[\varepsilon_{PAR,\lambda_i}+\varepsilon_{IBU,\lambda_i}]}\right]=\frac{d}{d\lambda}\left[\frac{\varepsilon_{CAF_i}}{(\varepsilon_{PAR,\lambda_i}+\varepsilon_{IBU,\lambda_i})}\right]\frac{C_{CAF}}{C^0_{PAR}} \quad (7)$$

Eq. (7) is the mathematical foundation of multicomponent analysis which permits the determination of the concentration of each of the active compounds in solution without interference from the other components of the ternary system.

In practice, Eq. (7) corresponding to the first derivative ratio spectrum of CAF is obtained by dividing the absorption spectrum of the ternary mixture of PAR, IBU and CAF by the standard spectrum of two of the compounds in the ternary mixture. Also, in Eq. (7), the derivative signal of the ratio spectrum of the ternary mixture is dependent only on the

concentration values of CAF and $C^0_{PAR}$, but is independent of the concentration values $C_{PAR}$ and $C_{IBU}$ in the ternary mixture. In the developed method, the concentration of $C_{CAF}$ in the ternary mixture is proportional to the first derivative signals corresponding to a maximum or minimum point.

For matrix effect suffering, a standard addition graph is obtained by recording and storing the spectra of solutions of different added concentrations of pure CAF into the ternary mixture, and the spectrum of a solution of a binary mixture of pure PAR and IBU of concentrations $C^0_{PAR}$ and $C^0_{IBU}$. The stored spectra of the ternary mixture after addition of pure CAF are divided by the standard spectrum of the mixture of PAR and IBU by using MATLAB software. As explained here, this technique can be used for determination of other compound (PAR). In this research, simultaneous additions of three compounds of PAR, IBU and CAF have been presented into the ternary mixture and analysis of PAR and CAF is possible by using the doubled-divisor ratio derivative method for the first time. In contrast HPSAM used for determination of IBU contents in the ternary mixture. This technique is very simple and fast in comparison with individual standard addition method.

## Experimental

*Reagents*

Paracetamol, ibuprofen hydrochloride and caffeine were kindly provided by Iranian Pharmaceutical Companies (Tehran, Iran). Analytical grade phosphoric acid, boric acid, acetic acid and sodium hydroxide supplied from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Britton–Robinson (B-R) buffer (0.1mol $L^{-1}$) in the pH range of 2-10 was used throughout.

A $1.0 \times 10^{-3}$ mol $L^{-1}$ paracetamol solution was prepared daily by dissolving 0.0148 g PAR (99.0%) in ethanol (96%) and was diluted in a 100 ml volumetric flask to the mark. A $1.0 \times 10^{-3}$ mol $L^{-1}$ ibuprofen hydrochloride solution was prepared daily by dissolving 0.0206 g of IBU (99.5%) in ethanol (96%) and diluted in a 100 ml volumetric flask. A $1.0 \times 10^{-3}$ mol $L^{-1}$ caffeine solution was prepared daily by dissolving 0.0194 g of CAF (99.5%) in double distilled water and diluted into a 100 ml volumetric flask. These solutions kept in a refrigerator at $4^oC$ in dark. More dilute solutions were prepared by several dilutions with double distilled water.

*Instrumentation and software*

UV-Visible absorption spectra were recorded by a spectrophotometer (PerkinElmer) model Lambda 25, with the use of 1.0 cm quartz cells.

A Pentium IV (2.53 GHz) computer controlled all the setting and data processing. All spectra transformed to Matlab program (R2008a) to calculate double-divisor ratio derivative spectra.

A pH-meter (Metrohm, Model 827) with a double junction glass electrode was used to adjust pH of the solutions.

*Preparation of Real Samples*

To assay Novafen capsule containing paracetamol (325 mg), ibuprofen (200 mg) and caffeine (40 mg) in each capsule, the content of five capsules mixed together. The quantity of 0.0263 g of the powder was accurately weighted and then dissolved in 100 mL of ethanol (96%). After mixing completely, the solution was diluted to the mark with ethanol. This solution was kept in refrigerator at 4 $^{o}$C and finally diluted 10 times in the determination step.

*General Procedure*

The general procedure for analysis of PAR, IBU and CAF in a ternary mixture was as follows. To approximately 2.5 ml of sample solution in a 25.0 ml volumetric flask, 1.0 ml B-R buffer (pH 11.0) added and the final volume diluted to the mark with distilled water after successive standard additions of three components (PAR, IBU and CAF) at the same mole ratio. The spectrum of each solution was recorded in the wavelength range of 200-320 nm and saved as text files. For applying HPSAM, the absorbance of the mixture after each standard addition plotted via standard concentrations of IBU at both wavelengths of 226 and 260 nm. The coordinate of the intersection at x axis is equal to the concentration of IBU in the ternary mixture. In contrast, for determination of PAR and CAF, the spectra of ternary mixture after each standard addition divided to the standard binary samples of CAF+IBU and PAR+IBU in the same concentrations of 10 □mol $L^{-1}$ for each species. Then the ratio spectra transformed to Matlab software and converted to derivative ratio spectra and the values of PAR and CAF determined by standard addition plots at wavelengths 255 and 290 nm respectively.

## Results and Discussions

*Initial investigation*

To demonstrate the analytical applicability of the proposed method for the analysis of ternary mixtures, three pure spectra of PAR, IBU and CAF recorded separately. As it has shown in Fig. 2, the spectra are too overlapped in the range of 210–300 nm. The absorbances at wavelengths 226 and 260 nm are equal for PAR and CAF but differ as much as possible. Fig. 3 shows successive standard addition of three analytes (PAR, IBU and CAF) into an unknown mixture. The H-Point standard addition plot for a typical sample solution has shown in Fig. 4 at the appropriate wavelength pairs. As it has shown, the coordination of the H-point at x-axis is approximately equal to the concentration of IBU in the ternary mixture.

For determination of PAR in the presence of IBU and CAF, the absorption spectra of the ternary mixture after simultaneous standard additions at different concentrations of PAR, IBU and CAF prepared and divided by the spectrum of the standard mixture solution of IBU and CAF (10 μ mol $L^{-1}$ each in B-R buffer) and the ratio spectra were obtained (Fig. 5). First derivatives of the ratio spectra were plotted with $\Delta\lambda$= 5 nm (Fig. 6). The amount of PAR was determined by measuring the amplitude at 255 nm corresponding to a minimum in the first derivative of the ratio spectra in the spectral region selected (200.0-280.0 nm) base on the standard addition method. In a similar way, the concentration of CAF in the ternary mixture determined using the double divisor (PAR and IBU) ratio spectra derivative method at 290 nm corresponding to a minimum in the first derivative of the ratio spectra (Fig. 7).

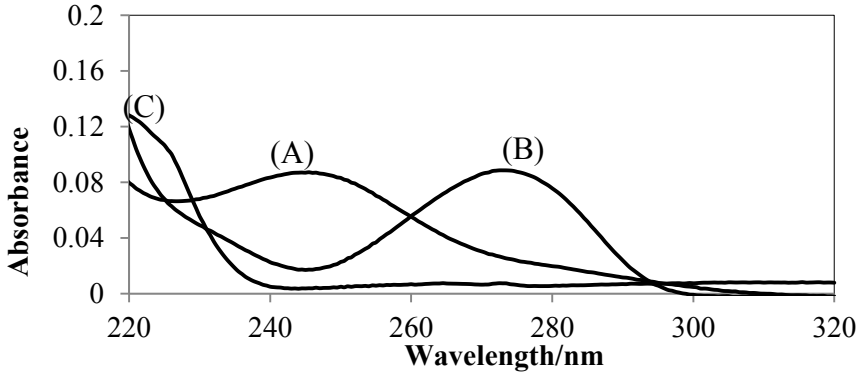


**Figure 2:** Absorption spectra of paracetamol (A), ibuprofen (B) and caffeine (C) at B-R buffer pH 11.0. The concentration of each component is 10 μmol $L^{-1}$.

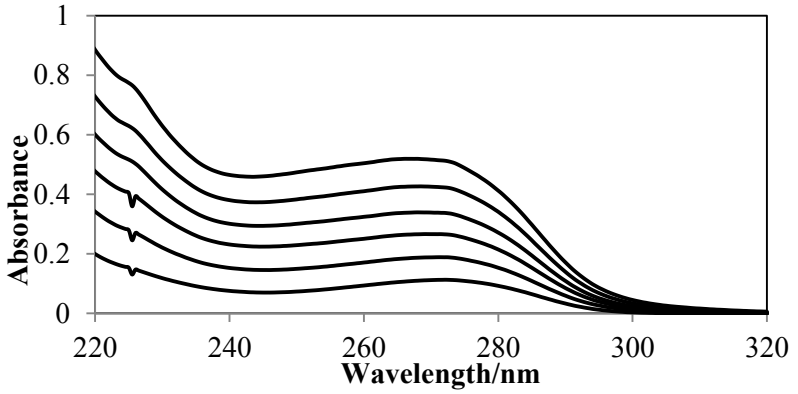


**Figure 3:** The spectra of the ternary mixture after addition of standards PAR, IBU and CAF in the same mole ratios in the concentration range of 10-60 μmol $L^{-1}$ for spectra 1-6. The initial solution contains a ternary mixture of PAR (10 μmol $L^{-1}$), IBU (10 μmol $L^{-1}$) and caffeine (10 μmol $L^{-1}$).

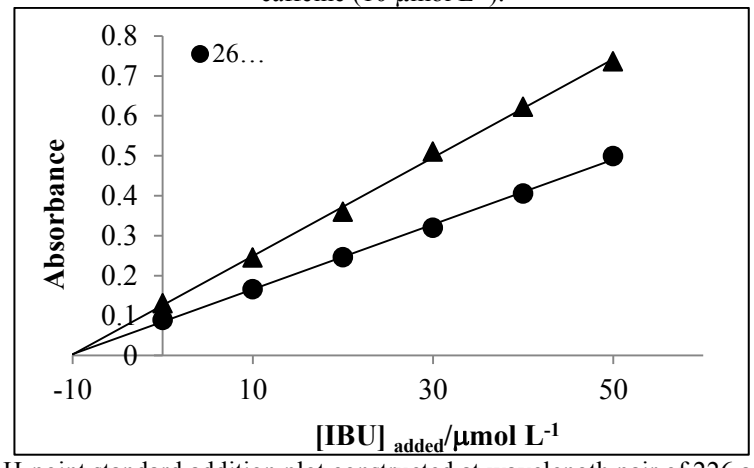


**Figure 4:** H-point standard addition plot constructed at wavelength pair of 226 and 260 nm. Conditions: B-R buffer (pH 11.0), PAR (10 μmol $L^{-1}$), IBU (10 μmol $L^{-1}$) and caffeine (10 μmol $L^{-1}$).

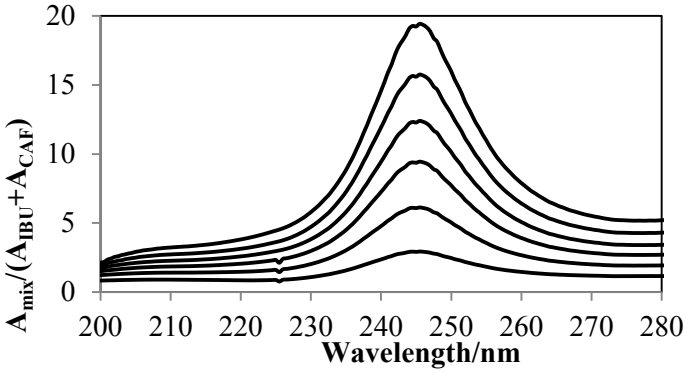


**Figure 5:** Double divisor of the ratio spectra for a ternary mixture of paracetamol, caffeine and ibuprofen in 10 µmol $L^{-1}$ for each component after simultaneous standard addition of components in the concentration range of 10-60 µmol $L^{-1}$. Conditions: double divisors (IBU, 10 µmol $L^{-1}$+CAF, 10 µmol $L^{-1}$), B-R buffer pH 11.0.

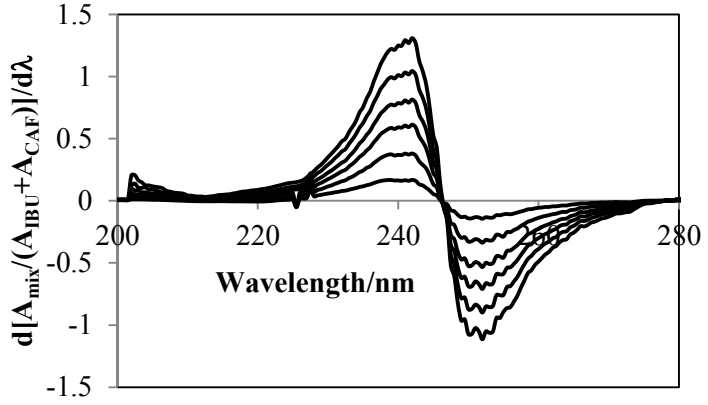


**Figure 6:** First-derivative ratio spectra for a ternary mixture of paracetamol, caffeine and ibuprofen in 10 µmol $L^{-1}$ after simultaneous standard addition of components in the concentration range of 10-60 µmol $L^{-1}$. Conditions: double divisors (IBU, 10 µmol $L^{-1}$+CAF, 10 µmol $L^{-1}$), $\Delta\lambda$= 5 nm, B-R buffer pH 11.0.

*Effect of pH*

It has shown that the maximum absorbances for PAR, IBU and CAF are independence to the pH of the solution in terms of sensitivity and overlapping. Consequently, alkali media (pH 11.0, B-R buffer) was selected as an optimum media to obtain higher selectivity in the presence of cationic ions.

*Reproducibility*

To check the reproducibility of the proposed method, three replicate experiments for the analysis of paracetamol, ibuprofen and caffeine in ternary mixtures have been designed (Table 1). As it has shown the relative standard deviations are in the range of 0.11 to 1.20 %.

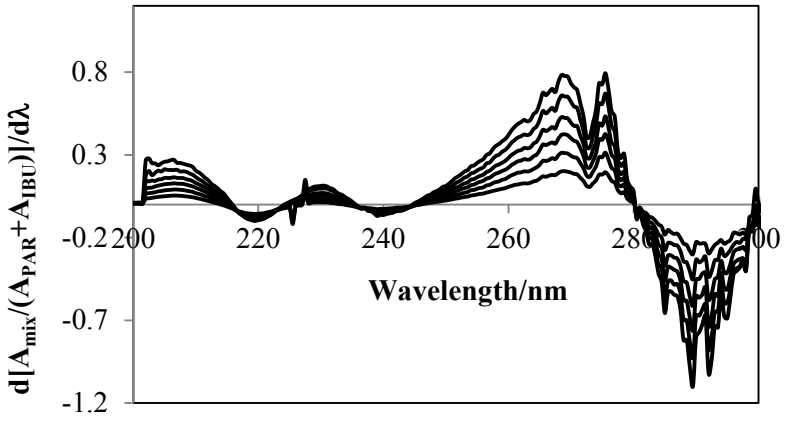


**Figure 7:** First-derivative ratio spectra for a ternary mixture of paracetamol, caffeine and ibuprofen in 10 µmol L-1 after simultaneous standard addition of components in the concentration range of 10-60 µmol L-1. Conditions: double divisors (PAR, 10 µmol L-1+IBU, 10 µmol L-1), □□= 5 nm, B-R buffer pH 11.0.

**Table 1.** Replicative determination of paracetamol, ibuprofen and caffeine in some ternary mixtures by combination of double-divisor ratio spectra derivative method and HPSAM.

| Mixture | Added (µmol $L^{-1}$) | | | Found (µmol $L^{-1}$) | | | RSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | PAR | IBU | CAF | PAR | IBU | CAF | PAR | IBU | CAF |
| 1 | 10 | 10 | 10 | 10.1 | 10.0 | 9.9 | 0.59 | 1.20 | 0.11 |
| | 10 | 10 | 10 | 10.0 | 10.2 | 10.0 | | | |
| | 10 | 10 | 10 | 9.9 | 9.9 | 10.0 | | | |
| 2 | 50 | 50 | 50 | 50.1 | 49.6 | 50.0 | 0.84 | 0.23 | 0.95 |
| | 50 | 50 | 50 | 49.3 | 49.4 | 50.0 | | | |
| | 50 | 50 | 50 | 49.9 | 50.4 | 49.8 | | | |
| 3 | 10 | 50 | 20 | 10.1 | 50.1 | 20.0 | 0.46 | 0.25 | 0.55 |
| | 10 | 50 | 20 | 10.0 | 50.0 | 20.0 | | | |
| | 10 | 50 | 20 | 10.1 | 49.8 | 20.0 | | | |

*Accuracy*

In order to test the quality of the proposed method, the combination of HPSAM and double divisor ratio spectra derivative method were applied to predict the concentrations of paracetamol, ibuprofen and caffeine in ten synthetic mixtures (Table 2) and Novafen

capsules (Table 3). All of the recoveries were satisfactory in the range of 99.7 % to 106.9 %. With the aim of studying, the concentration of the other components has not any influence on the predictions for other compounds at low concentration levels. Therefore the concentrations of PAR, IBU and CAF were predicted satisfactory by combination of HPSAM and double-divisor ratio spectra derivative method.

**Table 2.** Determination of paracetamol, ibuprofen and caffeine in some ternary mixtures.

| Mixture | Added (µmol $L^{-1}$) | | | Found (µmol $L^{-1}$) | | | Recovery (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | PAR | IBU | CAF | PAR | IBU | CAF | PAR | IBU | CAF |
| 1 | 40 | 20 | 30 | 38.11 | 20.00 | 30.10 | 97.6 | 107.5 | 102.3 |
| 2 | 10 | 20 | 10 | 9.76 | 21.50 | 10.23 | 105.0 | 105.0 | 102.3 |
| 3 | 8 | 10 | 20 | 8.40 | 10.50 | 20.18 | 105.0 | 105.0 | 100.9 |
| 4 | 8 | 30 | 10 | 8.00 | 29.60 | 9.49 | 100.0 | 98.7 | 94.9 |
| 5 | 30 | 40 | 20 | 29.46 | 40.0 | 20.18 | 98.2 | 100.0 | 100.9 |
| 6 | 50 | 50 | 50 | 49.50 | 48.93 | 50.00 | 99.0 | 97.9 | 100.0 |
| 7 | 30 | 60 | 30 | 28.17 | 60.0 | 29.91 | 93.9 | 100.0 | 99.7 |
| 8 | 20 | 70 | 30 | 20.80 | 70.10 | 30.00 | 104.0 | 100.1 | 100.0 |
| 9 | 10 | 10 | 10 | 9.90 | 10.00 | 9.61 | 99.0 | 100.0 | 96.1 |
| 10 | 40 | 10 | 40 | 41.20 | 9.42 | 39.28 | 103.0 | 94.2 | 98.2 |

## Conclusions

This work formulates a new approach to the simultaneous analysis of ternary mixtures of paracetamol, ibuprofen and caffeine which have overlapping spectra. In the double-divisor ratio spectra derivative method, for each compound in ternary mixture, without searching for the critical point for the separated peaks, the maximum amplitude of the separated peaks can be measured. This can be considered as an advantage of the new method over alternative methods for the resolution of ternary mixtures. In the case of H-point standard addition method, an optimum wavelength pair is necessary for the determination of analyte in the presence of interference. As an exception case, the absorbances at wavelength pair of 226 and 260 nm were approximately equal for both interferents PAR and CAF and as differ as possible for IBU as analyte. In this case HPSAM could be applied satisfactory for determination of IBU in the presence of PAR and CAF. Our new method has great promise for the routine determination of two or more compounds in mixtures and for the analysis of pharmaceutical preparations containing these mixtures.

**Table 3.** Determination of paracetamol, ibuprofen and caffeine in some ternary pharmaceutical formulations.

| Sample* | Added (mg) | | | Found (mg) | | | Recovery (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | PAR | IBU | CAF | PAR | IBU | CAF | PAR | IBU | CAF |
| 1 | ---- | ---- | ---- | 325.33 | 45.11 | 99.89 | 99.89 | 106.87 | 100.79 |
| | 65.00 | 83.42 | 44.34 | 392.24 | 130.27 | 256.00 | 103.76 | 100.73 | 99.70 |
| 2 | ---- | ---- | ---- | 335.95 | 41.83 | 201.92 | 102.40 | 109.90 | 100.09 |
| | 65.00 | 83.42 | 44.34 | 392.72 | 130.20 | 247.47 | 91.33 | 100.01 | 104.53 |
| 3 | ---- | ---- | ---- | 332.75 | 46.45 | 203.85 | 101.88 | 106.12 | 101.89 |
| | 65.00 | 83.42 | 44.34 | 392.34 | 130.81 | 249.58 | 93.27 | 99.51 | 99.18 |
| 4 | ---- | ---- | ---- | 319.81 | 43.80 | 212.13 | 98.25 | 102.00 | 104.24 |
| | 65.00 | 83.42 | 44.34 | 398.47 | 124.87 | 246.32 | 106.87 | 97.26 | 82.67 |
| **Average** | | | | | | | 99.71 | 102.80 | 99.14 |
| **RSD (%)** | | | | | | | 5.27 | 4.23 | 7.00 |

*Brands 1 to 4 are Novafen capsules containing paracetamol (325 mg), ibuprofen (200 mg) and caffeine (40 mg) in each capsule from some pharmaceutical companies.

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