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ORIGINAL PAPER

Simultaneous Determination of Methocarbamol and Ibuprofen by First Derivative Synchronous Fluorescence Spectroscopic Method in Their Binary Mixture and Spiked Human Plasma

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Abstract Methocarbamol is formulated with Ibuprofen for treatment of alleviated pain associated with muscle spasm. This manuscript describes a sensitive and selective first derivative synchronous spectrofluorimetric method for simultaneous determination of both drugs. Factors affecting method selectivity were studied where best results were obtained upon using $\Delta\lambda$=20 and water as a solvent. Methocartbamol was determined at 283 nm while Ibuprofen at 285.5 nm in the concentration ranges of 0.4–5 and 0.2–4.8 μg/mL, respectively. The applicability of the proposed method was ascertained by application to different laboratory prepared mixtures and marketed formulation. The high sensitivity achieved by the proposed method permitted its application for determination of the drugs in human plasma spiked with pure drugs and their combined tablets. The proposed method showed no significant difference when compared with the reported HPLC method using student's t-test and F-ratio test.

Keywords Synchronous spectrofluorimetry · Methocarbamol · Ibuprofen · Human plasma

Introduction

Methocarbamol (MET) is chemically known as 2-hydroxy-3-(2-methoxyphenoxy) propyl carbamate [1]. It is a centrally acting skeletal muscle relaxant whose action may be due to its general depressant effect on the CNS. Ibuprofen (IBU) is 2-(4-isobutylphenyl) propionic acid [1] which is a non steroidal drug with anti-inflammatory, antipyretic and analgesic properties [2]. They are co-formulated together for treatment of pain associated with muscle spasm.

After exhaustive literature review, There are many reports have been published for determination of MET and IBU mixture either in their binary mixture or in mixtures with other drugs. The drugs have been analyzed by different spectrophotometric [3, 4], HPTLC-densitometric [5] and HPLC methods [6–8].

To the best of our knowledge, no spectrofluorimetric method has been published for simultaneous determination of MET and IBU in their binary mixture. So it was necessary to develop a highly sensitive and selective synchronous spectrofluorimetric method for simultaneous determination of the studied drugs.

The normal fluorescence spectra of both MET and IBU are greatly overlapped, which hindered direct measurement of the studied drugs; this problem is more exaggerated if it is desired to analyze their combined dosage form. This case directs us to utilize derivative synchronous fluorescence spectroscopy (DSFS) to solve such problem of both drugs. Factors affecting method selectivity has been tested and optimized such as, diluting solvent, $\Delta\lambda$ and data points. The method has been validated according to ICH guidelines [9] and has been successfully applied to human plasma.

Experimental

Instrument

1- Jasco FP-6200 Spectrofluorometer equipped with a xenon lamp and 1 cm quartez cuvette (Japan), the following requirements are taken into consideration:
- Measurement mode: synchronous.
- Band width (Ex): 10 nm.

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- Band width (Em): 10 nm.
- Response: fast.
- Data pitch: 1 nm.
- Sensitivity: medium.
- Scanning speed: 500 nm/min.
- Delta wavelength: 20 nm.

2- Ultracentrifuge 80–2, 4000 rpm (China).
3- Sonix Tv ss-series ultrasonicator (USA).

Samples

Pure Samples

Pure MET was kindly supplied by October Pharma S.A.E., 6th of October city, Egypt with certified purity of 99.80 %. Pure IBU was kindly supplied by El Kahira Co. for Pharmaceutical and chemical industries, Cairo, Egypt with certified purity of 100.06 %.

Pharmaceutical Formulation

Ibuflex® tablets batch No (120602) were manufactured by Global Napi Pharmaceuticals, 6th of October city, Egypt. Each tablet is claimed to contain 750 mg of MET and 400 mg of IBU.

Chemical and Reagents

- Methanol (Merck, Sigma-Aldrich, Chromasolv®, Germany)
- Deionized water (SEDICO Pharmaceuticals Co., Cairo, Egypt).
- Sulfuric acid and sodium hydroxide (El- NASR Pharmaceutical Chemicals Co., Abu- Zabaal, Cairo, Egypt).
- Human plasma obtained from VACCERA.

Standard Solutions

- Stock standard solutions of MET and IBU (1 mg/mL)

They were prepared by accurately weighing 100 mg each of MET and IBU separately in two separate 100-mL volumetric flasks and dissolving in methanol.

- First working standard solutions of MET and IBU (0.2 mg/mL)

They were prepared by diluting 10 mL from their respective stock standard solutions (1 mg/mL) into two separate 50-mL volumetric flasks with de-ionized water.

- Second working standard solution of MET and IBU (0.02 mg/mL)

They were prepared by diluting 5 mL from their respective first working standard solutions (0.2 mg/mL) into two separate 50-mL volumetric flasks with de-ionized water.

Procedures

Construction of Calibration Curves

Calibration curves for both MET and IBU have been constructed by accurately transferring different volumes from their respective second working standard solutions (0.02 mg/mL) into two separate sets of 10 mL volumetric flasks to prepare solutions in the range of 0.4–5 μg/mL and 0.2–4.8 μg/mL for MET and IBU, respectively using water as a solvent.

Synchronous fluorescence spectra has been recorded for the prepared solutions using the instrumental parameters mentioned before in the wavelength range of 250–500 nm, then first derivative synchronous fluorescence spectroscopy (FDSFS) has been carried out using Savitzky-Golay algorithm and data points=3. MET was selectively measured at 283 nm (zero crossing for IBU) while IBU could be determined at 285.5 nm (zero crossing for MET) in the obtained FDSFS spectra. Calibration curves were then constructed and regression equations were computed.

Analysis of Laboratory Prepared Mixtures

Different laboratory prepared mixtures containing different concentrations with different ratios of MET and IBU including their ratio in the marketed formulation were prepared. Then the procedure under construction of calibration curves has been followed.

Assay of Pharmaceutical Formulation

Ten Ibuflex® tablets have been weighed, grinded and mixed well. An accurately weighed amount of the powdered tablets equivalent to 150 mg of MET and 80 mg IBU were transferred into 100-mL volumetric flask. 75 mL methanol was added and the solution was ultra-sonicated for 15 min, then filtered and the volume was completed with methanol. Working solutions (equivalent to 0.2 mg/mL and 0.02 mg/mL MET) were than prepared using water as a solvent then the method under construction of calibration curves was then followed to measure concentrations of both MET and IBU. To assess the validity of the method, standard addition technique has been done.

Application to Spiked Human Plasma

Into two sets of 10- mL volumetric flasks, accurately measured aliquots of MET and IBU were separately transferred,

1 mL blank (drug free) plasma was added to each flask, then the volume was completed to the mark using methanol to prepare concentrations in the range of 4–50 μg/mL for both MET and IBU. *Set (1)*.

Each solution of set (1) was shaken for 5 min, centrifuged for 20 min to separate the precipitated plasma protein. Aliquots of 1-mL from the clear supernatant were accurately transferred into another two series of 10-mL volumetric flasks, and then the volume was completed using water to obtain final concentrations from (0.4–5 μg/mL) for both drugs. *Set (2)*.

The synchronous fluorescence spectra of the prepared solutions of set (2) were recorded against a blank prepared by the same manner and the method mentioned under linearity was followed. Calibration curves and regression equations were constructed.

Validation of Spiked Human Plasma Application To check applicability of the method, human plasma was spiked with Ibuflex® tablets and the concentration of the studied drugs in the spiked plasma were then calculated.

Results and Discussion

Spectrofluorimetric technique is less tedious and less cumbersome compared to HPLC and other methods which require long run time and suffer from tedious operation procedures. Moreover the sensitivity offered by this method is far higher than that of HPLC [10].

Synchronous fluorescence spectroscopy (SFS) has several advantages over conventional fluorescence spectroscopy, including high selectivity and low interference [11]. Because of its sharp, narrow spectrum, SFS serves as a very simple, effective method for achieving data for quantitative determination in a single run [12]. It was used for simultaneous determination of

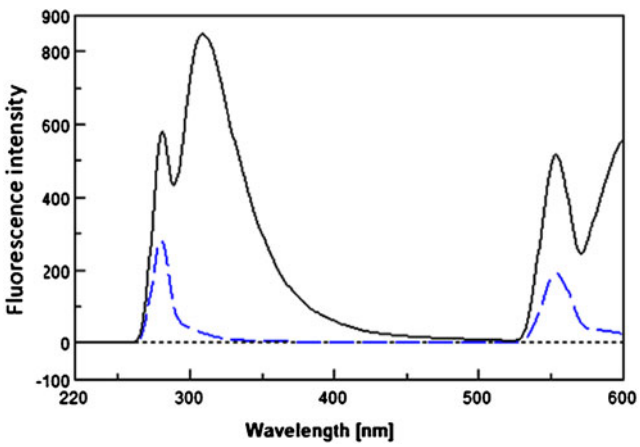

Fig. 1 Emission spectra of 1 μg/mL each of Methocarbamol (____) and Ibuprofen (- - - - -) excited at 277 nm using water as a solvent

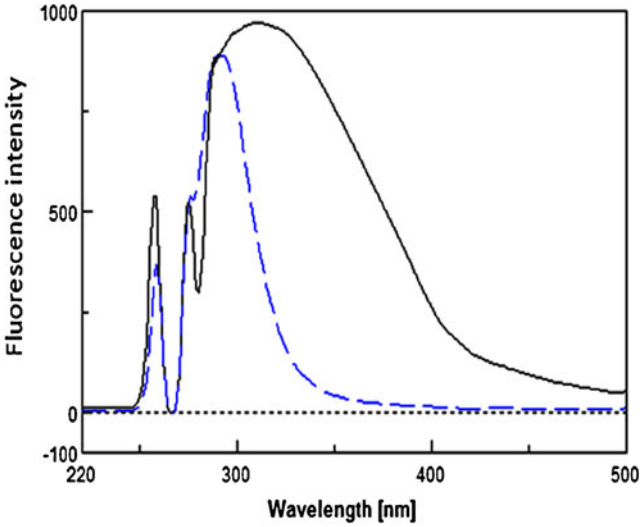

Fig. 2 Emission spectra of 1 μg/mL each of Methocarbamol (_____) and Ibuprofen (- - - - -) excited at 263 nm using water as a solvent

different binary mixtures and was applied to biological fluids [13–16].

Methocarbamol exhibits native fluorescence at λ_{em}=308 and 516 nm after excitation at 277 nm, Fig. 1, while IBU shows native fluorescence at λ_{em}=290 after excitation at 263 nm, Fig. 2. As shown from these figures, spectra of MET and IBU are severely overlapped which inhibited their simultaneous determination. In order to resolve this overlapping, SFS has been used.

As shown in Fig. 3, SFS spectra of both MET and IBU still have great spectral overlap, hence first derivative synchronous fluorescence spectroscopy (FDSFS) has been applied where MET can be determined at 283 nm and IBU at 285.5 nm upon using $\Delta\lambda$=20 nm and data points=3, Fig. 4.

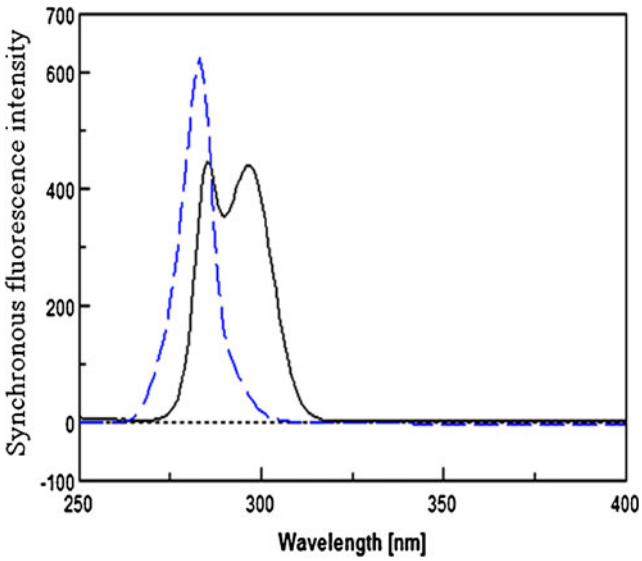

Fig. 3 Synchronous fluorescence spectra of 4 μg/mL each of Methocarbamol (____) and Ibuprofen (- - - - - - -) using water as a solvent

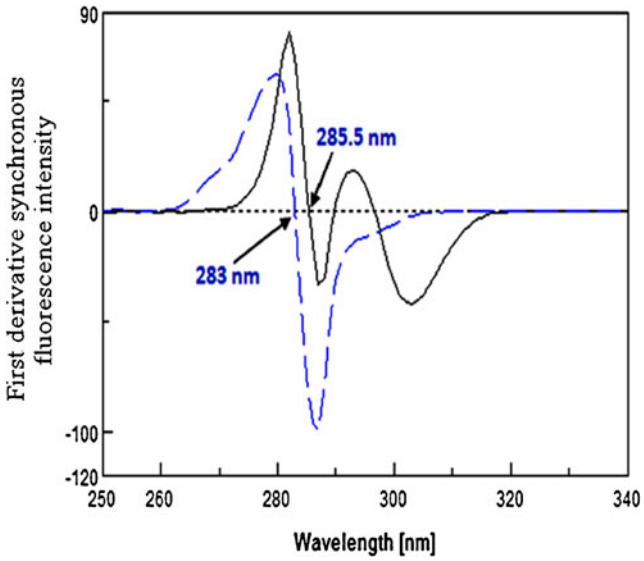

Fig. 4 First derivative synchronous fluorescence spectra of 4 μg/mL each of Methocarbamol (____) and Ibuprofen (- - - - - - -) using water as a solvent

Method optimization

In order to improve the selectivity of the developed method, different experimental parameters were carefully studied and optimized. Univariate method optimization approach has been followed during optimization of the method.

– Effect of the diluting solvent

Different solvents were tried such as methanol, water, 0.05 M sulfuric acid and 0.05 M sodium hydroxide. Upon using water as a solvent the best sensitivity for the studied drugs has been attained, Fig. 5, also it provides the highest selectivity between the studied drugs.

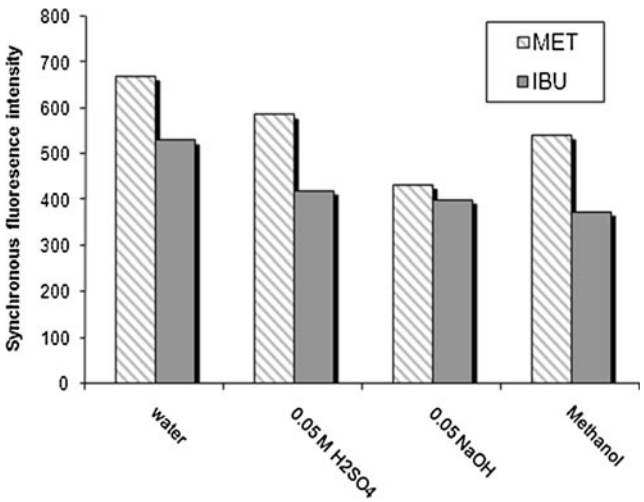

Fig. 5 Effect of diluting solvent on synchronous fluorescence intensity of Methocarbamol and Ibuprofen

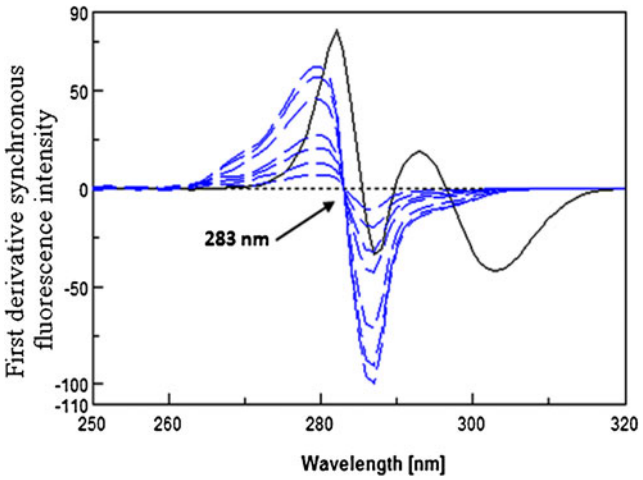

Fig. 6 First derivative synchronous fluorescence spectra of IBU (- - - - -) in the concentration range of (0.2–4.8 μg/mL) overlaid with first derivative synchronous fluorescence spectra of 4 μg/mL of MET (_____)

– Effect of instrumental parameters

Selection of Δλ and band width values significantly affected the spectral shape, signal value and resolution between the studied components. Different Δλ (10.15, 20, 25 and 30 nm) and band width (5, 10 and 20 nm) values were tried. Good resolution was achieved using Δλ=20 nm with band width=10 nm.

– Effect of derivative parameters

To attain the highest selectivity between the derivative spectra of MET and IBU, different data points values (3, 7, 13, 25) were studied. Applying data points=3, MET showed a peak at 283 nm corresponding to zero crossing for IBU while IBU gave a peak at 285.5 nm at which no interference from MET has been observed.

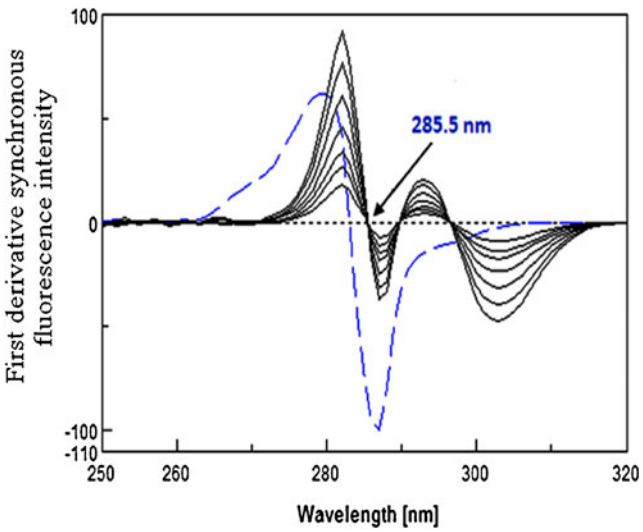

Fig. 7 First derivative synchronous fluorescence spectra of MET (_____) in the concentration range of (0.4–5 μg/mL) overlaid with first derivative synchronous fluorescence spectra of 4 μg/mL of IBU (- - - - -)

Table 1 Regression and analytical parameters of the proposed first derivative synchronous fluorescence spectroscopic method for determination of Methocarbamol (MET) and Ibuprofen (IBU)

Parameters	MET	IBU
Linearity		
Range (μg/mL)	0.4–5	0.2–4.8
Slope	17.6173	21.2360
Intercept	4.2537	0.6856
Correlation coefficient	0.9998	0.9999
Standard error of slope	0.130	0.063
Confidence limit of slope	17.311–17.924	21.060–21.412
Standard error of intercept	0.362	0.188
Confidence limit of intercept	3.397–5.110	0.163–1.208
Accuracy (mean±SD)	99.44±1.419	100.79±1.770
Precision (SD)		
Repeatability[a]	0.804	1.408
Intermediate precision[b]	1.293	1.457

[a] The intraday (n=3), average of three different concentrations of both MET and IBU (1, 2 and 3 μg/mL) repeated three times within day

[b] The interday (n=3), average of three different concentrations of both MET and IBU (1, 2 and 3 μg/mL) repeated three times in three successive days

Application of the method

In order to determine MET and IBU using the developed FDSFS, firstly calibration curves were constructed. Synchronous fluorescence spectra of different concentrations of MET and IBU in the range of 0.4–5 μg/mL and 0.2–4.8 μg/mL, respectively were recorded in the wavelength range of 250–500 nm using water as a solvent, Δλ=20 nm and band width =10 nm. First derivative of the recorded synchronous spectra has been obtained using data points=3 and Savitzky-Golay algorithm. Calibration curves were constructed by plotting the intensity at 283 and 285.5 nm for MET and IBU versus their corresponding concentrations, Figs. 6 and 7, and regression equations have been computed and found to be:

$$P_{MET} = 17.6173C_{MET} + 4.2537 \quad r = 0.9998$$

$$P_{IBU} = 21.2360C_{IBU} + 0.6856 \quad r = 0.9999$$

Where P is the first derivative synchronous fluorescence intensity at the selected wavelengths, C is the concentration in μg/mL and r is the correlation coefficient. Other regression equations parameters are shown in Table 1.

In order to test the suitability of the developed FDSFS method, it was used to determine the concentrations of MET and IBU in Ibuflex® tablets. Acceptable results were obtained which agreed with the labeled amounts, Table 2. As well, standard addition technique has been carried out in order to confirm the validity and accuracy of the method where good percentage recoveries were obtained and given in Table 2.

Statistical analysis of the results obtained by applying the developed FDSFS method and the reported RP-HPLC one [6] using student's-t and F-ratio tests, showed no significant difference, within probability of 95 % regarding both accuracy and precision, results have been displayed in Table 3.

Furthermore, the high sensitivity of the recommended FDSFS method permitted its application for determination of MET and IBU in spiked human plasma. The two drugs have been measured in the concentration range of 0.4–5 μg/mL and the concentrations of MET and IBU, Table 4, were calculated from the following regression equations:

$$P_{MET} = 16.9755C_{MET} + 3.9348 \quad r = 0.9998$$

$$P_{IBU} = 21.2180C_{IBU} + 6.521623 \quad r = 0.9999$$

Table 2 Determination of Methocarbamol and Ibuprofen in Ibuflex® tablets by the proposed first derivative synchronous fluorescence spectroscopic method and results of standard addition technique

Pharmaceutical formulation	Component	Taken	Found	% Found[a] ± SD	Standard addition technique	
					Pure added (μg/mL)	% Found[b]
IIbuflex® tablets (B. N. 0611108) claimed to contain 750 mg MET and 400 mg IBU/tablet	MET	3.00	2.98	99.33±0.849	0.80	101.21
					1.00	99.87
					1.20	102.05
		Mean ± SD				101.04±1.010
	IBU	1.60	1.66	103.75±1.874	1.00	98.55
					1.50	101.45
					2.00	100.88
		Mean ± SD				100.30±1.536

[a] Average of 6 determinations

[b] Average of 3 determinations

Table 3 Statistical comparison of the results obtained by applying the proposed first derivative synchronous fluorescence spectroscopic method and the reported RP-HPLC for determination of Methocarbamol and Ibuprofen in pure forms

Items	FDSFS method		Reported method[b] [6]	
	MET at 283 nm	IBU at 285.5 nm	MET	IBU
Mean	99.44	100.79	100.00	99.90
SD	1.419	1.770	1.000	0.900
Variance	2.014	3.133	1.000	0.810
n	7	7	6	6
Student's t- test	0.647 (2.101)[a]	0.770 (2.101)[a]		
F- value	2.014 (4.387)[a]	3.868 (4.387)[a]		

[a] Figures between parenthesis represent the corresponding tabulated values of t and F at P=0.05.

[b] HPLC: using Bondapak C18 column and 0.2 % orthophosphoric acid: methanol (45:55, v/v) as a mobile phase and UV detection at 215 nm

Where P is the first derivative synchronous fluorescence intensity at the selected wavelengths, C is the concentration in μg/mL and r is the correlation coefficient.

To prove the applicability of the proposed method for determination of MET and IBU in human plasma after oral administration of their pharmaceutical formulation, human plasma were spiked with Ibufex® tablets and concentration of both drugs were measured, reliable results were obtained as given in Table 4.

Table 4 Determination of Methocarbamol and Ibuprofen in spiked human plasma by proposed first derivative synchronous fluorescence spectroscopic method with application to pharmaceutical formulation

MET			IBU		
Added (μg/mL)	Found[a]	% Found[c]	Added (μg/mL)	Found[b]	% Found[c]
0.40	0.40	100.00	0.40	0.40	100.00
1.2	1.17	97.50	1.0	1.01	101.00
2.0	2.01	100.50	2.0	2.02	101.00
3.6	3.64	101.11	2.8	2.79	99.64
4.0	4.02	100.50	3.6	3.57	99.17
4.8	4.83	100.63	4.8	4.78	99.85
5.0	4.94	98.80	5.0	5.04	100.80
Mean ± SD		99.86±1.271	Mean ± SD		100.21±0.728
Pharmaceutical formulation (Ibuflex® tablets)[d]					
3.00	3.02	100.67±1.763	1.60	1.57	98.13 ± 1.976

[a] Using the equations: P_{MET}=16.9755 C_{MET}+3.9348

[b] Using the equations: P_{IBU}=21.2180 C_{IBU}+6.521623

[c] Average of 6 determinations

[d] Average of 6 determinations

Methods Validation

Method validation was performed in accordance with the international conference on harmonization (ICH) guidelines [9].

Linearity of the recommended method was evaluated and it was evident in the range of 0.4–5 and 0.2–4.8 μg/m for ME and IBU, respectively. Statistical evaluation of the calibration curves, regarding standard errors of the slope and intercept and their confidence limits are given in Table 1. Good linearity was revealed from the high values of the correlation coefficients, low values of standard errors of the slope and intercept and high values of their confidence limits.

Precision of the method was assessed as SD at different levels, repeatability and intermediate precision. The obtained values of the calculated SD, Table 1 proved the high precision of the developed method.

Accuracy of the suggested FDSFS was calculated as % recovery and it was estimated by its application for determination of pure samples of MET and IBU. Accuracy of the method was found to be 99.44 % and 100.79 % for MET and IBU, respectively, Table 1. Additionally, results of standard additions technique verified accuracy of the method and revealed that there was no interference from tablets excepients, Table 2.

Specificity of the method was checked by its application for analysis of laboratory prepared mixtures containing different ratios of intact MET and IBU. Satisfactory results were found,

Table 5 Determination of Methocarbamol and Ibuprofen in laboratory prepared mixtures by the proposed first derivative synchronous fluorescence spectroscopic method

Ratio MET: IBU	MET at 285.5 nm			IBU at 283 nm		
	Taken (μg/mL)	Found	% Found[a]	Taken (μg/mL)	Found	% Found[a]
3 : 1.-6[b]	3.00	2.98	99.33	1.60	1.64	102.50
1 : 1	1.00	0.97	97.00	1.00	1.03	103.00
2 : 3	2.00	2.02	101.00	3.00	3.03	101.00
3 : 2	3.00	3.01	100.33	2.00	1.94	98.50
3 : 1	2.40	2.34	97.50	0.80	0.97	98.75
1 : 3	0.80	0.77	96.25	2.40	2.47	102.92
Mean			±SD			98.57 ± 0.927
		10-1.-1-1±-2.-058				

[a] Average of 3 determinations

[b] The ratio of the two drugs in the marketed formulation

98.57 % and 101.11 % for MET and IBU, respectively. Results in Table 5 proved that each of the cited drugs could be successfully determined without interference from the other.

Conclusion

This manuscript demonstrated the development and validation of sensitive, specific, accurate and precise first derivative synchronous fluorescence spectroscopy (FDSFS) method for simultaneous determination of MET and IBU in their binary mixture and combined dosage form. The recommended method is time and money saving, sample and data treatments do not require complicated steps. The high sensitivity of the method encourages its application to human plasma spiked with MET, IBU and Ibuflex® tablets.

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