# Quantification of tamsulosin hydrochloride and solifenacin succinate by discriminative derivative synchronous emission spectroscopy

# Tamsulosinhidroklorürve solifenasinsüksinat'ın Senkronize türev emisyonspektrometrisi yöntemiyle eşzamanlı miktar tayinleri

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**Short title:** Synchronous quantification of tamsulosin hydrochloride/solifenacin succinate (English)

Tamsulosinhidroklorürve solifenasinsüksinat'ın Senkronize türev emisyonspektrometrisi yöntemiyle eşzamanlı miktar tayinleri (Turkish)

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**ABSTRACT**

The present study describes a simple, reliable and reproducible first derivative synchronous spectrofluorimetric method for the simultaneous quantification of tamsulosin hydrochloride and solifenacin succinate. Tamsulosin hydrochloride was quantified at a wavelength of 322 nm (zero-crossing wavelength point of solifenacin succinate) and solifenacin succinate was measured at 570 nm (zero-crossing wavelength point of tamsulosin hydrochloride). Calibration plots were constructed over the concentration range of 2-10 µg/mL for tamsulosin hydrochloride and 30-150 µg/mL for solifenacin succinate. The method gave satisfactory results when it is validated for linearity, specificity, accuracy, precision, LOD and LOQ as per International Conference on Harmonization (ICH) guidelines. The assay values in commercial formulation were found to be in the percentage range of 95.0 for tamsulosin hydrochloride and 103.5 for solifenacin succinate by the proposed method. These results were well in agreement with their label claimed. The proposed synchronous analytical method can be employed for routine quality control analysis of tamsulosin hydrochloride/ solifenacin succinate in tablet dosage forms.

**Key words:** Tamsulosin hydrochloride, Solifenacin succinate, Synchronous spectrofluorimetry, Method validation.

Bu çalışmada, tamsulosinhidroklorürve solifenasinsüksinat'ın eşzamanlı miktar tayini için basit, güvenilir ve tekrarlanabilir birbirinci türev senkronize spektroflorimetrik yöntem önerilmiştir.Tamsulosinhidroklorürtayini, 322 nmdalga boyunda (solifenasinsüksinatiçin sıfır noktası) ve solifenasinsüksinattayini, 570 nmdalga boyunda (tamsulosinhidroklorüriçin sıfır noktası) gerçekleştirilmiştir. Kalibrasyon doğruları, tamsulosinhidroklorüriçin 2-10 µg/mL ve solifenasinsüksinatiçin 30-150 µg/mLkonsantrasyonaralığında oluşturuldu.Yöntem valide edildiğinde doğrusallık, özgünlük, doğruluk, kesinlik, LOD ve LOQ açısından ICH kurallarına göre tatmin edici sonuçlar verdi. Ticari formülasyonlarda önerilen yöntemle elde edilen deney sonuçları, tamsulosinhidroklorüriçin %95.0 ve solifenasinsüksinatiçin %103.5 aralığında bulundu.

Bu sonuçlar, belirtilen etiket miktarlarıyla uyumludur. Önerilen senkronize analitik yöntem, tablet dozaj formlarında tamsulosinhidroklorürve solifenasinsüksinat'ınrutin kalite kontrol analizi için kullanılabilir.

**Anahtar kelimeler:** Tamsulosinhidroklorür, Solifenasinsüksinat, senkronize spektroflorimetri, yöntemvalidasyonu.

## Introduction

Tamsulosin hydrochloride (TMH) is chemically 5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl] amino}propyl]-2-methoxybenzene-1-sulfonamide and used as selective antagonist of α-1A-adrenergic receptors (1). Solifenacin succinate (SFS) is chemically butanedioic acid (3R)-1-azabicyclo[2.2.2]octan-3-yl (1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate and used as competitive muscarinic acetylcholine receptor (M3) antagonist (2). Vesomni, a marketed combined tablet dosage form of these drugs was used in the treatment of lower urinary tract symptoms associated with BPH prostate (benign prostatic hyperplasia) (3). The structures of both TMH and SFS were depicted in Figure 1.

A survey of literature on TMH revealed several methods, such as visible spectrophotometric methods using Folin reagent, sodium nitroprusside-acetaldehyde (4) and bromophenol blue (5), UV spectrophotometric method (6), fluorimetry utilizing sodium dodecyl sulphate micellar system (7) and methanol (8). The methods reported for SFS were mainly based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and UV spectrophotometry (9, 10). Some spectrophotometric methods using alkaline potassium permanganate, formation of ternary complex with copper (II)/eosin, ammonium molybdate in ammonium thiocyanate and ion-pair complex formation with bromocresol green (11) also described for the analysis of SFS. Spectrophotometric methods are considered to be inappropriate for simultaneous analysis of drugs in multi-component dosage form due to lack of specificity.

To the best of our knowledge, no method has been reported yet for simultaneous quantification of TMH and SFS except RP-HPLC method (12). However chromatographic methods are complex with costly instrument set up, skilled operators and expensive solvents limit the application of simultaneous quantification of above drugs. Spectrofluorimetry has assumed a special status in drug analysis due to its greater sensitivity and specificity than spectrophotometry using at two wave-lengths, excitation and emission. In accustomed fluorescence, an emission spectrum is attained by scanning the monochromator of emission at various wavelengths ($\lambda_{em}$), at an appropriate excitation wavelength ($\lambda_{ex}$) but in synchronous fluorescence scan both the

monochromators varies simultaneously. When accustomed spectra are over-lapped, the synchronous technique is used to reduce the extent of overlapping. The derivative spectrofluorimetry is a powerful approach for resolution of analytes when an analytical peak is overlapped by a large peak of another analyte, particularly in multi-component analysis (13-21).

The development of a suitable method for simultaneous analysis of TMH and SFS is a challenge, because the drugs are present in the ratio of 1:15 in tablet dosage form. If SFS is diluted, the quantification of second drug (TMH) analysis may become difficult. So, simultaneous quantification of TMH and SFS was attempted. A first derivative synchronous spectrofluorimetric method has been developed based on their native fluorescence and validated as per current ICH guidelines (22). The emission spectra of TMH and SFS were overlapped hence it was difficult to analyze and quantify their contents by conventional fluorimetry. This overlap need to be endeavored by copacetic modification, so first derivative synchronous spectrofluorimetric method was contemplated.

## MATERIALS AND METHODS

### *Chemicals and reagents*

All chemicals and reagents used in the present investigation were of analytical grade. TMH and SFS gift samples were provided by Orchid Pharma Ltd, Chennai, India and the marketed tablet dosage form, Vesomni was procured from local pharmacy.

### *Instrumentation*

The fluorescence spectra and measurements were recorded using a Shimadzu (Japan) RF-5301 PC spectrofluorimeter, equipped with 150 watt Xenon arc lamp, quartz cell (1 cm) and connected to RFPC software. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm.

### *Preparation of standard stock solutions*

Stock solution containing 1000 µg/mL of drug was prepared by dissolving 10 mg of TMH/ SFS in 10 mL of distilled water separately. Aliquot of 1 mL solution from stock

was diluted up to 10 mL with distilled water to attain an end concentration 100 µg/mL of each drug.

*Analytical method development*

*Scanning of drugs by conventional-spectrofluorimetry*

TMH or SFS (100 µg/mL) was diluted to 10 mL with distilled water to attain an end concentration of 10 µg/mL. Spectrofluorimetry mode was used for the scanning of the sample against distilled water to get the excitation and emission wavelengths, anon excitation wavelength was fixed and solutions were further scanned to get the emission spectra. TMH exhibited inveterate fluorescence at emission wavelength 328 nm after excitation at 292 nm, similarly SFS exhibited fluorescence at emission wavelength 294 nm after excitation at 256 nm in distilled water. The emission and excitation spectra of TMH and SFS were shown Figure 2 and 3. Though the excitation and emission wavelengths were disparate for both the drugs, whist the fluorescence spectra exhibited protruding of intensity, so that the conventional spectrofluorimetric method does not permit the simultaneous estimation of both drugs.

*Synchronous fluorescence spectra*

An attempt was made to attain synchronous spectra of TMH and SFS by maintaining a constant interval of 50 nm between the emission and excitation wavelengths (Figure 4). There was a large overlap of the spectra of TMH and SFS, hence synchronous spectrofluorimetry was found to be inappropriate. This overlap need to be endeavored by copacetic modification, so first derivative synchronous spectrofluorimetric method was contemplated.

*First derivative synchronous spectrofluorimetry*

The synchronous zero order emission spectra were transformed into consonant first order spectra in the range of 350-700 nm. Zero crossing criterions can be used for estimations that are proportional to the concentrations of TMH and SFS.

*Analytical method validation*

The method was validated for linearity, specificity, accuracy, precision (both intra and inter-day), limit of detection and limit of quantification as per International

Conference on Harmonization (ICH) guidelines to prove that the analytical method can be useful for quality control of both the drugs.

*Linearity*

The standard concentrations of TMH (2-10 µg/mL) and SFS (30-150 µg/mL) were quantified by first derivative synchronous spectrofluorimetric technique, anon fluorescence intensities were recorded and calibration curve was contrived by plotting the analyte intensities paradoxical to the drug concentrations.

*Specificity*

The method specificity was assessed by comparing the spectra obtained from the placebo, commercial formulations and synthetic mixture of standard solutions. Synthetic mixture was prepared by adding 0.6 mL of TMH standard stock solution (100 µg/mL) and 0.9 mL of SFS standard solution (1000 µg/mL) to the 10 mL volumetric flask, volume was made up to the mark with distilled water to obtain final concentration of 6 µg/mL TMH and 90 µg/mL SFS. Then same concentrations of sample solution were prepared using marketed tablets. The method was applied to analyze blank, synthetic mixture and formulation solutions in order to check if any component of the formulation could generate a response or a read with emission band similar to the drugs.

*Accuracy*

Acquisition studies were conducted using standard addition method where the known amount of TMH/SFS was added to the pre-analyzed sample according to 80, 100 and 120% levels of labeled claim further subjected to contemplated analytical method, anon percentage recovery and relative standard deviation (RSD%) were calculated for each concentration.

*Precision*

The intra-day and inter-day precision of the proposed first derivative spectrofluorimetric simultaneous method was ascertained by estimating the corresponding response three times on the same day (intra-day precision) and on 3 different days over a period of 1 week (inter-day precision) for three different

concentrations of TMH (2, 6 and 10 µg/mL) and SFS (30, 90 and 150 µg/mL).The results were reported in terms of relative standard deviation (RSD%).

*Limit of detection (LOD) and limit of quantification (LOQ)*

The limit of detection (LOD) and limit of quantitation (LOQ) for the proposed method were performed on sample containing very low concentrations of analyte (TMH and SFS) as per the ICH guidelines and calculated based on calibration curve.

*Assay of TMH and SFS in their fixed dose formulation*

Twenty tablets of marketed formulation (Vesomni) were accurately weighed and powdered. A quantity of powder equivalent to 0.4 mg of TMH and 6.0 mg of SFS was dissolved in methanol (5 mL) and sonicated for 15 min. The flask was shaken and volume was made up to 10.0 mL with distilled water. The above solution was filtered through Whatmann filter paper (No.41). From the filtrate 1.0 mL was transferred into volumetric flask and the volume was made up to 10.0 mL with distilled water to give a solution containing 4 µg/mL of TMH and 60 µg/mL of SFS. This solution was analyzed using proposed method for the simultaneous quantification of TMH and SFS. The amount of drugs present in the sample solution were determined by substituting derivative responses into the equation of the linear line representing the calibration curves for TMH and SFS, with correction for dilution.

## RESULTS AND DISCUSSION

*Analytical method*

Synchronous scanning spectrofluorimetry alliance with derivative techniques is expedient in locution of sensitivity, spectral discrimination and decisive identification of chemical species in multi-component analysis. TMH molecule contains two aromatic rings, namely ethoxy phenyl and methoxy benzene-1-sulfonamide rings, in which more number of π electrons were available to put on view of fluorescence, likewise SFS also put on view due to the presence of tetrahydroisoquinoline ring. Different solvent systems were tested in furtherance of the best predicaments, like solubility and fluorescence activity of both the drugs. TMH and SFS exhibited the indigenous fluorescence at emission wavelength 328 nm subsequent to excitation at 292 nm and

emission wavelength 294 nm subsequent to excitation at 256 nm, respectively in distilled water as solvent. The accustomed and synchronous fluorescence spectra of these drugs overlapped substantially indicating that these methods do not permit the simultaneous determination of both the drugs followed by this overlap problem was overcome using first derivative spectrum (Figure 5), revealed that TMH gave zero intensity at 570 nm, while SFS gave significant derivative response likewise SFS gave zero intensity at 322 nm, where TMH gives significant derivative response. Therefore, 322 and 570 nm were elected for reckoning of TMH and SFS respectively in synthetic mixture and tablet dosage forms.

*Analytical method validation*

*Linearity*

The calibration curve for TMH and SFS recorded at 322 and 570 nm, respectively. The linearity was evaluated by the least square regression method. The regression analysis of the calibration curves were shown in Figure 6 and 7. The responses for TMH at 322 nm were found to be linear in the concentration range of 2-10 µg/mL with a correlation co-efficient ($r^2$) value of 0.9996. Similarly the responses for SFS at 570 nm were linear in the concentration range of 30-150 µg/mL with a correlation coefficient ($r^2$) value of 0.9992. From the Figure 8, it was observed that with the increase in TMH concentration, the derivative response at 322 nm was increased. Similarly, the derivative response for SFS at 570 nm was proportional to its concentration.

*Specificity*

Derivative synchronous spectrum obtained from the commercial formulation solution was compared with the spectrum of synthetic mixture of standard solutions (TMH and SFS) and blank. The spectra of both commercial formulation and the synthetic mixture were found to be similar. Specificity of the method was shown in Figure 9, which revealed that there was no interference from the excipients in the tablets with derivative response of either of the drugs (TMH and SFS) at their respective analytical wavelengths (322 and 570 nm). Hence, the method was proved to be specific.

*Accuracy*

The accuracy of the analytical method was determined by standard addition method. Three different levels (80, 100 and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and RSD % values were calculated and reported in Table 1. The % recoveries of TMH and SFS were found to be in the range 102.27–113.75 and 97.22–101.0, respectively which are found to be satisfactory.

*Precision*

The repeatability (intra-day precision) and intermediate precision of the method was determined by three concentrations for both TMH (2, 6 and 10 µg/mL) and SFS (30, 90 and 150 µg/mL). The results were summarized in Table 2. The RSD % of repeatability was less than 2.0 for both the drugs, indicating good precision of the developed method.

*Limit of detection (LOD) and limit of quantitation (LOQ)*

From the linearity plot the LOD and LOQ of TMH and SFS were calculated. The LOD and LOQ for TMH was 0.210 and 0.639 µg/mL and SFS was found to be and 2.640 and 8.0 µg/mL. The summary of the system suitability parameters was represented in Table 3. The results obtained from validation evidenced that the proposed method is scientifically sound.

*Assay of drugs from commercial tablets*

The accuracy of proposed method was evaluated by the assay of commercially available tablets (VESOMNI) containing 0.4 mg of TMH and 6 mg of SFS. The results obtained were compared with the corresponding labeled amounts and reported in Table 4. The amount of TMH and SFS in formulation was found to be 0.38±0.006 and 6.21±0.024 mg, respectively. The % assay in commercial formulations was found to be 95.0 for TMH and 103.5 for SFS by the proposed method. The RSD% for formulation was less than 2, which indicates the accuracy of the proposed method.

## CONCLUSION

A simple and rapid first derivative synchronous spectrofluorimetric method for the simultaneous quantification of TMH and SFS was developed and validated as per ICH guidelines. The results of validation studies denoted the immense scope of sensitivity, accuracy, precision, and system suitability of the analytical method. The proposed method is successfully adopted for the assay of TMH and SFS and the results were found to be in good agreement with their respective label claim, which suggested that there is no interference of formulation excipients in the estimation. The contemplated spectrofluorimetric method has been found to be superior, because of its high specificity, spectral discrimination, economical, eco-friendly and readily available solvent and lack of extraction procedure. These advantages endorse the developed method can be habitually employed in quality control for simultaneous analysis of TMH and SFS in tablet dosage forms.

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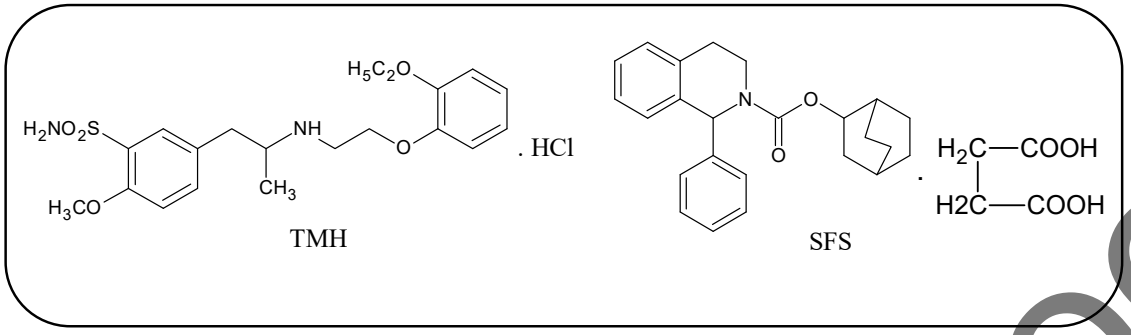


**Figure 1.** Structures of TMH and SFS

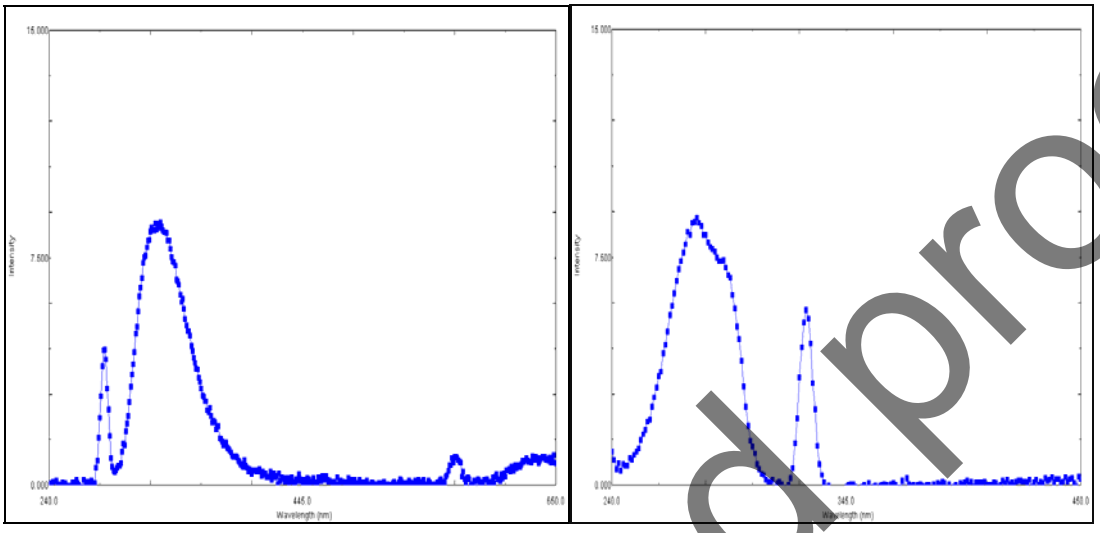

**Figure 2.** Emission (328 nm) and excitation (292 nm) spectra of TMH (10 µg/mL) in distilled water

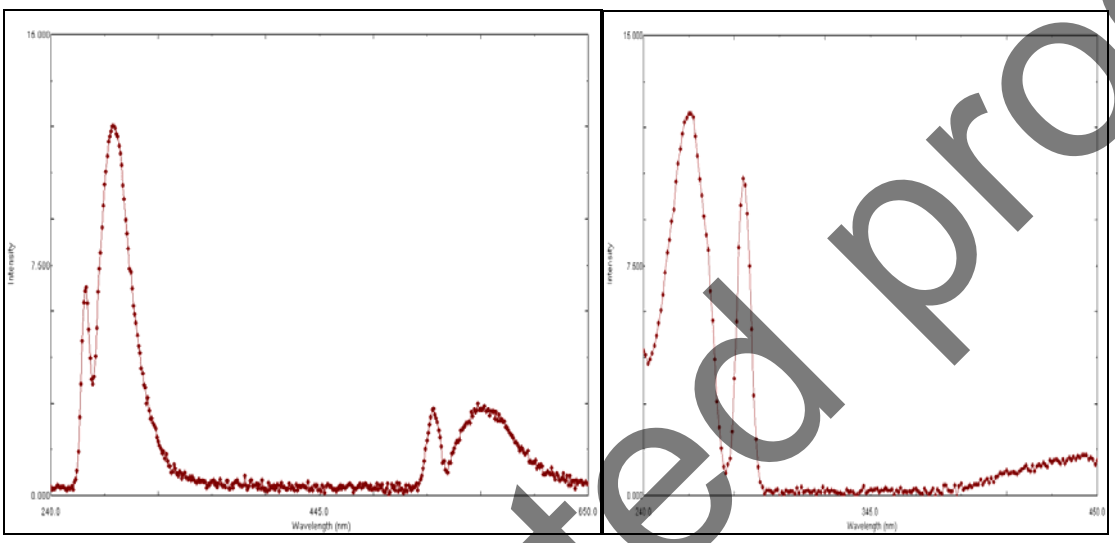

**Figure 3.** Emission (294 nm) and excitation (256 nm) spectra of SFS (10 µg/mL) in distilled water

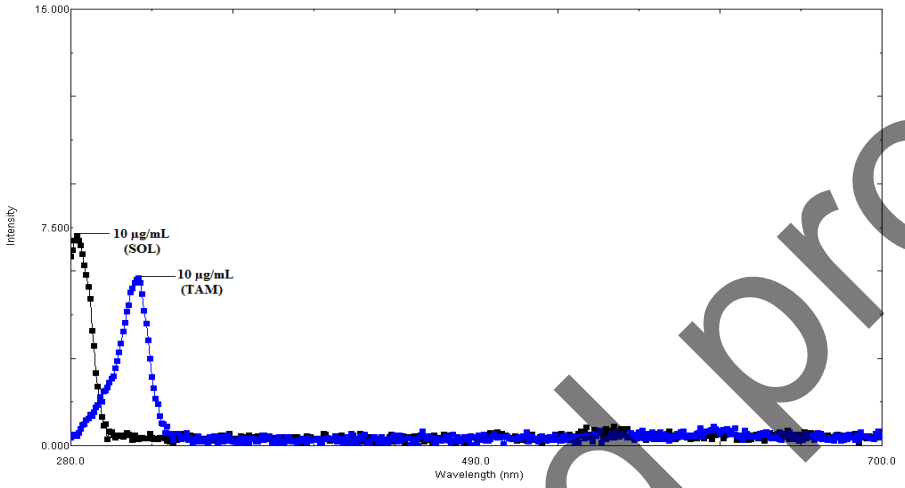


**Figure 4.** Zero-order synchronous overlaid emission spectra of TMH (10 µg/mL) and SFS (10 µg/mL) in distilled water

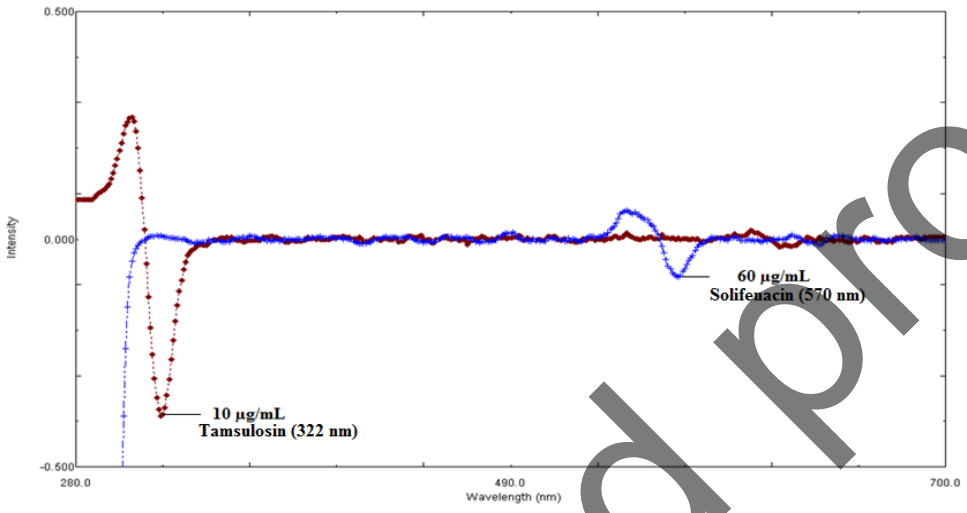


**Figure 5.** First-order synchronous overlaid emission spectra of TMH (10 µg/mL) and SFS (60 µg/mL) in distilled water

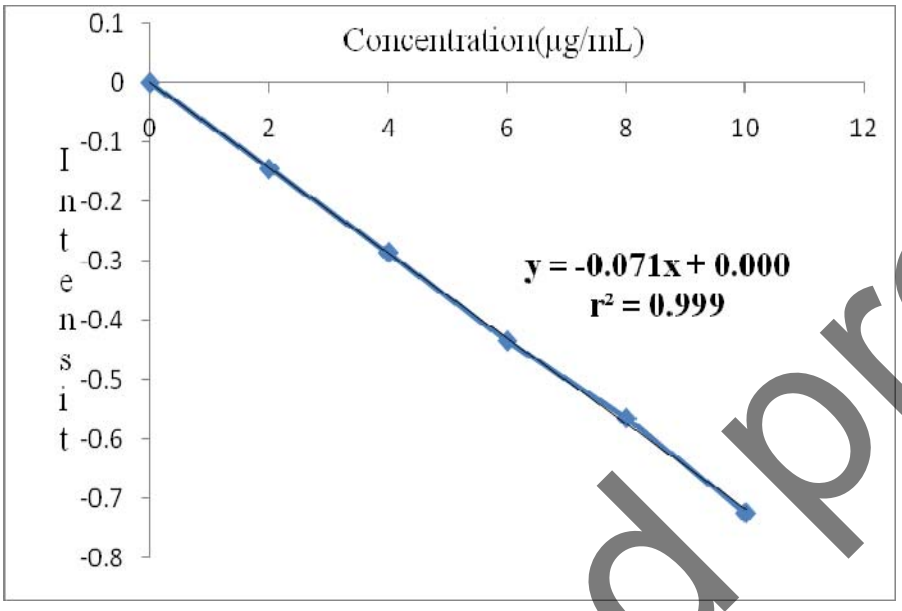


**Figure 6.** Linearity plot of TMH (2-10 µg/mL) in distilled water at 322 nm

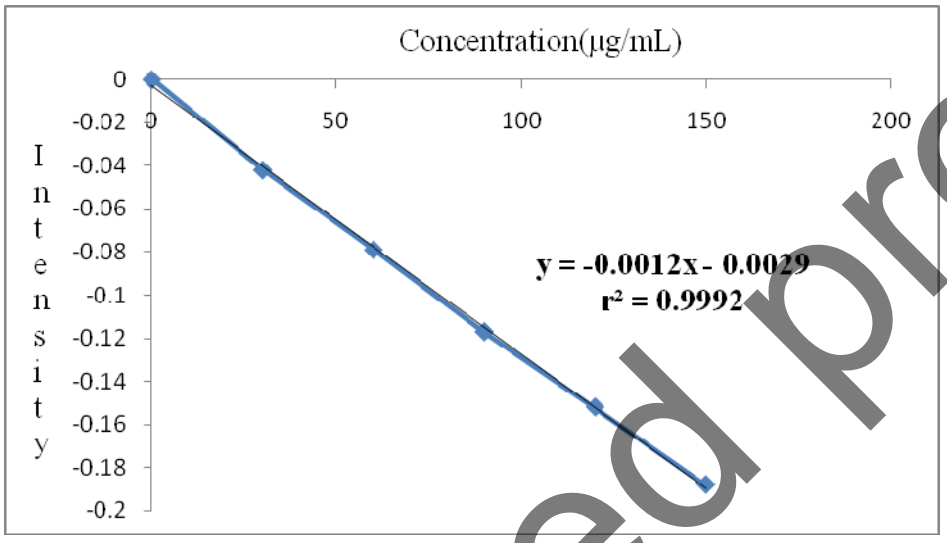


**Figure 7.** Linearity plot of SFS (30-150 µg/mL) in distilled water at 570 nm

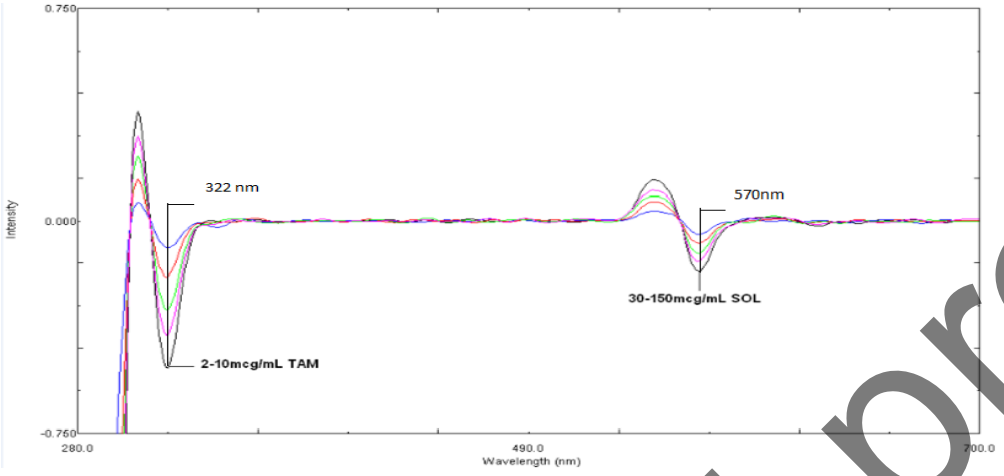


**Figure 8.** First-derivative linearity spectra of TMH (2-10 µg/mL) and SFS (30-150 µg/mL)

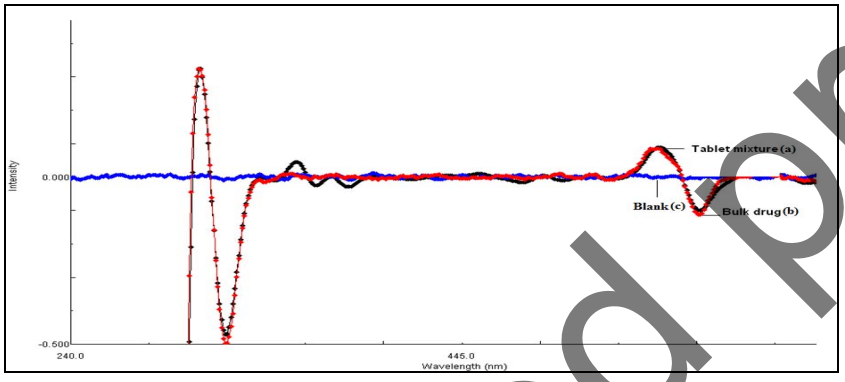


**Figure 9.** First-order synchronous overlaid spectra of commercial formulation (a), synthetic mixture (b) and blank (c)

**Table 1.** Accuracy data of the analytical method

| Analyte | Recovery level % | Amount of standard (µg/mL) | Conc of sample spiked (µg/mL) | Total amount (µg/mL) | Amount recovery (AM±SD) (µg/mL) (n=3) | % Recovery | RSD % |
|---|---|---|---|---|---|---|---|
| **TMH** | 80 | 0.4 | 0.32 | 0.72 | 0.75±0.012 | 104.16 | 1.60 |
| | 100 | 0.4 | 0.4 | 0.8 | 0.91±0.007 | 113.75 | 0.76 |
| | 120 | 0.4 | 0.48 | 0.88 | 0.90±0.011 | 102.27 | 1.20 |
| **SFS** | 80 | 6.0 | 4.8 | 10.8 | 10.52±0.078 | 97.22 | 0.74 |
| | 100 | 6.0 | 6.0 | 12.0 | 12.12±0.026 | 101.0 | 0.21 |
| | 120 | 6.0 | 7.2 | 13.2 | 13.00±0.054 | 98.48 | 0.41 |

**Table 2.** Data for precision of the analytical method

| Drug | Concentration (µg/mL) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|---|
| | | Concentration estimated (µg/mL) (AM ± SD) (n=3) | RSD % | Concentration estimated (µg/mL) (AM ± SD) (n=3) | RSD% |
| **TMH** | 2 | 2.01 ± 0.005 | 0.24 | 2.12 ± 0.004 | 0.18 |
| | 6 | 6.21 ± 0.012 | 0.19 | 6.05 ± 0.022 | 0.36 |
| | 10 | 10.07 ± 0.025 | 0.24 | 10.05 ± 0.034 | 0.33 |
| **SFS** | 30 | 31.28 ± 0.064 | 0.20 | 31.02 ± 0.046 | 0.40 |
| | 90 | 91.28± 0.029 | 0.03 | 90.15 ± 0.084 | 0.09 |
| | 150 | 148.08± 0.226 | 0.15 | 150.22 ± 0.152 | 0.10 |

**Table 3.** System suitability parameters of TMH and SFS

| **Parameter** | **TMH** | **SFS** |
|---|---|---|
| Emission wavelength (nm) | 322 | 570 |
| Beer's Law Limit (µg/mL) | 2-10 | 30-150 |
| Slope (m) | -0.0720 | -0.0010 |
| Intercept (c) | 0.0002 | -0.0030 |
| Correlation coefficient ($r^2$) | 0.9992 | 0.9996 |
| LOD (µg/mL) | 0.210 | 2.64 |
| LOQ (µg/mL) | 0.639 | 8.0 |
| Regression equation | y = -0.07196X+0.00024 | y = -0.00125x-0.00290 |

**Table 4.** Assay data of TMH and SFS in marketed formulation

| Formulation | Drug | Label claim (mg) | Amount found (mg) (AM ± SD) (n=3) | % Assay | RSD% |
|---|---|---|---|---|---|
| Vesomni | TMH | 0.4 | 0.38 ± 0.006 | 95.0 | 1.57 |
| | SFS | 6 | 6.21 ± 0.024 | 103.5 | 0.38 |