



Quantification of Tamsulosin Hydrochloride and Solifenacin Succinate by Discriminative Derivative Synchronous Emission Spectroscopy

Tamsulosin Hidroklorür ve Solifenasin Süksinatın Senkronize Türev Emisyon Spektrometrisi Yöntemiyle Eşzamanlı Miktar Tayinleri

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ABSTRACT

Objectives: The present study was undertaken with the objective to develop and validate a simple spectrofluorimetric method for the simultaneous quantification of tamsulosin hydrochloride and solifenacin succinate.

Materials and Methods: First-derivative synchronous spectrofluorimetry was attempted for the simultaneous quantification of the analytes. 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).

Results: 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 the ICH guidelines. The assay values in the 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 claim.

Conclusion: The proposed synchronous analytical method can be employed for routine quality control analysis of tamsulosin hydrochloride/solifenacin succinate in tablet dose forms.

Key words: Tamsulosin hydrochloride, solifenacin succinate, synchronous spectrofluorimetry, method validation

ÖZ

Amaç: Bu çalışmanın amacı tamsulosin hidroklorür ve solifenasin süksinatın aynı anda miktar tayininin yapılabilmesi için basit bir senkronize spektrofiorimetri yöntemi geliştirmek ve valide etmektir.

Gereç ve Yöntemler: Bahsedilen analitlerin aynı anda miktar tayinlerinin yapılabilmesi için birinci türev senkronize spektrofiorimetri yöntemi kullanılmıştır. Tamsulosin hidroklorür 322 nm de ölçüm yapılarak (bu dalga boyu solifenasin süksinatın sıfır kesim noktasıdır) solifenasin süksinat ise 570 nm de ölçüm yapılarak (bu dalga boyu tamsulosin hidroklorürün sıfır kesim noktasıdır) miktar tayinleri gerçekleştirilmiştir.

Bulgular: Kalibrasyon eğrileri tamsulosin hidroklorür için 2-10 µg/mL, solifenasin süksinat için ise 30-150 µg/mL konsantrasyon aralığında hazırlanmıştır. Geliştirilen yöntemle; ICH kılavuzlarına göre hesaplanan doğrusallık, seçicilik, doğruluk, kesinlik ve LOD ve LOQ değerleri kullanılarak başarılı sonuçlar elde edilmiştir. Önerilen yöntemle yapılan analiz sonuçları ticari formülasyon içerisinde tamsulosin hidroklorürün %95.0 solifenasin süksinatın ise %103.5 oranında bulunduğunu göstermiştir. Bu sonuçlar preparatın üzerinde belirtilen değerler ile iyi bir uygunluk göstermektedir.

Sonuç: Önerilen analitik yöntemin tablet dozaj formlarında tamsulosin hidroklorür ve solifenasin süksinatın rutin kontrol analizlerinde kullanılabileceği anlaşılmıştır.

Anahtar kelimeler: Tamsulosin hidroklorür, solifenasin süksinat, senkronize spektrofiorimetri, yöntem validasyonu

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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 dose form of these drugs was used in the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia.³ The structures of both TMH and SFS are depicted in Figure 1.

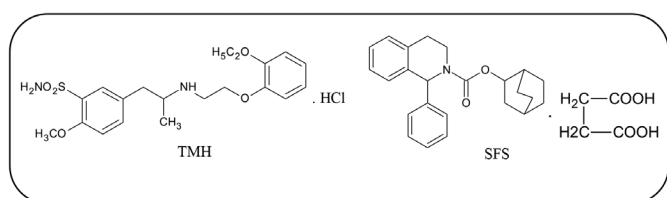


Figure 1. Structures of TMH and SFS

TMH: Tamsulosin hydrochloride, SFS: Solifenacin succinate

A survey of the literature on TMH revealed several methods, such as visible spectrophotometric methods using Folin reagent, sodium nitroprusside-acetaldehyde⁴ and bromophenol blue⁵, ultraviolet (UV) spectrophotometry⁶, fluorimetry using a sodium dodecyl sulphate micellar system⁷ and methanol⁸. 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 green¹¹ have also been described for the analysis of SFS. Spectrophotometric methods are considered to be inappropriate for simultaneous analysis of drugs in multi-component dose forms 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 reverse-phase high-performance LC (RP-HPLC).¹² However, chromatographic methods are complex with costly instrumentation, require skilled operators, and expensive solvents limit the application of simultaneous quantification of above the drugs. Spectrofluorimetry has assumed a special status in drug analysis due to its greater sensitivity and specificity than spectrophotometry using two wave-lengths, excitation, and emission. In accustomed fluorescence, an emission spectrum is attained by scanning the monochromator of emission at various wavelengths (λ_{em}), at an appropriate excitation wavelength (λ_{ex}), but in synchronous fluorescence scans both monochromators vary simultaneously. When accustomed spectra are over-lapped, the synchronous technique is used to reduce the extent of overlapping. Derivative spectrofluorimetry is a powerful approach for the 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 form. If SFS is diluted, the quantification of second drug (TMH) analysis may become difficult. Therefore, 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 International Conference on Harmonization (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 needed to be endeavored by copacetic modification, so a 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

A stock solution containing 1000 $\mu\text{g}/\text{mL}$ of drug was prepared by dissolving 10 mg of TMH/ SFS in 10 mL of distilled water separately. A 1-mL aliquot of stock solution was diluted up to 10 mL with distilled water to attain an end concentration 100 $\mu\text{g}/\text{mL}$ of each drug.

Analytical method development

Scanning of drugs by conventional-spectrofluorimetry

TMH or SFS (100 $\mu\text{g}/\text{mL}$) was diluted to 10 mL with distilled water to attain an end concentration of 10 $\mu\text{g}/\text{mL}$. Spectrofluorimetry mode was used for the scanning of the sample against distilled water to obtain the excitation and emission wavelengths, the anion excitation wavelength was fixed, and solutions were further scanned to obtain 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 are shown Figure 2 and 3. Though the excitation and emission wavelengths were disparate for both the drugs, and although the fluorescence spectra exhibited protruding intensity, conventional spectrofluorimetry 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 needed 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

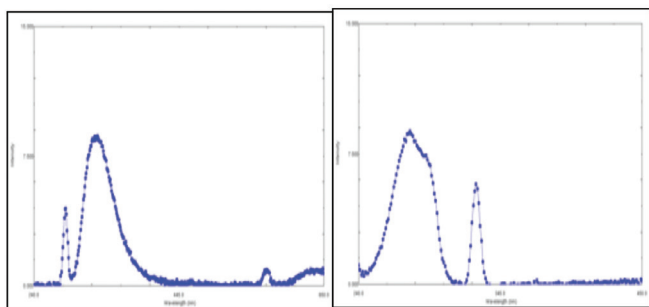


Figure 2. Emission (328 nm) and excitation (292 nm) spectra of TMH (10 µg/mL) in distilled water

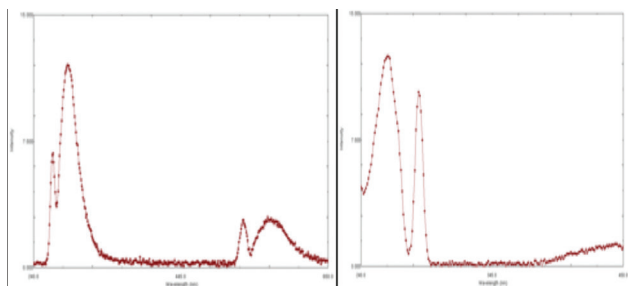


Figure 3. Emission (294 nm) and excitation (256 nm) spectra of SFS (10 µg/mL) in distilled water

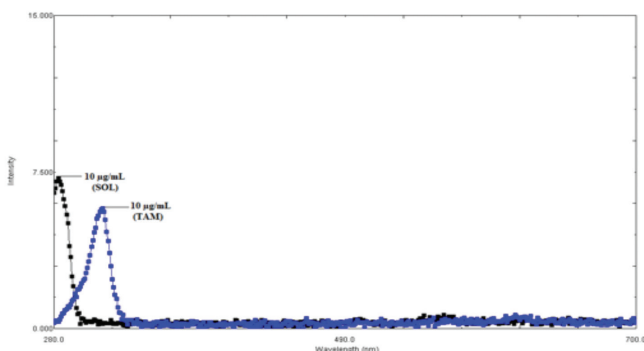


Figure 4. Zero-order synchronous overlaid emission spectra of TMH (10 µg/mL) and SFS (10 µg/mL) in distilled water

TMH: Tamsulosin hydrochloride

350-700 nm. Zero crossing criteria 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 (LOD), and limit of quantification (LOQ) as per the ICH guidelines to prove that the analytical method could be useful for the quality control of both drugs.

Linearity

The standard concentrations of TMH (2-10 µg/mL) and SFS (30-150 µg/mL) were quantified using first-derivative synchronous spectrofluorimetry, and fluorescence intensities were recorded and a 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 a synthetic mixture of standard solutions. The 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 a 10 mL volumetric flask, the volume was made up to the mark with distilled water to obtain a 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 components of the formulation could generate a response or a read with an emission band similar to the drugs.

Accuracy

Acquisition studies were conducted using a 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 the label claim, and further subjected to the contemplated analytical method, and anion 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 are reported in terms of RSD%.

LOD and LOQ

The LOD and LOQ for the proposed method were performed on samples containing very low concentrations of analyte (TMH and SFS) as per the ICH guidelines and calculated based on the 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 the 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 a 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 the 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 in alliance with derivative techniques is expedient in the locution of sensitivity, spectral discrimination, and decisive identification of chemical species in multi-component analysis. TMH molecules contain two aromatic rings, namely ethoxy phenyl, methoxy benzene-1-sulfonamide and tetrahydroisoquinoline rings, in which greater numbers of π electrons were available to put on view of fluorescence. Different solvent systems were tested in furtherance of the best predicaments, such as the solubility and fluorescence activity of both 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 did not permit the simultaneous determination of both drugs due to this overlap problem, which was overcome by using first-derivative spectrum (Figure 5), revealing that TMH gave zero intensity at 570 nm, whereas SFS gave a significant derivative response, likewise, SFS gave zero intensity at 322 nm, but TMH gave a significant derivative response. Therefore, 322 and 570 nm were elected for the determination of TMH and SFS, respectively, in the synthetic mixture and tablet forms.

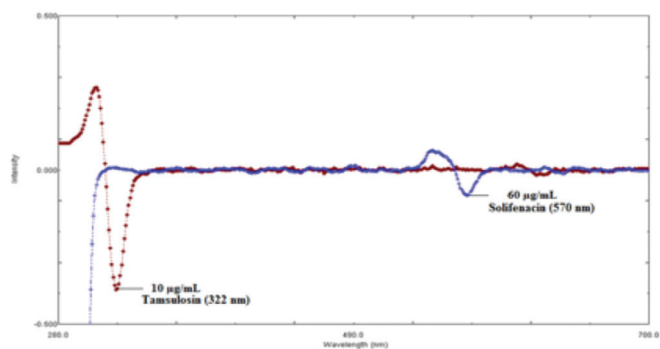


Figure 5. First-order synchronous overlaid emission spectra of TMH (10 µg/mL) and SFS (60 µg/mL) in distilled water

Analytical method validation

Linearity

The calibration curve for TMH and SFS was recorded at 322 and 570 nm, respectively. The linearity was evaluated by the least square regression method. The regression analysis of the calibration curves is shown in Figures 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 can be observed that with the increase in TMH concentration, the derivative response at 322 nm increased. Similarly, the derivative response for SFS at 570 nm was proportional to its concentration.

Specificity

The 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 the commercial formulation and the synthetic mixture were found to be similar. Specificity of the method is shown in Figure 9, which revealed that there was no interference from the excipients in the tablets with derivative response of either drug (TMH and SFS) at their respective analytical wavelengths (322 and 570 nm). Hence, the method was proved to be specific.

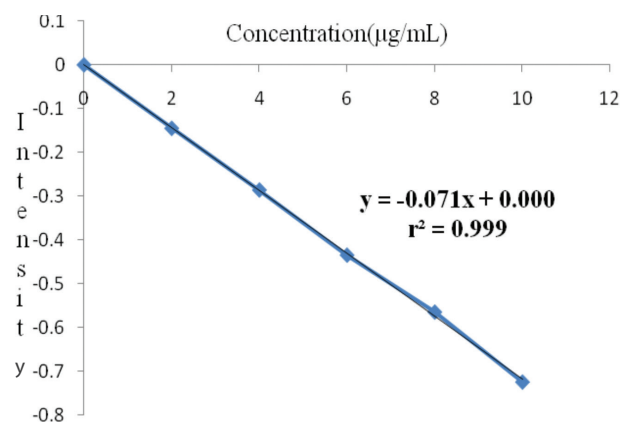


Figure 6. Linearity plot of TMH (2-10 µg/mL) in distilled water at 322 nm

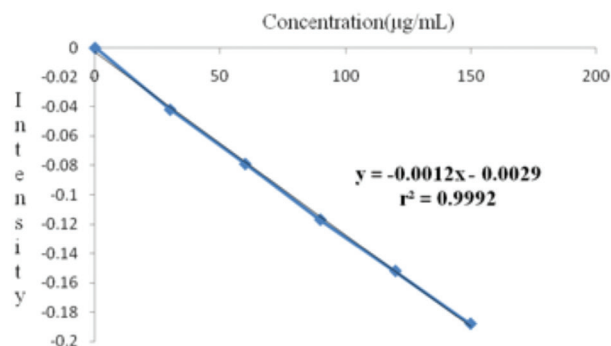


Figure 7. Linearity plot of SFS (30-150 µg/mL) in distilled water at 570 nm

Accuracy

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

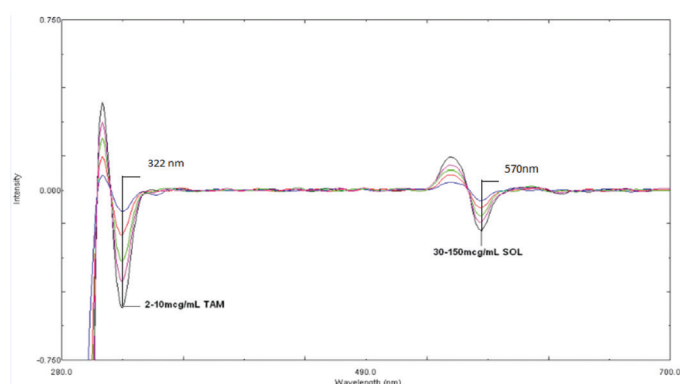


Figure 8. First-derivative linearity spectra of TMH (2-10 µg/mL) and SFS (30-150 µg/mL)

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 are summarized in Table 2. The RSD% of repeatability was less than 2.0 for both drugs, indicating good precision of the developed method.

LOD and 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

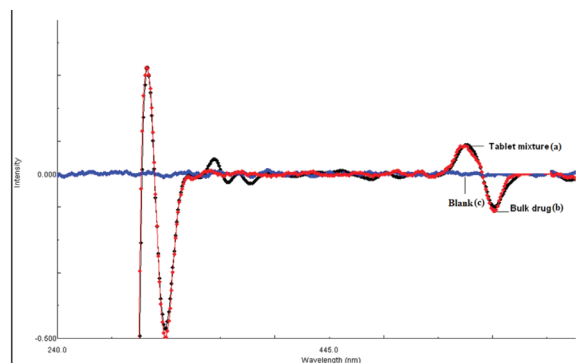


Figure 9. First-order synchronous overlaid spectra of commercial formulation (a), synthetic mixture (b) and blank (c)

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

SFS: Solifenacin succinate, TMH: Tamsulosin hydrochloride, RSD: Relative standard deviation

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

SFS: Solifenacin succinate, TMH: Tamsulosin hydrochloride, RSD: Relative standard deviation

Table 3. System suitability parameters of TMH and SFS

Parameter	TMH	SFS
Emission wavelength (nm)	322	570
Beer's law limit ($\mu\text{g/mL}$)	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 ($\mu\text{g/mL}$)	0.210	2.64
LOQ ($\mu\text{g/mL}$)	0.639	8.0
Regression equation	$y = -0.07196X + 0.00024$	$y = -0.00125x - 0.00290$

SFS: Solifenacin succinate, TMH: Tamsulosin hydrochloride, LOD: Limit of detection, LOQ: Limit of quantification

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

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

RSD: Relative standard deviation, SFS: Solifenacin succinate, TMH: Tamsulosin hydrochloride

$\mu\text{g/mL}$ and SFS was found to be 2.640 and 8.0 $\mu\text{g/mL}$. The summary of the system suitability parameters is represented in Table 3. The results obtained from validation evidenced that the proposed method was scientifically sound.

Assay of drugs from commercial tablets

The accuracy of the proposed method was evaluated through 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 \pm 0.006 and 6.21 \pm 0.024 mg, respectively. The percentage assay in the 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 the 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 was successfully adopted for the assay of TMH and SFS and the results were found to be in good agreement with their

respective label claims, which suggested that there was no interference of formulation excipients in the estimation. The contemplated spectrofluorimetric method was found to be superior because of its high specificity, spectral discrimination, economic, eco-friendly, and readily available solvent and lack of extraction procedure. These advantages endorse that the developed method can be habitually employed in quality control for simultaneous analysis of TMH and SFS in tablet dose forms.

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Conflict of Interest: No conflict of interest was declared by the authors.

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